Mechanisms underlying apathy in health and Parkinson's disease

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Abstract

Apathy or lack of motivation is increasingly recognized to be a major factor affecting quality of life and prognosis in many neurological conditions. It is particularly prevalent in Parkinson's disease, impacting on every disease stage, including *de novo* cases, and has been reported to affect up to 70% of cases. Despite the pervasiveness of apathy, challenges remain in its detection, clinical assessment and treatment. Several lines of evidence have implicated fronto-striatal reward related neural pathways in the genesis of apathy but the precise processes remain to be fully explained.

This thesis examines the potential mechanisms of apathy using Parkinson's disease as a model to study the condition. Novel oculomotor tasks that used eye movement and pupillary responses were developed to help assess if insensitivity to incentives could be an underlying component of apathy. This was examined in healthy young and elderly participants as well as in patients with Parkinson's disease. Patients were tested both ON and OFF their normal dopaminergic medication so that the effect of dopamine could be assessed and the association with apathy determined. This was also performed in a pharmacological study in young participants with the use of Haloperidol, a dopaminergic D2-selective antagonist.

Insensitivity to rewards modulated by dopamine was regarded to be a contributory mechanism of apathy in Parkinson's disease and also applicable to general mechanisms of motivation in healthy populations.

Contents in Brief

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1. General Introduction

1.1 Introduction

Apathy, often considered to be a disorder characterized by lack of motivation, is a common neuropsychiatric syndrome that is increasingly appreciated to be a major problem in many neurodegenerative conditions. These include Parkinson's disease (PD) (Chaudhuri and Schapira, 2009; den Brok *et al.*, 2015), Alzheimer's disease (Landes *et al.*, 2001a), vascular dementia, small vessel disease (Hollocks *et al.*, 2015), frontotemporal dementia and amyotrophic lateral sclerosis (Lillo *et al.*, 2012; Mioshi *et al.*, 2014; O'Callaghan, Bertoux and Hornberger, 2014; Bertoux *et al.*, 2015). Its importance has become more apparent over the past two decades as awareness of its clinical impact has grown (Duffy, 2000; Landes *et al.*, 2001a). Apathy also poses a significant health burden for patients and their carers. It is associated with several adverse outcomes and reduced quality of life (Benito-León, Cubo and Coronell, 2012; Hollocks *et al.*, 2015), yet its underlying mechanisms are poorly understood. Understanding is further complicated by a lack of objective measures and overlap between apathy and other neuropsychiatric illness such as depression and anhedonia which often occur concurrently (Kaji and Hirata, 2011; Marianna Amboni, Gabriella Santangelo, 2015).

1.2 Epidemiology of apathy in Parkinson's disease

Apathy is particularly prevalent in Parkinson's disease (Chaudhuri and Schapira, 2009; den Brok *et al.*, 2015). The reported prevalence in PD ranges from 7% to 70% (Pluck, 2002; van Reekum, Stuss and Ostrander, 2005; Aarsland, Marsh and Schrag, 2009; Dujardin and Defebvre, 2012; Sinha, Manohar and Husain, 2013). This large variability is likely to be due to differences in the assessment tools used and might also reflect the heterogeneity of the PD population. On average approximately 40% of PD cases (den Brok *et al.*, 2015) and up to one quarter of newly diagnosed drug naïve patients (Pedersen *et al.*, 2010) are affected. Dividing PD patients further into phenotypic subgroups demonstrates that both tremor-dominant and akinetic-rigid variants of PD suffer from apathy. The rates however are not equal; akinetic-rigid phenotypes are worse affected (Moretti *et al.*, 2012) and the addition of apathy

contributes to the poor disease progression normally observed in this subpopulation. Age is also an associated factor: patients with apathy tend to be older (den Brok *et al.*, 2015) and those given a PD diagnosis at an older age may also have increased risk of developing the disorder (Sinha, Manohar and Husain, 2013).

Other demographic differences such as gender, disease duration and Hoehn and Yahr scores (which index disease severity) do not seem to have a definite association with apathy but increased motor severity is related (den Brok *et al.*, 2015). PD cases with worse motor disease progression at 4 year follow up are more likely to develop apathy (Pedersen *et al.*, 2009). Importantly, the evidence suggests that this motor association is not a secondary psychological reaction to the physical disability, but instead is linked to the severity of neurodegeneration (Pluck, 2002), implying distinct pathological processes in the development of apathy.

1.3 Theoretical framework of goal directed behaviour

Given the heterogeneity of PD and overlap of apathy with other neuropsychiatric conditions there are likely to be a range of different underlying mechanisms leading to reduced motivation. It is unlikely that there is one simple cause. As a result, more targeted and personalized objective methods for the diagnosis and treatment of apathy are needed. Surprisingly, despite the clinical significance, the mechanisms underlying apathy are poorly understood. Apathy is defined as a reduction of motivation due to decreased goal directed behaviours (Marin, 1991), therefore understanding the processes of goal directed behaviour is important in establishing the mechanisms of apathy.

Theories of goal directed behaviour conceptualise the processes underpinning motivation as a sequence of stages, by which an internal state is translated into the completion of a goal through an action (Schultz, 1999). This process may occur sequentially, however it is also possible that several components occur in parallel. Dysfunction at any one or more of these stages might lead to the manifestations of apathy, and also provide us with discrete ‘compartments’ to probe the mechanisms underlying the syndrome.

It is useful to examine goal directed behaviours within the broader framework of decision-making. Decision making involves integration and evaluation of sensory inputs with information about the cost and benefit of potential actions, including rewarding and aversive outcomes, and learning from previous experiences (Fellows, 2004). Therefore, a simple model of goal directed behaviour includes identification of options for behaviour, evaluation of these options and then execution of an action based on the choice made (Baron, 2008) (see **Fig. 1.1**). These components will be considered in more detail below, and evidence for potential associations with apathy will be given for each.

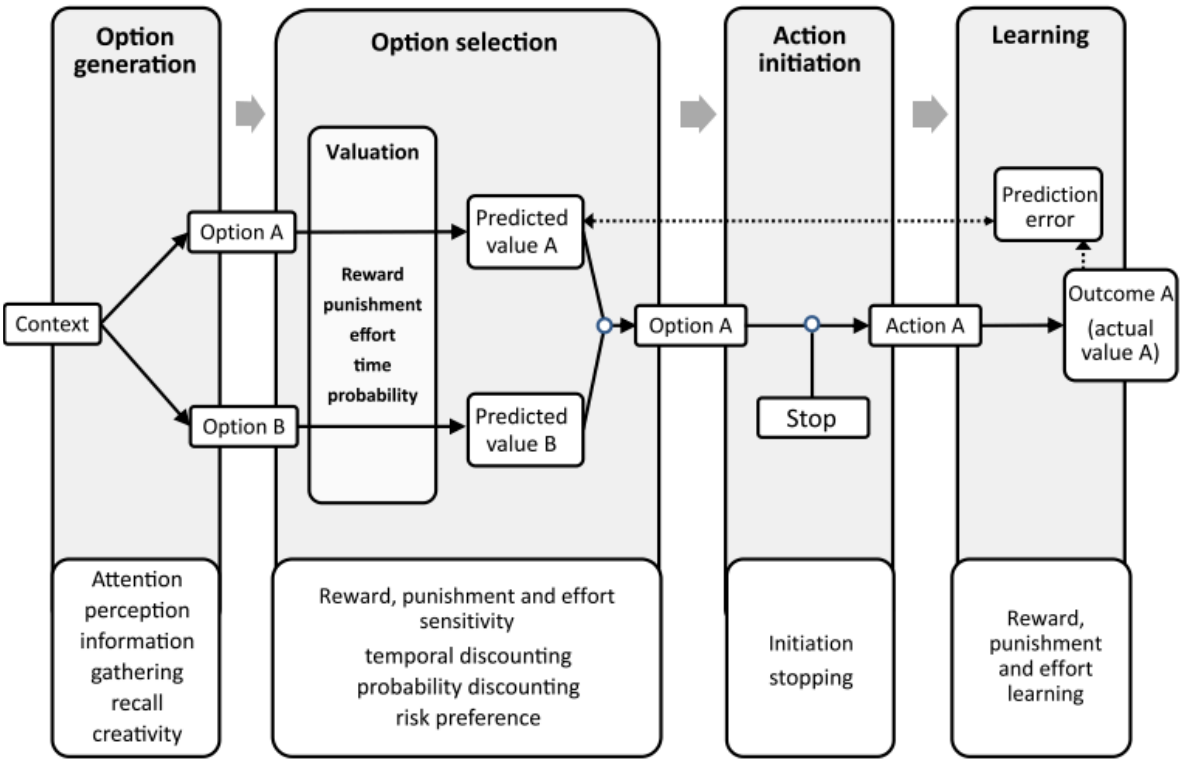

Figure 1.1 ***Schematic of potential processes involved in motivated goal directed behaviour.***

Attention and perceptual mechanisms in the environment are used to generate possible behavioural options. These are then evaluated based on features including predicted reward and punishment. The chosen option is then linked to an appropriate action, and finally the outcomes of motivated behaviours are compared with predictions made prior to the action and learned from. Adapted from Sinha et al (2013).

Option generation

The initial stages of goal directed behaviours might be considered to be the generation of options for behaviour. Before any goal can be achieved, the choice of behaviours needed to obtain a goal must first be identified or generated. These options are produced through assimilation of information in the environment as well as an individual's creativity and perceptual processes (Sinha, Manohar and Husain, 2013). If humans or animals do not produce potential alternatives for action, then a lack of self-initiated behaviour might occur, which in turn might manifest as apathy (Dujardin *et al.*, 2007). Option generation in the context of goal directed behaviour is seldom studied directly. However, some insights can be obtained from performance on fluency tests used in clinical neuropsychological assessment. Examples include verbal fluency (Thurstone, 1938) where participants must generate as many words as possible in a given time period beginning with a certain letter, or design fluency (Jones-Gotman and Milner, 1977) where as many drawings as possible must be produced which fit specific rules. These processes are impaired in patients with damage to frontal-dorsomedial brain areas, where reductions in initiation and sustaining of responses are evident (Stuss, 2011) and may therefore also be important in apathetic states.

Goal evaluation

Once options are generated, evaluation of the preferred goal is required. Many factors might influence the *value of a goal,* including how rewarding or aversive it might be, the likelihood of being able to achieve it, the timing of outcomes and the effort required to obtain it (Rangel, Camerer and Montague, 2008). This encapsulates the *utility* of the goal and is highly variable between individuals, potentially reflective of differences in motivation level (Salamone *et al.*, 2016). In animal studies, value is defined in terms of how much *effort* is willing to be expended in order to obtain an outcome (Walton *et al.*, 2006). Animals and humans tend to avoid effortful behaviours, which are perceived as aversive in nature, and choose options which minimize effort (Salamone *et al.*, 2007; Croxson *et al.*, 2009; Kool, Mcguire and Botvinick, 2010; Chong *et al.*, 2017; Morel, Ulbrich and Gail, 2017).

Walton *et al* (2006) demonstrated this behaviour in rats using a T-maze study where effort was operationalized as the number of lever presses needed to obtain a reward (Walton *et al.*, 2006). When rats were given a high reward option in one arm of the maze compared to

a low reward option in the other, animals switched their preferences away from higher rewards as the effort to obtain them increased. Therefore, when deciding whether to perform a goal, the value of potential rewards is weighed against the amount of effort required to obtain them, a process referred to as *effort discounting* (Botvinick Matthew, Huffstetler S, 2009).

Effort discounting has been investigated in humans using computerized tasks. Participants are normally presented with different monetary incentives and the effort required to obtain them is dependent on the force they exert using handgrip devices. One such study by Schmidt *et al* (2008) indicated that patients with apathy following lesions of the basal ganglia, produce less effort force modulation in response to different rewards offered when compared to controls (Schmidt *et al.*, 2008). This suggested that apathy was associated with normal valuation of reward but increased sensitivity to effort. Another study that used a similar task to index effort in young participants revealed that reduced willingness to exert effort also correlated with apathy level (Bonnelle, Veromann, *et al.*, 2015).

Similarly, from behavioural work in PD patients, Chong *et al* (2015) demonstrated that when given the option, patients engage less in choices that required larger effort compared to controls for the same amount of reward (Chong *et al.*, 2015). Interestingly however, when ON dopaminergic medication, the amount of effort PD patients were willing to expend for rewards increased, reflecting enhanced motivated behavior in the presence of increased dopaminergic stimulation. Changes in sensitivity to reward have been linked to patients with basal ganglia strokes using tasks that evaluate reaction time differences towards various reward cues. Patients who had slower responses for rewards also had greater apathy self-report scores (Rochat *et al.*, 2013), again demonstrating a potential link between motivation and incentive evaluation. The findings from these studies demonstrate that reward and effort sensitivity are key components of goal directed behaviour and may also be modulated by dopamine.

As discussed, the evaluation of incentives is vital in decision-making. However, the degree to which rewards are valued also depends on an individual's *reward sensitivity*. Reward sensitivity is the extent to which motivation is modulated by a change of reward, regardless of any effort requirement (Bonnelle, Veromann, *et al.*, 2015). Decision-making

tasks are able to assess the balance between reward and effort but do not allow direct evaluation of reward sensitivity independent of effort. One potential way of monitoring reward sensitivity may be through the use of objective physiological changes to incentives. For example, galvanic skin responses have been used to reflect affective evaluation of monetary incentives through autonomic sympathetic arousal (Schmidt *et al.*, 2008). Likewise, sensitivity to incentives has also been explored using pupil modulation to anticipated rewards in patients with PD (Manohar and Husain, 2015). Crucially however, no associations with apathy have been made using pupillometry and its potential for this will be discussed in depth later.

Evaluation of losses and aversive stimuli are also important for goal directed behavior. Indeed losses are considered to influence decisions and motivated behaviour even more than rewards (Kahneman and Tversky, 1983). Therefore, it is possible that apathy might also be due to decreased sensitivity towards incentives that are aversive in nature. Whether an individual is insensitive to reward, loss or both, might vary for many reasons. This might include individual differences in levels or type of neurotransmitter involvement or dysfunction in brain regions associated with aspects of motivation, including the ventral striatum, ventral tegmental area and prefrontal cortex (Tom *et al.*, 2007; Guitart-Masip *et al.*, 2011). The role of these regions is discussed further below.

Action initiation and learning

After the utility of a goal is established, an action to obtain it needs to be initiated and energy expended. In a study of patients with bilateral striato-pallidal damage which resulted in behavioural apathy, a disconnect was revealed between motor output and reward evaluation (Schmidt *et al.*, 2008). In this group, evaluation of potential stakes on offer using galvanic skin responses remained intact. However, less effort was subsequently produced to obtain the reward compared to controls. Similarly, in a brain imaging study of healthy people, those with higher apathy levels had reduced connectivity between areas considered to play a key role in incentive processing such as the anterior cingulate cortex (ACC) and regions associated with motor output including the supplementary motor area (SMA), suggesting that the translation of intentions into actions might also be contributory to behavioral apathy (Bonnelle, Manohar, *et al.*, 2015).

Action valuation and initiation of actions have been linked to limbic and associative pathways in the brain (Syed *et al.*, 2015). These include ventral regions of the basal ganglia, such as the nucleus accumbens (NAcc), and their projections to frontal, including anterior cingulate, brain regions (Obeso *et al.*, 2008). In animals, stimulation of these regions produces actions including attack, defence and feeding responses (Mogenson, Jones and Yim, 1980). However when these areas are lesioned, such actions are reduced (Walton, Bannerman and Rushworth, 2002). Apathy, may therefore involve dysfunction in the transformation of intention to act through to the action itself via limbic-motor interactions (Salamone and Correa, 2012; Sinha, Manohar and Husain, 2013). This will be discussed further in the next section under animal studies of motivation and neural correlates of apathy.

Finally, following the action, the outcome of the motivated behaviour is compared with prior predictions of potential results, providing a basis for learning for future goal directed behaviours (Sinha, Manohar and Husain, 2013). Using this conceptual framework, motivation can be considered in discrete components and, most importantly, the contribution of each can potentially be examined when they go wrong in disorders such as apathy (see **Fig. 1.1** for a summary).

1.4 Animal studies of motivation

Many important insights into the brain systems underlying goal directed behaviour have emerged from research in the animal literature. Lesions or dopamine depletion in the limbic system have been shown to reduce motivated behaviour, similar to that observed in humans with apathy (Chong and Husain, 2016). A key structure implicated in this process is the NAcc which is located in the ventral striatum and receives many dopaminergic inputs (Levy, 2006; Yager *et al.*, 2015). Animal studies that directly deplete dopamine within the NAcc have been conducted to aid understanding of processes involved in reward related behaviours and determine the mechanisms that incentivize actions. One example by Salamone *et al* (1994) examined the choices of rats following administration of the dopamine antagonist haloperidol as well as post-neurotoxic ablation of the NAcc (Salamone, Cousins and Bucher, 1994). Responses made appeared to be based on the *effort requirements* of the response. Effort was varied using physical barriers that needed to be overcome to obtain rewards. Animals who were dopamine depleted selected higher rewards less often when

effort required to obtain them was greatest. Correspondingly, animals also increased the selection of lower reward choices when alternative higher reward options were more effortful.

Many other animal studies have also examined the consequences of dopamine disruption (Salamone *et al.*, 1991; Salamone, Cousins and Bucher, 1994; Walton, Bannerman and Rushworth, 2002; Denk *et al.*, 2005; Walton *et al.*, 2006; Salamone and Correa, 2012; Cummings *et al.*, 2015), all typically demonstrating behaviour suggestive of reduced sensitivity to reward and often requiring greater incentives to act. Indeed, in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) NAcc lesioned monkeys, significant increases in apathetic behaviours were demonstrated, with dopaminergic inputs from the ventral tegmental area implicated (Brown *et al.*, 2012). Experiments like this demonstrate that dopamine can modulate reward and effort sensitivity, as well as probing the involvement of these processes in goal directed behaviour.

As discussed, brain mechanisms underpinning responses to potential rewards are crucial for goal directed behaviours. The neuronal mechanisms underlying this will now be considered. When a reward is obtained that is different from expected, a prediction error signal in the midbrain is produced (Schultz, 2007). Pioneering work by Schultz *et al (1986-present)*, using single cell recordings in primates, established that unexpected rewards are encoded by phasic activation of dopamine neurons, allowing subsequent learning from an action to occur (Schultz, Dayan and Montague, 1997). In monkey and rodent studies, if animals are unaware of choice outcomes but a reward is unexpectedly given, a positive error is produced in the prediction of reward. Subsequently, after learning the outcome of certain choices, when reward occurs that match an animal's prediction, no reward prediction error is demonstrated. If however, after learning that a stimulus predicts a reward and *no reward* is received, activity of dopamine neurons becomes depressed, known as a negative prediction error (Schultz, Dayan and Montague, 1997; Schultz, 2007). Importantly, such responses did not occur for *aversive stimuli* like air puffs or saline drops (Howe *et al.*, 2013), but they did occur for cues that predicted rewards even before they were received (Schultz, 2016). This result appears to associate dopamine neurons within the basal ganglia with *reward expectation*, a process crucial in goal directed behaviours (Schultz, 1999).

Recent work by Syed *et al*, (2015) confirmed that phasic dopamine processes are not just reliant on reward expectancy alone. In addition, goal directed motor *actions* are also necessary. The authors demonstrated that NAcc dopamine in rats increased for reward cues only when a movement was simultaneously initiated in order to obtain reward (Syed *et al.*, 2015) (**Fig. 1.2**). Dopamine release in the NAcc may therefore be contingent on initiation of motor actions and not only on reward evaluation. Thus, the degree to which action determines an outcome is likely to be important in goal directed behaviour and perhaps modulated through ventral striatal areas like the NAcc.

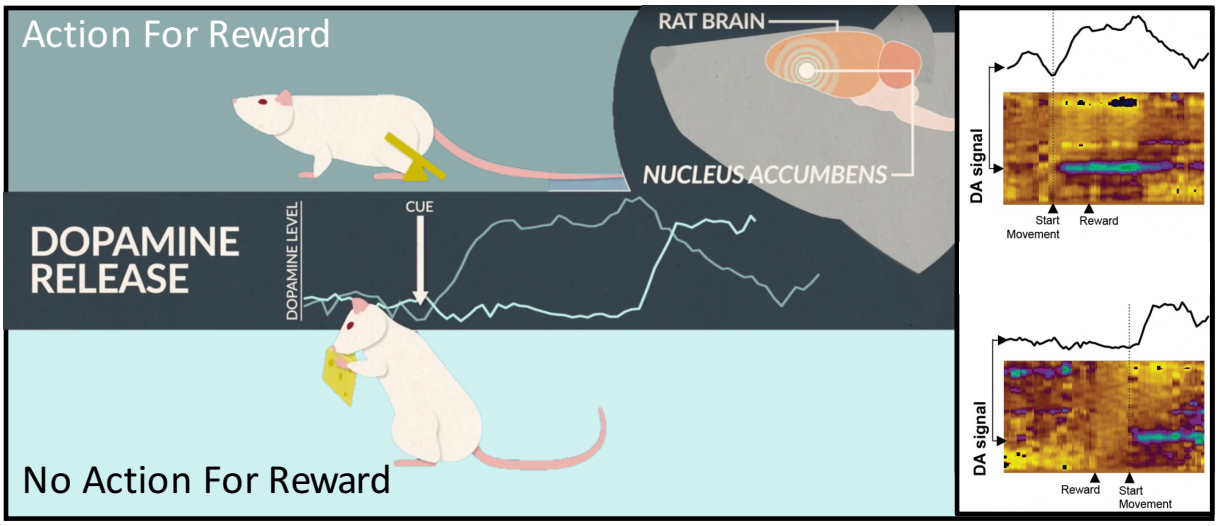

Figure 1.2. Action initiation in rodents modulates Nucleus accumbens dopamine encoding of future rewards. *Using cyclic voltammetry in rats, initiation of an action for reward by pressing a lever demonstrated increased dopamine signals in the nucleus accumbens at the time of reward cue onset. This was not the case when no action for the reward cue was made and only occurred subsequently when movements were eventually initiated. Adapted from Syed et al (2015).*

1.5 Neural correlates of apathy

A variety of brain imaging techniques have been used in the literature to examine changes associated with apathy in humans. Although it is becoming more recognized that a consistent anatomical basis for apathy across many neurological disorders might exist (Le

Heron, Apps. and Husain, 2017), studies that specifically examine apathy and PD will be mainly focused on here. Modalities including positron emission tomography (PET), functional MRI, structural and diffusion imaging will be covered and anatomical associations with apathy from lesions studies will also be explored.

To examine potential differences in metabolic function in those who suffer from apathy, a study by Robert *et al* (2014) used PET imaging in a cohort of PD patients undergoing subthalamic nucleus deep brain stimulation (STN-DBS) (Robert *et al.*, 2014). They found that patients with *pre*-operative reductions in ventral striatal metabolism were more likely to develop apathy *post*-operatively, suggesting that underlying striatal neuronal loss may be to blame. Similarly, the results of a PET study by Lawrence *et al* (2011), suggested that striatal dysfunction in apathy was related to reward. Using a task where monetary versus non-monetary outcomes were presented, the authors demonstrated that apathetic PD patients had reduced cerebral blood flow responses to incentives in the striatum as well as midbrain and frontal cortex (Lawrence, Goerendt and Brooks, 2011). These findings, along with other PET studies, appear to associate apathy with mesolimbic rather than nigrostriatal pathways (Thobois *et al.*, 2010; Dujardin and Defebvre, 2012) and highlight the importance of fronto-striatal brain areas in reward processing and motivated behaviour. They also suggest that reward value encoding might be an important function that becomes dysfunctional in PD patients with apathy.

Structural PD imaging studies have identified subcortical anatomical differences within the basal ganglia that further implicates striatal involvement in apathy (Carriere *et al.*, 2014). Carriere *et al* (2014), reported that patients with apathy have significant levels of atrophy within the NAcc (**Fig. 1.3**), which as seen from animal studies, is a key ventral striatal structure linked to reward related behaviour (Salamone *et al.*, 2007). Morphological shape changes in the NAcc were also found to correlate with apathy severity on an individual level. Therefore, potential imbalances in reward valuation due to ventral striatal degeneration might explain some of the behavioural manifestations of apathy found in PD.

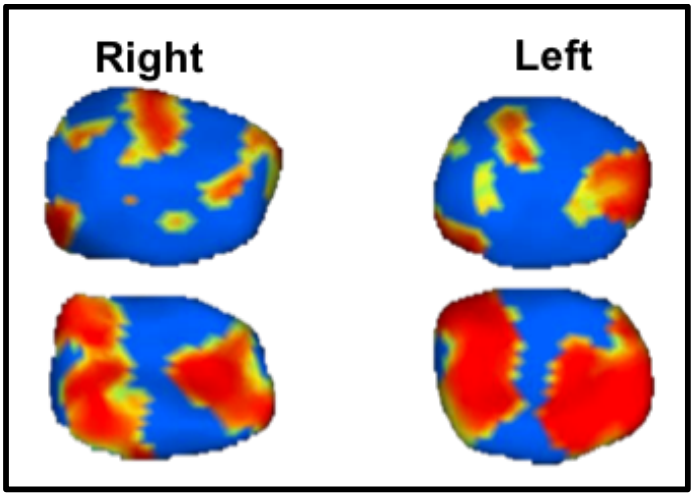

Figure 1.3. Subcortical shape analysis of the nucleus accumbens in PD.

Regions of inward deformation within the nucleus accumbens indicated in yellow and red (therefore presumed atrophy when comparing apathetic patients to healthy controls). This is superimposed on the mean shape of the nucleus accumbens from healthy and PD subjects, indicated in blue. Adapted from Carriere et al (2014).

At the cortical level, grey matter voxel-based morphology imaging in frontotemporal degeneration patients with apathy, has shown correlations with caregiver ratings of apathy and atrophy in fronto-striatal circuits (Lansdall *et al.*, 2017). However, in PD patients, grey matter volume in apathy has not been found to be reduced (Carriere *et al.*, 2014; Baggio *et al.*, 2015). Instead, associations with reduced resting state functional connectivity in limbic components of fronto-striatal pathways have been reported (Baggio *et al.*, 2015). These findings suggest connectivity related dysfunction might be an important factor in PD patients with apathy.

Exploration of the potential associations with connectivity further, a study by Bonnelle *et al* (2015) involving young participants, revealed that the degree of apathy on questionnaire scores, correlated with white matter connectivity within cingulum bundle tracts (Bonnelle, Manohar, *et al.*, 2015) (**Fig. 1.4**). This was reported to signify inefficient communication between areas of the brain involved with goal processing, including the cingulate cortex, and motor regions such as the supplementary motor area. As discussed in earlier sections, such a scenario might lead to dysfunction in circuits involved in the translation of intentions into actions, and thereby to apathy (Sockeel, 2006).

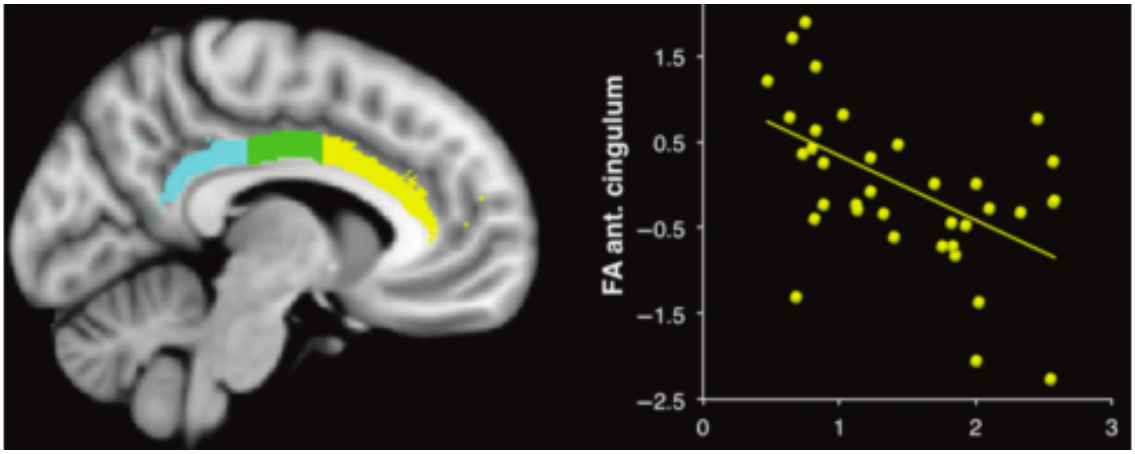

Figure 1.4. The association between the strength of structural connectivity and apathy severity in a young population.

A significant correlation in the anteriour cingulum white matter connectivity (yellow area) using fractional anisotropy (FA) with the degree of behavioural apathy on questionnaire scores of young healthy participants. Adapted from Bonnelle et al (2015).

Further evidence for dysfunction in converting motivation into motor output in apathy comes from findings on the functional organization of basal ganglia circuits. Through the use of imaging and animal tracer studies, functional domains within the striatum have been suggested (Tremblay *et al.*, 2015). Event related fMRI has associated ventral striatal areas such as the NAcc with motivational information such as reward cues, encoding incentive anticipation (Knutson *et al.*, 2001). While imaging during motor planning and execution of button press tasks has implicated more dorsal striatal structures including the caudate nucleus and putamen (Gerardin *et al.*, 2004). Thus, travelling anatomically through the striatum in a ventral to dorsal fashion seems to be associated with a gradient of different functions for goal directed behaviours (Tremblay *et al.*, 2015). More ventral areas are associated with motivation towards goals and reward anticipation, while dorsal regions appear to convert this into the motor output required to obtain it. The striatum may therefore act as an interface between motivation and action, with any dysfunction at this gateway resulting in apathetic behaviour (Mogenson, Jones and Yim, 1980; Groenewegen, Wright and Beijer, 1996; Tremblay *et al.*, 2015) (**Fig 1.5**).

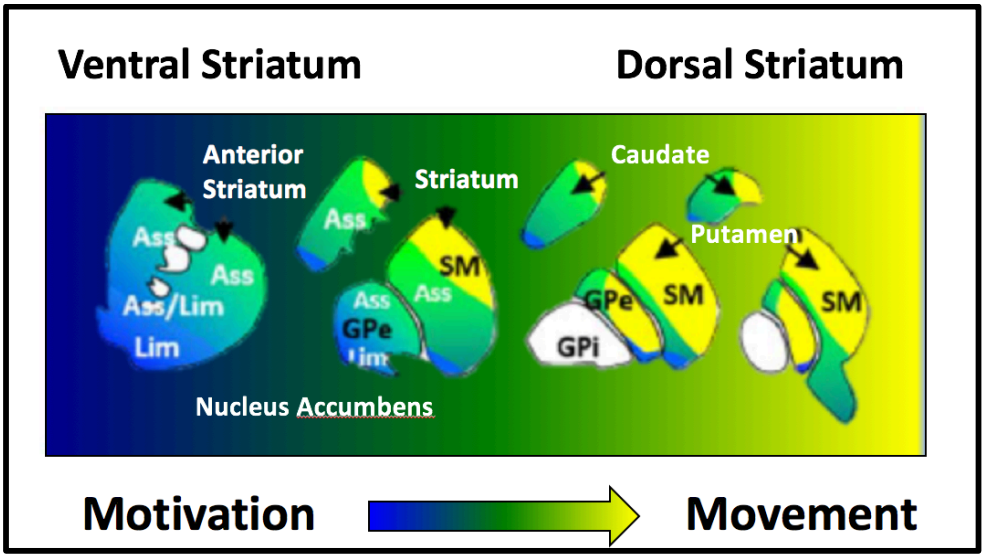

Figure 1.5. Illustration of the presumed functional gradient within the striatum.

Schematic representing a gradient of function through the striatum, with ventral areas more associated with dopaminergic neuron projections from the ventral tegmental area and associated with motivation, through to the dorsal striatum with substantia nigra inputs being more associated with movement and action. Adapted from Tremblay et al (2015).

Finally, the three-way relationship between fronto-striatal brain regions, apathy and incentive processing has also been suggested through clinical case reports of patients who have developed amotivated behavioural changes secondary to vascular infarcts. This includes akinetic mutism following large anterior cingulate lesions (Barris and Schuman, 1953) (**Fig. 1.6B-D**) and another case of profound apathy post bilateral basal ganglia strokes (Adam *et al.*, 2013) (**Fig. 1.6A**). Interestingly, the latter also developed reduced sensitivity to rewards as assessed using a behavioral oculomotor task which examined reaction time responses to monetary incentives. In this particular case, when a dopamine agonist, ropinirole, was administered, clinical improvements in motivation were reported as well as improvements in reaction time responses in anticipation for rewards (Adam *et al.*, 2013).

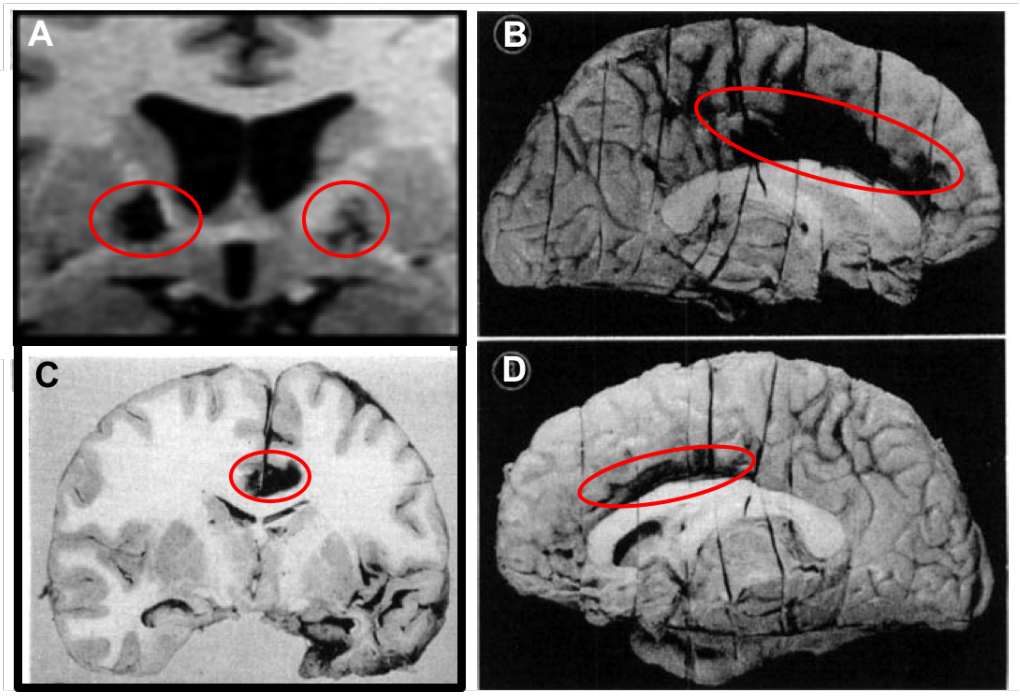

Figure 1.6. Clinical lesion studies of apathy following cerebrovascular events in patients.

A. Bilateral basal ganglia infarcts leading to profound apathetic behaviour and reduction in reward sensitivity on behavioural testing. Adapted from Adam et al (2012).

B. Bilateral anterior cingulate infarcts producing akinetic mutism in a previously motived individual. Adapted from Barris & Schumann (1953).

The clinical response to dopaminergic therapies in patients with apathy suggests that dopamine is necessary for reward evaluation and motivated behaviour. In PD, disparities in how fronto-striatal brain regions are affected by degenerative processes might lead to specific differences in the clinical manifestations of apathy (Levy, 2006). For example, the development of behavioural versus emotional or cognitive apathy (Stuss, Van Reekum and Murphy, 2000). Different types of clinical apathy will be discussed further in subsequent sections.

In summary, disruption within frontal and basal ganglia circuits – especially anterior cingulate and ventral striatal regions like the NAcc (Carriere *et al.*, 2014; Le Heron, Apps. and Husain, 2017) – appear to be most associated with apathy in PD (Levy, 2006). Evidence from functional and structural imaging also suggests that these brain areas are involved in incentive processing, strengthening the link between reward, apathy and motivated goal directed behaviour.

1.6 Neurochemistry of motivation

Unlike other neuropsychiatric conditions such as depression, the neurochemical substrate of apathy in humans has been poorly characterized (Dujardin *et al.*, 2007). Potential neurotransmitters that have been implicated include dopamine, acetylcholine, noradrenaline and serotonin (van Reekum, Stuss and Ostrander, 2005). The strongest candidate remains the catecholamine dopamine. Dopamine modulates a number of behavioural mechanisms including reward prediction, vigour of action, and initiation of effortful behaviour (Schultz, 2007; Kurniawan, Guitart-Masip and Dolan, 2011; du Hoffmann and Nicola, 2014; Chong *et al.*, 2015). Given the importance of these properties in goal directed behaviour and the association of dopamine with neurotransmission in reward-related mesolimbic brain pathways, there is a strong basis for its involvement in motivation. Apathy may therefore occur when dopamine is deplete within brain areas associated with goal directed behaviour, notably ventral striatal (Brown *et al.*, 2012; Salamone and Correa, 2012) and frontal brain regions (Levy, 2006; Manohar and Husain, 2016).

The importance of dopamine in reward-guided behaviour also makes it a potential treatment option in disorders of diminished motivation (Chong and Husain, 2016). Indeed, depletion of dopamine in neurodegenerative conditions like PD may explain the high prevalence of apathy, and is evidenced further by fluctuations of apathy severity between ON and OFF states, suggestive of a dopamine-dependent syndrome (Czernecki *et al.*, 2002). Clinically, improved apathy scores are also found in PD patients on greater doses of dopaminergic drugs (den Brok *et al.*, 2015) and therapeutic benefits have been reported post stroke following administration of dopamine agonists (Adam *et al.*, 2013).

Given dopamine's involvement in goal directed behaviours, it may be plausible that motivation lies on a spectrum, with apathy on one extreme due to hypo-dopaminergic states, and impulsivity on the other secondary to higher levels of dopamine (Sinha, Manohar and Husain, 2013) (**Fig. 1.7A**). Clinically this may explain the prevalence of conditions such as impulse control disorders which occur secondary to dopamine agonist therapy in PD (Garcia-Ruiz *et al.*, 2014), and apathy which can occur in *de novo* PD cases (Pedersen *et al.*, 2010). However, it appears that dopamine function is more complex and increases and decreases of levels can have varying behavioural outcomes. For example, in a study of working memory,

increased levels of dopamine actually led to impairment of cognitive performance rather than improvement (Cools and D'Esposito, 2011). The relationship between dopamine and performance appears to be represented by an inverted-U shaped function rather than being linear in nature (Cools and D'Esposito, 2011). Excessive and insufficient levels seem to hinder performance, with responses to dopamine administration varying across people due to a number of individual differences (**Fig. 1.7B**). These differences may include receptor up and down regulation influencing dopamine's mechanism of action and also the variability of baseline dopamine levels within prefrontal and basal ganglia brain areas, both of which will be discussed below.

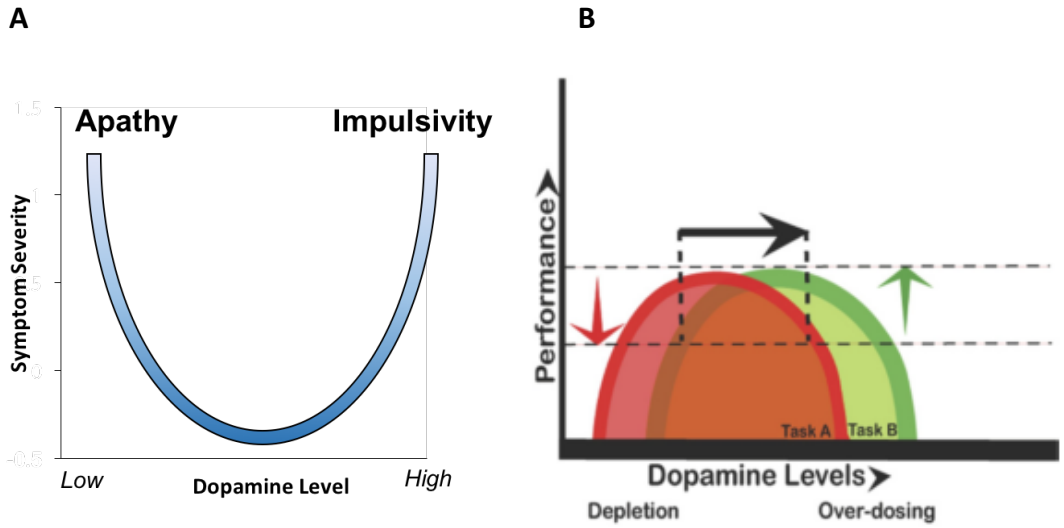

Figure 1.7A-B. Dopamine and its association with motivational disorders and cognitive performance.

A. *Possible spectrum of symptom severity related to dopamine level, with apathy due to hypo-dopaminergic states and impulsivity secondary to high levels of dopamine.*

B. *Inverted U-shaped curve of cognitive performance as a function of dopamine, indicating an optimal level for peak performance which can be shifted depending on the cognitive task being performed. Adapted from Cools et al, 2011.*

The ability to assess basal levels of dopamine within striatal areas of the brain often requires PET imaging. However, interesting associations between working memory capacity and dopamine synthesis have also been made in the literature (Cools *et al.*, 2008). A PET imaging study by Cools *et al* (2008) reported a significant correlation between dopamine synthesis capacity in the striatum of young subjects and working memory assessments using listening span (**Fig. 1.8**). Participants with low working memory capacity, as indexed by

memory spans, also had lower dopamine tracer uptake and thus reduced synthesis rates in the striatum, whereas subjects with higher working memory capacity displayed higher rates of striatal dopamine synthesis. Therefore, working memory scores may be useful as a proxy for baseline dopamine in assessing behavioural responses to incentives in the context of motivation, a strategy that has been used successfully in a variety of studies (Kimberg, D'Esposito and Farah, 1997; Frank and O'Reilly, 2006; van der Schaaf *et al.*, 2012, 2013; Spronk *et al.*, 2016).

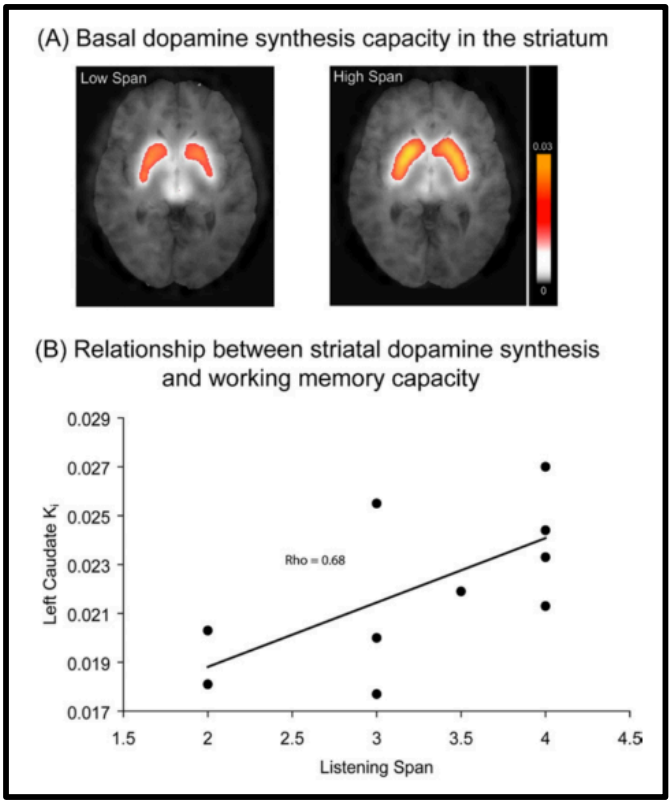

Figure 1.8. Striatal dopamine synthesis capacity and its association with working memory.

A. *Basal dopamine synthesis capacity in the striatum using whole-brain 6-[18F] fluoro-L-m-tyrosine PET. Those with low digit span working memory capacity had reduced uptake of tracer in the striatum compared to those with high span scores, predictive of dopamine synthesis.*
B. *Significant correlation between working memory capacity and dopamine synthesis capacity in the left caudate nucleus. Adapted from Cools et al (2008).*

The mechanism of action of dopaminergic neurons are dependent on activation of different receptor subtypes, including those which are crucial for cognitive function and influencing behaviours (Chong and Husain, 2016). There are five known dopamine receptor

types, D1 to D5. These are classified as D1-like (which includes D1 and D5 receptors) and D2-like (including D2, D3 and D4 receptors) (Civelli *et al.*, 1991). D2-like receptors, specifically the D3 subtype, appears most associated with motivation due to their distribution in the mesolimbic system (Chong and Husain, 2016). They are also most associated with enhancement of motivated behaviours when activated, reversing PD related motivational deficits in both human and rodent studies (Guttman and Jaskolka, 2001; Thobois *et al.*, 2013; Carnicella *et al.*, 2014; Favier *et al.*, 2014)

Although evidence suggests dopamine's strong involvement in reward and goal directed behaviours, it is also important to consider other neurotransmitter involvement in motivation. In apathy for example, a PET study that examined *de novo* PD patients who were dopamine deplete, revealed that the severity of clinical apathy was related to degeneration of serotonergic pathways within the anterior caudate nucleus and the orbitofrontal cortex (Maillet *et al.*, 2016). Serotonergic pathways have commonly been implicated in encoding of *aversive stimuli* and punishments, rather than rewards (Daw, Kakade and Dayan, 2002; Denk *et al.*, 2005; Guitart-Masip *et al.*, 2014; Hu, 2016). However, there is more recent evidence that serotonin and its 5-HT neuronal receptors may also be involved in reward encoding, including optogenetic and single unit recordings in mice studies (Miyazaki *et al.*, 2014; Li *et al.*, 2016). This suggests that the neurochemical basis underlying behavioural disorders including apathy may be multifactorial.

Crucially, it might be the context of incentives as well as their valence which determines which neurotransmitter system is involved, and the behavioural and physiological responses towards them. Conflicting evidence exists between the impact incentive type has on behavioural outcomes. Rewards and punishments appear to have differential effects depending on the end outcome measured and their context. For example, in a study by Wachter *et al* (2009), punishments – but not rewards – significantly reduced action response times, whereas learning rates were greatest for rewards (Wachter *et al.*, 2009). In another motor learning study, by contrast, punishments increased learning rates compared to rewards (Galea *et al.*, 2015). It appears that differences in responses between rewards and losses across studies may depend upon the context, particularly whether outcomes are performance dependent (Cooper and Knutson, 2008).

When outcomes are a result of an action, the context may have *motivational salience*; therefore all incentives are encoded and represented equally, irrespective of whether they are aversive or appetitive in nature (Bissonette *et al.*, 2014). On the other hand, *motivational value* might occur when there are fixed and certain outcomes, independent of the nature of the response, attributing larger value and responsiveness to rewards compared to aversive stimuli (**Fig. 1.9**). The use of different incentive types such as rewarding and aversive outcomes may help better understand which processes become dysfunctional in motivational disorders such as apathy.

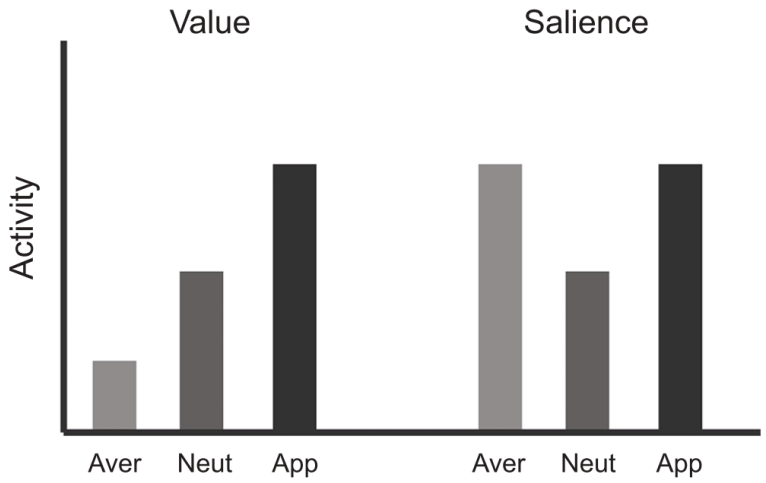

Figure 1.9. Dissociating motivational value signals from motivational salience signals.
If activity in a brain region or from a behavioural or physiological response to incentive represents "value" signals, then activation for appetitive (App) stimuli should be greater than aversive (Aver) stimuli, and neutral (Neut) responses between the two. If activity represents salience and not the intrinsic value of the incentive, then appetitive and aversive stimuli should be greater than neutral stimuli. Adapted from Bissonette et al (2014).

Mixed results have been reported from EEG studies that attempt to assess brain activity related to dopaminergic modulation. For example in PD patients presented with neutral, aversive and rewarding cues, event related potentials were larger for *both* rewarding and aversive cues compared to neutral conditions, suggestive of motivational salience (Talmi, Atkinson and El-Deredy, 2013; Renfroe *et al.*, 2016). However, in another task, greater positivity for punishment than for rewards was observed (Löw *et al.*, 2008), perhaps indicative of value encoding of incentives. If these signals are indeed related to phasic dopaminergic neuronal activation, then it may mean dopamine signals are associated with several aspects of

stimuli including value, salience, reward and punishment (Bromberg-Martin, Matsumoto and Hikosaka, 2010; Schultz, 2010).

Further evidence for the differential role of various neurotransmitters and behavioural outcomes comes from investigations of both non-human models and PD patients. An animal study that used the D2/D3 receptor dopamine agonists pramipexole to correct motivational deficits following substantia nigra lesions reported positive effects on motivation (Favier *et al.*, 2014), whereas serotonin modulating therapies such as citalopram did not improve responses for rewards (Magnard *et al.*, 2016). Association with dopamine and incentive types have also been shown in PD studies when drug therapy is manipulated. When patients are OFF dopaminergic therapy they are better at learning to avoid negative outcomes, while when ON dopamine they appear more responsive to positive outcomes (Frank, 2004). These findings suggest more dopamine dependent pathways for reward related behaviours and perhaps involvement of serotonin in other non-rewarding incentives such as punishment and effort (Meyniel *et al.*, 2016). The entire process, however, may be far more complex as evidenced by monkey studies where dopamine D2 receptor antagonists injected into the caudate nucleus actually increased reward modulation of saccadic motor behaviour rather than decrease it (Hikosaka, 2005). This might potentially be related to dose dependent pre or postsynaptic dopaminergic receptor activity (Frank and O'Reilly, 2006).

Noradrenaline may also play a role in energisation of motivated behaviour (Varazzani *et al.*, 2015). Dopamine and noradrenaline are both tyrosine-related catecholamines. Using PET imaging and a tracer specific to transporter binding for both, it has been reported that depression and apathy in PD are associated with the loss of dopamine and noradrenaline innervation within the limbic system (Remy *et al.*, 2005). These deficits appeared specific to the locus coeruleus noradrenergic (LC-NA) pathways and the ventral striatum, areas associated with arousal processes and motivation respectively (Rajkowski, Kubiak and Aston-Jones, 1994). Noradrenergic involvement in apathy might therefore be related to reductions in salience signal encoding from incentives, contributing to reduced actions for goals in apathy (Loued-Khenissi and Preuschoff, 2015). Others studies have proposed a less prominent role for noradrenaline in apathy, instead suggesting that it may cross-talk with dopamine in brain regions linked to goal directed behaviour (Guiard, El Mansari and Blier, 2008) potentially linking reward to motivation (Manohar and Husain, 2015).

Finally, the cholinergic system has also been proposed to play a role in the mechanisms of apathy. Cholinergic pathways are often disrupted in PD (Bohnen and Albin, 2011) and evidence from clinical studies have suggested an association with apathy. In the only double-blinded placebo controlled randomized trial available for the pharmaceutical treatment of apathy in PD, rivastigmine, an acetylcholinesterase inhibitor was found to improve clinical apathy scores in PD patients who were free from dementia and depression (Devos *et al.*, 2014). The drug also improved caregiver burden and activities of daily living (ADL) suggesting a significant benefit after treatment.

1.7 Pharmacology and anatomy of saccadic and pupillary responses

There are many difficulties in assessing motivation, these include lack of objective methods for measuring it. Most clinical assessments require physician interpretation and subjective questionnaire scoring. These do not necessarily provide an in-depth understanding of deficits suffered by patients nor explain the mechanisms behind them. Behavioural paradigms that assess reward-based decision making have improved our understanding and revealed deficits in conditions like PD (Czernecki *et al.*, 2002; Cools *et al.*, 2003; Schmidt *et al.*, 2008; Torta and Castelli, 2008; Torta *et al.*, 2009; Chong *et al.*, 2015), but only one investigation has attempted to link this with motivational deficits like apathy (Martinez-Horta *et al.*, 2014). Although useful, these tasks provide binary scores and are not able to measure the dynamic reward response processes likely to be active during evaluation of decisions. The use of other effectors like eye movements and pupil response might potentially provide a more informative assessment tool (Leigh and Kennard, 2004).

There is an extensive literature on the study of saccadic eye movements in PD - reviewed in (Anderson and MacAskill, 2013; Terao *et al.*, 2013). For example, it has been demonstrated that patients may have hypometric saccades and particularly saccades to remembered locations (Crawford, Henderson and Kennard, 1989). A growing number of human and monkey studies have also started to measure responsiveness to reward through the use of eye movement and pupillary indices (Hong and Hikosaka, 2008; Chen *et al.*, 2014; Rudebeck *et al.*, 2014), but this has occurred only recently in PD (Manohar *et al.*, 2015). Saccades provide a relatively pure measure of action with reduced degrees of freedom compared to limb movements while pupillometry allows assessment of physiological

responses through autonomic modulation of pupil size. The aim of using these techniques would be to assess differences in the modulation of oculomotor and physiological properties by environmental factors such as incentives. This might increase understanding of goal directed behaviour and also aid in the objective assessment of motivation.

For example, rewarded saccades have been shown to increase in velocity compared to unrewarded ones in both monkeys as well as humans, including in patients with PD (Tachibana and Hikosaka, 2012; Chen *et al.*, 2014; Manohar *et al.*, 2015; Manohar and Husain, 2016). These findings are quite surprising to many researchers because the optimal speed of saccades has been thought to follow a highly stereotyped relationship with the amplitude of eye movements. Velocity normally increases predictably with the amplitude of eye movements, a relationship referred to as the *main sequence* (Bahill, Clark and Stark, 1975). This relationship is believed to reflect optimization of the trade-off between accuracy and duration of eye movements. Larger saccades require larger neural signals to increase speed and as a result generates extra neural noise, thereby reducing accuracy (Harris and Wolpert, 1998, 2006).

Primate studies, however, have revealed that oculomotor properties including velocity, accuracy and reaction time can be modulated by reward (Takikawa *et al.*, 2002; Chen *et al.*, 2013), and appear to be associated with motivation and the value of a goal. Indeed, this has now also been demonstrated in humans (Chen *et al.*, 2013, 2014) with evidence that rewards are able to break the speed-accuracy trade-off through the facilitation of dopamine (Manohar *et al.*, 2015). But as of yet, no links with apathy have been made in any of these study types. The findings that incentives are able to modulate motor outputs may also have implications for PD. For example, in furthering understanding of the motor phenomenon 'Kinesia paradoxa' which is the energisation of motor abilities via exposure to motivating environmental factors (Glickstein and Stein, 1991). Patients with PD can suddenly move rapidly in the context of an aversive motivating factor such as imminent danger or threat. Understanding if certain incentives types are able to produce this phenomenon may have clinically useful applications.

Pupillometry is the measurement of pupil diameter. It has been used as a psychological assessment tool for over 50 years and provides a continuous measure without

participants being aware of any changes (Laeng, Sirois and Gredebäck, 2012). Studies of pupil responses in PD have begun to show that these might be modulated by reward (Manohar and Husain, 2015; Manohar *et al.*, 2017). However, the exact neural pathways orchestrating pupillary and cognitive processes are not clearly defined. LC-NA projections originating in the pons may facilitate this connection, as demonstrated by single-cell recording in LC neurons in monkeys. Changes in pupil diameter were correlated with baseline firing rate of LC neurons (Aston-Jones and Cohen, 2005) potentially via arousal (Rajkowski, Kubiak and Aston-Jones, 1994) or decision making processes (Nieuwenhuis, Aston-Jones and Cohen, 2005). Dopaminergic projections also share common connections with the LC-NA pathways, so other neurotransmitter systems are also likely to be involved (Wang and Munoz, 2015). Indeed, there is experimental evidence for dopamine and noradrenaline interactions (Delaville, Deurwaerdère and Benazzouz, 2011). One study which examined noradrenaline deplete mice, found that in the absence of noradrenaline, decreased levels of dopamine were released, suggesting a strong relationship between the two neurotransmitters (Rommelfanger and Weinshenker, 2007). Other investigations have also shown that rewards modulate pupil responses (Delaville, Deurwaerdère and Benazzouz, 2011; Manohar *et al.*, 2017). Therefore, pupil responses to incentives in apathy might help to reveal underlying brainstem processes in which dopamine and noradrenaline interact to link reward to motivation (Manohar and Husain, 2015).

Through the observation of motor and physiological responses it might be possible to understand which brain areas are associated with their modulation, and crucially which structures are implicated when the system is dysfunctional, particularly in disorders of motivation. Midbrain (vertical saccade) and pontine (horizontal saccade) burst neurons have been linked to the generation of saccades and are considered to play a key role in the control of their velocity (Sparks, 2002). These neurons receive afferents from striatal neurons, which are sensitive to expected rewards as demonstrated through neural recordings (Hikosaka, 2007). Frontal, parietal and striatal areas might also play a role in stimulating the superior colliculus so that rewarded saccades are invigorated further (Munoz and Everling, 2004). The superior colliculus in the midbrain also evokes transient pupil dilation and may be associated with preparatory processes in the generation of saccades (Wang, Brien and Munoz, 2015). Furthermore, top-down inputs from the fronto-parietal cortex, LC-NA system and dopaminergic basal ganglia via the intermediate layers of the superior colliculus, are able to

alter pupil response in reward related cognitive tasks (Wang and Munoz, 2015) (**Fig 1.10**). These associations may allow us to index dysfunction of these brain areas in patients who suffer from disorders of reward evaluation, which may be the case for apathy.

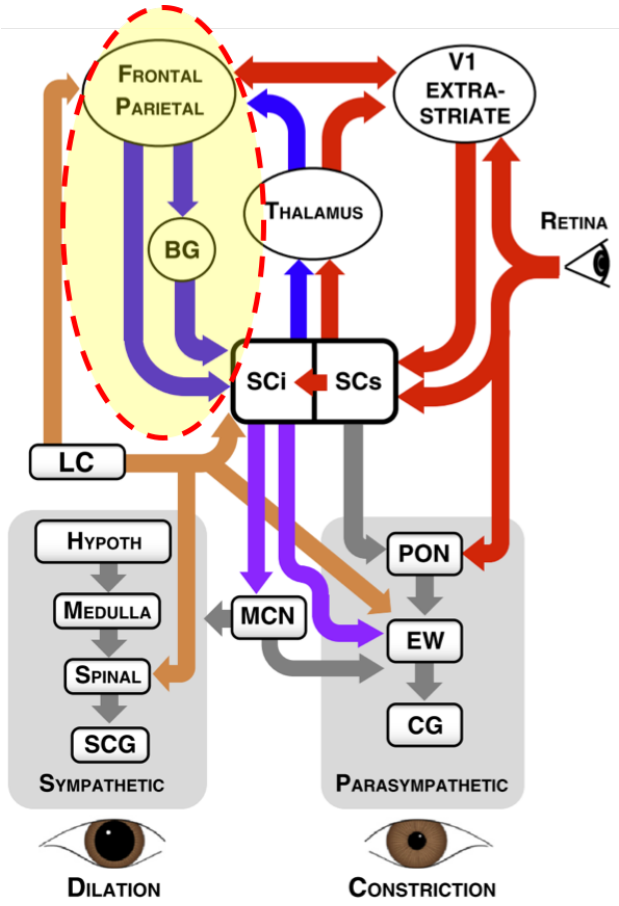

Figure 1.10. Brain circuitry for pupil modulation.

Top down influences from frontal parietal and basal ganglia areas (highlighted in yellow) to intermediate layers of the superior colliculus (SCi) may modulate pupil dilation and constriction, providing a gateway to objectively visualising underlying deficits in motivated behaviour. BG, basal ganglia; CG, ciliary ganglion; EW, Edinger–Westphal nucleus; Hypoth, hypothalamus; LC, locus coeruleus; MCN, mesencephalic cuneiform nucleus; PON, pretectal olivary nucleus; SCi, intermediate layers of the superior colliculus; SCs, superficial layers of the superior colliculus; SCG, superior cervical ganglion; V1, primary visual cortex. Adapted from Wang et al, 2015.

1.8 Apathy and prognosis in Parkinson's disease

There is mixed opinion regarding the clinical impact that apathy has in PD (Bogart, 2011) but overwhelming evidence points towards a negative prognosis. The difficulty lies in

attributing specific causality. Few longitudinal studies have compared the outcomes associated with PD and concurrent apathy directly, but in those that have, an association with more rapid disease progression and cognitive decline has been suggested (Aarsland *et al.*, 1999). Other cross-sectional studies have found apathy in PD to be associated with a number of adverse outcomes, including communication difficulties (McKinlay *et al.*, 2008), increased motor symptoms and worse physical disability (den Brok *et al.*, 2015). Perhaps the most significant and consistent finding is the association with cognitive decline in PD (Bogart, 2011). Apathy may in fact herald the onset of dementias (Dujardin *et al.*, 2009) and therefore function as an important prognostic factor particularly as dementia increases mortality in PD (Hughes *et al.*, 2004).

1.9 Impact of apathy on quality of life

The presence of apathy in healthy elderly populations correlates with reductions in satisfaction with life and in general health status (Vallerand, O'Connor and Hamel, 1995). This clearly reflects the importance of motivation and the negative consequences in its absence. In AD and other dementias, apathy is accompanied by worse functional disability, reduced rehabilitation success (Resnick *et al.*, 1998) and larger care giver burden and stress (Landes *et al.*, 2001b). Similar findings also hold true in PD, with lower quality of life scores reported in patients who suffer from apathy (Oguru *et al.*, 2010; Benito-León, Cubo and Coronell, 2012). Results however do vary, and the distinction between effects of apathy and co-existing neuropsychiatric disorders such as depression or cognitive impairment can lead to changeable measured outcomes (Skorvanek *et al.*, 2013). Mood disturbance and apathy together appear to have the greatest negative impact on quality of life (Martinez-Martin *et al.*, 2011). Indeed, non-motor symptoms generally in PD are a frequent cause of institutionalization and increased care cost (Chaudhuri *et al.*, 2006).

Caregivers are also negatively affected by the presence of apathy in PD. This is apparent even in the early stages of the disorder as the care burden becomes significantly greater, even in comparison to other neuropsychiatric illness such as depression (Leiknes *et al.*, 2010). ADL scores are also lower, so a functional impact on day to day life is also apparent when suffering from apathy (den Brok *et al.*, 2015). These reduced ADL scores do seem to improve with treatment of apathy in PD, as does the extent of caregiver burden, but quality of life does not recover (Devos *et al.*, 2014). Consequently, therapies to improve motor function

are only one part of restoring quality of life for patients and neuropsychological disorders like apathy should also be addressed in order to improve outcomes (Shulman, 2000).

1.10 Overlap with depression and anhedonia

PD patients can suffer from a large range of neuropsychiatric symptoms. It has been reported that 61% of PD patients have at least one psychiatric symptom, and 45% two or more (Aarsland *et al.*, 1999). Depression is frequently described, occurring at approximately double the level of the general population (Lieberman, 2006) and also appears to be the most important association with apathy in elderly non-demented PD patients (Skorvanek *et al.*, 2013). Patients suffering with depression often have symptoms of apathy. Indeed loss of motivation forms part of the diagnostic criteria for depression (American Psychiatric Association, 2013)**.** Therefore, there has been much debate in the past if apathy is a distinct entity in PD or just a symptom of depression, given the strong relationship. In a number of other neurological disorders, including AD, Huntington's disease and frontotemporal dementia, apathy is deemed as a specific neuropsychiatric syndrome, distinct from depression (Levy *et al.*, 1998).

In PD, consensus points in a similar direction (Oguru *et al.*, 2010; Lindsey Kirsch-Darrow *et al.*, 2011; Ziropadja *et al.*, 2012). About one half of PD patients with apathy do not suffer from concomitant depression (Brown and Pluck, 2000) (**Fig. 1.11**). These findings suggest that both disorders are likely to be frequent features of PD but can exist independently of one another (Kirsch-Darrow *et al.*, 2006; Kaji and Hirata, 2011). This distinction is important clinically as misdiagnosis can result in inadequate treatments and also worse outcomes for patients (Barnhart, Makela and Latocha, 2004; Drijgers *et al.*, 2009). Depression and apathy are also strongly associated with fatigue which is another disabling non-motor symptom in PD (Skorvanek *et al.*, 2015).

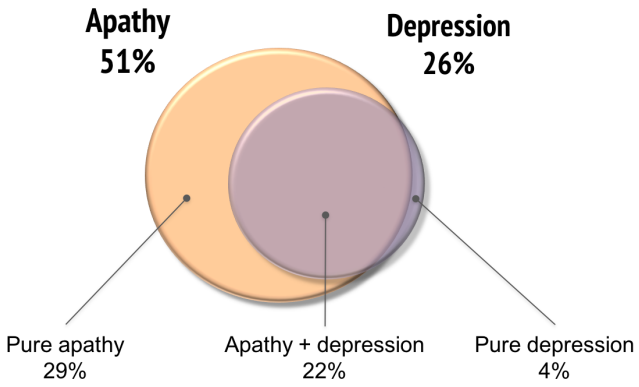

Figure 1.11. An example of overlap and prevalence of apathy and depression in a cohort of PD patients.

Adapted from Kirsch-Darrow et al, 2006.

The syndrome of anhedonia also has considerable overlap with apathy and is a key component of various neuropsychiatric conditions including depression, where it can be a diagnostic feature. Anhedonia is traditionally defined as reduced ability to experience pleasure (Fawcett, 1983) but more recently it has been conceptualised as a disorder that affects motivation mechanisms, including anticipatory *'wanting'* (the desire to obtain a reward) and consummatory *'liking'* (the satisfaction obtained when consummation of a behaviour occurs) (Thomsen, Whybrow and Kringelbach, 2015). This can be likened to the desire for food when hungry which motivates actions to seek food, versus the sensation of satisfaction when the food is finally eaten. These components of pleasure may have separate neural pathways. 'Wanting' has been associated with fronto-striatal dopaminergic circuits which have also been linked with motivation and apathy (Berridge, 2004; Levy, 2006), while 'liking' has been associated with NAcc, ventral pallidum and projections to the orbitofrontal cortex (Berridge and Kringelbach, 2008; Muhammed and Husain, 2016) (**Fig. 1.12**).

Although these two systems are inter-related, they are associated with dissociable behavioral features (Kringelbach and Berridge, 2012). Apathy might be more related to the anticipatory component of anhedonia which is dopamine responsive, while some suggest the consummatory component is more associated with depression (Loas, Krystkowiak and Godefroy, 2012; Jordan *et al.*, 2013). The similarities between apathy and anhedonia are seemingly closer than those between apathy and depression. Anhedonia accompanies both and might be associated with deficits in 'liking' and 'wanting' (Kaji and Hirata, 2011), although

this is a controversial area. Like apathy, anhedonia in PD appears to be independent of motor symptoms, has been attributed to frontal lobe dysfunction (Assogna *et al.*, 2011) and is also responsive to PD therapies (Lemke *et al.*, 2005).

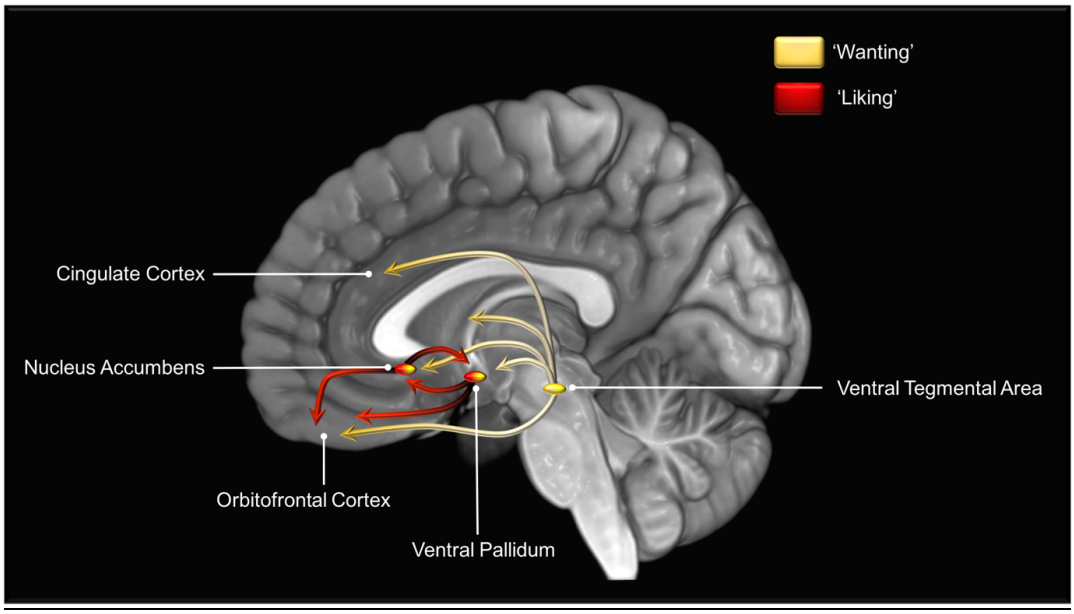

Figure 1.12. Proposed neural projections involved in the 'wanting' and 'liking' brain circuits involved in motivation and pleasure.

Hedonic hotspots include the nucleus accumbens and the ventral pallidum, with strong associations to orbitofrontal brain areas and reward processing areas. Adapted from Muhammed et al, 2016.

1.11 Detection and measurement of apathy

A variety of clinical questionnaires have been designed to assess apathy, but no gold standard has been agreed upon. The Movement Disorder Society Unified Parkinson's disease rating scale (MDS-UPDRS) (Goetz *et al.*, 2008) attempts to identify apathy in patients through a single questionnaire component; however this should only really be considered for screening purposes (Leentjens *et al.*, 2008). More detailed instruments validated for the assessment of apathy in PD take the form of self-report clinical questionnaires and semi-structured interview screens. Perhaps the most extensively used from a research perspective is the Lille Apathy Rating Scale (LARS) (Sockeel, 2006) which examines different apathy subdomains (Leentjens *et al.*, 2008). For everyday clinical practice an abbreviated short form of the LARS (Dujardin *et al.*, 2013) has shown reliability in evaluating apathy in PD and a

caregiver version also exists (Dujardin *et al.*, 2008). The Apathy Scale (Starkstein *et al.*, 1992), Apathy Evaluation Scale (Marin, Biedrzycki and Firinciogullari, 1991) and the Apathy Inventory (Robert *et al.*, 2002) have also all been validated in PD. These instruments, when combined with other objective behavioral measures, will hopefully give rise to a more accurate and better diagnosis of apathy in PD.

As highlighted above, the most globally accepted interview questionnaire developed from a research perspective is the LARS (Sockeel, 2006). This is the assessment used throughout the thesis and its subcomponents will be expanded on here. The LARS is a 33 item semi structured clinical interview, performed by the assessing clinician. Common features classically used to sub-divide apathy, and which are also features of the LARS, include reduced goal-directed behaviour encompassing *self-initiated action*, *intellectual curiosity*, *emotional indifference*, and *self-awareness*. The LARS subdomains were based on the main conceptual principles of apathy proposed originally by Marin *et a*l and by the clinical experience of the authors (Marin, Biedrzycki and Firinciogullari, 1991).

Dissecting each domain in turn suggests that apathetic behaviour can firstly encompass a motor component *action initiation* which is also termed behavioural apathy or so-called auto-activation deficit (Levy, 2006). This is the lack of physical "get up and go" that many patients describe. The neural networks proposed to be involved in this form of apathy include the supplementary motor area and the basal ganglia, comprising bilateral caudate, putamen and pallidum (Pagonabarraga *et al.*, 2015). The *intellectual curiosity* domain of apathy refers to the cognitive inquisitiveness of an individual, as apathetic patients can suffer from a loss of spontaneous ideas and normal curiosity for routine and new events. From a neural networks perspective, it has been speculated that ventrolateral prefrontal cortex and posterior parietal dysfunction are thought to be the underlying cause (Pagonabarraga *et al.*, 2015). *Emotional apathy* refers to indifference in emotion; a loss of spontaneous emotion and blunted affect in response to either negative or positive stimuli. Here, it has been proposed that more mesolimbic pathway involvement is disrupted, with areas including the orbitofrontal cortex and subgenual cingulate impaired (Pagonabarraga *et al.*, 2015). The LARS *self-awareness* domain refers to a manifestation of apathy which was first described by Stuss *et al* (Stuss, Van Reekum and Murphy, 2000). It has been linked to cognitive decline (Sockeel, 2006) and relates to a reduction in self-criticism and changing of behaviour in order to meet

social requirements in the interest of ourselves. There is evidence that those with apathy also have a reduced awareness of their condition with lack of significant insight (van Reekum, Stuss and Ostrander, 2005).

1.12 Constructs of apathy in Parkinson's disease and challenges in diagnosis

There are many overlapping descriptions of apathy but no clear consensus on an exact definition or formal diagnostic criteria (Marin, 1991; Cummings *et al.*, 1994; Stuss, Van Reekum and Murphy, 2000; Robert *et al.*, 2002; Levy, 2006; Starkstein and Leentjens, 2008). The syndrome is generally defined as a disorder of motivation with central common features. These include a reduction in goal directed behavior encompassing cognitive, emotional and self-initiated motor domains. One challenge that gives rise to the difficultly in its classification is the distinction of apathy as a syndrome in itself or as a symptom of the underlying disease it is associated with (Marin, 1991; Starkstein and Leentjens, 2008).

In PD it is often difficult to recognize and distinguish between symptoms of apathy and the phenomenology of the neurodegenerative condition, as there are many features common to PD and apathetic syndromes. For example, indifference and lack of effort caused by apathy can overlap with the masked facial expression and paucity of movement observed in Parkinson's disease (Shulman, 2000). The need therefore for reliable and reproducible criteria is paramount for accurate diagnosis and appropriate treatment. Diagnostic criteria for apathy in AD and other neuropsychiatric disorders have been proposed but they have yet to be implemented formally in current DSM or ICD classification systems and there are no definite standards for diagnosing apathy specifically in PD (Marin, 1991; Starkstein *et al.*, 2001; Robert *et al.*, 2009; Mulin *et al.*, 2011). Current suggested criteria for apathy are summarised in **Table 1.**

For a diagnosis of apathy, the patient should fulfil the criteria A, B, C and D
A Loss of or diminished motivation in comparison to the patient's previous level of functioning and which is not consistent with his/her age or culture. These changes in motivation may be reported by the patient or by the observations of others.
B Presence of at least one symptom in at least two of the three following domains for a period of at least four weeks and present most of the time. Domain B1 (Behaviour): Loss of, or diminished, goal-directed behaviour as evidenced by at least one of the following: Initiation symptom: loss of self-initiated behaviour (for example: starting conversation, doing basic tasks of day-to-day living, seeking social activities, communicating choices). Responsiveness symptom: loss of environment-stimulated behaviour (for example: responding to conversation, participating in social activities). Domain B2 (Cognition): Loss of, or diminished, goal-directed cognitive activity as evidenced by at least one of the following: Initiation symptom: loss of spontaneous ideas and curiosity for routine and new events (i.e, challenging tasks, recent news, social opportunities, personal/family and social affairs). Responsiveness symptom: loss of environment-stimulated ideas and curiosity for routine and new events (i.e, in the person's residence, neighbourhood or community). Domain B3 (Emotion): Loss of, or diminished, emotion as evidenced by at least one of the following: Initiation symptom: loss of spontaneous emotion, observed or self-reported (for example, subjective feeling of weak or absent emotions, or observation by others of a blunted affect). Responsiveness symptom: loss of emotional responsiveness to positive or negative stimuli or events (for example, observer-reports of unchanging affect, or of little emotional reaction to exciting events, personal loss, serious illness, emotional-laden news).
C These symptoms (A - B) cause clinically significant impairment in personal, social, occupational, or other important areas of functioning.
D The symptoms (A - B) are not exclusively explained or due to physical disabilities (e.g. blindness and loss of hearing), to motor disabilities, to diminished level of consciousness or to the direct physiological effects of a substance (e.g. drug of abuse, a medication).

Table 1. Proposed diagnostic criteria for apathy in clinical practice in neurodegenerative disorders.
Adapted from Mulin et al. (2011)

As discussed, there are many assessments that attempt to cover the different dimensions of apathy. However, several items included in the assessments are highly abstract in nature and therefore limit their clinical application. Adding to the difficulty in breaking down the constructs of apathy in PD is the overlap with other cognitive and neuropsychiatric disorders. **Fig 1.13** provides an illustrative example of the overlap between apathy, depression and cognitive impairment in a representative sample of PD patients.

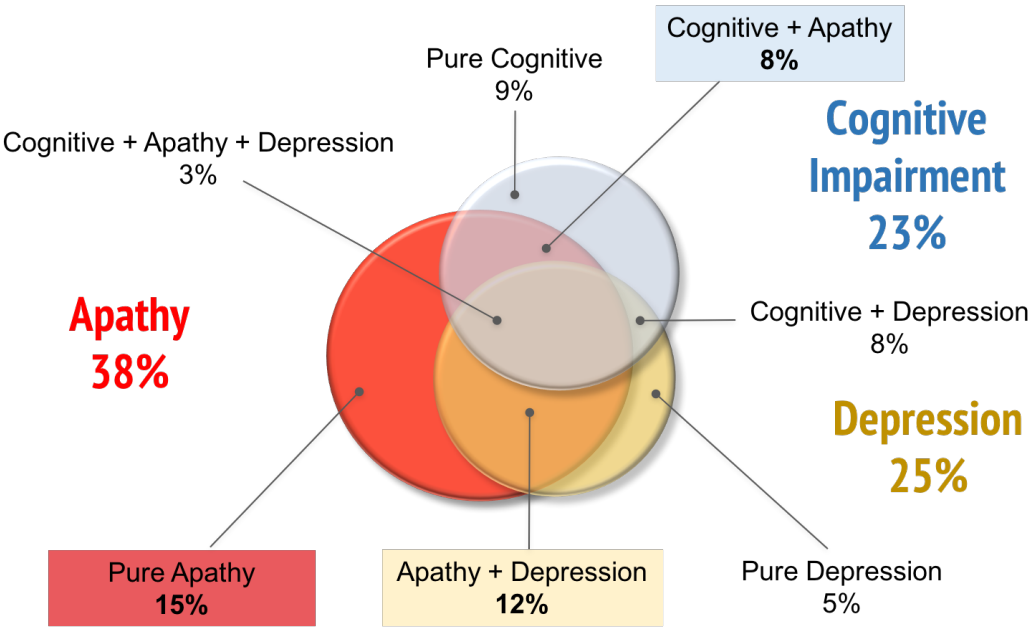

Figure 1.13. Prevalence of apathy, depression and cognitive impairment in a group of 65 patients with idiopathic PD.

Apathy was assessed using the LARS, with a cut off ≤22, Cognitive function was assessed using the Montreal cognitive assessment (MoCA) and a score of 26 or less indicated impairment and depression was assessed using the Beck depression inventory) BDI-II with a score equal or greater than 16 indicating significant mood disturbances. (Muhammed et al 2017 - Unpublished).

To help address this problem, recently it has been proposed that apathy could be divided into more broad and perhaps clinically relevant subtypes in PD, that allow for different neural substrates and potential targeted therapies (Pagonabarraga *et al.*, 2015). Given that apathy itself in PD has been shown to herald the onset of dementia (Dujardin *et al.*, 2009) and shares common but dissociable features with depression (Kaji and Hirata, 2011; Lindsey Kirsch-Darrow *et al.*, 2011; Robert *et al.*, 2012; Skorvanek *et al.*, 2015), a simplified 3-domain classification of apathy in PD has been proposed which has been adapted from the work of

Pagonabarraga *et al* (Pagonabarraga *et al.*, 2015) (**Fig 1.14)**. This divides PD patients with diminished motivation in isolation (*behavioural apathy*), when associated with depression (*emotional apathy*) or when in the presence of executive dysfunction or cognitive impairment (*cognitive apathy*). It remains to be established whether this will provide a more clinically useful assessment and allow for more tailored therapy choices when treating patients.

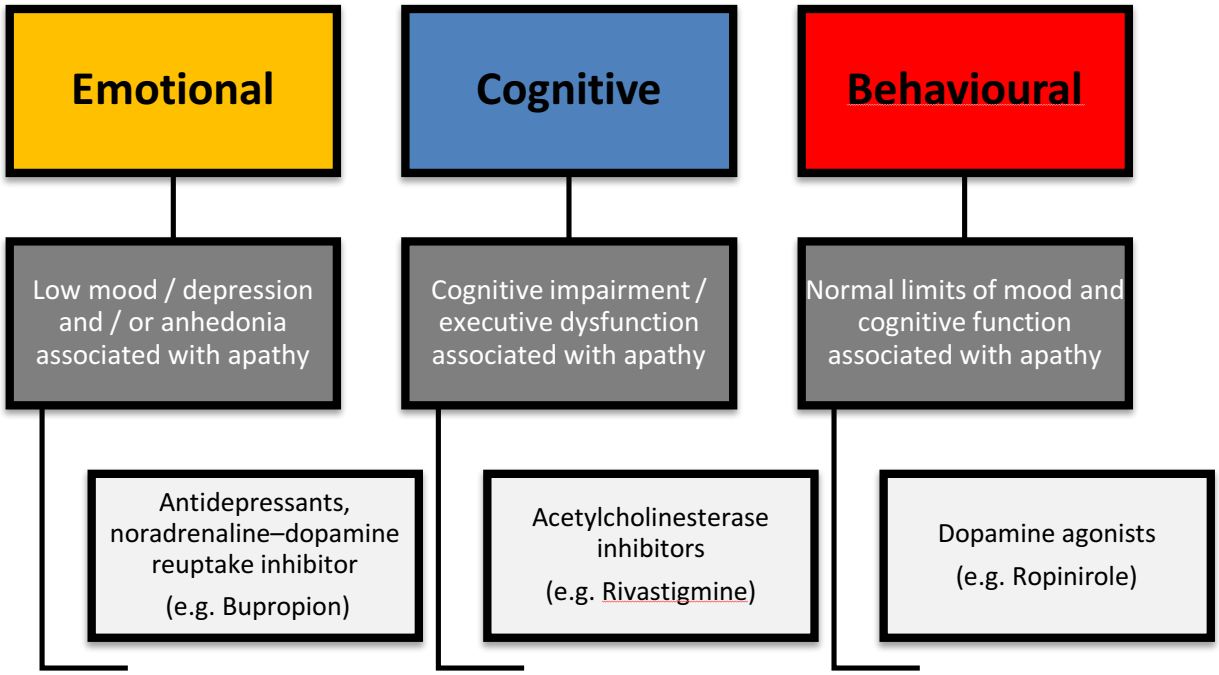

Figure 1.14. Clinically operative categorisation of apathy.

Based on symptoms related to diminished motivation irrespective of its cause. Patients may have apathetic symptoms with 1) low mood and symptoms of depression, 2) symptoms suggestive of cognitive impairment or 3) the absence of both depressive and cognitive symptoms, known as behavioural apathy. Each group may benefit from targeted therapeutic interventions which may show more beneficial outcomes based on potentially different underlying dysfunctions. Adapted and simplified from Pagonabarraga et al (2015).

1.13 Other neurodegenerative disorders and apathy

The body of this thesis focuses on apathy in Parkinson's disease however apathy is a common feature of many neurodegenerative disorders in addition to PD, such as Alzheimer's disease (AD) (Landes *et al.*, 2001b), vascular dementia and small vessel disease (Hollocks *et al.*, 2015), frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) (Lillo *et al.*, 2012; Mioshi *et al.*, 2014; Bertoux *et al.*, 2015). Across disorders, there appears to be consistent neural correlates of apathy, with dysfunction particularly in frontostriatal circuits

including the dorsal anterior cingulate cortex and ventral striatum (VS) (Le Heron, Apps. and Husain, 2017).

Although dopamine is important in motivation particularly in PD, other neurotransmitters are also believed to play a role. In FTD, dopamine treatment does not appear to have a significant effect on apathy therefore dysfunction of other pathways including the serotonergic and noradrenergic systems are believed to be involved (Lansdall *et al.*, 2017)**.** Further, noradrenergic structures such as the Locus Coeruleus demonstrate degenerative changes on post mortem in those with apathy and FTD (Irwin *et al.*, 2016).

Interestingly, the behavioural profile of other neurodegenerative disorders such as ALS, appear to mirror those of FTD, particularly the behavioural variant of FTD, with apathy being an early and important symptom, independent of mood disturbance (Lillo *et al.*, 2012). Indeed, in groups of patients with both ALS and FTD the most common neuropsychiatric change was apathy, observed in 60% of patients (Mioshi *et al.*, 2014). Given the overlap in behavioural and cognitive characteristics between FTD and ALS, it would be plausible that similar mechanisms of motivational dysfunction occur in both.

Similarly, other neurodegenerative condition such as AD have an exceptionally high prevalence of apathy. Apathy in AD is suggested to be one of the most common changes in behaviour (Landes *et al.*, 2001b). Cholinergic treatments for AD such as donepezil have shown benefits in treating motivational deficits, indicating a possible role for acetylcholine rather than dopamine in this patient group (Mega *et al.*, 1999).

1.14 Potential therapies for apathy

Given the different domains of apathy and the variety of potential mechanisms contributing to its clinical phenotype, it may be necessary to adopt an individualized symptom specific approach in the treatment of each patient (Pagonabarraga *et al.*, 2015). A variety of pharmacological approaches have been used (Chase, 2011), each with varying success. However at present there are no formally approved drugs (Santangelo *et al.*, 2013).

The severity of apathy level in PD tends to be lower in patients on higher doses of dopaminergic drugs (Leroi *et al.*, 2012; den Brok *et al.*, 2015). Dopamine agonists including pramipexole (Guttman and Jaskolka, 2001) and piribedil (Thobois *et al.*, 2013) can have beneficial effects on apathy, as well as mood, in PD. Pramipexole can also lead to improvements in associated anhedonia and depression (Lemke *et al.*, 2005). Likewise, the ability of dopamine receptor agonists to improve motivation has been observed in other patients with prefrontal or basal ganglia lesions (Kohno *et al.*, 2010; Adam *et al.*, 2013). Apathy in PD, however, is not always dopamine responsive. Indeed, there are suggestions of two types of syndrome: dopamine-sensitive and dopamine-resistant apathy. The former is often reported to occur post deep brain stimulation (DBS) a number of months *after dopamine withdrawal* following the procedure, and it can be reversed after reintroduction of the medication (Czernecki *et al.*, 2008; Thobois *et al.*, 2010). Dopamine-resistant apathy on the other hand seems to be related to the progression of PD and may be due to structural atrophy in deep brain structures such as the nucleus accumbens and the caudate nucleus (Carriere *et al.*, 2014). Therefore, which class a patient falls into may have significant repercussions on drug responsiveness and subsequent prognosis.

Although dopamine dysfunction is likely to be contributory, it is not necessarily the solitary cause. Dysfunction of non-dopaminergic systems has also been shown to predict the development of apathy (Pedersen *et al.*, 2009). As mentioned earlier, acetylcholine is a possible candidate implicated in the process (Devos *et al.*, 2014). Other drug therapies such as methylphenidate (which increases both dopamine and noradrenaline levels) have also been tried in neurological conditions such as AD (Herrmann *et al.*, 2008; Rosenberg *et al.*, 2013). Although there have been no controlled trials in PD there is a case report of apathy in PD improving on methylphenidate (Chatterjee and Fahn, 2002), and a methylphenidate trial comparing gait hypokinesia and freezing which reported a notable improvement in apathy severity (Moreau *et al.*, 2012).

The use of antidepressant therapies in apathy is controversial. There is evidence to suggest they can actually lead to apathy in some individuals (Barnhart, Makela and Latocha, 2004; Wongpakaran *et al.*, 2007). However, this may be agent-specific, with selective serotonin reuptake inhibitors faring worse. Conversely, antidepressants with a dopamine

component such as bupropion have been more effective (Corcoran, Wong and O'Keane, 2004), and this may be more suitable for patients who have significant overlap with mood disorders, but like most therapies for apathy, further validations are needed.

No firm evidence for DBS as a treatment of apathy has been found. In fact, DBS has been reported to directly lead to apathy in some studies (Drapier *et al.*, 2006), but improve motivation in others (Czernecki, 2005), all with no apparent association to stimulator location (L. Kirsch-Darrow *et al.*, 2011). Instead, as noted above, apathy accompanying DBS may be secondary to weaning of dopamine medication as motor symptoms improve from stimulation. This is particularly plausible as apathy is associated with reduced striatal metabolism in PET imaging prior to STN-DBS implantation (Robert *et al.*, 2014).

Non pharmaceutical interventions such as exercise and cognitive training have also been proposed (Stanton and Carson, 2015) but current evidence is limited. There have been results pointing to increased physical activity in PD being associated with lower levels of apathy (Abrantes *et al.*, 2012; van der Kolk and King, 2013), however whether this is due to greater motivation to perform activity in the first place remains to be established.

1.15 Conclusion

Apathy in PD greatly impacts upon the quality of life of patients and their carers. Although much headway has been made in recent years, it is a challenging disorder to study – and treat. Associations with other neuropsychiatric conditions make diagnosis difficult and lack of mechanistic understanding has made choice of appropriate therapy a challenge. Yet there are positive prospects. More objective assessment methods, better understanding of underlying mechanisms and greater rigor in trial design are all required to advance the field and improve management of patients.

1.16 Outline of experimental chapters

Chapter 2

In this thesis, I first investigated whether it might be possible to measure sensitivity to reward using physiological measures. Two novel eye-tracking paradigms were developed to investigate whether increasing monetary rewards modulate saccadic properties and pupillary response. This was tested in groups of young and elderly healthy participants. Correlations between oculomotor metrics and questionnaire measures of motivation were performed. Potential rewards were offered at the beginning of each trial. The quicker participants initiated a saccade towards a target, the greater the proportion of reward obtained. Saccadic peak velocity, reaction time, accuracy and pupillary dilation was assessed in response to different reward magnitudes.

Chapter 3

Next, using a simplified version of the eye tracking paradigm described in chapter 2, *insensitivity to reward* was examined in relation to pathological apathy in patients by measuring pupillary responses to anticipated incentives. This approach was tested in 40 patients with idiopathic PD and compared with age matched controls. 30 patients were examined ON and OFF their dopaminergic medication in two counterbalanced sessions, so that the effect of dopamine on reward sensitivity could also be assessed. An additional 10 drug naïve patients were also included in the OFF group analysis.

Chapter 4

The link between motivation and motor output was explored using a GO/NO GO task. To distinguish between pupillary responses in anticipation of reward versus those associated with motor preparation, a novel version of the oculomotor task described in chapter 3 was developed. Performance was examined in a group of young participants, elderly controls and 22 patients with PD were tested ON and OFF dopamine. Comparisons between the effect on pupil reward sensitivity when action was needed versus when no action was required was performed, and the relationship to apathy assessed.

Chapter 5

Other types of incentive including *avoidance of punishment* contribute to goal directed behaviours. It is not fully understood how or if dopamine modulates occulomotor and pupillary responses to loss. A new eye tracking paradigm was developed to assess these properties and to compare them to responses in anticipation of reward. 26 PD patients ON and OFF dopamine took part as well as healthy young and elderly controls. They were instructed to make fast eye movements for three new conditions: to obtain money, avoid losing money or for a neutral baseline condition. Oculomotor properties, pupil responses to incentives and the effect of dopamine in relation to baseline dopamine levels were assessed.

Chapter 6

A double blinded placebo controlled drug study in 30 healthy young participants was conducted using the eye tracking paradigm developed in chapter 5. This was performed to further understand physiological and behavioural responses towards incentives when under the influence of the D2 dopamine receptor antagonist, haloperidol versus placebo. The evaluation of responses to rewards and losses was assessed using pupillary changes to the cues while also taking into consideration working memory as a proxy for individuals' baseline dopamine. Drug differences and associations with apathy were made with pupillary responses to incentives.

Participants

For PD patient group and age matched healthy controls, in all behavioural tasks, for effect size (Cohen's *d*) = 0.85 (alpha = 0.05; beta = 0.2), sample size of N=18 was required per group. Due to potential attrition, a minimum of 22 subjects were recruited per group. All groups tested were larger than the minimal sample size needed for effects.

Chapter 2 contained young and elderly participants, the same elderly controls were also used in chapter 3 in combination with a group of Parkinson's disease patients tested ON and OFF dopamine. Chapter 4 contained a new group of young and elderly control participants and a new cohort of patients with Parkinson's disease. Chapter 5 also included a new group of young and elderly controls and a further new group of patients with Parkinson's disease. Chapter 6 included a new group of young participants.

2. Novel oculomotor and pupillary reward sensitivity metrics in healthy people

2. Abstract

The effects of rewards on eye movements and pupillary dilation was measured using a novel oculomotor paradigm. At the beginning of each trial, healthy participants heard one of three maximum monetary reward levels available for making a saccade to a subsequent target. In study 1, the targets were at one of three different eccentricities from fixation and in study 2, there was just one distance. Using an exponentially decaying reward function based on participant reaction time, the quicker participants initiated a saccade towards the target, the greater the proportion of reward obtained. There was a significant increase in saccadic peak velocity as reward magnitude increased, together with an associated improvement in target landing accuracy for bigger rewards. Pupillary response to rewards was also greater for the larger incentive and scaled in a linear fashion. The sensitivity to reward as measured by the difference in pupil response between no incentive and the maximum reward level correlated with questionnaire assessments of motivation.

These findings demonstrate that saccadic properties, including peak velocity, accuracy and pupil response are influenced by rewards. The results suggest that there is greater flexibility in execution of saccades than previously thought. Furthermore, modulation of saccadic properties and particularly pupil response, might potentially be used as a marker of an individual's motivation and reward sensitivity. This may provide an objective method for probing disorders of motivation, such as pathological apathy, in neurological conditions like Parkinson's disease.

2.1 Introduction

Recent studies have revealed that the decision to make movements is complex and has many influencing factors (Turner and Desmurget, 2010; Opris, Lebedev and Nelson, 2016). Expected rewards not only affect the decision to make a movement but can also alter movement properties, such as speed and accuracy (Leon and Shadlen, 1999). Saccades

provide a good model system to study as they are relatively well-characterized compared to other effector responses (R. Carpenter, 2004). The optimal speed and accuracy of a saccade has long been considered to be represented by the 'main sequence' (Bahill, Clark and Stark, 1975), defined as the stereotypical relationship between peak velocity (PV) and amplitude. Dogma regarding the main sequence has proposed that saccadic velocity should vary with only amplitude of movement and is otherwise fixed. One of the suggested reasons for the stereotypical relationship between PV and amplitude is to optimize the trade-off between the accuracy of an eye movement and the duration of the movement (Harris and Wolpert, 2006). This compromise is believed to exist due to increased neuronal noise associated with generating larger signal to achieve faster saccades. More recently, however, primate studies have begun to suggest that movement parameters (such as peak velocity and reaction time) can be modulated by reward (Takikawa *et al.*, 2002; Chen *et al.*, 2013). Indeed, even the tradeoff between speed and accuracy can be broken when rewards are at stake (Manohar *et al.*, 2015).

In addition, reward expectation causes increased spiking of neuronal activity prior to making a saccade in the dorsolateral prefrontal cortex of macaques (Leon and Shadlen, 1999) and the basal ganglia (Kawagoe, Takikawa and Hikosaka, 1998), while the firing of cells in the lateral intraparietal area (LIP) scale monotonically with the value of planned saccades according to reward magnitude (Sugrue, Corrado and Newsome, 2005). These associations between encoding of reward and motor action highlights the interplay between goal evaluation and the resultant behaviour. Therefore, the saccadic system can be used as a possible model to explore how rewards modulate our behavioural response and how motivation may differ depending on incentives. Furthermore, in conditions like the neurodegenerative disorder Parkinson's disease (PD), saccadic eye movements provide a useful action measure as they are not affected in the same way as limb movements by bradykinesia and fatigue (Flowers and Downing, 1978). This is crucial if tasks are to be developed which are to be assessed in patients with PD. These differences in saccadic properties are only now being reported in human research (Reppert *et al.*, 2012; Chen *et al.*, 2014; Gray *et al.*, 2014; Manohar *et al.*, 2015) and the associations made between increased motor commands for rewards and intrinsic value of visual information (Xu-Wilson, Zee and Shadmehr, 2009).

Pupillary dilation has been associated with various cognitive processes including arousal (Bradley *et al.*, 2008), effort (Moresi *et al.*, 2008), decision making (Satterthwaite, Green and Myerson, 2007), uncertainty and surprise (Preuschoff, T Hart and Einhäuser, 2011). More recent studies have also demonstrated that reward expectation can influence pupil properties. Work in healthy participants revealed increases in pupillary dilation to incentives that were both rewarding and aversive in nature (Manohar *et al.*, 2017). This autonomic pupil response was also examined in patient groups with medial prefrontal cortex lesions (Manohar and Husain, 2016) and in PD (Manohar and Husain, 2015). Although pupil dilation for reward was apparent in both patient studies, they also demonstrated a blunted response compared to healthy control participants. Therefore, greater incentives to carry out an action appears consistent with larger pupillary responses and may be reflective of differences in an individual's 'reward sensitivity', a potential index of an individuals appraisal of reward.

To investigate further what modulatory effect reward has on saccadic and pupil properties, two novel eye-tracking paradigms were performed in healthy control participants. These oculomotor properties included saccadic peak velocity, reaction time, accuracy and pupil size during the anticipation of a reward. The first study assessed these properties over three different distances to test if the main sequence would influence findings and the second focused specifically on the effect of reward on saccadic and pupillary properties, while also examining the effects of aging.

The most extensively used assessment for apathy is the Lille Apathy Rating Scale (LARS) which examines different apathy subdomains (Leentjens *et al.*, 2008). Patients were assessed for apathy using the LARS as it has been validated for identification of apathy in PD (Sockeel, 2006). Using the clinical interview LARS, a score of ≥ -22 was set as the cut off for apathy, encompassing mild to severe apathy symptoms (Sockeel, 2006). The LARS was used throughout the thesis to assess apathy in PD patients and elderly controls, it is a 33 item semi structured clinical interview, performed by the assessing clinician and is divided into four subcomponents. Common features used to sub-divide apathy, and which are also features of the LARS, include reduced goal-directed behaviour encompassing *self-initiated action*, *intellectual curiosity*, *emotional indifference*, and *self-awareness*. The four LARS subdomains were based on the main conceptual principles of apathy proposed originally by Marin *et a*l

and by the clinical experience of the authors (Marin, Biedrzycki and Firinciogullari, 1991) these components of apathy have been further expanded on in chapter one.

The Extended Lille Apathy Rating Scale (LARS-e) questionnaire (Bonnelle, Veromann, *et al.*, 2015) was performed in young controls. This is an adapted 51-point item questionnaire designed to measure apathy levels in non-patient populations, it assesses different domains of apathy based on a participant's view of his/her life over the previous two weeks. The LARS-e is based on The Lille Apathy Rating Scale (LARS) (Sockeel, 2006) which was developed for the assessment of clinical apathy in patients with PD. It is comprised of 4 subdomains, each linked with different domains of motivation, including: reduced intellectual curiosity, emotional indifference, reduced action initiation, and lack of self-awareness.

2.2 Methods

Study One: *Three target amplitudes and three reward levels*

2.2.1 Consent

All studies were approved by the local ethics committee and written consent was obtained from all subjects in accordance with the Declaration of Helsinki. Participants were informed that they would be paid according to task performance, a minimum of £8 and maximum of £12 was paid and transport costs were reimbursed.

2.2.2 Demographics

Twenty-one young healthy participants were recruited from an online advert (10 males, average age 27 years (SD+/- 5 years, 19 right handed). All had normal or corrected to normal vision. Participants were screened for any history of neurological or psychiatric conditions. They all also completed the Extended Lille Apathy Rating Scale (LARS-e), questionnaire (Bonnelle, Veromann, *et al.*, 2015). This is an adapted 51-point item questionnaire designed to measure apathy levels in non-patient populations, which assess different domains of apathy based on a participant's view of his/her life over the previous two weeks. The LARS-e is based on The Lille Apathy Rating Scale (LARS) (Sockeel, 2006) which was developed for the assessment of clinical apathy in patients with PD. It is comprised of 4 subdomains, each linked with different domains of motivation, including: reduced intellectual curiosity, emotional indifference, reduced action initiation, and lack of self-awareness.

2.2.3 Experimental paradigm

An infrared eye tracker (Eyelink 1000, SR Research, Ottawa) was used to monitor pupil diameter and eye position. At the start of each trial in the experiment, participants heard a voice over the loud speakers informing them of the maximum monetary reward level available for making a saccade to an illuminated target disc: 0p, 10p or 50p maximum (**Fig. 2.1A**).

The targets measured 4° in diameter and were presented randomly in the horizontal mid plane at one of three different eccentricities (5.7°, 11.4°, 17.1°) to the right or left from a central fixation disc. As soon as the target appeared, participants made an eye movement towards it. They were told that the faster they moved their eyes the more money they would make. Participants were required to fixate on the central disc for 500ms to start the trial. Simultaneously with the start of the reward voice recording the fixation disc changed colour from pale green to pale yellow to indicate the beginning of the trial. Disc luminance was matched so as not to change pupil dilatation.

After a randomly variable fore period of 1400, 1500 or 1600ms the central fixation disc disappeared and concurrently a target disc would appear and remain on the screen until the participant's gaze arrived. Participants were rewarded based on reaction time using an exponential fall off function (**Fig. 2.1B**) and this reward was displayed on top of the target disc in pence at the end of each trial. Participants performed 15 blocks of 54 trials each, with a 5-minute break between each 5 blocks, resulting in 810 trials in total with 45 saccades to each of the six target positions for each of the three reward levels.

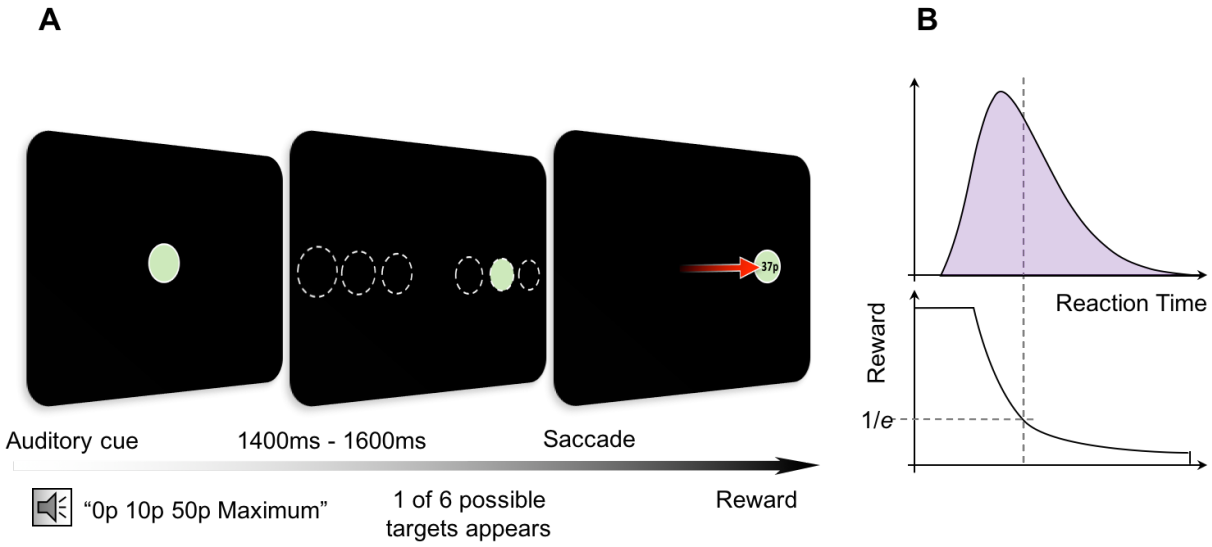

Figure 2.1. ***Study 1: Experimental paradigm - Multiple target distances.***

A. *Participants heard a recorded auditory cue that informed them of the maximum reward available for each trial: '0p/10p/50p maximum'. After a randomly variable fore-period of 1400, 1500 or 1600ms the central fixation disc disappeared and concurrently a new target disc appeared. Targets appeared at one of three eccentricities from fixation, 5.7°, 11.4° and 17.1°. Participants were rewarded according to reaction time, with the reward obtained displayed within the target disc in pence (e.g. 37p).*

B. *The absolute amount of reward earned varied with reaction time, but crucially was dynamically adjusted according to mean reaction time of each participant at any point during the experiment. To calculate the reward obtained for each trial, an adaptive exponential fall-off based on the mean reaction time of the preceding 20 trials was used. Participants received a proportion of the maximum amount on offer dependent on performance. This adaptive procedure allowed difficulty level to be maintained consistently over the experiment and importantly provided equal overall reward amounts to all participants.*

2.2.4 Reward criteria

Participants received a proportion of the maximum reward on offer dependent on performance. The absolute amount of reward earned varied with reaction time, but crucially was dynamically adjusted using an *adaptive* exponential fall-off based on average reaction time of the *preceding twenty trials*. This allowed difficulty level to be maintained consistently over the experiment, factored in different individual fatigue rates and baseline reaction times (RT) and importantly provided equal total reward amount to all participants (**Fig. 2.1B**). Thus, any differences in performance between groups could not be attributed to differences in overall rewards earned.

RT was taken as the time from target onset until a saccade reached the target, and was employed to calculate the reward. This was computed to the nearest pence using the following formula:

$$R\,(t) = R_{max} \cdot \min\left(e^{\frac{\tau 2 - 1}{\tau 1}}, 1\right)$$

R is the reward obtained for the current trial. *t* is the time taken to reach the target. *Rmax* is the maximum reward value that could be won on a given trial. $\tau 1$ and $\tau 2$ are adaptive reward criteria which were adjusted using the quantiles of the RT distribution for the preceding 20 trials. 10% of trials were kept faster than $\tau 1$ and 30% of trials slower than $\tau 2$. This adaptive process was irrespective of trial type and unknown to participants, it was designed to maintain constant and comparable reward rates across participants. This meant that any difference observed in performance between comparison groups could not be attributed simply to overall reward obtained.

2.2.5 Reward feedback

To ensure participants were equally extrinsically motivated by the amount of reward they earned, feedback was displayed within the target disc in pence at the end of each trial. Disc luminance was matched across all trials so as not to affect pupil dilation. If the reward obtained was 10p or more an audible bell sound was also played, and if greater than 30p a 'cash register' sound was played after reward delivery at the end of the trial; no sound was heard for less money.

Behavioural indicators of task performance included saccadic speed and saccadic variability. In debriefing, all participants said that they had attempted to maximize their gains, although people varied in strategy with some saying they tried to speed up for bigger rewards, while others said they tried to slow down on no reward trials.

2.2.6 Eye tracker data recording

Eye tracking was performed in a dimly lit room 60 cm in front of a 21" CRT monitor (1024x768 pixels; 100 Hz refresh). Stimuli were presented on a Windows computer running Matlab (The MathWorks) and Psychophysics Toolbox. The frame-mounted infrared tracker monitored left eye position and sampled at 1 kHz. Eye movements were measured online by the Eyelink computer and transferred directly to the presentation computer to provide immediate feedback. 9-point calibration was performed at the start of each experiment. Eye movement analysis was carried out using custom-made Matlab code. An eye movement was classified as a saccade if it was > 2°, and the accepted landing area to register completion of the saccade was 5° in radius from target centre. The first landing point within the target area was used to classify saccade completion. Reaction time was calculated from target onset to the time when a saccade was registered as complete, and peak saccadic velocity computed as maximum velocity recorded during the saccade. Residual saccadic velocity was also calculated in order to factor out any effects of the main sequence, amplitude of the saccades made were regressed out from the measured velocities. Saccadic accuracy was defined as the variability of saccades, by calculating the average standard deviation of saccadic amplitudes for each condition. Using saccadic variability as a measure of accuracy takes into account any changes in starting position due to eye tracker drift and also variability in the end point error of each saccade.

Pupil dilation was calculated as a proportional change from average baseline pupil size measured in Eyelink units. Eyelink units are arbitrary units of visual angle calculated by the number of pixels that form the image of the pupil and provides a raw measure of pupil size. Recordings were time locked to the reward cue onset and normalised using a 200ms baseline subtraction for each trial. Pupil traces lost due to blinks were interpolated. A moving average smoothing window of 100ms was applied to the final recordings.

2.2.7 Eye tracker data analysis

Analysis was performed using repeated measures ANOVA, with trials grouped according to reward (0p, 10p, 50p) and amplitude (small, medium, large). To account for any non-sphericity in the data, where appropriate, statistics are reported with Greenhouse-Geisser correction. Significant main effects and interactions were decomposed with Bonferroni corrected pairwise comparisons. Significance was taken as p-values of less than 0.05. Correlations with questionnaires were performed using Spearman rank non-parametric testing and Pearson correlations were used for parametric behavioural outcome comparisons. Statistics were completed using Matlab and SPSS. To find associations between motivation and incentivized oculomotor outcomes (including saccadic peak velocity, accuracy and pupil responses to reward) correlation analysis was performed with the LARS-e subdomains.

2.2.8 Pupillometry criteria

Pupillary modulation by reward was taken as mean proportional change in pupil size from baseline pupil diameter at the beginning of each trial time, locked to the onset of the auditory reward cue. Mean pupillary size from 1400 – 2400ms after the auditory cue was used as the epoch of interest. This window was based on the known sluggish response dynamics of the pupil (Lehmann and Corneil, 2016), allowing adequate time for the pupil to vary in size after hearing the auditory reward cue in each trial. It was 1000ms in length to sufficiently encompass a large proportion of the pupil response and did not include the last 100ms of the trial to account for any effects of the moving average smoothing window. An individual's *pupillary reward sensitivity* was defined as the difference in mean proportional pupil change

between the maximal 50p reward and 0p reward conditions, over the time epoch defined above. A larger difference indicated greater reward sensitivity.

2.3 Results

Study One: *Three target amplitudes and three reward levels*

The main objective of the first study was to investigate whether external monetary rewards would influence saccadic and pupil properties.

2.3.1 Amplitude of eye movement increases with rewards

Three different eccentricities of 5.7°, 11.4° and 17.1° were used in the task as this formed equal spacing on the 21" display. To ensure that the saccades made to the three distances were consistently different from each other, an ANOVA was performed between the actual amplitude of the eye movement recorded at each target location and the reward level of that trial. There was a main effect of amplitude, $F(2,40)=5947.8$, $p<0.001$) and a main effect of reward, ($F(2,40)=9.0$, $p<0.01$) but no interaction. Significant differences were present between each target eccentricity pairwise group comparison $p<0.001$ and each reward pairwise comparison $p<0.03$, showing that the average distance for eye movements between each of the three targets locations was significantly different from one other as would be expected, (**Fig. 2.2A**)

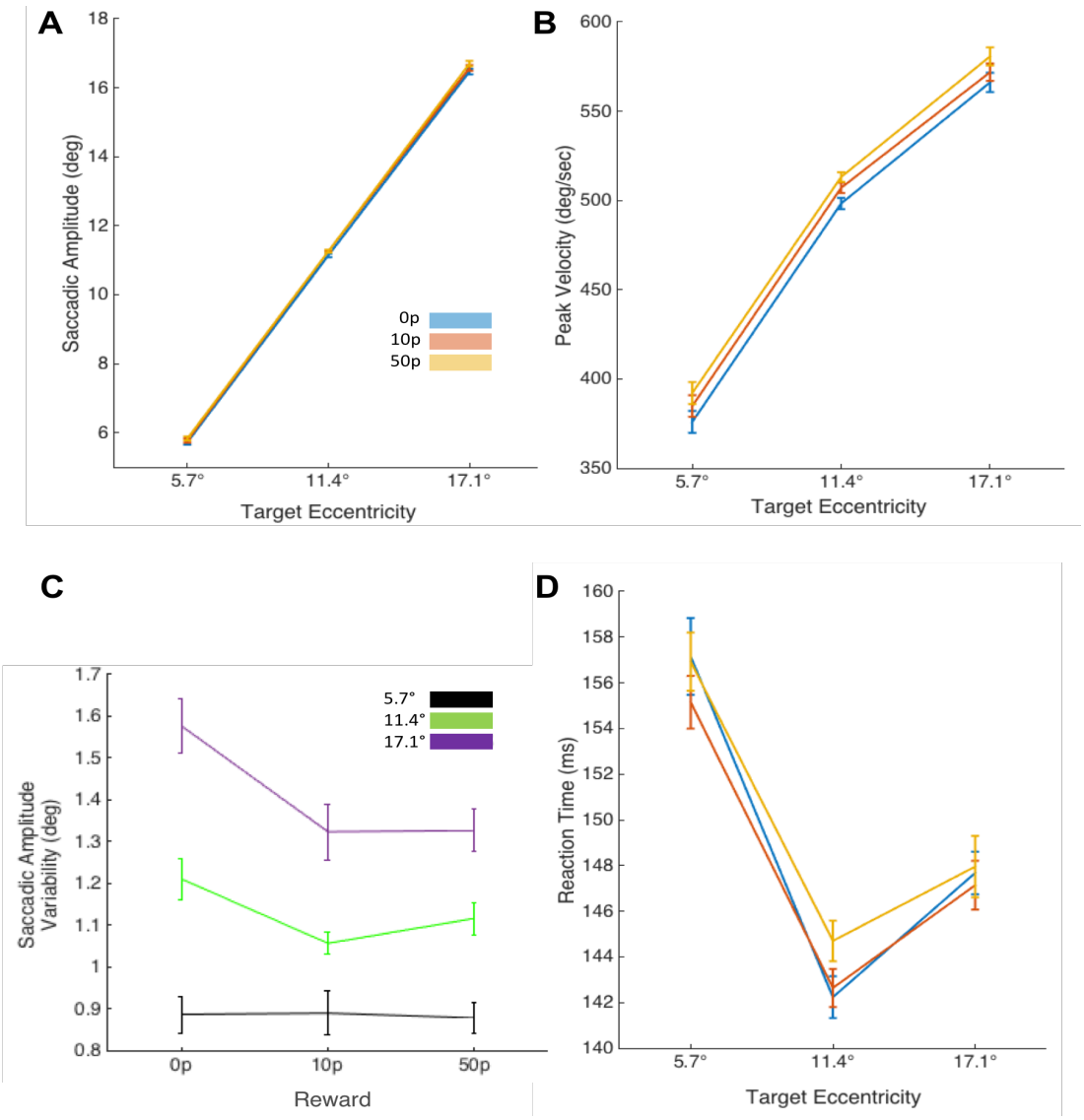

Figure 2.2A-D. Effects of target distance and reward on saccade metrics.

- ***A.*** *Average size of the saccades made for each reward level for small (5.7°), medium (11.4°) and large (17.1°) target eccentricities. Target locations were sufficiently spaced apart to produce a significant difference in saccadic amplitude.*
- ***B.*** *Average saccadic peak velocity for each reward at each target eccentricity. Speeds in general were greater as the eccentricities of targets increased, and faster peak velocities for larger rewards were also apparent at every eccentricity.*
- ***C.*** *Variability (accuracy) for each reward and target eccentricity. Calculated as the standard deviation of saccadic amplitude to take eye tracker drift and starting eye position into consideration. Overall worsening of accuracy was seen for increasing amplitude of eye movements. However, greater improvement of accuracy was seen for larger rewards the greater the eccentricity of the target from fixation.*
- **D.** *Average reaction time in milliseconds for each reward level at each target eccentricity, fastest RT was for the middle 11.4° target location.*

2.3.2 Rewards invigorate saccades

In keeping with the literature on the main sequence, peak velocity increased systematically with saccadic amplitude. (**Fig. 2.2B**) and there were no differences in velocities between left and rightward saccades. Crucially however, the bigger the magnitude of reward at each of the three target positions, the greater the PV. There was an overall main effect of reward ($F(2,40)=20.6$, $p<0.001$) as well as of target eccentricity ($F(2,40)=283.1$, $p<0.001$) with no significant interaction ($F(4,80)=0.734$, $p=0.57$). The invigoration of PV by reward occurred at each of the three distances tested. A pairwise comparison between the three reward levels showed significant differences at the $p<0.005$ level.

These findings suggest that saccadic velocity can be modulated directly by the rewards on offer. However, an alternative possibility is that rewards increase saccadic amplitude and, given the principles of the main sequence, it is this effect which leads to increased PV with reward. To assess if rewards directly modulated PV, the effect of the main sequence on saccadic peak velocity was factored out: residual velocities for each saccade were calculated by regressing out the amplitude from the peak velocity. Importantly, this resulted in the same outcome with a main effect of reward ($F(2,40)=17.2$, $p<0.001$), and significant differences between each reward level comparison ($p<0.02$). Thus, even after removing any amplitude effects of reward, saccadic velocity still increased with increasing incentives.

2.3.3 Rewards increase accuracy

Saccadic accuracy, indexed by movement variability (standard deviation of saccade size), worsened with larger target eccentricities (**Fig. 2.2C**). A main effect of reward ($F(2,40)=6.42$, $p<0.005$) and amplitude ($F(2,40)=42.1$, $p<0.001$) was present, with a significant interaction ($F(4,80)=3.12$, $p=0.019$). Pairwise comparisons showed differences in accuracy between all three target locations ($p<0.001$) with worsening accuracy as target distance increased. The interaction suggested that although accuracy worsened with bigger eccentricities, rewards improved accuracy the most the larger the distance of the eye movement. Individual comparisons between reward levels for each target distance were made. No significant differences for the smallest 5.7° eccentricity were found. There were significant differences between the 0p and 10p reward in the medium 11.4° amplitude

(p=0.014) and differences between all reward levels in the largest 17.1° eccentricity (p<0.02, except between 10p and 50p rewards; p=0.95), such that accuracy improved with greater rewards the larger the target eccentricity. Note therefore that both PV and accuracy improved with incentives; hence a speed-accuracy trade-off cannot account for these data.

2.3.4 Rewards and saccade reaction time

Reaction time (RT) was slowest at the smallest amplitude closest to fixation (5.7**°**) and fastest at the medium 11.4**°** target location. There was a main effect of amplitude ($F(2,40)=29.2$, $p<0.001$) and a trend to a main effect with reward ($F(2,40)=3.0$, $p=0.064$, with no significant interaction ($F(4,80)=1.9$, $p=0.117$). Pairwise comparisons showed that there was a significant difference between all target location comparisons ($p<0.002$; **Fig. 2.2D**).

2.3.5 Rewards influence pupil response

Pupil dilation was measured from the onset of the auditory stimulus (incentive cue) for each reward level to the time a saccade was initiated. Pupil size was significantly greater for the 50p reward cue compared to the 0p reward, with the 10p reward in between (**Fig. 2.3**). This was performed across all the different target eccentricities. Comparisons were made using sequential repeated measures permutation tests to account for multiple comparisons. There was a significant difference between the 0p and 50p condition from approximately 1200ms to the end of the trial. (**Fig. 2.3** – black bar)

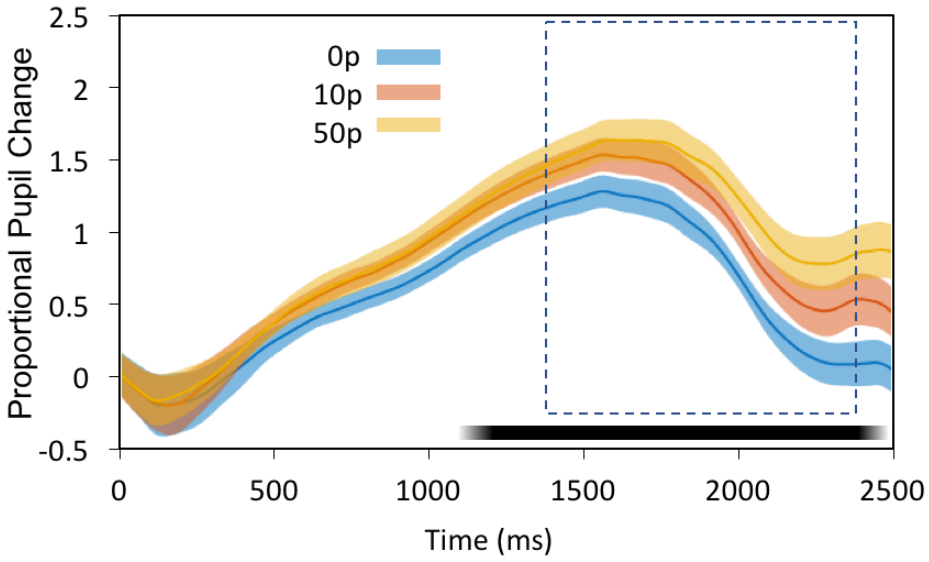

Figure 2.3. Pupil response to reward over time.

Group average pupil dilation after hearing the reward cue at time 0ms for the three reward levels. Shaded area denotes standard error of the mean and black bar indicates area of significant difference between the 0p reward and 50p reward over time (p<0.05). Dashed line indicates epoch of interest used in subsequent analysis.

Average pupil change was calculated over the period 1400ms to 2400ms (highlighted by dashed area in **Fig. 2.3**). As discussed in the methods, this window was selected in order to provide adequate time for the pupil to vary in size after hearing the auditory reward cue due to the known sluggish time course of the pupil. This average change over the epoch of interest is shown in **Fig. 2.4**. There was a main effect of reward ($F(4,80)=10.8$, $p<0.001$) and pairwise comparisons were significant between 0p vs 10p and 0p vs 50p at the $p<0.002$ level.

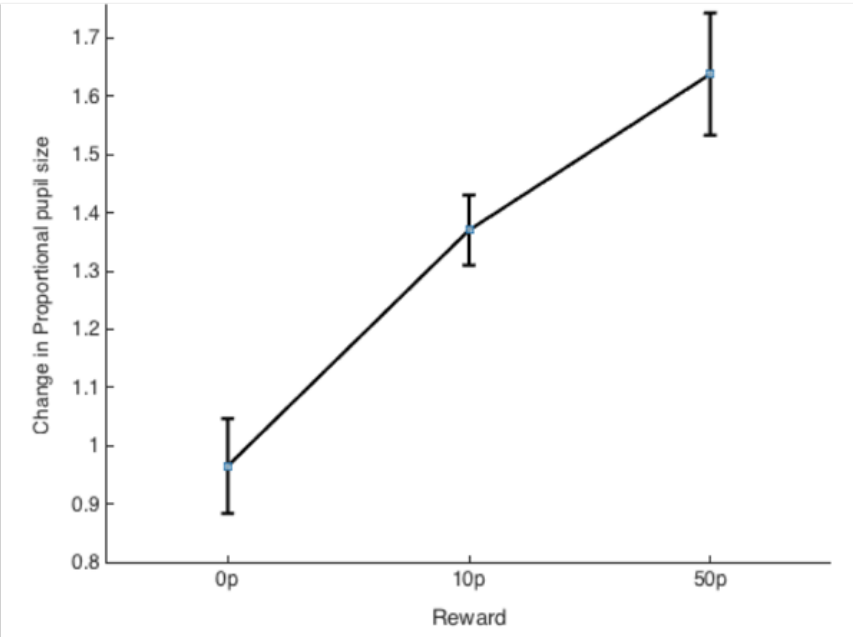

Figure 2.4. Average pupil modulation by reward in the young.

Average pupil dilation over 1000ms period from 1400ms to 2400ms (as indicated by dashed box area in Fig.2.3). There is greater pupil dilation for rewards of larger magnitude, this is significant between 0p vs 10p and 0p vs 50p (p<0.002).

2.3.6 Reward sensitivity and the possible link to motivation

In order to find planned associations between motivation and incentivized oculomotor outcomes (including saccadic peak velocity, accuracy, amplitude and RT) correlations were performed with the LARS-e subdomains. None of the stated saccadic properties demonstrated a correlation with the LARS-e motivation questionnaire subscales. Interestingly however, a link was present with motivation and the pupil response to reward. Using the 1400ms to 2400ms average time area of interest and obtaining a single reward sensitivity score for each participant, a significant correlation with the Intellectual curiosity motivation LARS-e subscale was present (r_s=0.448, p=0.042; **Fig. 2.5)**.

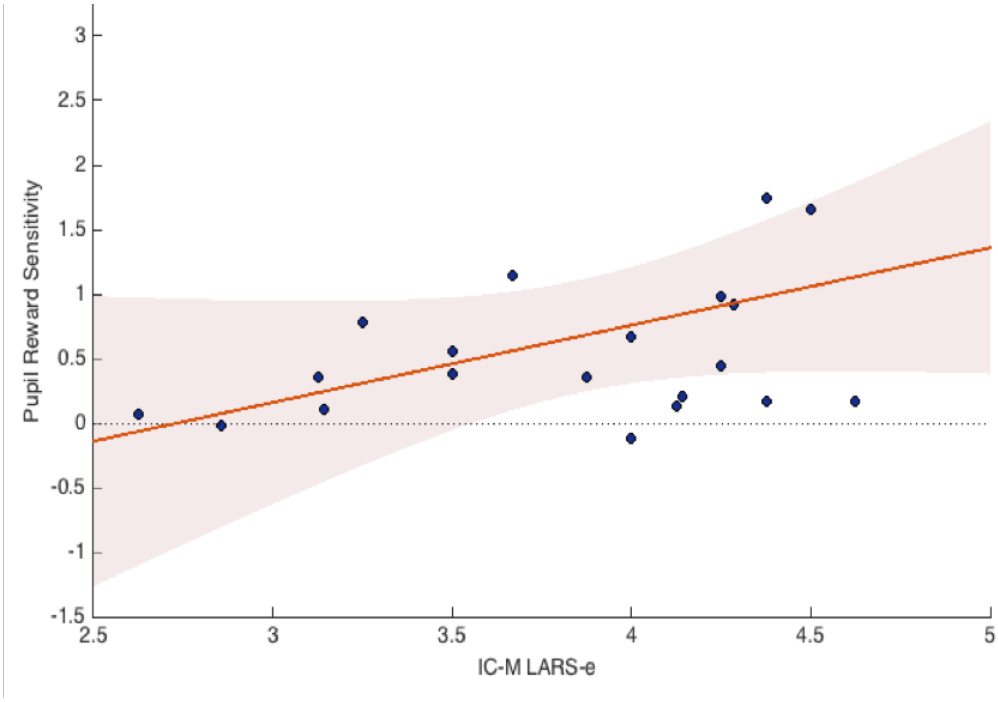

Figure 2.5. Correlation with pupil reward sensitivity and LARS-e motivation questionnaire in the young.

Significant Spearman Rank correlation between pupil reward sensitivity and the Intellectual curiosity – motivation subscale of the LARS-e. rs=0.448, p=0.042. The more Intellectually curious participants were on the LARS-e (IC-M LARS-e) the greater the pupil reward sensitivity of an individual.

2.4 Conclusion

Study One: *Three target amplitudes and three reward levels*

This novel oculomotor task provides evidence that rewards are able to modulate behavioural and physiological responses in saccadic eye movements and pupillary dilation.

The eye movement amplitudes between each of the three targets locations were significantly different from one other (**Fig. 2.2A**) and peak velocity increased systematically with amplitude as per the main sequence. The bigger the reward on offer the greater the PV, even when factoring out the effects of amplitude on saccades (**Fig. 2.2B**). Rewards also improve accuracy for larger eccentricity saccades, breaking the speed accuracy tradeoff (**Fig. 2.2C**). The most interesting result was in pupil responses to anticipated rewards. There was a larger pupil dilation for 50p rewards compared to 0p rewards and this remained significant over a prolonged period of time (**Fig. 2.3**). In addition, there is some evidence that individual differences in participants' pupil response to rewards may represent differences in motivation

level (**Fig. 2.5**). The weak association with motivation is likely due to the narrow range of motivation scores in the healthy population studied. No participant was clinically apathetic so any differences within this normal group would be anticipated to be small. It is likely that we would need to study a population with a larger spread of apathy measures in order to expand these differences in pupillary reward sensitivity and establish if this methodology could determine differences in responses between apathetic and non-apathetic individuals.

Given that the three target location differences did not add significantly to the findings, and to simplify the experiment with patient testing in mind, a less complex single amplitude version was developed and tested in young and elderly control participants.

2.5 Methods

Study Two: *One target amplitude and three reward levels*

2.5.1 Control demographics

Twenty young participants were examined, average age 25.75 years, SD+/- 4.53, 19 right handed, 8 male. Thirty-one elderly healthy participants were also tested in a single session, 13 were male and 27 right handed, average age was 67.5 years, SD+/- 8.5. They were recruited from a volunteer database and were also screened for any history of neurological or psychiatric conditions. All participants had normal, or corrected to normal vision at the time of testing. Young participants completed the LARS-e questionnaire, while the elderly participants were assessed for apathy using the Lille Apathy Rating Scale (LARS) (Sockeel, 2006); they were also screened for depression with the Beck Depression Inventory-II (BDI-II) (Beck, Steer and Brown, 1996) and for cognitive impairment on the Montreal Cognitive Assessment (MoCA) (Nasreddine *et al.*, 2005).

2.5.2 Experimental paradigm

Like the previous task, an infrared eye tracker (Eyelink 1000, SR Research, Ottawa) was used to monitor pupil diameter and eye position. The same instructions were given as before to participants. The faster they looked at a target, the greater the proportion of reward on offer they would obtain (**Fig. 2.6**). Each trial commenced with the onset of a disc at screen centre. After they had fixated this for 500ms, participants heard a recorded voice that informed them of the maximum reward available for that particular trial: "0p/10p/50p maximum". Subsequently, after a randomly variable period of 1400, 1500 or 1600ms the central fixation disc disappeared and concurrently a new target disc appeared randomly either to the left or right. Like in study 1, the targets measured 4° in diameter but this time appeared only at 11° eccentricity. One target location was used in this study, either on the left or the right of fixation. The distance of 11° eccentricity was chosen as this approximately equated to the optimal distance which maximized differences in amplitude, velocity and reaction times by reward as calculated from the previous experiment.

Participants performed five blocks of 54 trials each, with a 5-minute break between the first 3 and last 2 blocks, resulting in 270 trials in total with 90 trials for each of the three reward levels. Behavioural indicators of task performance included saccadic speed and saccadic variability. In debriefing, all participants said that they had understood the instructions and attempted to maximize their gains. The same reward criteria and feedback procedure was used as per study 1.

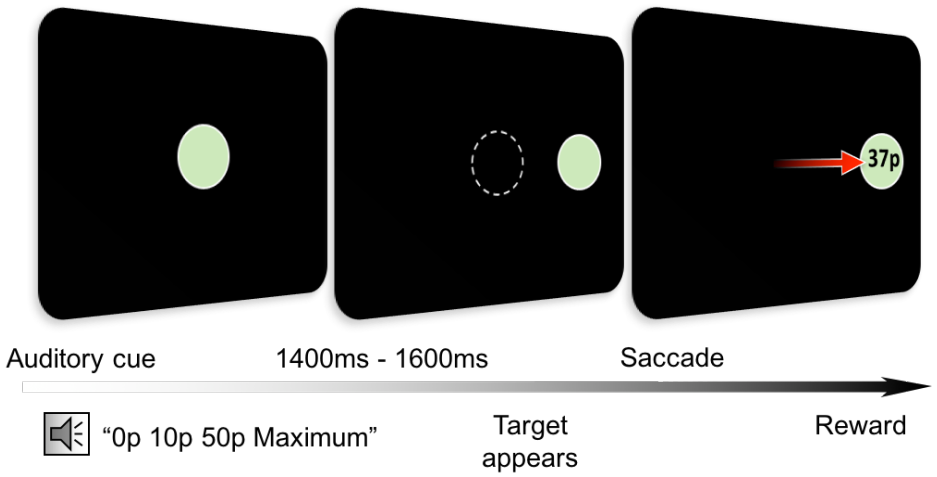

Figure 2.6. ***Study 2: Experimental paradigm - Single target distance.***

A recorded auditory cue was heard that informed participants of the maximum reward available for each trial: '0p/10p/50p maximum'. After a randomly variable fore-period of 1400, 1500 or 1600ms the central fixation disc disappeared and concurrently a new target disc appeared. In this study, only one target location (11° eccentricity to the right or left of fixation) was used. Participants were rewarded according to reaction time, with the reward obtained displayed within the target disc in pence (e.g. 37p).

2.5.3 Eye tracker data analysis and pupillometry criteria

The same eye tracking and recording analysis criteria for assessing the pupillary response was used as per study 1. An example of the saccadic paths that were generated from a single participant over the course of multiple trials can be seen in **Fig. 2.7**.

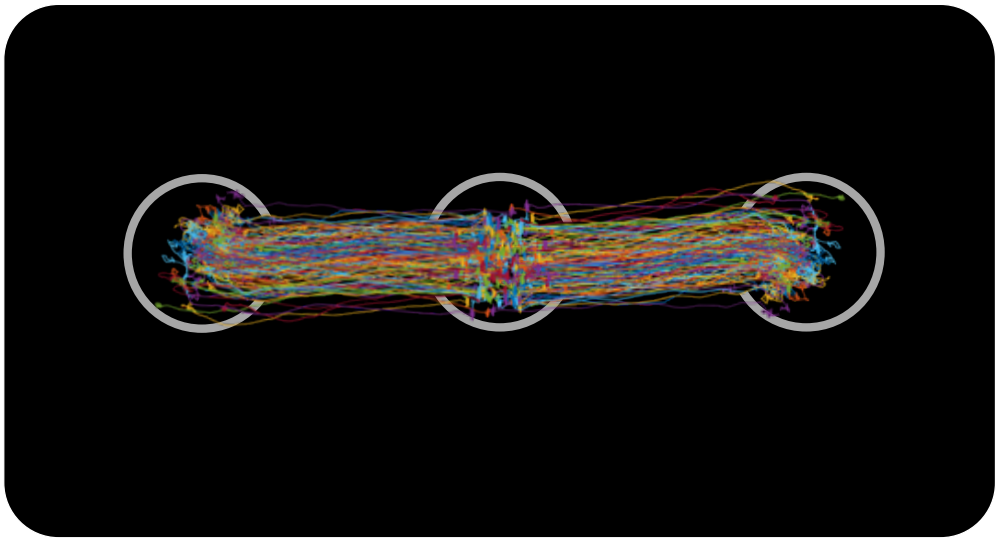

Figure 2.7. Example of saccadic trajectories during the task.

An example recording from one participant of the saccadic paths and trajectory made over one experiment for all reward types.

2.6 Results

Study Two: *One target amplitude and three reward levels*

2.6.1 Rewards invigorate saccades in the young and elderly

Like the previous three amplitude task in study 1, this single amplitude version showed that reward could modulate saccadic peak velocity. This was the case in both young and elderly participants (main effect of reward ($F(2,98) = 30.6$, $P < 0.001$). The young were also more responsive to rewards (steeper slope), with faster saccadic peak velocity for larger rewards on offer in comparison to elderly participants (reward by group interaction ($F(2,98) = 4.9$, $P < 0.01$; **Fig. 2.8A**). Pairwise comparisons between the reward levels showed significant differences between each reward level in young controls ($p<0.0001$) and in the elderly differences were only present between the 0p vs. 50p and 10 vs. 50p condition ($p<0.02$) but not for the 0p vs. 10p condition ($p=0.08$). Although the groups appeared to differ in overall velocities, statistically this was not the case and there was no main effect of group, ($F(1,49) = 2.6$, $P<0.115$).

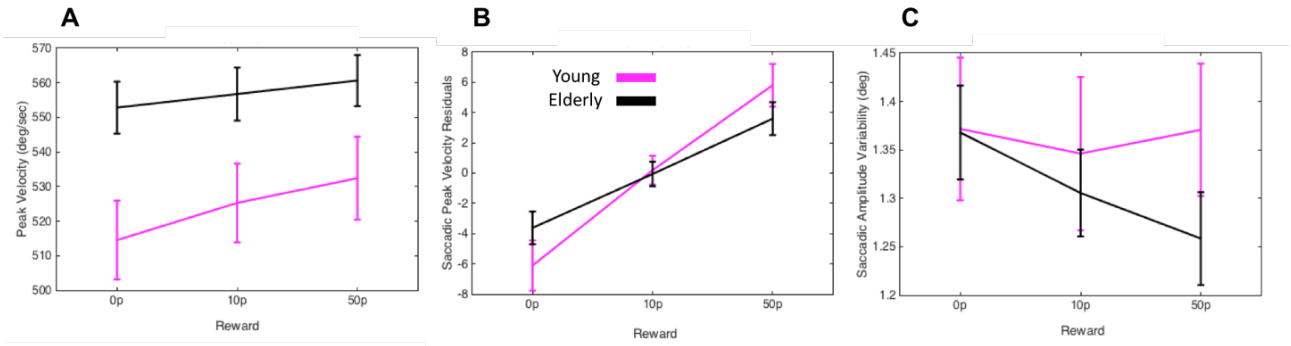

Figure 2.8A-C. Effects of reward on saccade metrics.

- ***A.*** *Young and elderly healthy participant's peak velocity as a function of reward. Faster speed saccades were present for larger rewards in both young and elderly participants, with the younger group becoming even faster for increasing reward levels compared to the elderly. Although when plotted, it appeared that elderly participants had faster saccades in general compared to young controls, no significant overall group difference was present.*
- ***B.*** *Residual velocities of young and elderly participants with amplitude regressed out of peak velocity for each reward level. Saccades were faster for larger rewards on offer and no differences in the effect of reward was present between young and elderly.*
- ***C.*** *Saccadic variability (standard deviation of amplitude) as a metric of accuracy for each reward on offer. Saccadic variability did not change significantly with increasing rewards on offer.*

Factoring out any changes in the amplitude of the saccades made by the two groups abolished the appearance of any overall difference (**Fig. 2.8B**). This was done by performing multiple linear regression. Any change in saccadic peak velocity accounted for by increases in saccadic amplitude were removed, leaving only the peak velocity residuals. When comparing peak velocities residuals, although the main effect of reward remained ($F(2,98) = 22.0$, $P < 0.001$), there was no longer a group by reward interaction ($F(2,98) = 1.3$, $P < 0.266$), so the differences in velocity for reward in young and elderly are likely to have been driven by differences in the amplitude of the saccades.

2.6.2 Rewards increase accuracy

On comparison of saccadic accuracy between young and elderly controls, overall there was no main effect of group. Analysis of the data from the elderly controls alone did demonstrate a trend towards a main effect of reward, ($F(2,60) = 3.1$, $P = 0.051$), which was driven mainly between differences in the 0p vs. 50p condition ($p=0.019$). (**Fig. 2.8C**).

Therefore, it seems that rewards are able to increase saccadic speed while simultaneously not worsening accuracy, thereby breaking current notions of speed accuracy tradeoffs, much like in the study 1. Further, in the elderly group it seems that reward goes as far to increase both speed and accuracy simultaneously.

2.6.3 Rewards do not influence saccadic latency

There were no significant main effects of group between young and elderly in reaction time, nor were there any effects of reward on reaction time in this study.

2.6.4 Rewards influence pupil response

The expectancy of increasing rewards on offer resulted in increased pupillary responses in young healthy controls, much like in study 1. The difference between the 0p and the 50p reward condition appeared from ~630ms after hearing the reward cue and remained until the end of the trail (**Fig. 2.9A**). The same occurred for the pupil response in elderly controls. There was, however, a temporal delay in significance between the 0p and 50p condition in the elderly compared to the young controls, with significant differences emerging later at ~1200ms and remaining until the end of the trail (**Fig. 2.9B**).

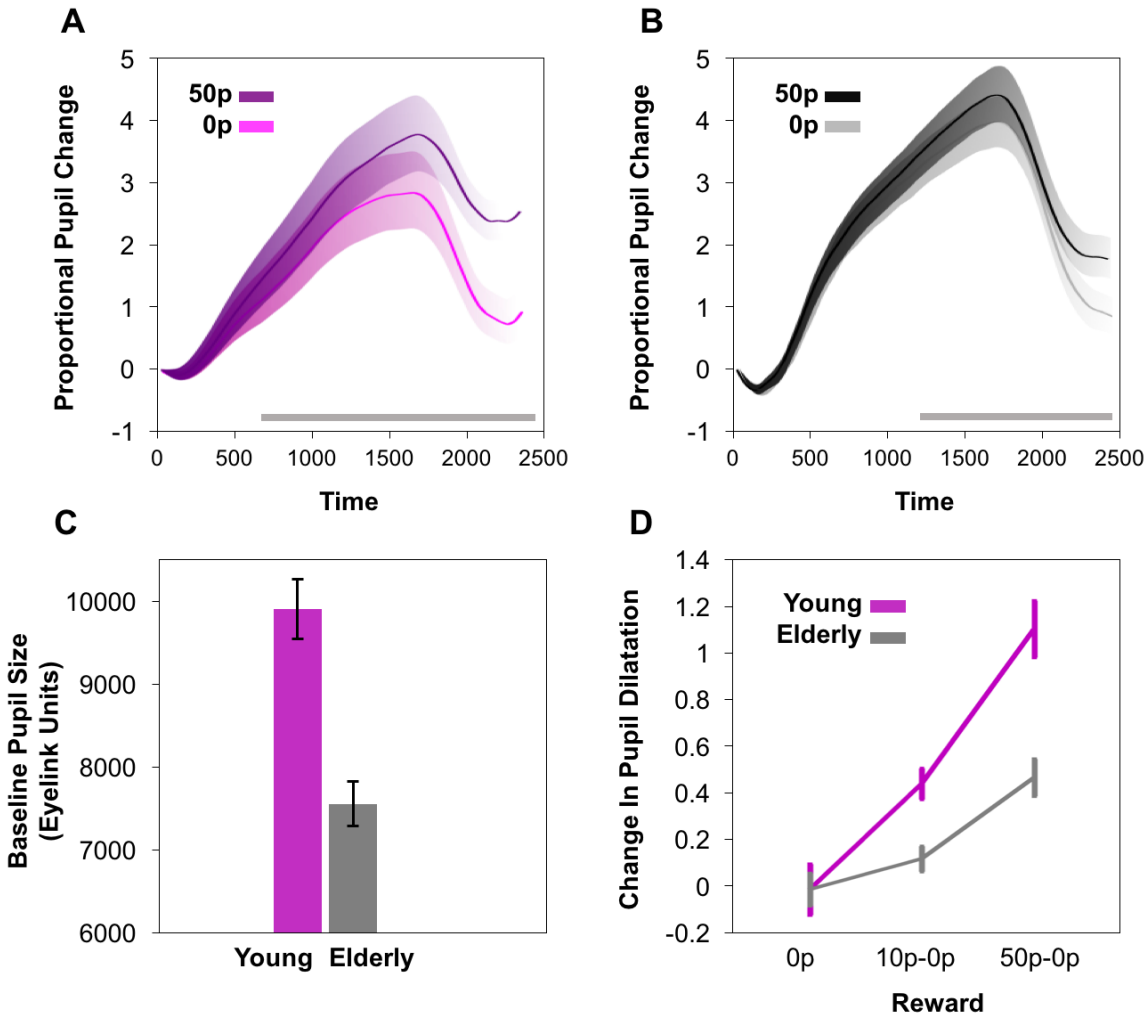

Figure 2.9A-D. Pupillary responses in control participants.

- ***A.*** *Mean proportional pupillary trace in young control participants after onset of reward cue at time 0ms to end of trial. Greater proportional change was observed for larger rewards compared to 0p reward. The 10p condition has been removed from the diagram for simplification. A significant difference between the 50p maximal reward (dark purple) and 0p reward level (light purple) was present from ~630ms to the end of the trial (P<0.05), denoted by grey bar at bottom of plot. Shaded areas represent standard errors of the mean (SEM).*
- ***B.*** *Mean pupillary trace in elderly control participants after onset of reward cue. A significant difference between 50p maximal reward (black) and 0p reward (grey) was present from ~1200ms to the end of the trial (P<0.05), denoted by grey bar at bottom of plot.*
- ***C.*** *Mean pupil baseline size across all rewards taken at the start of each trial and measured using raw Eyelink units. Young controls had significantly larger baseline pupil size compared to elderly controls.*
- ***D.*** *Proportional pupillary change as a function of reward level in young compared to elderly controls, taken as mean pupil dilation between 1400–2400ms. Plots have been normalized to the 0p baseline to demonstrate the reward sensitivity slopes between young (purple) and elderly control participants (grey). Although both groups demonstrated greater pupillary dilation with increasing reward magnitude, there was reduced pupil reward sensitivity in elderly compared to young participants despite elderly controls having a smaller baseline pupil size and therefore more capacity to dilate for rewards.*

2.6.5 Pupil reward sensitivity declines with age

In young and elderly controls, pupillary dilation was significantly increased by the magnitude of monetary reward on offer. As previously, reward sensitivity was defined as the difference in pupil response between the maximal 50p reward on offer and the 0p reward. Repeated measures ANOVA in the young controls over the time epoch of interest (1400-2400ms) demonstrated a significant main effect of reward ($F(1.5, 28.2) = 20.2$, $P < 0.001$) and post hoc comparisons between rewards (0p vs. 10p, 10p vs. 50p and 0p vs. 50p) revealed significant differences in pupillary response between each level ($P < 0.01$).

In elderly controls there was also a main effect of reward, ($F(1.7, 49.6) = 9.0$, $P < 0.001$). However, the extent of reward sensitivity was not as great, as shown by the shallower slope of the pupil size between 0p and 50p against reward on offer (**Fig. 2.9D**). In the elderly, post hoc comparisons showed a change in pupillary size between each reward at the $P <0.01$ significance level, except between 0p and 10p where no significant difference was found. The reduction in reward sensitivity with age was apparent with direct comparison between young and elderly controls. Here, there was no significant overall main effect of group demonstrated ($F(1, 49) = 1.1$, $P = 0.3$). However, a significant main effect of reward was still evident ($F(1.6, 78.6) = 30.8$, $P < 0.001$) and crucially there was also a significant reward by group interaction, so the extent of pupillary response to reward for increasing reward magnitudes was reduced in the older group ($F(1.6, 49) = 4.8$, $P < 0.02$).

These findings were evident despite young controls having significantly larger mean baseline pupil size compared to elderly controls and therefore less capacity to dilate for reward (**Fig. 2.9C**). Although elderly participants had on average smaller pupils compared to young people, and therefore more scope for proportional change, their pupillary response did not modulate with reward to the same degree, i.e. they had less change in pupil size between the 0p and 50p conditions.

On average the young and elderly controls both had a degree of reward sensitivity as measured by the pupil over time, but this decreased with age and was apparent between ~1570ms to ~2400ms as calculated by multiple permutation testing ($p<0.05$), (**Fig. 2.10**).

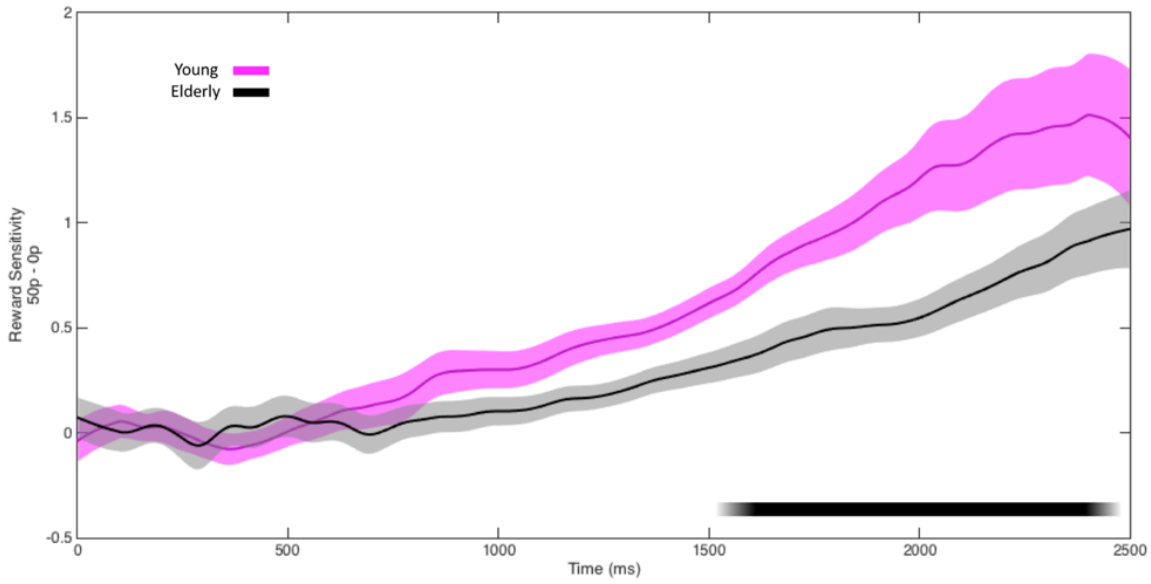

Figure 2.10. Young and elderly participant's pupil reward sensitivity over time.

Pupil reward sensitivity was calculated as the difference in pupil dilation for the 50p reward and the 0p reward and this was plotted over time. The black bar denotes area where young participants reward sensitivity is significantly greater than the elderly (p<0.05). Significance occurs from ~1570ms to the end of the trial.

2.6.6 Reward sensitivity may be linked to motivation

Interestingly, like in study 1, there were no associations with saccadic metrics (including peak velocity, RT, accuracy, amplitude and velocity residuals) and motivation in the young and elderly. Having demonstrated associations with pupil response for anticipated reward and motivation in study 1, this comparison was again made. Reliably, there was again a significant correlation present between the LARS-e Intellectual Curiosity Motivation sub-score and pupillary reward sensitivity (as calculated by the average change over 1400ms to 2400ms) in young controls (r_s =-0.461, p=0.041).

2.7 Conclusion

Study Two: *One target amplitude and three reward levels*

This second oculomotor task replicated findings from study 1 and provides a simpler paradigm which might be suitable for use in patient groups. Rewards invigorate saccades in the young and elderly (**Fig. 2.8A**) even when the effect of amplitude, as per the main sequence, is factored out (**Fig. 2.8B**). Rewards are also able to increase saccadic velocity while

simultaneously not worsening accuracy in the young and actually improving accuracy in the elderly (**Fig. 2.8C**) again breaking current concepts of speed accuracy tradeoffs. No effects on saccadic latency were demonstrated, but like in study 1, the link with motivation and pupil reward sensitivity remained. There were also differences with ageing, including a reduction in the pupil reward sensitivity in the elderly (**Fig. 2.9D**) and a lengthening of the temporal onset (grey bar denoting significance in **Fig. 2.9A** compared to **Fig. 2.9B**) while also taking baseline pupil sizes into consideration (**Fig. 2.9C**).

2.8 Discussion

These two novel oculomotor tasks demonstrate that monetary incentives can positively modulate saccadic properties, including invigoration of saccadic velocity in young healthy participants (**Fig. 2.2B**) as well as in the elderly (**Fig. 2.8A**). This is apparent even when the effects of larger amplitudes on velocity are regressed out (**Fig. 2.8B**). This phenomenon is known as the main sequence (Bahill, Clark and Stark, 1975) and refers to the relationship between the peak velocity and amplitude of saccadic eye movements. The larger the eccentricity of an eye movement, the faster the saccadic speed generated, possibly to optimize tradeoffs between the accuracy and duration of movements (Harris and Wolpert, 2006). To confirm that the effects of the main sequence were present in our data, the stereotypical response of the main sequence was reproduced in study 1. Peak velocity increased systematically with larger eccentricities of eye movements (**Fig. 2.2B**). It has been argued that the speed and timing of movements may be chosen to maximise the amount of reward obtained (Niv *et al.*, 2007). The invigoration of saccadic peak velocity by reward observed here fits this prediction. This suggests that there is more flexibility in the main sequence than previously thought: saccades are not simple stereotyped, ballistic movements but can be modulated by reward.

Rewards are also able to consistently reduce variability in saccadic movements, either maintaining or even increasing accuracy (**Fig. 2.8C**) breaking speed accuracy tradeoffs. This replicates findings from other saccadic and motor studies that showed apparent limits of performance can be overcome by incentives (Mazzoni, 2007; Chen *et al.*, 2014; Manohar *et al.*, 2015). This improvement of accuracy by reward was only present when saccadic eccentricities were larger than 5.7°, as there were no differences for accuracy between rewards at the smallest target distance (**Fig. 2.2C**). This results challenges the idea that optimal speeds and accuracy are always selected for saccadic movements (Harris and Wolpert, 2006), because reward seemingly optimizes movements further with no apparent cost to either speed or accuracy. One possible explanation for this might be that rewards reduce intrinsic neural noise associated with making a movement. As per motor control theory, the speed-accuracy tradeoff is believed to occur because faster movements are subject to greater motor noise in neural control signals (Harris and Wolpert, 1998). However,

when the receipt of reward is also incorporated into the action, there appears to be a break in this tradeoff through the reward paying the cost of noise reduction (Manohar *et al.*, 2015).

Pupil responses to anticipated rewards consistently demonstrated larger dilation responses for greater rewards on offer, this was present in young participants across study 1 (**Fig. 2.3**) and study 2 (**Fig. 2.9A**) and also in elderly participants (**Fig. 2.9B**). However, the extent of this reward sensitivity declined with age (**Fig. 2.10**). Crucially, there was a significant relationship between the pupil response to anticipated rewards and motivation level – even in healthy participants with a narrow range of apathy scores (**Fig. 2.5**). Since behavioural responses and pupil diameter are influenced by the salience of a stimulus, timings and auditory volume of all the cues, target luminance and reward levels were all matched, leaving only individuals' physiological response to the valuation of the rewards as the variable factor. Of course, larger rewards might be perceived to have greater salience and therefore produce increased arousal and more pupillary change. This response would nevertheless still index the sensitivity that individuals have for each reward magnitude. In addition, increased arousal may place individuals in a heightened state ready to perform goal directed actions (Ressler, 2004). By this reasoning, reward sensitivity simply describes the degree to which incentives modulate physiological response.

To clarify our view on the definition of reward sensitivity: we take "reward sensitivity" to be an empirical construct that describes the degree to which incentives modulate physiological response. Less pupillary modulation by reward might occur for several reasons: reduced attention to the incentives, reduced overall motivation or maximal energy output, or higher costs for exerting effort (a saccade) or processing the cue. According to this broad view, attentional costs might be a factor in decision-making to initiate action and thereby contribute to apathy and motivation in general. Therefore, if an individual's behaviour is less affected by reward for any reason, for example because they are less alert or attentive, this might still potentially contribute to reduced reward sensitivity, and inform us of their motivational state.

There were no associations with reaction time and reward in either of the two experimental studies. This may be due to the adaptive reward function which was designed to keep the reward rate constant throughout the experiment and allow fair comparisons between different groups (**Fig. 2.1B**). Using this adaptive reward ensured each group would

receive the same total reward, while remaining performance dependent. Thus, crucially it cannot be argued that any difference in observed performance between any groups tested in these tasks are simply due to one group receiving less reward than another and were thereby less motivated. This is a key part of the design and allowed comparable results across participants whilst maintaining average reward rate and total amount received. Following on from this, a variable fore-period was also built into the task so that anticipation and pre-emptive movement towards the target could be avoided, as temporal expectancy may have biased the results.

Expanding further on the reaction time result from the multiple amplitude task from study 1, the influence of eccentricity (the distance from the central fixation point to the target as measured by visual angle in degrees) on saccade reaction time has been previously studied in depth (Kalesnykas and Hallett, 1994). Small saccades that were around 1° of visual angle increased saccade latency. On the other hand, for large saccades greater then 15° there were also increases in saccade latency, mimicking the U-shaped RT result that was found in study one (**Fig. 2.2D**). Perhaps the slower reaction time for the smallest (5.7°) target distance could be explained by more fixation neurons in the rostral pole of the superior colliculus (Everling *et al.*, 1998) remaining activated when the targets closest in proximity to the fixation circle appeared (Kalesnykas and Hallett, 1994). However, given the design of the task, reaction time was not used as an indicator of behaviour and therefore does not influence our findings.

In debriefing, all participants said that they had attempted to maximize their gains, although people varied in strategy with some saying they tried to speed up eye movements for bigger rewards, while others said they tried to slow down on no reward trials. The implications of this are of interest as saccadic velocity normally increases predictably with the amplitude of eye movements, a relationship referred to as the *main sequence* (Bahill, Clark and Stark, 1975). However, from results of unpublished work by Muhammed *et al,* it appears participants are able to voluntary speed up and slow down saccadic peak velocity, contrary to the stereotypical predictions of the main sequence. If participants in this task adopted the strategy of slowing down saccades for zero reward, instead of going faster to obtain reward, then we may not see the full invigoration response of saccadic velocity when these participants were presented with rewards. This would reduce the potential effect of incentive on saccadic vigour and weaken the results. It may also explain why no association with apathy

scores were seen in saccadic peak velocity. To assess this in further detail, it may have been useful to include a visual rating scale at the end of each experiment to determine the subjective value placed on each outcome by participants and the actual strategy adopted by individuals.

Overall, these findings suggest that monetary rewards can modulate saccadic and pupil properties. They also provide behavioural correlates to previous imaging and electrophysiology studies where reward related responses in brain areas such as the ventral striatum, lateral intraparietal area and basal ganglia scale proportionally to reward magnitude (Kawagoe, Takikawa and Hikosaka, 1998; Elliott, Friston and Dolan, 2000; Sugrue, Corrado and Newsome, 2005). Having tested the paradigm in healthy young and elderly participants, it would be useful to investigate this association with motivation further in a population that suffers from clinical apathy. Exploring the differences that dopamine may have on reward sensitivity would also be of benefit as we know it to be an important neurotransmitter in reward related processes (Schultz, 2006, 2007). Therefore, the simpler single amplitude task from study 2 was taken forward to test in patients with Parkinson's disease, with or without pathological apathy, both ON and OFF their dopamine medication.

3. Reward sensitivity in Parkinson’s disease and associations with apathy

3. Abstract

Apathy is an under-recognised condition that has a significant impact in a host of neurodegenerative disorders. In Parkinson's disease, it adds significantly to health costs and prolongs disease burden while also reducing quality of life for patients and their caregivers. However, despite its clinical importance, there remains limited understanding of mechanisms underlying apathy. In this chapter, we investigated if insensitivity to reward might be a contributory factor to apathy and examined how this relates to the severity of clinical symptoms. Using the pupillary and saccadic metrics developed in study 2 to index motivation level, reward sensitivity was evaluated objectively. This approach was tested in 40 patients with Parkinson's disease and compared with the 31-elderly age matched control participants discussed in the last chapter. 30 patients were examined ON and OFF their dopaminergic medication in two counterbalanced sessions, so that the effect of dopamine on reward sensitivity could be assessed, while an additional 10 drug naïve patients were also included in the OFF-group analysis.

As eluded to in the previous chapter, pupillary dilation to increasing levels of monetary reward on offer provided quantifiable metrics of motivation in patients. Apathy severity was associated with reduced pupillary modulation by incentives in Parkinson’s disease, and was independent of motor impairment and autonomic dysfunction as assessed using overnight heart rate variability measures. Dopaminergic state also modulated reward sensitivity, with blunted sensitivity when patients were OFF dopaminergic drugs. This modulation was present both in pupillary and saccadic peak velocity response to reward. These findings suggest that reward insensitivity may be a contributory mechanism to apathy. It also provides a potential new clinical assessment for improved diagnosis and monitoring of apathy.

3.1 Introduction

Apathy is a debilitating disorder of reduced motivation for goal directed behaviour (Marin, 1991; Cummings *et al.*, 1994; Stuss, Van Reekum and Murphy, 2000; Robert *et al.*, 2002; Levy, 2006; Starkstein and Leentjens, 2008). It has many dissociable attributes from other neuropsychiatric disorders such as depression (SE Starkstein, SE Mayberg, TJ Preziosi, 1992; Lindsey Kirsch-Darrow *et al.*, 2011; Jordan *et al.*, 2013) . Apathy can be categorised into different sub types which are grouped either as reduced self-initiated motor actions, also known as auto-activation disorder (Levy, 2006); blunted cognitive curiosity; or emotional indifference (Stuss, Van Reekum and Murphy, 2000). Clinical apathy is defined as impairment in at least two of the three dimensions of apathy (Mulin *et al.*, 2011). It is a common feature of many neurodegenerative disorders such as Alzheimer's disease (Landes *et al.*, 2001a), vascular dementia and small vessel disease (Hollocks *et al.*, 2015), frontotemporal dementia and amyotrophic lateral sclerosis (Lillo *et al.*, 2012; Mioshi *et al.*, 2014; Bertoux *et al.*, 2015). It is particularly prevalent in Parkinson's disease (PD), affecting up to 70% of patients (SE Starkstein, SE Mayberg, TJ Preziosi, 1992; Pluck, 2002; van Reekum, Stuss and Ostrander, 2005; Dujardin *et al.*, 2007; Aarsland, Marsh and Schrag, 2009; Ahearn *et al.*, 2012; Cubo *et al.*, 2012; Santangelo *et al.*, 2013; Sinha, Manohar and Husain, 2013; den Brok *et al.*, 2015), including approximately 1 in 4 drug naïve cases (Pedersen *et al.*, 2010).

Non-motor symptoms such as apathy have a debilitating impact in PD (Martinez-Martin *et al.*, 2011; Duncan *et al.*, 2014). In fact, motivational deficits are one of the major determinants of quality of life (Barone *et al.*, 2009; Benito-León, Cubo and Coronell, 2012) which can often precede the onset of motor disturbances (Pont-Sunyer *et al.*, 2015) whilst being completely unrelated to physical disability (Pluck, 2002). Many in the field have started to recognize the extent of the problem faced by those suffering apathy and its adverse effects on day to day function (den Brok *et al.*, 2015; Pagonabarraga *et al.*, 2015), but unfortunately we still understand little about the potential underlying mechanisms (Marin, 1996; Dujardin and Defebvre, 2012; Sinha, Manohar and Husain, 2013).

Recent studies have suggested that reward sensitivity might be an important component of apathy, both in healthy individuals and in disease states (Czernecki *et al.*, 2002; Adam *et al.*, 2013; Bonnelle, Manohar, *et al.*, 2015; Bonnelle, Veromann, *et al.*, 2015). As

discussed in the previous chapter, a few human and monkey studies have measured responsiveness to reward through the use of eye movement and pupillary indices (Hong and Hikosaka, 2008; Chen *et al.*, 2014; Rudebeck *et al.*, 2014; Manohar and Husain, 2015; Manohar *et al.*, 2015). It is hoped that the eye-tracking paradigm developed in study 2 can be used to index reward sensitivity in PD using saccadic and pupillary properties. The aim would be to determine if dysfunctional reward processing contributes to clinical apathy. To date, most work has quantified apathy using questionnaire and clinical interview measures, correlating scores with other disease states including dementia (Dujardin *et al.*, 2009) and depression (Lindsey Kirsch-Darrow *et al.*, 2011), or linking outcomes to functional and structural imaging findings (Reijnders *et al.*, 2010; Carriere *et al.*, 2014; Baggio *et al.*, 2015).

In the past, paradigms used to assess reward-based decision making, including effort-based and gambling tasks, have demonstrated deficits in PD (Czernecki *et al.*, 2002; Cools *et al.*, 2003; Schmidt *et al.*, 2008; Torta and Castelli, 2008; Torta *et al.*, 2009; Chong *et al.*, 2015), but only one study has demonstrated any relationship to apathy (Martinez-Horta *et al.*, 2014). Although informative, these tasks give a binary assessment and cannot dynamically measure reward related processes which are likely to be active during the evaluation and execution stage of the decision itself. Galvanic skin responses have been used as a proxy for value encoding (Schmidt *et al.*, 2008), however it remains to be determined how this might relate specifically to apathy in PD. Other studies have shown shortfalls in reward-based learning in PD, particularly reversal learning (Swainson *et al.*, 2000; Czernecki *et al.*, 2002; Frank, 2004; Cools, Altamirano and D'Esposito, 2006; Peterson *et al.*, 2009; Schmidt *et al.*, 2014; Sharp *et al.*, 2016). But performance on such tasks is likely to rely on numerous cognitive processes, of which reward sensitivity might be only one. Furthermore, these investigations either did not examine the relationship to clinical apathy or were not adequately powered to make any inferences on this issue.

PD is a unique model to study apathy due to the dysfunction in frontrostriatal brain regions and dopamine depletion, both of which are implicated in motivation. The brain areas of interest in apathy include the ventral striatum, ventral tegmental area (VTA) and substantia nigra pars compacta (Robbins and Everitt, 1996; Levy, 2006; Drui *et al.*, 2013; Manohar and Husain, 2016). Dopamine modulates a number of mechanisms including reward prediction, vigour of action, and initiation of effortful behaviour (Schultz, 2007; Kurniawan, Guitart-Masip

and Dolan, 2011; du Hoffmann and Nicola, 2014; Chong *et al.*, 2015). However, based on anecdotal and clinical evidence, it is also likely to exhibit a modulatory function in apathy. For example, patients who have suffered basal ganglia infarcts and subsequently developed apathy, show improvement when treated with dopamine (Laplane *et al.*, 1989; Schmidt *et al.*, 2008; Adam *et al.*, 2013) and PD patients with subthalamic nucleus deep brain stimulation (STN-DBS) can also develop apathy when their dopaminergic drug dose is reduced post-operatively (Drapier *et al.*, 2006; Czernecki *et al.*, 2008; Thobois *et al.*, 2013). Yet the precise processes that dopamine alters motivation in these patient groups remains to be fully characterised.

In this chapter, we investigate whether reduced reward sensitivity might be a key mechanism underlying apathy in PD. We ask the question, does insensitivity to rewards cause subjective devaluation of incentives, thereby contributing to the behavioural phenotype of apathy? Based on the work in the field, we hypothesize that pupillary dilation and invigoration of motor actions would be increased for larger rewards, that this would be dependent on dopamine and, most importantly, reduced in apathetic patients. In order to control for any autonomic dysfunction effects on the pupillary response to rewards, PD patients also wore a single lead portable ECG monitor while at rest overnight to measure heart variability which is an important index of autonomic function (Rajendra Acharya *et al.*, 2006).

3.2 Methods

3.2.1 Participants

Patients and elderly participants were assessed for apathy using the Lille Apathy Rating Scale (LARS) which has been validated in PD (Sockeel, 2006); they were screened for depression with the Beck Depression Inventory-II (BDI-II) (Beck, Steer and Brown, 1996) and for cognitive impairment on the Montreal Cognitive Assessment (MoCA) (Nasreddine *et al.*, 2005). The severity of PD was measured using the Movement Disorder Society Unified Parkinson's disease rating scale (UPDRS) (Goetz *et al.*, 2008). No participant had a history of psychiatric illnesses or autonomic dysfunction.

3.2.2 Parkinson's disease demographics

Forty patients with PD were included in the study, twenty-six patients were male, and thirty-four right handed. All had a clinical diagnosis of idiopathic PD and no history of other major neurological or psychiatric illnesses. They were recruited from clinics in the Oxfordshire area. Ten were drug naïve and thirty were currently established on levodopa and dopamine agonist combinations. Nine patients were on levodopa only, two on dopamine agonists only and nineteen on a combination of both. The thirty patients on medication were tested in two counter balanced morning sessions, one session having taken their dopaminergic medication as normal (ON) and the other after overnight withdrawal (OFF).

Using the clinical interview LARS, a score of ≥ -22 was set as the cut off for apathy, encompassing mild to severe apathy symptoms (Sockeel, 2006). Non-drug naive patients were divided into two groups; those suffering with apathy (n = 14)
mean LARS -15.3, SD 6.7 and those without (n = 16) mean LARS -28.5, SD 2.8. Drug naïve patients were also divided based on their LARS score and included in the full hierarchical analysis with the PD patients who were OFF, half were allocated to each apathy and non-apathy groups taking the total to 19 and 21 respectively. On break down of the LARS, the apathetic patients scored significantly less than the non-apathetic patients in all three apathy domains. This included behavioural apathy (action initiation), cognitive apathy (intellectual

curiosity) and emotional apathy ($P < 0.01$). They did not, however, show any differences in the self-awareness component of the LARS.

There were no significant differences between apathetic and non-apathetic patients in age, motor severity (UPDRS part III), cognitive impairment, depression scores or levodopa equivalent dose (**Table 3.1**). On breakdown of the UPDRS components, apathetic patients had significantly worse non-motor symptoms (UPDRS part I). This difference was driven by a lower score on the apathy assessment question ($P < 0.05$). Disease duration was significantly longer in the non-apathetic patient group and their average age at diagnosis trended towards an earlier onset but there were no correlations with apathy level (LARS) and age in PD $r_s = 0.045$, $P = 0.813$. None of the patients had a current diagnosis or previous history of impulse control disorder.

	Healthy Elderly Controls	Parkinson's Disease (Medicated)	Controls vs Parkinson's Disease p-value	Non Apathetic Patients (LARS<-22)	Apathetic Patients (LARS≥-22)	Non Apathy vs Apathy p-value	Parkinson's Disease (Drug Naïve)
n	31	30		16 ON/OFF	14 ON/OFF		10 (5 Apathetic)
Age (years)	67.5 (±8.5)	66.5 (±5.9)	0.6	65.9 (±5.6)	67.3 (±6.4)	0.52	65.8 (±6.3)
Apathy (LARS)	-29 (±4.3)	-22.3 (±8.3)	<0.0001*	-28.5 (±2.8)	-15.3 (±6.7)	<0.0001*	-22.5 (±7.9)
Depression Score (BDI)	4.9 (±5)	13.3 (±6.8)	<0.0001*	11.7 (±5.5)	15.2 (±7.8)	0.16	12.2 (±8.6)
MoCA	28 (±1.5)	28 (±1.9)	0.5	28.2 (±1.6)	27.8 (±2.3)	0.57	26.1 (±3.3)
UPDRS I	n/a	8 (±4.8)	n/a	6.1 (±3.9)	10.1 (±4.9)	0.02*	7.8 (±5.9)
UPDRS III ON	n/a	19.4 (±9.8)	n/a	18.4 (±2.6)	20.4 (±2.4)	0.59	24.4 (±9.3)
Hoehn & Yahr Stage	n/a	1.3 (±0.7)	n/a	1.4 (±0.6)	1.2 (±0.8)	0.54	1.4 (±1.4)
Disease duration (years)	n/a	5.0 (±4)	n/a	6.8 (±4.6)	3.2 (±2.2)	0.02*	1.1 (±1.5)
Average age at diagnosis (years)	n/a	61.4 (±7.5)	n/a	59.2 (±7.6)	64 (±6.7)	0.08	64.9 (±6.6)
Hours since last dose ON vs OFF	n/a	2.5 (±2.2) vs 14.2 (±4)	n/a	2.1 (±1.1) vs 15.2 (±3.9)	2.9 (±3) vs 13.1 (±4)	0.32 0.24	n/a
Levodopa equivalent dose (mg/24 hours)	n/a	507 (±61.2)	n/a	493.9 (±75.8)	522.7 (±101.4)	0.82	n/a

Table 3.1. Demographics

*Demographics of healthy elderly age matched controls, total PD patients, drug naïve PD patients and patients divided into apathetic and non-apathetic subgroups. Numbers in brackets represent standard deviations and standard error of the mean where appropriate. * Indicates a significant result. LARS = Lille Apathy Rating Scale; MoCA = Montreal Cognitive Assessment; BDI = Beck Depression Inventory II. UPDRS = Unified Parkinson's disease rating scale.*

3.2.3 Elderly control demographics

The same elderly controls were used from study 2 in the previous chapter. There were no differences in age between the patients and elderly control participants or any differences in cognitive ability on the MoCA. PD patients had significantly worse total LARS scores than elderly controls and also higher BDI scores, however average depression scores in both groups were not classified as depressed and did not exceed mild mood disturbance/borderline depression (cut off > 20). Only one elderly control participant fell in the apathetic range, with a LARS score of -15. PD patients and elderly controls also had MoCA scores in the normal range. (**Table 3.1**).

3.2.4 Experimental paradigm and eye tracking recording and analysis

Eye tracking procedures were the same as in study 2, chapter 2. Pupillometry analysis was performed using split-level ANOVA, with within-subject factors of Drug (ON, OFF) and Reward (0p, 10p, 50p), and between-subjects factor of Apathy (apathetic, non-apathetic). To account for any non-sphericity in the data, where appropriate, statistics are reported with Greenhouse-Geisser correction. Bonferroni corrected pairwise comparisons were made if significant interactions were present. Significance was taken as p-values of less than 0.05. Correlations with questionnaires were performed using Spearman rank non-parametric testing and Pearson correlations were used for parametric behavioural outcome comparisons. Statistics were completed using Matlab and SPSS.

3.2.5 Heart rate variability recording and analysis

On the day of carrying out the eye tracking experiment, all PD patients also wore a portable Actiwave Cardio single channel ECG recorder and tri-axis accelerometer (CamNtech ltd) **(Fig. 3.2)**. This was placed on the mid-chest using adhesive electrodes and worn overnight while sleeping. Recordings were sampled at 128Hz and a 30-minute epoch for each patient between the hours of 1am and 4am was selected when completely at rest, as detected by no accelerometer activity. Heart rate variability (HRV) analysis was performed using time domain and frequency domain methods as performed by other studies of HRV in PD (Haapaniemi *et*

al., 2001). HRV data were not normally distributed, therefore comparisons between apathetic PD patients and non-apathetic PD patients were made using non-parametric Mann Whitney-U tests.

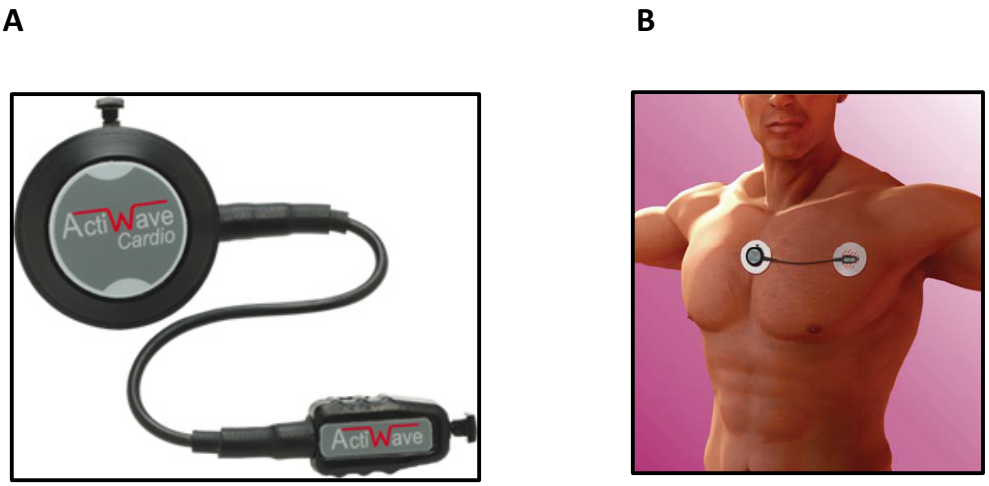

Figure 3.2. Accelerometer and heart rate variability equipment.

A. *The portable CamNTech accelerometer device to measure heart rate variability and movement.*

B. *The location on the chest that the device was worn by participants overnight.*

3.3 Results

3.3.1 Dopaminergic medication increases reward sensitivity in Parkinson's disease

Similar to controls, there was a main effect of reward on pupil size in the PD group, with larger rewards on offer eliciting greater pupillary dilation over time **(Fig. 3.3A and 3.3B)**. A main effect of drug state was also present, revealing PD Patients ON dopamine had greater pupillary response to rewards compared to when OFF **(Fig. 3.3D**; main effect of drug state $F(1, 28) = 17.6$, $P < 0.001$; main effect of reward $F(1.4, 39.5) = 15.0$, $P < 0.0001$). There was also a significant interaction between drug state and reward, with reduced reward sensitivity when OFF dopaminergic medication as indicated by a shallower sensitivity slope, ($F(1.9, 53.8) = 5.7$, $P < 0.01$). Post hoc pairwise comparisons emphasised this further by revealing significantly larger pupillary changes for increasing reward levels when ON dopamine (0p vs. 10p, $P < 0.01$; 10p vs. 50p, $P < 0.001$; 10p vs. 50p, $P < 0.0001$). However, this was blunted in the OFF state, with significant differences only between 0p vs. 50p ($P < 0.01$) and 10p vs. 50p ($P < 0.01$) and not between 0p vs. 10p.

In order to ensure that age differences in the PD group did not affect pupil reward sensitivity, a correlation analysis was performed. No significant correlation was found in either drug state between pupil reward sensitivity and age, ON $r = -0.31$, $P = 0.1$, and OFF $r = -0.24$, $P = 0.2$. There was also no significant difference in baseline pupil size between each of the reward levels prior to incentives being offered. This is an important observation as it means baseline pupil size did not influence the changes in pupil dilation for different reward levels. This was true both in the ON and OFF drug state with no significant drug state by reward interaction for baseline pupil size.

There was however, a significant difference in baseline pupil size between drug states. PD patients ON had on average larger pupils at baseline compared to those OFF (main effect of drug state ($F(1,29) = 20.7$, $P < 0.001$; **Fig. 3.3C)**. This further strengthens the hypothesis that dopamine increases reward sensitivity. When ON dopamine, the pupil was more dilated at baseline, implying less scope for subsequent pupillary dilation for reward compared to the OFF state in which the pupil had greater capacity to enlarge. Contrary to this, we actually found that larger rewards on offer lead to greater proportional pupil dilation in the ON state

compared to the OFF state **(Fig 3.3D)**. Therefore, despite the changes dopamine has on baseline pupil size, it still led to increased pupillary reward sensitivity (difference in dilation between the 0p and 50p condition). There was no significant difference in baseline pupil size between PD patients ON and elderly controls, or PD patients OFF and elderly controls.

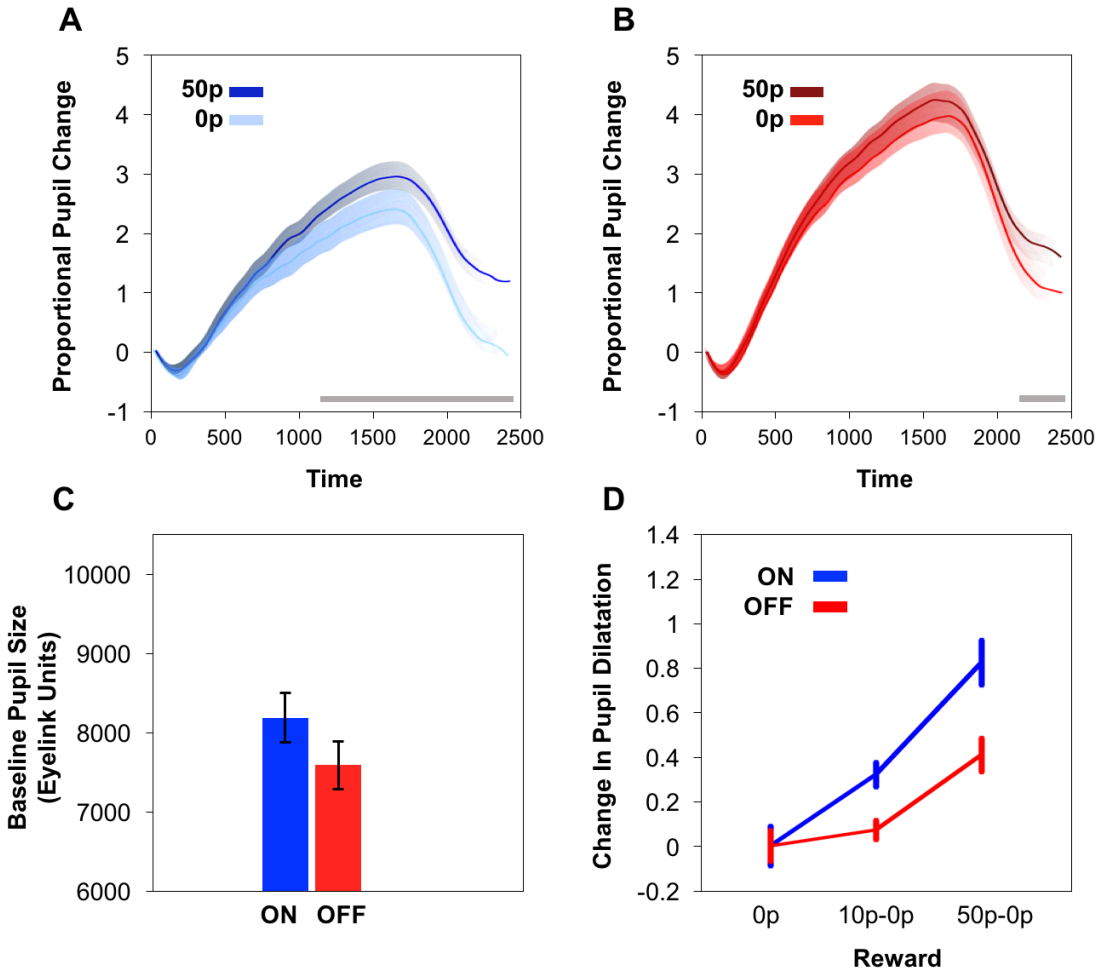

Figure 3.3A-D. Pupil measures in Parkinson's disease.

A. *Mean pupillary trace in PD patients ON dopaminergic medication. Greater proportional change was observed for the larger reward, with a significant difference between 50p maximal reward (dark blue) and 0p reward level (light blue) present from ~1100ms ($p<0.05$), denoted by grey bar at bottom of plot. The 10p trace was omitted from the figure for clarity, if included it would sit between the 0p and 50p trace.*

B. *Mean pupillary trace in PD OFF dopaminergic medication after onset of reward cue. A significant difference between the 50p maximal reward (dark red) and 0p reward (light red) was observed but only from ~2100ms onwards ($p<0.05$), denoted by grey bar at bottom of plot. Again, the 10p trace was omitted from the figure for clarity. This differential effect of reward was significantly delayed in onset compared to when ON dopaminergic medication. Moreover, the difference in response to the two reward levels was less in the OFF-drug state.*

C. *Mean pupil baseline size as taken at the start of each trial and measured using Eyelink arbitrary units. PD ON had significantly larger baseline pupil size compared to PD OFF.*

D. *Proportional pupillary change as a function of reward level in PD ON (blue) compared to PD OFF (red) dopaminergic medication, taken as the mean pupil dilation between 1400-2400ms. Changes have been normalised to the 0p baseline to demonstrate the relationship between reward sensitivity slopes. There was a reduced reward sensitivity in the OFF-drug state compared to ON, despite increased scope for dilation when OFF due to smaller baseline pupil sizes.*

3.3.2 Reward sensitivity is blunted in apathetic Parkinson's disease cases

Does reward sensitivity alter with PD compared to controls or with apathy in PD on this task? Taking into account all the data (control participants and PD patients ON and OFF dopamine and drug naïve) a linear mixed effects model was performed on average pupil reward sensitivity (difference between 0p and 50p reward level over the 1000ms epoch) for each individual participant. This being the highest level of analysis, *overall* there was no difference between PD patients and elderly controls ($F(1,96) = 0.02$, $P = 0.9$). There was however, a main effect of apathy ($F(1,96) = 11.2$, $P < 0.01$) with the apathetic PD group demonstrating significantly reduced pupillary responses to reward than the non-apathetic group (**Fig. 3.4**). Furthermore, while all comparisons between reward levels in the non-apathetic group were significant ($P < 0.003$ between each reward magnitude), none were so in the apathetic group, indicating that pupillary response to reward is blunted in apathy.

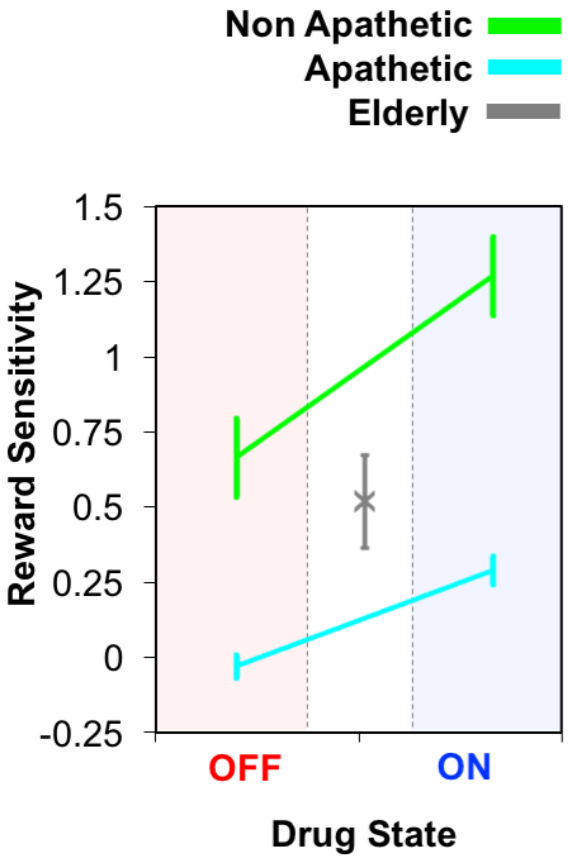

Figure 3.4. Pupillary responses in apathetic vs non-apathetic Parkinson's disease cases and controls.

Mean pupil reward sensitivity over 1400– 2400ms in apathetic Parkinson's disease patients (cyan) was significantly reduced compared to non-apathetic patients (green) both ON and OFF dopaminergic medication. There was an increase in reward sensitivity in both apathetic and non-apathetic patients when ON dopamine, but no significant interaction between the drug state and apathy level. Comparison of average pupil reward sensitivity between apathetic and non-apathetic Parkinson's disease patients to elderly controls (grey) showed a significant reduction in reward sensitivity in apathetic Parkinson's disease patients when OFF dopamine and greater reward sensitivity in non-apathetic Parkinson's disease patients when ON.

There was also a main effect of drug on reward sensitivity ($F(1,96) = 10.6$, $P < 0.01$) with reduced sensitivity when OFF, but no significant interaction between apathy and drug state. Planned Bonferroni corrected comparisons were also made. There were no *overall* significant differences between non-apathetic patients and elderly controls and also none *overall* between apathetic patients and elderly controls. However, when comparing elderly controls to apathetic cases OFF dopamine, apathetic PD patients were significantly less reward sensitive ($F(1,96) = 8.9$, $P = 0.03$). Non-apathetic patients OFF dopamine were no different from elderly controls, nor were apathetic ON. However, non-apathetic PD patients ON were significantly more reward sensitivity than elderly controls ($F(1,96) = 15.3$, $P = 0.001$).

These findings were also reflected in the time course of pupillary reward sensitivity as measured using multiple permutation testing to account for multiple comparison bias, with differences in pupillary response over time performed at each millisecond (**Fig. 3.5**). When OFF dopamine, apathetic PD patients had significantly less reward sensitivity compared to non-apathetic patients from ~1000ms to the end of each trial (denoted by light purple horizontal bar in **Fig. 3.5A**). They also showed some significant reduced reward sensitivity over time compared to elderly controls (denoted by light red horizontal bar), but no differences were apparent between non-apathetic and elderly participants.

In the ON state, reward sensitivity increased over the trial in both apathetic and non-apathetic patients compared to when OFF (**Fig. 3.5B**). However apathetic patients remained significantly less sensitive than non-apathetic patients from ~1100ms even when ON dopamine. Indeed, when ON dopamine, apathetic patients rose to the same reward sensitivity level as controls, whilst the non-apathetic patients displayed, for a short time period (grey horizontal bar), reward sensitivity that surpassed that of elderly controls. Although our groups are age-matched, we wished to ensure that age could not account for any of our effects. We therefore performed a repeat analysis with the effect of age regressed from our results. The outcomes reported above remained unchanged.

Next, we analysed the results across all patients, rather than dichotomizing into apathetic and non-apathetic groups. A mixed ANCOVA was completed, with z-scored total LARS apathy score for each PD patient used as a covariate. There was a significant main effect of drug state and of reward, an interaction between reward and apathy, as well as an interaction between drug state and reward (respectively, drug state $F(1, 28) = 18.3$, $P < 0.0001$; reward $F(1.4, 39.0) = 15.9$, $P < 0.0001$; apathy x reward $F(1.4, 39) = 5.1$, $P < 0.02$; drug state x reward $F(1.9, 54.2) = 6.1$, $P < 0.005$), but no significant three way interaction between drug state, reward and apathy. Decomposing these results revealed that being ON dopamine led to a greater reward sensitivity and being apathetic was associated with reduced reward sensitivity.

To explore this effect further, a two-tailed Spearman's Rank correlation between pupil reward sensitivity in ON and OFF state was performed as a function of total LARS score. A significant correlation was present both ON and OFF dopamine. The more apathetic a patient

the less pupillary reward sensitivity they exhibited (PD ON r_s = -0.56, $P < 0.001$, **Fig. 3.6A**; PD OFF r_s = -0.41, $P < 0.01$; **Fig. 3.6B)**. There was no significant difference in total levodopa equivalence doses between apathetic and non-apathetic groups (**Table 3.1**). Although it did not reach statistical significance (P=0.6), there was a trend towards a correlation between the difference in reward sensitivity when ON compared to OFF (**Fig. 3.6C**). This hinted that those patients who were most apathetic, also exhibited the least response to dopamine compared to those who were more motivated and had a larger increase in reward sensitivity when ON dopamine medication compared to when OFF.

Finally, comparisons were made to ensure that there were no differences in baseline pupil size in apathetic and non-apathetic PD groups prior to hearing the reward on offer. Both ON and OFF dopamine, there was no significant difference between baseline pupil size across the two groups for any of the reward conditions, nor was there any significant interaction between reward and apathy group. Overall, these analyses show a blunting of pupil dilation by reward as a function of apathy, which cannot be accounted for by baseline pupil size.

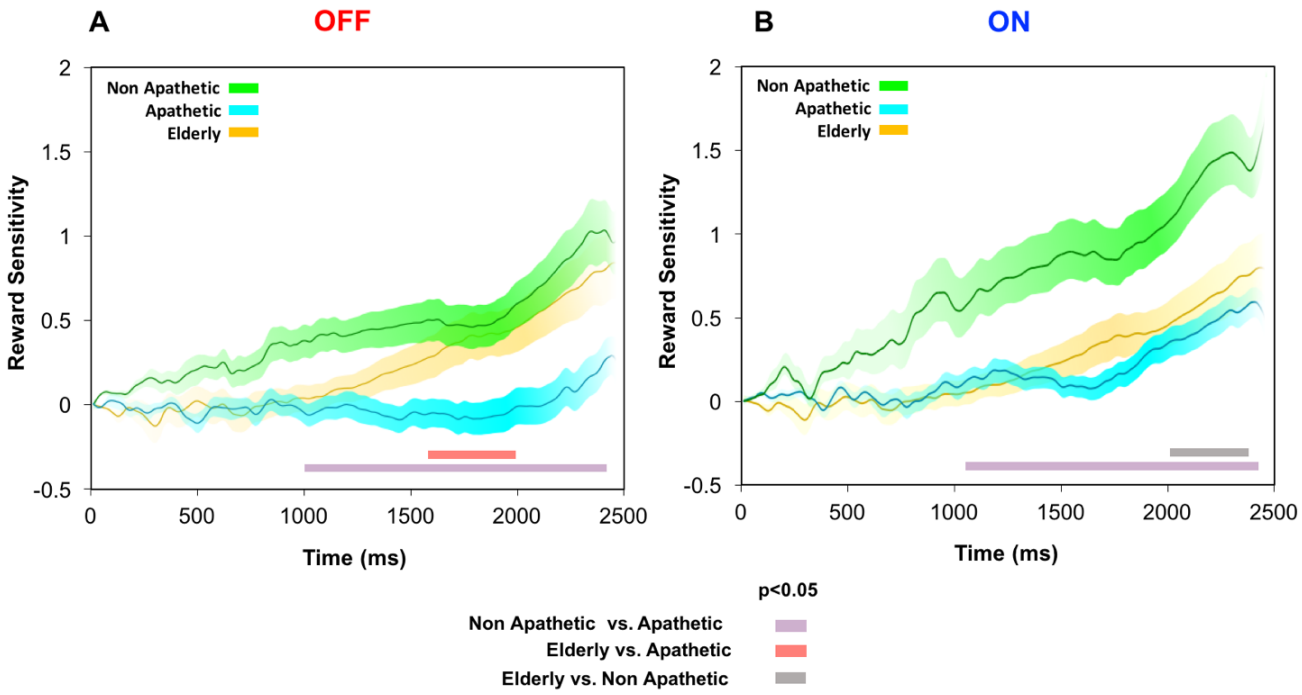

Figure 3.5A-B. Pupillary reward sensitivity in apathetic vs non-apathetic PD patients, ON / OFF dopamine and elderly age matched controls.

A. ***OFF state.*** *Mean pupil reward sensitivity (50p pupil change minus 0p pupil change) plotted over time between apathetic PD patients (cyan) and non-apathetic PD patients (green) when OFF dopamine or drug naïve. A significant difference in sensitivity occurred from ~1000ms to the end of the trial, indicated by light purple bar (p<0.05). There was also a significant time period where apathetic patients had reduced reward sensitivity compared to elderly controls, indicated by light red bar.*

B. ***ON state.*** *Mean pupil reward sensitivity (50p pupil change minus 0p pupil change) plotted over time between apathetic PD patients (cyan) and non-apathetic PD patients (green). A significant difference in sensitivity occurred from ~1100ms to the end of the trial, indicated by light purple bar (p<0.05). There was also a significant time period where non-apathetic patients ON dopamine had increased reward sensitivity compared to elderly controls, indicated by grey bar.*

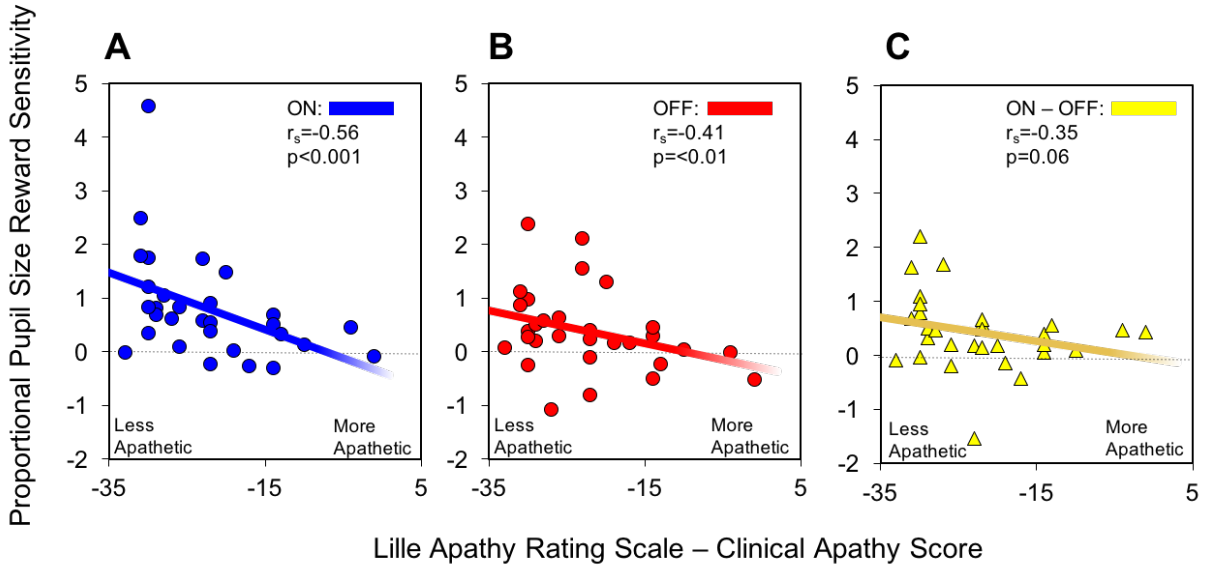

Figure 3.6A-C. Pupillary responses in Parkinson's disease cases as a function of apathy level on the Lille Apathy Rating Scale (LARS).

- ***A.*** *A significant correlation between average pupil reward sensitivity in Parkinson's disease patients ON and their clinical interview LARS score. More apathetic patients exhibit less pupillary reward sensitivity compared to more motivated patients.*
- ***B.*** *A significant correlation between pupil reward sensitivity and clinical interview LARS in Parkinson's disease OFF.*
- ***C.*** *The difference in reward sensitivity between ON and OFF (the interaction term) – demonstrating that the more apathetic patients are less influenced by dopamine compared to the motivated patients who have a bigger change in reward sensitivity when ON dopamine compared to OFF.*

3.3.3 Are pupil effects accounted for by generalised autonomic dysfunction?

One potential confound regarding any interpretation of reward sensitivity based on pupillary data is that they might reflect a generalised autonomic deficit which may be greater in apathetic PD cases. To control for this we also examined heart rate variability (HRV) obtained from a 30-minute epoch when patients were asleep and completely at rest (as defined by no accelerometer activity detected during these periods), as in previous studies (Haapaniemi *et al.*, 2001; Pospíšil *et al.*, 2008; Maetzler *et al.*, 2015). There were no significant differences between apathetic and non-apathetic PD groups in any of the autonomic markers of HRV that were analysed, including mean number of R waves; mean or standard deviation of inter-beat interval; average root mean squared of the inter-beat interval; high, low or very low frequency power spectral analysis; or low/high frequency ratio (**Table 3.7**).

Heart Rate Variability Assessment	Non Apathetic Patients	Apathetic Patients	p-value
Mean Number of R Waves	1835 (237)	1774 (559)	0.65
Mean interbeat interval (seconds)	0.99 (0.14)	0.98 (0.15)	0.99
Mean standard deviation of interbeat interval	0.06 (0.05)	0.04 (0.03)	0.10
Root mean squared of interbeat interval (RMSSD)	0.07 (0.08)	0.04 (0.03)	0.65
Mean high frequency (HF) (ms^2)	149.8 (139)	114.9 (137)	0.29
Mean low frequency (LF) (ms^2)	199.7 (195)	153.9 (171)	0.25
Mean low frequency/High frequency ratio	2.83 (4.3)	1.89 (2.1)	0.84
Mean very low frequency (VLF) (ms^2)	161.9 (153)	180 (215)	0.44

Table 3.7. Heart rate variability data comparing apathetic and non-apathetic PD patients.

Numbers in brackets represent standard deviations. No significant differences were found in any of the HRV parameters between apathetic and non-apathetic patient groups.

3.3.4 Saccadic eye movement measures

Our next stage of analysis was to examine differences in eye *movement* parameters between groups. The major focus of interest was peak velocity, a marker of invigoration of response which has been shown to be sensitive to rewards in both human and monkey studies (Chen *et al.*, 2013, 2014; Manohar *et al.*, 2015) and from our results in chapter 2. We also analysed reaction times and saccadic variability which are discussed later.

3.3.5 Peak velocity increases with incentive on offer

A repeated measures ANOVA performed on saccadic peak velocity for the three reward levels in young controls from chapter 2 demonstrates a main effect of reward ($F(1.4, 26.6) = 18.24$, $P < 0.001$). There was a significant increase between each reward level (all comparisons between rewards were significant, $P < 0.01$). This effect was also present in

elderly controls (main effect of reward, $F(1.7, 52.0) = 9.2$, $P < 0.001$). Again, peak velocity significantly increased with each increment in reward (all comparisons $P < 0.05$; grey dashed line in **Fig. 3.8A**). It is well established that peak velocity scales with saccade amplitude – the so-called 'main sequence' as discussed previously (Bahill, Clark and Stark, 1975). It is possible that the increases in peak velocity we observed with reward reflect only increases in saccade amplitude: that eye movements become larger when more reward is on offer. To examine this issue, the amplitude of each saccade was factored out by performing a linear regression on the raw saccade data and obtaining residual velocities **(Fig. 3.8B)**. Sensitivity to reward remained even after factoring out amplitude changes for both controls and PD patients, so the effect of incentives on peak velocity cannot be attributed to an invigoration of saccade length.

In the ON state PD patients showed greater reward sensitivity than when OFF (**Fig. 3.8A)**. There was a significant main effect of both drug state and reward on peak velocity, as well as a drug state by reward interaction (drug state, $F(1, 28) = 6.0$, $P < 0.02$; reward, $F(1.6, 44.9) = 14.9$, $P < 0.0001$; drug state x reward, $F(1.7, 48.5) = 7.0$, $P < 0.003$). Of note, overall saccadic velocity was greater when OFF dopamine, despite having a reduced sensitivity to reward (shallower slope) compared to ON. This effect was due to larger amplitude saccades when OFF as the effect was abolished when amplitude is factored out **(Fig. 3.8B)**. Pairwise comparisons showed a significant increase in saccadic peak velocity between every reward level in PD ON (all reward level comparisons, $P < 0.01$). But in the OFF state there was only a significant difference between 0p vs. 50p ($P < 0.04$).

Next, saccadic reward sensitivity was calculated for each participant. This was taken as mean difference in peak velocity between the 50p and 0p conditions. A within subjects (drug state) and between subject (apathy/non-apathy group) ANOVA was performed. There were no significant effects of apathy, nor was there an interaction between drug state and apathy. There was however a main effect of drug state, with greater reward sensitivity in peak velocity when ON than OFF ($F(1, 28) = 10.8$, $P < 0.003$). There was no significant correlation between UPDRS-III motor severity score and peak velocity reward sensitivity ON or OFF dopamine. These analyses, therefore, reveal that saccadic peak velocity scales with incentive on offer and is greater in the ON state but, unlike the pupillometry findings, there was *no relationship to*

clinical apathy. Thus, there is a dissociation between pupil and saccadic measures with respect to apathy.

Finally, we also compared the PD group to elderly controls. In the ON state, patients appeared to have greater sensitivity to rewards compared to controls (main effect of reward $F(1.6, 93.4) = 26.8$, $P < 0.0001$; reward x group interaction $F(1.6, 93.4) = 5.5$, $P < 0.01$). Pairwise comparisons in PD ON revealed significantly greater increases in peak velocity at every increment in reward level ($P < 0.0001$). Elderly controls however, only showed a significant difference between the 0p vs. 50p conditions ($P < 0.02$); the remaining comparisons (0p vs. 10p and 10p vs. 50p) did not reach significance. Comparison of elderly controls to PD OFF showed a significant main effect of reward ($F(1.9,110.4) = 10.0$, $P < 0.0001$) but no significant interaction **(Fig. 3.8A).**

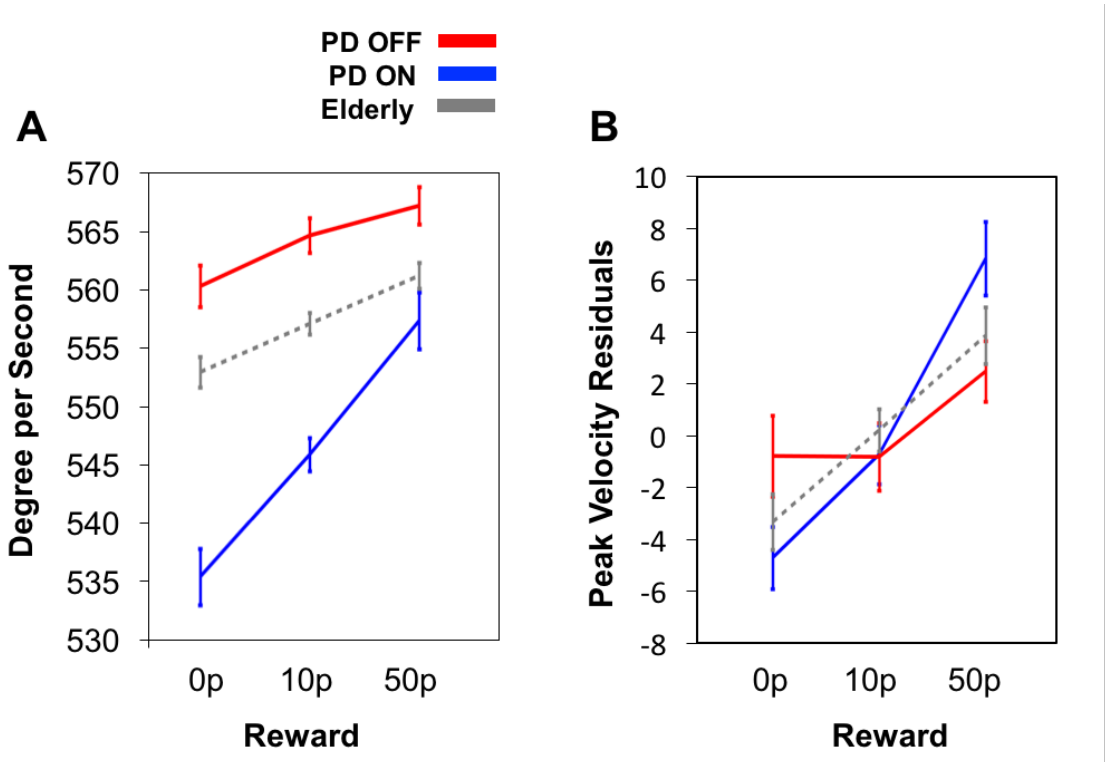

Figure 3.8A-B. Saccadic responses in PD patients and elderly controls.

- ***A.*** *Average saccadic peak velocity in degrees per second as a function of reward for elderly controls (dashed grey), PD ON (blue) and PD OFF (red). Significantly steeper saccade reward sensitivity slopes were present in PD ON compared to PD OFF and elderly controls. There was increased invigoration of movements for bigger rewards in all groups, with increased incline when ON dopamine.*
- ***B.*** *Visual representation of sensitivity slopes when changes in amplitude were accounted for by linear regression. Residual velocities plotted as a function of reward show that greater reward sensitivity is still present in PD ON when compared to PD OFF and compared to elderly participants at the largest level of reward.*

To ensure that participants understood the task and were performing as instructed, saccadic peak velocity and saccadic amplitude variability were also used as an indicator of task performance. As demonstrated above, saccadic velocity scaled with larger incentives signifying participants understood that moving faster would result in obtaining larger rewards. Accuracy (as measured by saccadic variability) was also assessed in the PD patients collapsed across drug state as there were no differences between ON and OFF. Increased accuracy in all groups for increasing reward magnitudes was observed, this demonstrates both an increase in accuracy as well as velocity when larger rewards were on offer. There was a main effect of group ($F(1,30) = 15.6$, $p<0.0001$) with PD (ON and OFF) being significantly more variable compared to elderly controls. There was also a main effect of reward with higher rewards resulting in less variable saccades ($F(2,60) = 6.3$, $p <0.01$). No significant interaction was present. (**Fig. 3.9**).

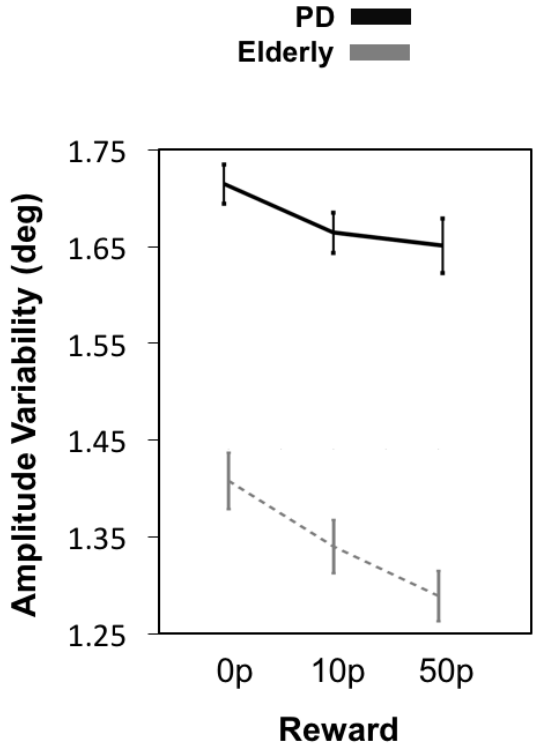

Figure 3.9. Saccade amplitude variability for PD (ON and OFF) compared to elderly controls.

A reduction in saccade amplitude variability was apparent with increasing rewards on offer. This demonstrates both an increase in accuracy as well as velocity when larger rewards were on offer in elderly as well as PD patients.

3.3.6 Saccadic reaction times and learning

In our task, reward did not significantly affect reaction time in any of the groups tested. To ensure the differential effects of reward observed in peak saccadic velocity and pupillary response were not attributable simply to faster learning of the task by particular groups or by being ON or OFF dopamine in PD patients, the average reaction time for each reward level was compared for the first versus second half of the experiment. As reward obtained was dependent on reaction time, if learning was a significant factor in the task design then reaction time should decrease as participants learn that the faster they react, the larger the reward obtained. In young and elderly controls, there was no significant difference between first and second halves of the experiment, nor an interaction between experiment half and reward. PD patients, both ON and OFF dopamine or when broken down to apathetic and non-apathetic groups, displayed the same pattern of results, with no significant learning effect detected.

The RT result in our experiment (**Fig. 3.10**) indicates that in general participants did not vary their RT across conditions, although velocity and pupillary response to reward were clearly strongly modulated. This was true for young and elderly control participants and also for PD ON and OFF medication. There was no main effect of drug state, reward level or interaction in the PD group ON versus OFF dopamine. There were also no significant differences in RT between reward levels in the young or elderly control groups in this experiment.

In many designs, RT is the key metric of interest. Our task, in contrast, allows us to examine different outcome measures. The pupil response has the benefit of being based on anticipation of the maximal value of reward available at the start of each trial and, in line with recent work, it seems that a key behavioural metric of how reward can invigorate or energize movement is saccadic velocity (Chen *et al.*, 2013; Manohar *et al.*, 2015). In our experiments, both these were modulated by incentives on offer while RT was tracked for the purposes of adjusting the reward function in an adaptive manner, to maintain comparable reward rates between participants and groups.

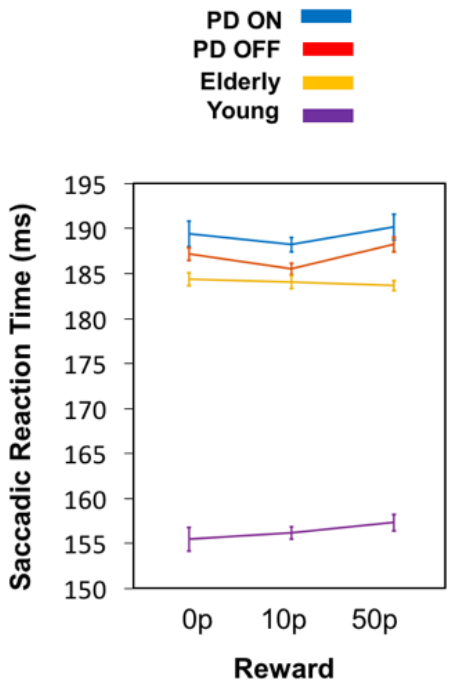

Figure 3.10. Reaction time for young, elderly and PD patients ON and OFF dopamine.

There was no difference in reaction time between any of the reward levels and group differences between the young and other groups did not reach statistical significance.

3.3.7 Pupil reward sensitivity is specific to apathy not depression

When dividing patients using a median split on Beck Depression Inventory (BDI-II) scores, no significant pupillary reward sensitivity difference was demonstrated over time between those with low vs. higher depression scores (**Fig. 3.11**). Although significant depression was not present in our cohort, when comparing the low and high depression PD groups, dopamine again significantly increased pupillary reward sensitivity when ON, but no difference was demonstrated between the groups (**Fig. 3.12**), unlike when the cohort was divided based on apathy score, (**Fig. 3.4**).

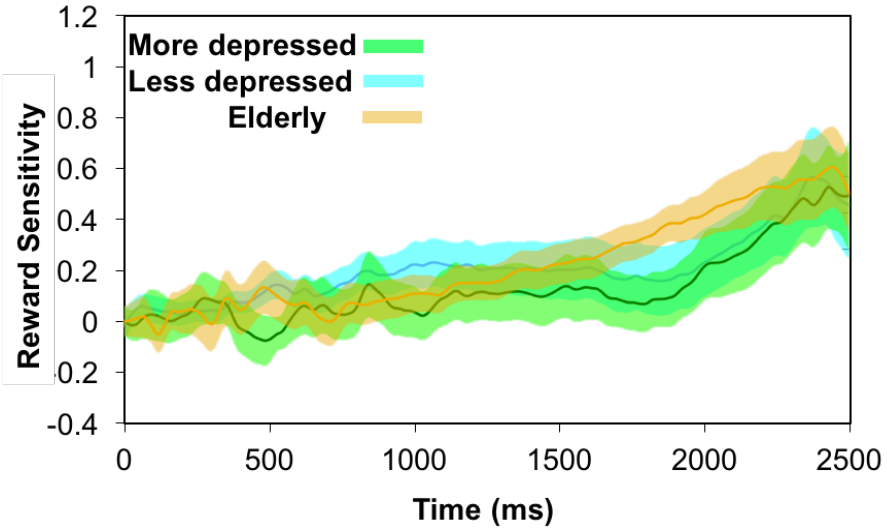

Figure 3.11. Pupillary responses over time in those with high and low depression scores in Parkinson's disease and elderly controls.

There were no differences in pupil reward sensitivity between the three groups when split into PD patients with higher and lower depression scores. Suggesting that the reward sensitivity metric is specific to apathy rather than other neuropsychiatric disorders.

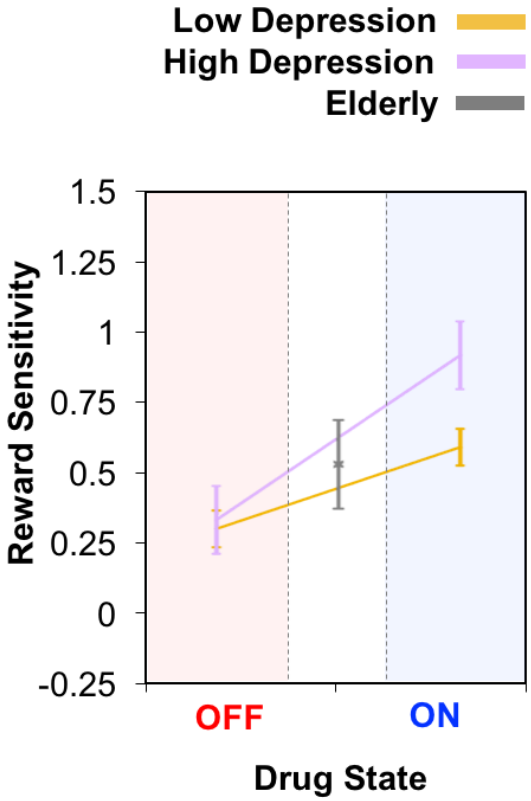

Figure 3.12. Pupillary responses and depression in Parkinson's disease cases and controls.

When splitting the patient into low and high depression scores and assessing pupil reward sensitivity over the 1400ms to 2400ms time period of interest, there was no significant difference between any of the groups, however when ON dopamine an increase in sensitivity was seen.

3.4 Discussion

3.4.1 Summary

The findings presented here reveal that apathetic individuals with PD exhibit significantly less pupillary response during the evaluation of reward compared to non-apathetic patients (**Fig. 3.4**). Dopamine was found to play a key role in this valuation, with blunting of pupillary reward sensitivity when patients were OFF and restoration of sensitivity when ON dopaminergic medication (**Fig. 3.5**). Although there were no significant associations between clinical apathy level and saccadic velocity, dopamine did influence oculomotor responses for rewards. Patients ON showed increased invigoration of saccadic velocity that scaled with reward magnitude, compared to when OFF (**Fig. 3.8**). All these findings were independent of total dopamine equivalent dose, autonomic dysfunction and severity of clinical motor symptoms.

3.4.2 Pupillary response sensitivity to reward

This study is the first to relate pupillary measures of reward sensitivity to clinical apathy. It demonstrates that reward insensitivity is dopamine dependent and may contribute to disorders of motivation (Robbins and Everitt, 1996; Sinha, Manohar and Husain, 2013). Pupillometry has been used to probe cognitive processes (Wang and Munoz, 2015), with modulation of pupil size associated with a range of non-luminance dependent factors, including arousal, salience and reward (Varazzani *et al.*, 2015; Joshi *et al.*, 2016). Various neurotransmitters are considered to be involved in this process, including dopamine (Korczyn and Keren, 1980; Manohar and Husain, 2015) with potential interactions occurring between dopaminergic and noradrenergic systems (Guiard, El Mansari and Blier, 2008). Moreover, dopamine related brain regions, including the basal ganglia, have been implicated in pupil size change (Wang and Munoz, 2015).

The findings presented here show that apathetic PD patients seemingly lose the ability to differentiate value of rewards of increasing magnitude, at least as indexed by pupillary reward sensitivity. Note though, that we would not argue that reward insensitivity completely

accounts for apathy. Rather it may be one component of the syndrome, with apathy in PD representing manifestations of an under-activated approach system (Shulman, 2000) secondary to dysfunctional arousal mechanisms linking reward to motivation. It may be the case that apathetic patients need a greater amount of dopamine to normalize their clinical symptoms, over and beyond the level indexed by our pupillary reward measure. Indeed, PD patients in general might require greater reward sensitivity compared to controls in order to motivate themselves to perform a goal directed task.

The exact neural pathways orchestrating pupillary and cognitive process, however, are not clearly defined. Locus coeruleus noradrenergic (LC-NA) projections may facilitate this connection via arousal (Rajkowski, Kubiak and Aston-Jones, 1994) or decision making processes (Nieuwenhuis, Aston-Jones and Cohen, 2005). Dopaminergic projections also share common connections to LC-NA pathways, so other systems are likely involved (Wang and Munoz, 2015) with evidence for a dopamine and noradrenaline interaction (Delaville, Deurwaerdère and Benazzouz, 2011). Abnormal pupillary reward sensitivity in PD patients with apathy may therefore arise through dysfunction of brainstem arousal processes, in which dopamine and noradrenaline interact to link reward to motivation (Delaville, Deurwaerdère and Benazzouz, 2011; Manohar and Husain, 2015).

PD patients often suffer from executive deficits (Dirnberger and Jahanshahi, 2013; Gratwicke, Jahanshahi and Foltynie, 2015). The use of physiological measures like pupillometry to index value placed on monetary incentives minimizes executive function requirements (Hong and Hikosaka, 2008; Chen *et al.*, 2014; Rudebeck *et al.*, 2014; Manohar and Husain, 2015; Manohar *et al.*, 2015). Our findings suggest that apathetic PD patients place less value on rewards in the evaluation stage of goal directed action. This may represent a reduction in the 'wanting' or motivation to obtain rewarding stimuli (Berridge, 2007), and account for some of the manifestations of apathy such as reduced, *self-initiated* goal-directed behaviour. Reassuringly, no association with reward sensitivity and depression was found (**Fig. 3.11** and **3.12**) suggesting that a different mechanism is at play in depression and that this is not associated with reward sensitivity and 'wanting', but instead may be more linked to pleasure and 'liking' (Berridge and Kringelbach, 2008).

Lastly, it is important to ensure that any differences in pupillary response to reward between apathetic and non-apathetic PD patients are not secondary to generalised systemic autonomic dysfunction. Investigations using ambulatory ECG recordings in PD patients has revealed that autonomic dysfunction can be detected using measures of heart rate variability (Haapaniemi *et al.*, 2001; Pospíšil *et al.*, 2008; Maetzler *et al.*, 2015). Using several different variability measures, we found that apathetic patients were not significantly different from non-apathetic patients. Thus, the pupillary response differences to reward between the two groups were not associated with differences in more global autonomic dysfunction.

3.4.3 Invigoration of saccadic velocity by reward

Within the PD group, an interesting dissociation was found between the physiological changes in pupillary response to reward incentivisation and the motor response performed to obtain it (Niv *et al.*, 2007; Kurniawan *et al.*, 2010; Kurniawan, Guitart-Masip and Dolan, 2011). Therefore, although pupillary reward sensitivity was reduced in apathetic patients, they still maintained invigorated movement, indexed by peak saccadic velocity, when making an *externally cued* goal-directed action. One explanation might simply lie in a larger dynamic range for the pupillary measure in comparison to the saccade metric. Pupil modulation for reward in the period of interest showed up to 59% increase between 0p and 50p levels in PD patients ON dopamine, whereas saccadic differences were only of the order 3.9%, perhaps explaining why no association with apathy was detected. Another possibility is that apathy in PD is a disorder of *intrinsic reward evaluation* rather than specifically energisation of motor actions, despite both seemingly being dopamine dependent. In daily life, behavioural apathy is manifest as a reduction of *self-initiated* goal-directed behaviour. A common complaint of carers is that patients with behavioural apathy will perform actions if prompted, but not of their own volition. In the experiments conducted here, participants were cued by an *extrinsic* stimulus (target onset) to make an eye movement and their saccadic velocities scaled with the magnitude of incentives on offer. It is possible that while the reward evaluation system linked to pupillary change is dysfunctional in apathy, externally cued actions remain less affected than self-initiated ones. Thus, when reward evaluation is used to guide self-generated actions there might be less invigoration of such behaviour compared to externally prompted ones. Intriguingly, a recent study has documented that pupillary dilation prior to making saccades

that require greater voluntary control (anti-saccades) is blunted in PD (Wang *et al.*, 2016), although this investigation did not seek to explore an association with apathy.

A key deficit in PD apathy may be associated with the ventral striatum, involved in encoding value of upcoming actions (Rowe, Hughes and Nimmo-Smith, 2010), and medial frontal areas including supplementary and pre-supplementary motor cortex (SMA and pre-SMA) (Nachev, Kennard and Husain, 2008). These medial frontal regions encode movements for reward in monkey single cell recordings (Scangos and Stuphorn, 2010) and may be associated with self-initiated movements. They are particularly related to internally-driven motivational processes (Bouret and Richmond, 2010) necessary for enhancing ventral striatum responses to the anticipation of reward (Pujara *et al.*, 2016). In our apathetic cohort, the action initiation sub-score of the LARS, which assesses voluntary action initiation, was significantly lower compared to non-apathetic PD patients. It has also been suggested that later onset PD has greater loss of ventral striatal dopamine, vital for maintaining reward sensitivity (Kish, Shannak and Hornykiewicz, 1988; Sinha, Manohar and Husain, 2013). This is consistent with imaging findings that show ventral striatal atrophy in PD cases with apathy (Carriere *et al.*, 2014).

In our study, saccadic peak velocity sensitivity to reward was reduced in the PD group overall when OFF dopamine compared to ON (**Fig. 3.8**). Blunted action speed for rewards, as demonstrated by reduced saccadic peak velocities in dopamine depleted states, may be representative of abnormal reward rate monitoring (Niv *et al.*, 2007; Manohar *et al.*, 2015) perhaps reflective of reduced dopamine levels within the basal ganglia (Levy, 2006; Niv, 2013). Our task maintained a fixed reward rate using an adaptive staircase that was based on individual performance to keep the amount obtained equal for each participant. PD patients ON dopamine appeared to be able to monitor the average rate of reward irrespective of apathy level, as demonstrated by increased saccadic vigour to maximise reward obtained, but their evaluation of this reward was disrupted in apathy, as indexed by the blunted pupillary response.

3.4.4 Reward sensitivity and apathy in PD

Other emerging evidence has suggested that impaired incentive processing may be contributory to apathy in PD. A recent study examined feedback related negativity (FRN) responses to rewards (Martinez-Horta *et al.*, 2014). PD patients with apathy display reduced amplitude differences in FRN signals whilst performing a gambling task, which may reflect compromised mesocorticolimbic reward pathways, and is consistent with the findings reported here. Indeed, apathy in Parkinson's disease appears to be associated with nucleus accumbens (NAcc) atrophy (Carriere *et al.*, 2014), an area implicated in reward evaluation (Berridge and Kringelbach, 2008). Apathetic behaviours have also been reported in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) monkey model, where dopaminergic depletion is associated with VTA as well as cortical disruption (Tian *et al.*, 2015). Overall, dopamine pathway dysfunction in NAcc and VTA are reported to be the strongest predictors of apathy in this animal model of PD (Brown *et al.*, 2012).

The influence of dopamine on reward sensitivity is also apparent in other work that examines its role in reward and effort-based decision-making in PD. ON dopaminergic medication, patients are more willing to exert effort for the same reward level compared to when OFF (Chong *et al.*, 2015), perhaps via dopamine's ability to reduce subjective effort costs and increase reward sensitivity. Similarly, reductions in dopamine therapy after implantation of STN-DBS for motor symptoms in PD can potentially unmask existing mesolimbic pathway degeneration that leads to dopamine-sensitive apathy (Thobois *et al.*, 2010).

The premise of the study was based on the hypotheses that reward sensitivity might be blunted in PD cases with apathy, and that dopaminergic medication might modulate reward sensitivity. Our *a priori* comparison of interest therefore was to compare PD patients with and without apathy and explore within patient effects of being ON or OFF dopaminergic medication. Therefore, the main comparisons we wished to make were between apathetic and non-apathetic patients. However, we also aimed to use the elderly control group as a reference point, rather than as a discriminating factor to compare between apathy and non-apathy in PD. The findings suggested that when ON dopamine, *non-apathetic* PD patients demonstrated greater reward sensitivity compared to elderly controls and when OFF,

apathetic patients were less reward sensitive versus controls. This appears to be a logical finding as it may be hypothesized that patients who do not have a reduction in motivation (non-apathetic), and who are given more dopamine may be overly sensitive to reward compared to controls, whereas those who are apathetic and also dopamine deplete would be less reward sensitive. Again, elderly controls in this study were used as a reference point in order to compare the differences between apathetic and non-apathetic PD patients, therefore their scores were taken into account during the overall higher-level group analysis.

In our study, there was no difference in the average levodopa daily dose between the apathetic and non-apathetic patients. One possible explanation for differences in reward sensitivity between the groups might be differential degeneration of ventral versus dorsal striatal regions. Later onset PD may be associated with greater loss of ventral striatal dopamine (Kish, Shannak and Hornykiewicz, 1988; Sinha, Manohar and Husain, 2013) while in comparison, dorsal striatal function, crucial for motor control, is better preserved (Tremblay *et al.*, 2015). Therefore, in later onset PD, replacement of dopamine might still be insufficient to overcome apathy, despite improving motor symptoms. In our cohort, apathetic patients were on average 5 years older at diagnosis consistent with this hypothesis. Although disease duration was significantly longer in the non-apathetic patient group, in population studies disease duration has not been shown to have a definite association with apathy (den Brok *et al.*, 2015). PD cases with worse motor disease progression at 4 year follow up however, are more likely to develop apathy (Pedersen *et al.*, 2009). Finally, the diminished response to dopaminergic medication in apathy also raises the possibility of dopamine resistance (Carriere *et al.*, 2014) and the potential that other neurotransmitter pathways could play a role in the disorder (Chaudhuri *et al.*, 2006). Although more work is needed to explore this further, a recent trial has reported effective treatment of apathy using the cholinesterase inhibitor, rivastigmine, in non-demented PD patients (Devos *et al.*, 2014).

3.4.5 Conclusion

This study demonstrates that reward sensitivity, modulated by dopamine, is blunted in PD patients suffering from clinical apathy. The findings raise the possibility that reward insensitivity may be a contributory mechanism to apathy and that it can be indexed through

pupillary changes in response to monetary incentivisation. The results highlight how personalised physiological assessment in PD can reveal potentially inadequate drug treatments of non-motor symptoms, and provide a basis for novel objective clinical markers of motivation in neurodegenerative conditions.

4. Reward sensitivity, motor preparation and action initiation

4. Abstract

Clinical apathy leads to dysfunction of goal directed behaviour, however a key component of this is the initiation of action to obtain a goal. The previous chapters highlight the blunting of reward sensitivity in apathy. However another component may be the disruption of initiation of action and its relationship with evaluation of rewards. In this chapter, the link between motivation and motor output it explored and its association with clinical apathy and dopamine assessed. A new group of 23 young participants, 18 elderly controls and 22 patients with Parkinson's disease tested ON and OFF dopamine were included. To distinguish between pupillary response to anticipated reward verses response associated with motor preparation, a 'go/no-go' version of the oculomotor task from chapter 3 was performed. In this study, only half of the trials required a saccade to be initiated to receive a reward and in the remaining trials no action was needed but a reward was still obtained.

No significant difference in pupil response was demonstrated when an action was made versus when it was not made for reward, suggesting that pupillary responses to rewards in this task were not secondary to motor preparation. Being ON or OFF dopamine did not influence this response either. Crucially, however, the link with apathy in PD patients was observed only when an action was required to receive a reward, and only in the ON state. These findings suggest that pupillary reward sensitivity is not a consequence of motor preparation, but that action initiation is key to eliciting a significant reward sensitivity association with apathy. It focuses the potential mechanisms of apathy towards a reduction in reward sensitivity but only when associated with initiation of actions and in the presence of adequate dopamine states.

4.1 Introduction

One of the proposed components of goal directed behaviour includes the initiation of action in order to obtain an end outcome (Sinha, Manohar and Husain, 2013). Animals exhibit these behaviours for basic survival including obtaining food, escaping from predators and seeking shelter but in humans it also forms the basis of our activities of daily living. As demonstrated in chapter 3, PD patients with clinical apathy have dysfunction in their evaluation of rewards possibly explaining the paucity of their actions, a key clinical characteristic of apathy (Dujardin *et al.*, 2007). However, impairments in the process of translating valuation into action may also be implicated in apathy and will be addressed further in this chapter.

Distinct models of cortico-basal ganglia circuits have been established using virus tracing in primate studies (Middleton and Strick, 2000; Saga *et al.*, 2011). These circuits are topographically organized and subdivided into three main pathways, each implicated in different functional processes, ranging from intention to act through to action execution (Tremblay *et al.*, 2015). Limbic and associative loops which include ventral regions of the basal ganglia, such as the NAcc, and their projections to frontal and anterior cingulate cortex (ACC) brain regions (Obeso *et al.*, 2008) appear to be associated with action valuation and initiation (Syed *et al.*, 2015). Electrical stimulation of these limbic regions in animals, produces complex behaviours including attack, defence and feeding responses (Mogenson, Jones and Yim, 1980). Conversely, when lesioned, evaluation of reward and effort costs are shifted (Walton, Bannerman and Rushworth, 2002). Damage to either the ACC or mesolimbic dopamine pathways in rats and monkeys, creates a bias in preference towards options that are low effort but relatively small reward (Walton *et al.*, 2006), resulting in devaluation of reward and increased sensitivity to the effort costs for actions. This link between valuation and initiation of actions for reward may occur through the interface between motor circuits in the dorsal striatum, including the caudate and putamen (Tremblay *et al.*, 2015). These basal ganglia regions project to cortical motor and supplementary motor areas (SMA) (Obeso *et al.*, 2008) as well as limbic structures, such as the NAcc (Groenewegen, Wright and Beijer, 1996). The NAcc receives dopaminergic inputs from the ventral tegmental area and has been associated with apathy in MPTP lesioned monkeys (Mogenson, Jones and Yim, 1980; Brown *et al.*, 2012). Interestingly, lesions affecting the caudate nucleus, putamen and globus pallidus also leads to

apathy across a large case study of human patients, additionally implicating these brain areas in the disorder (Bhatia and Marsden, 1994). Furthermore, in young volunteers with higher apathy scores, inefficient communication in white matter connectivity between the rostral cingulate areas and the SMA is also found following DTI imaging (Bonnelle, Manohar, *et al.*, 2015). Apathetic participants appear to incur larger costs in initiating effort and have deficits in converting motivation into motor output when tested on incentivized decision making tasks (Bonnelle, Manohar, *et al.*, 2015). Therefore apathy may encompass problems in the transformation of an intention (motivation to act) through to the action itself via limbic-motor interactions (Salamone and Correa, 2012; Sinha, Manohar and Husain, 2013).

It has been largely accepted that dopamine encodes predictions about future rewards (Howe *et al.*, 2013). A recent rodent study demonstrated that NAcc dopamine increases when a cue for a reward is heard and, crucially, a movement is needed to be initiated in order to receive the reward (Syed *et al.*, 2015). This phasic dopamine response peaks just before obtaining the reward and is not present when a movement is not needed to obtain it, even though the reward cue conveyed the same information about the upcoming incentive. Dopamine release in the NAcc is therefore contingent on initiation of motor actions and not just reward evaluation, implicating it again as a potential interface between limbic and motor systems (Groenewegen, Wright and Beijer, 1996). Therefore, the degree to which action determines an outcome is likely to be important in goal directed behaviour. Given that action follows the assignment of value to the end goal, the outcome should equal the reward to which the action leads (Rangel, Camerer and Montague, 2008). This was also demonstrated in in a recent oculomotor behavioural study performed on healthy participants that found increased pupil dilation was only present when reward was contingent on action (Manohar *et al.*, 2017). Here, autonomic arousal was dependent on reward outcome being based on participants performance and therefore dissociates contingency effects from arousal due to other factors like uncertainty.

Models of saccadic properties and motor preparation suggest signals rise towards a threshold for initiation of movements (Paré and Munoz, 1996; Gold and Shadlen, 2000, 2002; R. H. S. Carpenter, 2004; Rothkirch *et al.*, 2013) and recordings from oculomotor brain regions such as the frontal eye fields show ramping up of activity prior to saccade onset (Hanes and Schall, 1996). When movements are primed, in gap tasks for example, faster saccadic onset

occurs (Rolfs and Vitu, 2007). In these task designs latency is reduced when a fixation target is removed before a subsequent target appears and is thought to occur due to heightened preparation to make a saccade through the warning that an impending target is about to appear (Stevenson, Elsley and Corneil, 2008). Evidence for motor preparation signals in gap, pro and anti-saccade tasks (Jainta *et al.*, 2011; Wang, Brien and Munoz, 2015) and through our previous findings that saccades are faster for larger rewards, it is reasonable to hypothesize that motor preparation signals are being ramped up prior to a saccade being made. Saccades with shorter reaction times appear to occur when ramping activity reaches the threshold for action initiation earlier. If this is indeed the case, then it would be important to establish if changes in pupil response attributed to reward cues are indeed due to reward evaluation rather than a build up for motor action for upcoming saccades.

In this chapter, we explore the relationship between reward evaluation and movement initiation in healthy participants and those with Parkinson's disease, focusing on specific oculomotor responses to reward and their link to apathy. We aim to address the following points:

Firstly, do changes in pupil response to rewards (established in the previous two chapters) arise due to motor preparation signals linked to performing a saccade, or is this response dissociable from movement? Secondly, are pupil responses to reward cues synonymous with the striatal activity demonstrated in the animal literature that occurs only when an action is initiated? Finally, are reward sensitivity deficits in Parkinson's disease patients with clinical apathy linked to impairments in the evaluation of rewards only or, are the initiation of actions also crucial for association of reward cues, like in recent animal studies (Syed *et al.*, 2015). If so, are these responses detectable in the pupil and modulated further by dopaminergic state in PD?

In order to address these questions, an adaptation of the previous oculomotor study was performed in young and elderly healthy participants as well as a group of patients with idiopathic Parkinson's disease, assessed both ON their normal dopaminergic medication and while OFF. The task was a novel variation of a 'go/no-go' task which was designed to explore initiation of actions rather than response inhibition as no reflexive movement needed to be suppressed. It comprised of two separate arms, one where a motor action (saccade) was

made to receive a reward, and another where no motor action was needed for reward. Saccadic properties and pupillary response to reward cues were assessed in all the participant groups and comparisons to clinical apathy in the patients was also made.

4.2 Methods

4.2.1 Participants

Young, elderly and patients with idiopathic Parkinson's disease were recruited. Young participants were enlisted through the online recruiting system of the Department of Experimental Psychology at the University of Oxford. Elderly control subjects were sampled from a pool of volunteers registered with the Oxford Dementia and Ageing Research (OxDARE). Parkinson's disease patients were recruited from clinics in the Oxfordshire area. A minimum of £8 and maximum of £12 was paid and transport costs were reimbursed.

There were no significant differences in LARS score between healthy elderly and PD patients in this study. New cohorts of PD patients and controls were used for this study and as PD is a heterogeneous population, the total level of apathy in this group was less than in chapter 3. Also, elderly controls in this chapter on average scored worse on the LARS, this is likely due to the older age group of control participants tested in this study and may account for why no difference was found in apathy level between the patient and elderly groups. **Table 4.1.**

4.2.2 Young and elderly control demographics

Twenty-three young healthy volunteers and eighteen elderly control participants with no history of psychiatric or neurological disorders took part in the experiment (Young: Mean age 23.78 years (SD +/- 5.01) 7 male; Elderly: Mean age 70.4 years (SD +/- 6), 11 male).

4.2.3 Parkinson's disease demographics

Twenty-two patients with idiopathic Parkinson's disease were recruited, all were screened for any preexisting psychiatric disorders and any significant comorbidities. Average age 65.2 (SD+/- 8.9) and 14 males, see **Table 4.1** for full demographic breakdown.

	Healthy Young Controls	Healthy Elderly Controls	Parkinson's Disease (Medicated)	Controls vs Parkinson's Disease p-value
n	23	18	22	
Age (years)	23.8 (±5.01)	70.4 (±6)	65.2 (±8.9)	0.03*
Apathy (LARS)	n/a	-26 (±5.4)	-23.5 (±8.5)	0.2
Depression Score (BDI)	n/a	5.4 (±4.0)	11.3 (±7.0)	<0.01*
MoCA	n/a	27.5 (±2.2)	28.6 (±1.4)	0.06
UPDRS I	n/a	n/a	5.5 (±4.1)	n/a
UPDRS III ON	n/a	n/a	18.5 (±9.8)	n/a
Hoehn & Yahr Stage	n/a	n/a	1.4 (±0.6)	n/a
Hours since last dose ON vs OFF	n/a	n/a	2.4 (±2.4) vs 14.1 (±4.1)	n/a
Levodopa equivalent dose (mg/24 hours)	n/a	n/a	615 (±72.7)	n/a

Table 4.1. Demographic comparison of Parkinson's disease patients and elderly controls

*Numbers in brackets represent standard deviations and standard error of the mean where appropriate. * Indicates a significant difference. LARS = Lille Apathy Rating Scale; MoCA = Montreal Cognitive Assessment; BDI = Beck Depression Inventory II. UPDRS = Unified Parkinson's disease rating scale. 8 PD patients and 3 controls scored worse than the -22 apathy threshold on the LARS.*

4.2.4 Experimental paradigm

The task design was adapted from Chapter 1, study 2. Again, participants were instructed that the faster they looked at a target, the greater the proportion of reward on offer they would obtain (**Fig. 4.2**). Each trial commenced with the onset of a disc at screen centre. After they had fixated this for 500ms, participants heard a recorded voice that informed them of the maximum reward available for that particular trial: "0p/10p/50p

maximum". Simultaneously with the auditory reward cue a '+' or a '×' symbol would appear within the fixation target for 200ms. The '+' indicated a 'go' trial and the '×' a 'no-go' trial. '×' and '+' symbols were used as visual cues because they were the same graphical image rotated 45 degrees. This allowed luminance of the visual cue to be matched and direct comparisons to be made.

'Go' trials – A go trial was indicated by the appearance of a '+' symbol. After a randomly variable period of 2000, 2100 or 2200ms the central fixation disc disappeared and a target concurrently appeared either to the left or the right at 11° of visual angle. This was the same procedure as in study 2, however in this study the times at which the targets appeared was extended by 600ms. Increasing the delay between reward cue and target onset allowed effects of reward processing and any movement effect on pupil dilation sufficient time to uncouple. Rewards earned were displayed in the centre of the target after completion of each trial. The total amount of reward received on each trial was performance driven dependent on saccadic reaction time.

'No-go' trials – In this condition, if a 'x' was indicated at the start of the trial, then the target always remained in the centre of the screen and participants had to maintain fixation. Fixation was held for the same three variable fore periods of either 2000, 2100 or 2200ms. Subjects were instructed that the proportion of reward earned depended on their ability to stare centrally at the target. Earnings were displayed in the same way as in 'go' trials. Unbeknown to the participants, the average earnings in the last 20 trials of the 'go' condition determined the reward obtained in 'no-go' trials. This ensured that the same overall amounts of money were received in the two conditions over the course of the experiment.
The two types of trial occurred in equal frequency in a randomised order. Trials were separated by a 2500ms interval. Young participants completed 8 blocks divided into two sessions separated by a 10-minute break. Elderly controls and PD patients completed 6 blocks. Each block had 36 trials and lasted approximately 4.5 minutes.

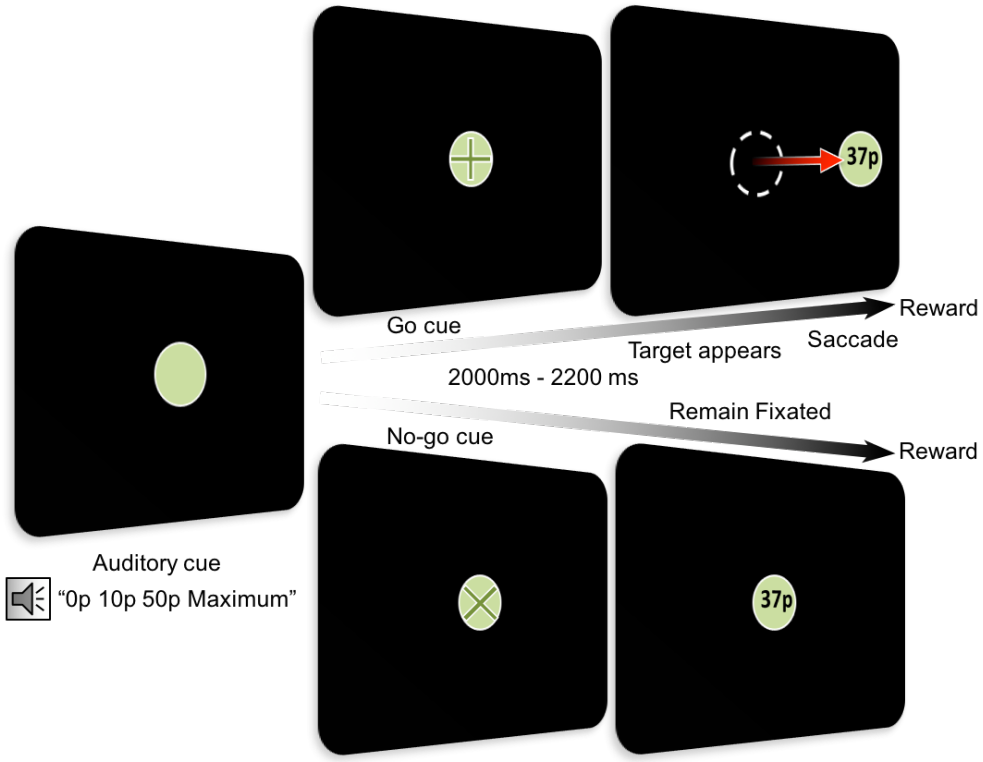

Figure 4.2. The experimental paradigm.

During the task, 50% of trials required a saccade to be made to obtain a reward ('Go' trials indicated by a '+' at the start of the trial). In the remaining 50% of trials, participants were required to maintain fixation centrally ('No-Go' trials indicated by an 'x' at the start of the trial). 'go' and 'no-go' trials were randomly intermixed. Reward obtained was based on reaction time in the 'Go' trials and reward rates in both arms were matched.

4.2.5 Eye tracking recording and analysis

Eye tracking procedures and analysis were the same as in chapter 2 (Sections 2.2.4 to 2.2.7).

4.3 Results

The results from the healthy participants will be explored first, followed by the PD patients and comparisons with age matched controls.

4.3.1 Young Control Participants

4.3.1.1 Saccadic peak velocity

To Ensure that reward was also having the same effect in this task as our earlier experiments, the saccadic properties in the 'Go' arm were analyzed. Saccadic peak velocity showed similar increases in speed for larger rewards compared to no rewards with a main effect of reward, ($F(2, 44) = 25.8$, $P < 0.0001$), and significant differences in velocities between the 0p versus 10p reward and the 0p versus 50p reward ($p<0.001$), but no differences between the 10p versus 50p condition were present ($p=0.21$). (**Fig. 4.3A**).

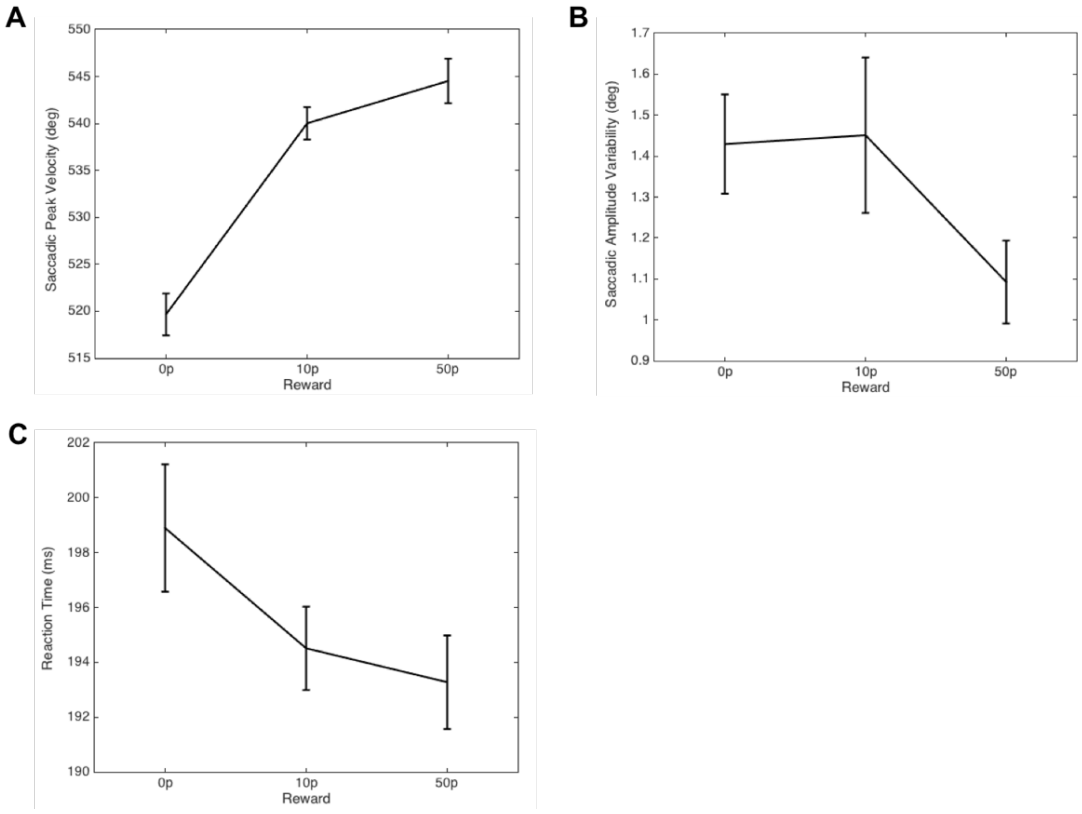

Figure 4.3A-C. Oculomotor responses from young healthy participants in the 'go' arm of the experiment.

A. *Saccadic peak velocity measured in degrees per second increased for larger magnitudes of reward on offer.*

B. *Accuracy as measured by saccadic variability as a function of incentive on offer. A significant reduction in saccadic variability for the largest reward level compared to no reward was present.*

C. *Reaction time for increasing reward levels did not reach statistical significance.*

4.3.1.2 Saccadic accuracy

Accuracy, as assessed by mean variability in saccadic amplitude, did not demonstrate a within subjects main effect of reward ($F(2, 44) = 1.36$, $P = 0.276$), however planned within subject contrasts demonstrated a significant linear improvement in accuracy for the higher 50p reward compared to no reward, ($F(1, 2) = 8.13$, $P = 0.009$), significant for the 0p versus 50p comparison. Therefore, accuracy (like in previous chapters) did not worsen despite saccadic velocity for bigger rewards being faster, again breaking speed accuracy tradeoffs. (**Fig. 4.3B**).

4.3.1.3 Saccadic reaction time

Like the previous experiments, there were no significant effects of reaction time in this task, ($F(2, 44) = 1.6$, $P = 0.2$). (**Fig. 4.3C**).

4.3.1.4 Pupillary response to rewards is present in 'go' and 'no-go' trials

Using the same 1400ms to 2400ms epoch of interest as the previous experiments, there was a main effect of reward ($F(2, 44) = 19.15$, $P < 0.0001$), this pupillary response to reward was present both when an action had to be made in the 'go' condition, and also when no action was needed in the 'no go' condition. There were no significant main effects of trial type ('go' or 'no-go'), however a trend towards 'go' trials having greater overall pupil responses was suggested, ($F(1, 22) = 3.7$, $P = 0.07$). No interaction was present ($F(2, 44) = 0.158$, $P = 0.85$). (**Fig. 4.4A**).

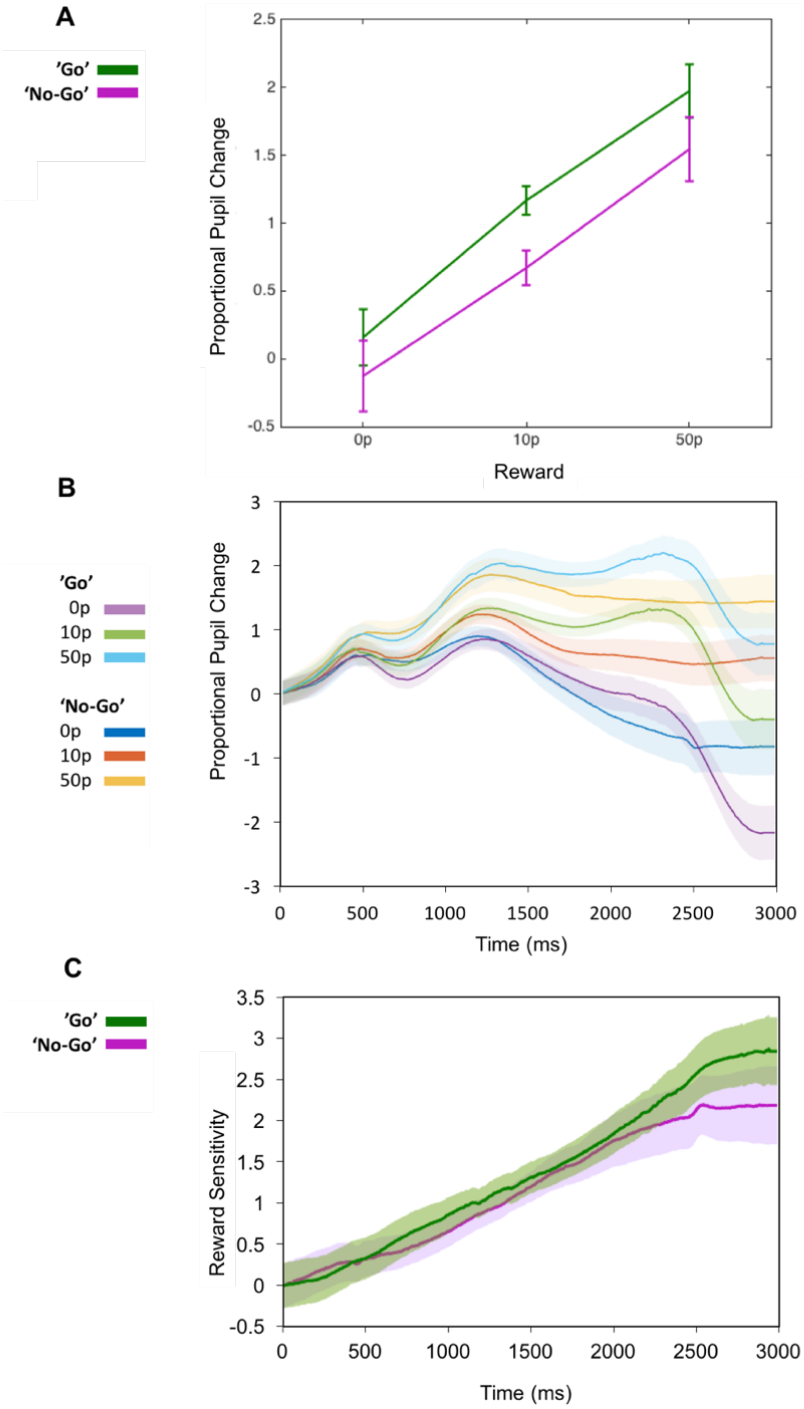

Figure 4.4A-C. Pupil responses in young participants across 'go' and 'no-go' trials.

A. *Pupil proportional change to reward taken as an average across a 1000ms epoch from 1400ms to 2400ms. Increased pupil response to reward was present as magnitude increased but no difference was seen between 'go' trials in green and 'no-go' trials in violet.*

B. *Average proportional pupil changes over the course of the trials in young healthy participants, broken down into responses to 'go' and 'no-go' cues. Cue onset was at time 0ms.*

C. *Average reward sensitivity over time, taken as the difference in the proportional pupil change between the 50p reward condition and the 0p reward. Broken into reward sensitivity for 'go' trials – green, versus 'no-go' trials – violet. No significant difference in reward sensitivity was present at any time point across the length of the entire trial.*

Pupil change over time demonstrated divergence of pupil response for the 3 reward levels from around 500ms (**Fig. 4.4B**). The pupil responses in the 'go' and the 'no-go' trials seemed to have slight differences in dilation and constriction responses for reward. In the 'go' arm, a tri-phasic dilation and constriction pattern was seen, with slightly greater peaks in pupil dilation overtime, whereas the 'no-go' trials had a more bi-phasic response, and overall flatter pupillary changes over time. Target appearance was from 2000 to 2200ms and reaction time was approximately 200ms, so saccadic onset ranged from approximately 2200ms to 2400ms. However, when comparisons were made over the 1000ms average epoch of interest (**Fig. 4.4A**), no overall difference was found statistically.

To determine if any association between pupil dilation and saccades were present, a correlation analysis was performed for each reward magnitude between saccadic velocity and subsequent pupil dilation. It seemed that there was a very weak association between pupil size and saccade velocity only in the 0p condition with r_s =0.4180 and p= 0.048. The remaining reward levels did not reach significance. Reaction time did not correlate with any changes in pupil size regardless of reward on offer.

4.3.1.5 Reward sensitivity

Despite a trend towards larger overall pupil responses to each reward level in the 'go' condition. On average, when comparing reward sensitivity (50p pupil response minus 0p pupil response) in the 'no-go' trials versus the 'go' trials, across the entire trial period, there were no significance differences detected in young participants. (**Fig. 4.4C**). Comparisons were made using multiple permutation tests at each millisecond time point.

4.3.2 Elderly Controls Participants

Next, we examined data for the elderly controls.

4.3.2.1 Saccadic peak velocity

Saccadic peak velocity in the elderly participants increased only for the largest 50p reward level, driving a significant main effect of reward (F(2, 34) = 6.8, *P* = 0.003). There was a significant difference in peak velocity between the 10p versus 50p reward and the 0p and 50p reward (p<0.02), but no differences between the 0p versus 10p condition (p=0.83). (**Fig. 4.5A**).

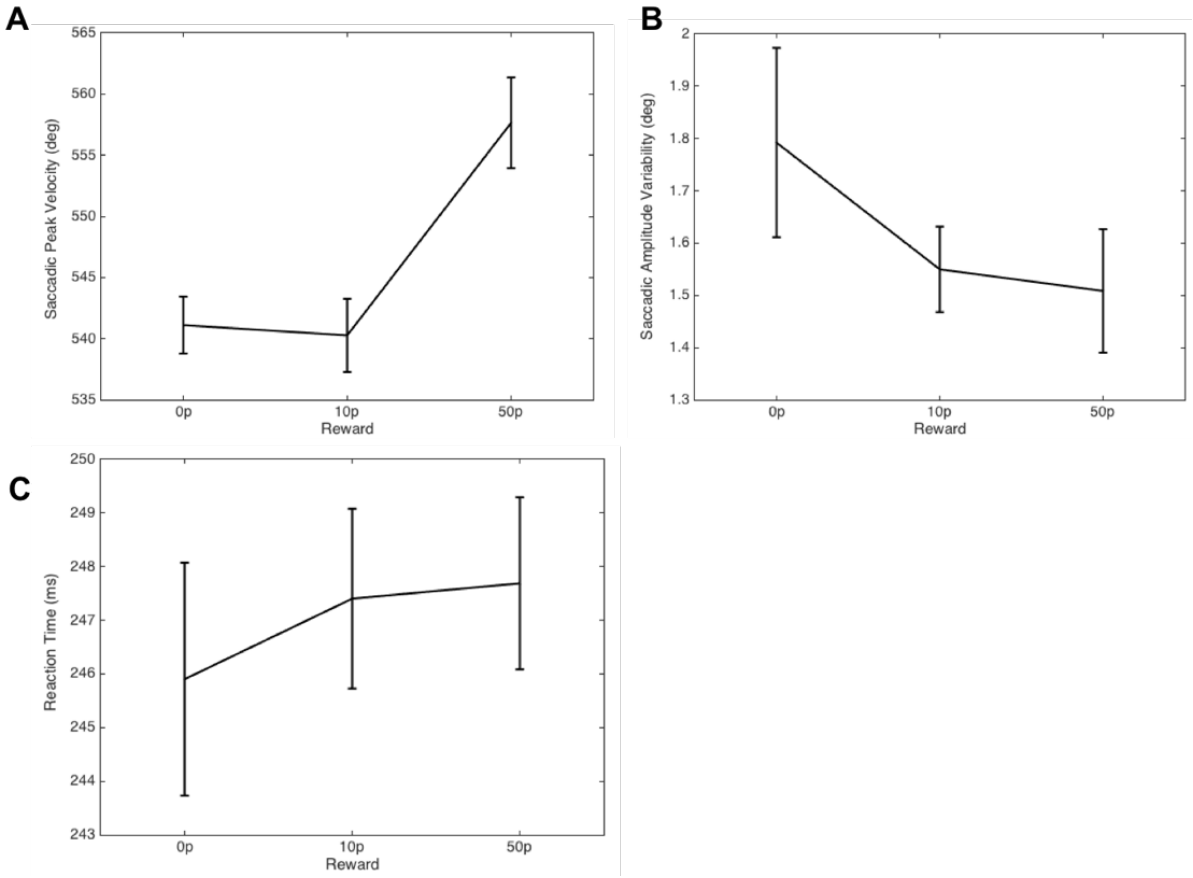

Figure 4.5A-C. Oculomotor responses from elderly healthy participants.

- ***A.*** *Saccadic peak velocity as a function of incentive. Saccadic velocity increased for the largest 50p level of reward on offer compared to the 0p and 10p level.*
- ***B.*** *Saccadic accuracy as assessed by variability of saccade amplitude. There was no significant difference in accuracy as reward levels increased in elderly participants.*
- ***C.*** *Reaction time for increasing reward levels did not differ between reward levels in the elderly group.*

4.3.2.2 Saccadic accuracy

Variability in saccadic amplitude did not demonstrate a main effect of reward in the elderly (F(2, 34) = 0.879, *P* = 425). Although there was no improvement in accuracy with

increasing reward, there was also no reduction in accuracy despite an increase in velocity being present with the largest reward, which also defies speed accuracy trade off predictions and is consistent with the previous findings in the young. (**Fig. 4.5B**)

4.3.2.3 Saccadic reaction time

Much like the young participants, there were no significant effects of reaction time in the task in the elderly controls, ($F(2, 34) = 1.8$, $P = 0.83$). (**Fig. 4.5C**)

4.3.2.4 Pupillary response to rewards is present in 'go' and 'no-go' trials

The pupillary response to reward was present both in the 'go' and the 'no-go' condition in the elderly group, main effect of reward ($F(2, 44) = 14.4$, $P < 0.0001$). Like in the young group, there was also no main effect of 'go' or 'no-go' trials ($F(1, 22) = 3.2$, $P = 0.09$) and no interaction ($F(2, 44) = 0.19$, $P = 0.83$), but a suggestion that the 'go' trials lead to larger pupil dilation overall was still evident. (**Fig. 4.6A**). No association between pupil dilation and saccadic velocity was present in elderly participants.

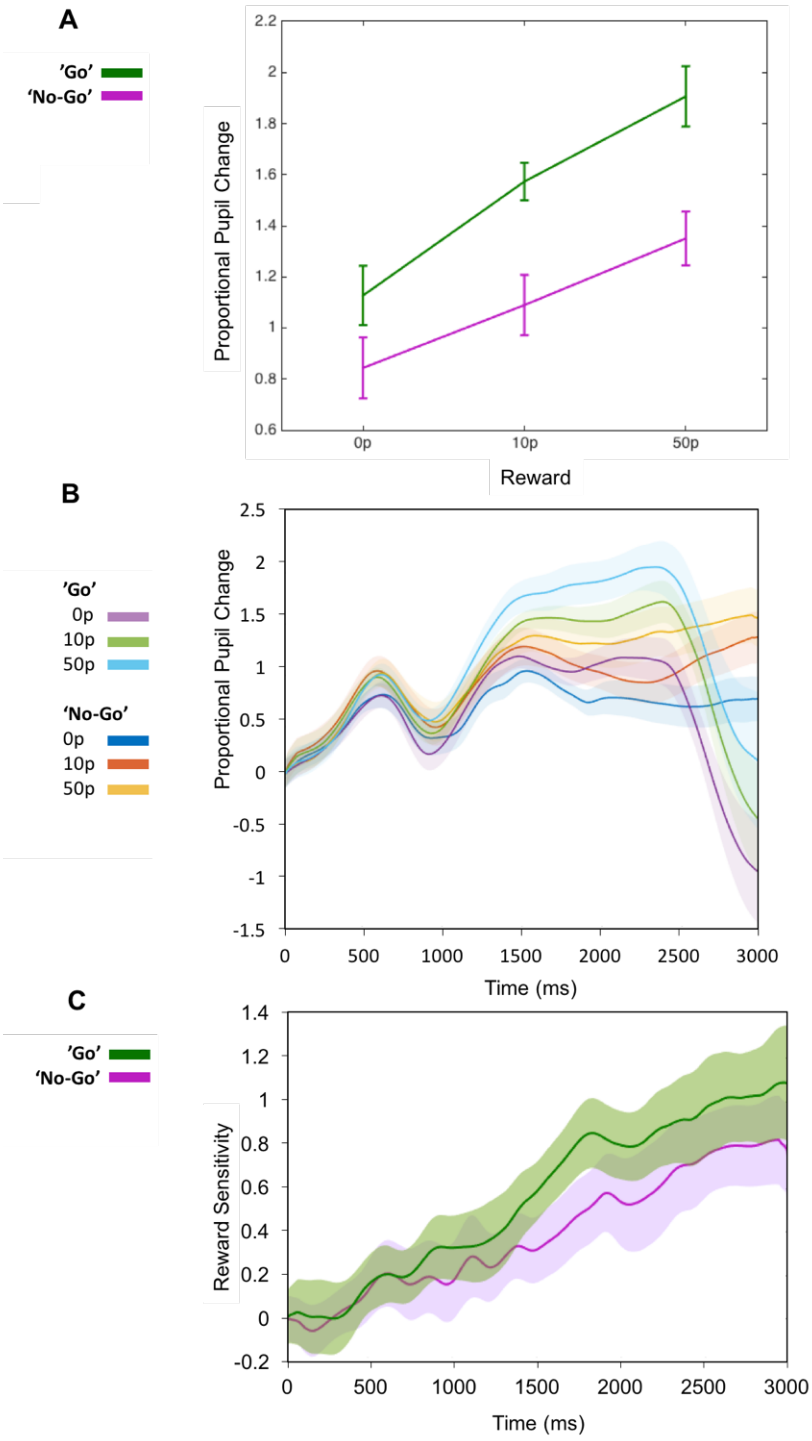

Figure 4.6A-C. Pupil responses in elderly participants across 'go' and 'no-go' trials.

A. *Pupil proportional change to reward taken as an average across a 1000ms period from 1400ms to 2400ms. Increased pupil response to reward was present as magnitude increased but like in the young, no difference was seen between 'go' trials (green line) and 'no-go' trials (violet line).*

B. *Average proportional pupil changes over the course of the trials in elderly participants, broken down into responses to 'go' and 'no-go' cues. Cue onset was at time 0ms.*

C. *Average reward sensitivity over time, taken as the difference in the proportional pupil change between the 50p reward condition and the 0p reward. Broken into reward sensitivity for 'go' trials (green), versus 'no-go' trials (violet). No significant difference in pupil reward sensitivity was present.*

Pupil change over time for the different conditions and reward levels in elderly participants, again revealed slightly different patterns of pupil dilation for reward when an action needed to be made versus when none was needed, (**Fig. 4.6B**). However, as shown above, no statistical significance for pupil responses to reward was reached between the 'go' condition compared to the 'no-go' condition.

4.3.2.5 Reward sensitivity

Like in the young, pupil reward sensitivity in 'no go' versus 'go' conditions across the whole trial period was not significant in elderly controls. (**Fig. 4.6C**).

4.3.3 Young versus Elderly Controls

4.3.3.1 Saccadic peak velocity

Comparing peak velocities in the 'go' arm of the experiment between the young and the elderly groups demonstrated a main effect of reward ($F(2, 78) = 21.3$, $P < 0.001$), with faster speeds between each reward level ($p<0.01$). Interestingly, there was also a reward by group interaction ($F(2, 78) = 5.8$, $P < 0.01$). Deconstructing this interaction revealed that the young controls showed the biggest difference in velocity between the 0p versus 10p reward and the 0p versus 50p reward ($p<0.001$), with no difference between the 10p versus 50p condition. Whereas the elderly group demonstrated the greatest difference between the 0p versus 50p and the 10p versus 50p but not the 0p versus 10p condition. This suggests that there may be a shift in the importance attributed to various reward sizes with aging, perhaps with more elderly individuals needing greater amounts of reward to speed up saccades and the young attributing the same value to all rewards greater than 0p. (**Fig. 4.7A**).

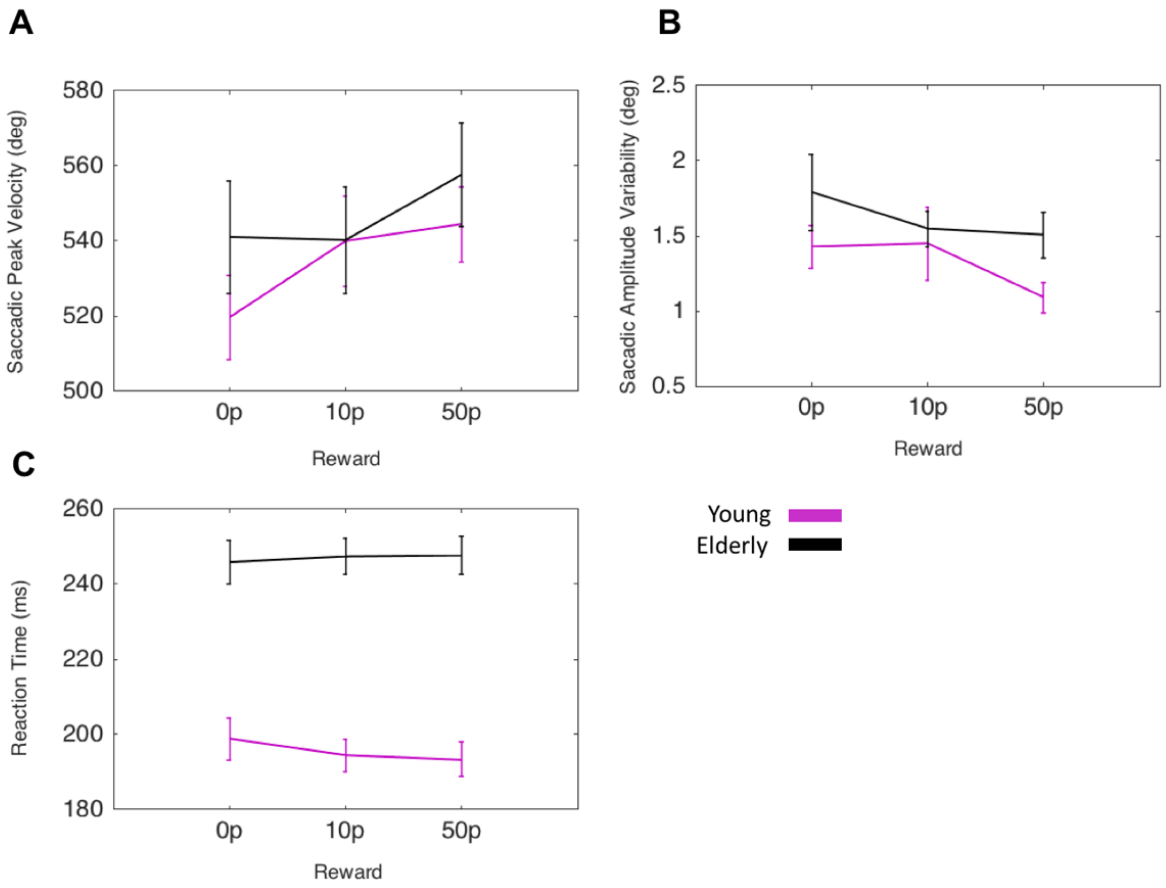

Figure 4.7A-C. Comparison of oculomotor responses between young and elderly participants.

A. *Saccadic peak velocity increased for the largest level of reward on offer compared to 0p in both young (violet) and elderly groups (black). Younger participants had a larger change in their pupil response from 0p to any reward amount, whereas elderly participants only demonstrated an effect with the largest reward level on offer.*

B. *Saccadic accuracy as assessed by variability of saccade amplitude. No worsening of accuracy was seen for increasing levels of reward. There was an increase in accuracy when comparing between the 0p and 50p reward level both in the young (violet) and elderly (black) groups.*

C. *Reaction time for increasing reward levels did not differ between reward levels in either group but elderly participants had significantly slower reaction times overall.*

4.3.3.2 Saccadic accuracy

Variability in saccadic amplitude showed no main effect of reward ($F(2,78) = 1.7$, $P = 0.199$), but there was a within subject linear contrast effect of reward ($F(1,39) =$, $P = 0.04$) suggesting that the difference between the largest 50p reward and 0p level are significantly different with greater accuracy for the biggest reward. The finding that accuracy improves going from 0p to the largest 50p reward level, is present in both groups (**Fig. 4.7B**).

4.3.3.3 Saccadic Reaction Time

There was no main effect of reward, (F(2,78) =0.38, *P* = 0.69) or any reward by group interaction in reaction times, (F(2,78) =1.4, *P* = 0.25). However, there was a main effect of group with elderly controls having significantly slower reactions times in general compared to young controls, (F(1,39) =26.7, *P* < 0.001). (**Fig. 4.7C**).

4.3.3.4 Pupillary comparisons

Pupil reward sensitivity was calculated as the pupil response to the 50p reward minus the 0p response over time. Pupil reward sensitivity in young controls compared to elderly controls in the 'no-go' trials using multiple permutation tests showed a small ~350ms time period of differences between the two groups, (p<0.05). (**Fig 4.8A**).

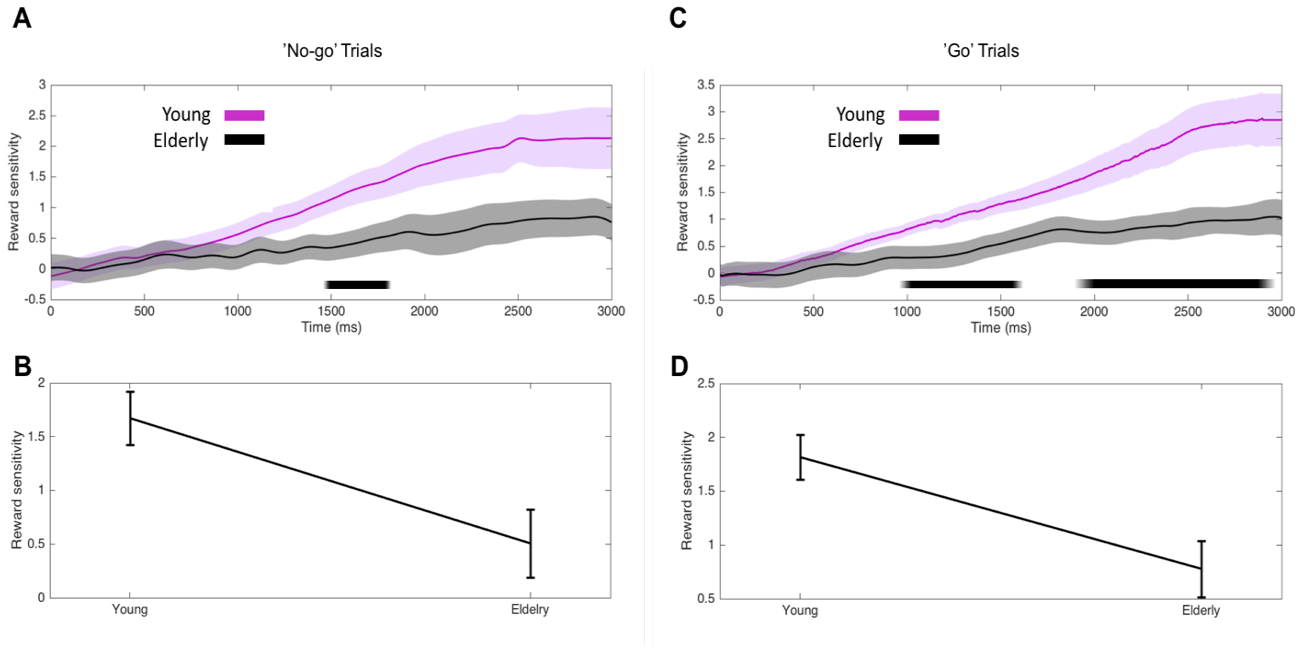

Figure 4.8A-D. Reward sensitivity in 'go' and 'no-go' trials compared between young and elderly groups.

A. *Average reward sensitivity over time in 'no-go' trials, taken as the difference in the proportional pupil change between the 50p reward condition and the 0p reward. Broken into reward sensitivity for young (violet) and elderly (black participants). Black bar denotes significant difference between the two groups over a short ~350ms period.*

B. *Pupil reward sensitivity (50p-0p reward) taken as an average across a 1000ms time epoch from 1400ms to 2400ms in the 'no-go' trials. Young participants had significantly larger pupil response to reward over the time epoch of interest compared to elderly participants.*

C. *Average reward sensitivity over time in 'go' trials, taken as the difference in the proportional pupil change between the 50p reward condition and the 0p reward. Broken into reward sensitivity for young (violet) and elderly (black participants). Black bar denotes significant difference between the two groups over a large period of the trial starting from ~1000ms post reward cue onset to the end of the trial.*

D. *Pupil reward sensitivity (50p-0p reward) taken as an average across a 1000ms period from 1400ms to 2400ms in the 'go' trials' Young participants had significantly larger pupil response to reward compared to elderly participants.*

The difference between the two groups became much more prominent when the 'go' trials were examined over time. (**Fig 4.8C**). A large area of difference from around ~1000ms to the end of the trial was evident using multiple permutation testing, with only short ~400ms period where this did not reach statistical significance at a two-tailed level ($p<0.05$).

Using ANOVA to compared the reward sensitivity of the young controls versus elderly controls in the 'No-go' trials over the 1400ms to 2400ms epoch of interest, there was a significant difference, ($F(1, 40) = 4.2$, $P = 0.048$). Young controls had a higher reward sensitivity compared to elderly controls, in the 'no go' condition. (**Fig 4.8B**). This difference was also true when comparing the two groups pupil reward sensitivity in the 'go' trials, ($F(1, 40) = 4.6$, $P = 0.038$), (**Fig 4.8D**) and replicates the result from chapter 2 where reward sensitivity decreased with age.

4.3.4 Parkinson's disease ON and OFF

4.3.4.1 Saccadic peak velocity

When analyzed separately, PD patients ON dopamine show a significant increase in peak velocity for rewards of larger magnitude ($F(2,42) =9.4$, $P < 0.001$), this was true between each reward level ($p<0.03$). When in the OFF state, the extent to which peak velocity increased with reward was reduced, although there was still a main effect of reward ($F(2,42) =8.0$, $P = 0.001$) significance was only present between the 0p vs. 10p and 0p vs. 50p reward level ($p<0.02$) and no difference between the 10p vs. 50p ($P=0.241$). (**Fig. 4.9A,** ON – blue line, OFF – red line).

When comparing ON and OFF together in a combined repeated measures ANOVA, with drug and reward level, there was a main effect of reward but no statistically significant main effect of drug or interaction was present. Larger rewards lead to increasing speeds ($F(2,42) = 17.5$, $P < 0.00001$), and this was significant at every reward level comparison (0p vs 10p, 10p vs 50p and 0p vs 50p, $p<0.02$). (**Fig. 4.9A**).

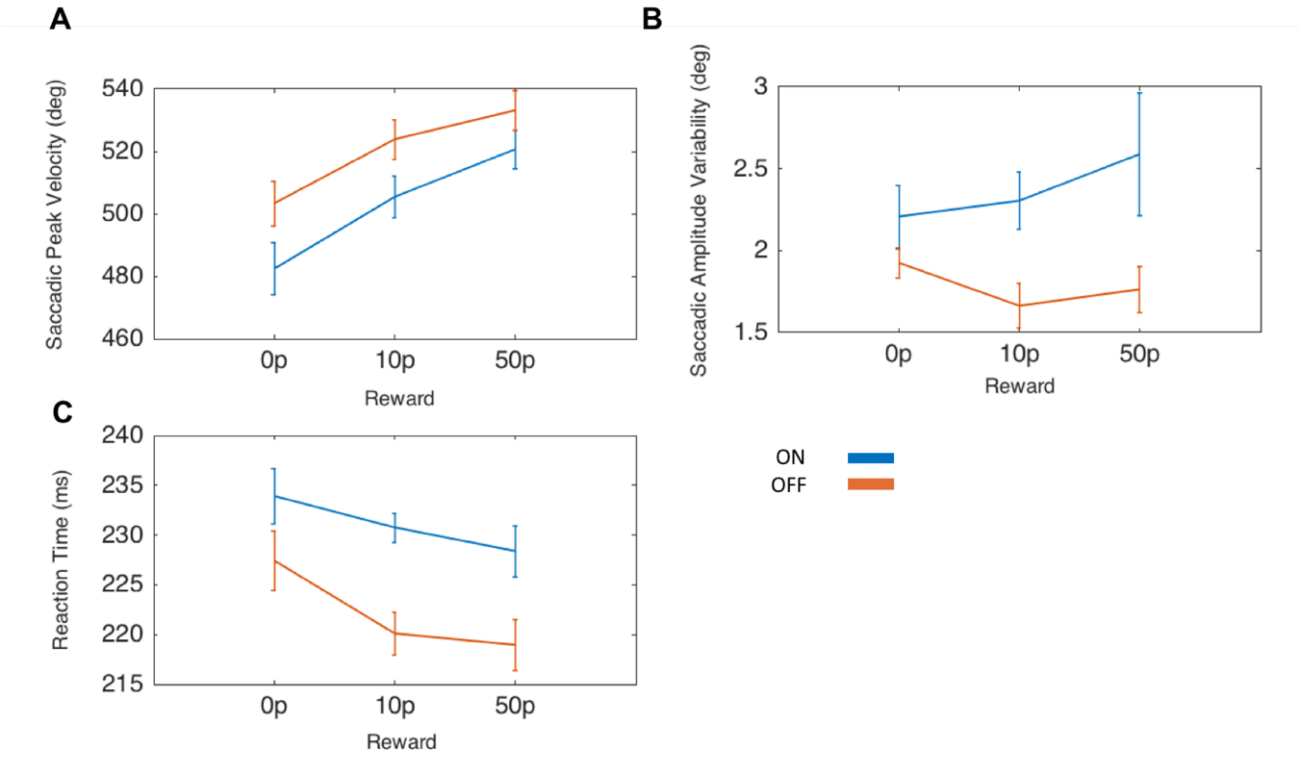

Figure 4.9A-C. ***Comparison of oculomotor responses between PD patients ON and OFF dopamine.***

- ***A.*** *Saccadic peak velocity increased for the increasing levels of reward on offer both when ON (blue) and OFF (red) dopamine.*
- ***B.*** *Saccadic accuracy as assessed by variability of saccade amplitude. No worsening of accuracy for increasing levels of reward when ON (blue) or OFF (red) was detected. However, when ON dopamine the variability of saccades seemed to be greater overall when compared to OFF.*
- ***C.*** *Reaction time for increasing reward levels did not differ between reward levels in either group but surprisingly patients ON (blue) dopamine had significantly slower reaction times overall compared to OFF (red).*

4.3.4.2 Saccadic accuracy

When performing separate comparisons, a significant main effect of accuracy in PD ON or PD OFF was not present, ($F(2,42) = 0.45$, $P = 0.643$ and $F(2,42) = 3.0$, $P = 0.06$ respectively). Interestingly, by performing a 2x3 repeated measures ANOVA comparing accuracy between reward levels and drug state, a significant main effect of drug emerged ($F(1,21) = 7.5$, $P =$

0.012) with those ON dopamine having on average worse accuracy (increased saccadic variability) compared to those OFF. (**Fig. 4.9B**).

4.3.4.3 Saccadic reaction times

There was no significant main effect of reaction time in PD ON ($F(2,42) = 1.2$, $P = 0.313$) or PD OFF ($F(2,42) = 2.6$, $P = 0.09$). When comparing drug and reward effects on reaction time together there was a trend for faster reaction time with increasing reward levels ($F(2,42) = 2.6$, $P = 0.09$) and a main effect of drug ($F(1,21) = 14.9$, $P = 0.001$) demonstrating that when OFF dopamine, reaction times in general were actually faster than when ON. Perhaps surprisingly, being ON dopamine medication seemed to increase saccadic latency in general. (**Fig. 4.9C**).

4.3.4.4 Pupillary response to rewards is present in 'go' and 'no-go' trials

Using average pupil changes over the 1000ms time period of interest (1400 – 2400ms), comparison of pupil response to rewards in the 'go' versus the 'no-go' condition was made. When ON dopamine a main effect of reward was present only, ($F(2, 42) = 8.4$, $P = 0.001$), with increasing pupil dilation for increasing reward magnitude. There was no main effect of trial condition ('go' and 'no-go', ($F(1, 21) = 0.68$, $P = 0.42$), nor any interaction, ($F(2, 42) = 0.31$, $P = 0.73$) when ON. (**Fig. 4.10A**).

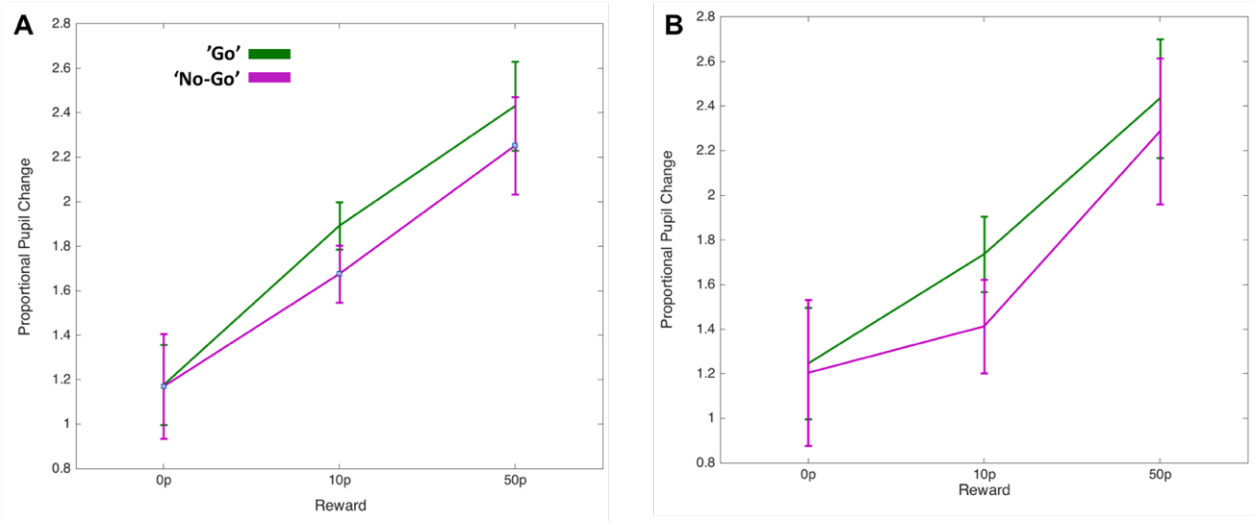

Figure 4.10A-B. PD pupil response to reward in 'go' and 'no-go' trials in the ON and OFF state.

- ***A.*** *When ON dopamine, pupil proportional change to reward taken as an average across a 1000ms period of time from 1400ms to 2400ms. Increased pupil response to reward was present as reward magnitude increased but no difference was seen between 'go' trials (green) and 'no-go' trials (violet).*
- ***B.*** *When OFF dopamine, pupil proportional change also increased as reward increased, with no difference in 'go' trials (green) and 'no-go' trials (violet).*

The same is true when OFF dopamine. A main effect of reward was present ($F(2, 42) = 3.6$, $P = 0.037$) but no main effect of the 'go' and 'no-go' conditions, ($F(1, 21) = 0.38$, $P = 0.54$), or any interaction ($F(2, 44) = 0.51$, $P = 0.61$), (**Fig. 4.10B**). Finally, no correlations between pupil dilation and saccadic velocity or reaction time was present in PD, both when ON or when OFF dopamine.

4.3.4.5 Dopamine does not change pupil response of 'go' vs 'no-go' trials

When comparing ON and OFF, 'go' and 'no-go' effects on pupil response using a repeated measures ANOVA, only a significant main effect of reward was present, ($F(2, 42) = 6.7$, $P = 0.003$), and no other main effect or interaction. The pairwise comparisons showed a significant difference between all reward levels ($p<0.02$) except between the 0p and 10p reward which was not statistically significant ($p=0.073$). Therefore, in this experiment, dopamine does not seem to change the pupil response to rewards when a saccade is made ('go' trials) versus when it is not ('no-go' trials).

4.3.4.6 Pupil Reward sensitivity and a link to motivation

As reported, pupil response to reward increased with greater incentives on offer. There was however, no difference in pupil reward sensitivity across the course of the trial despite if a 'go' or a 'no-go' trial was performed. This confirms that making a saccade does not influence pupillary reward sensitivity as measured by pupil response. It also strengthens our findings from the previous study, confirming that they were indeed due to reward sensitivity rather than just motor preparation. This was true both in the ON (**Fig 4.11A**) state as well as the OFF state (**Fig 4.11B**).

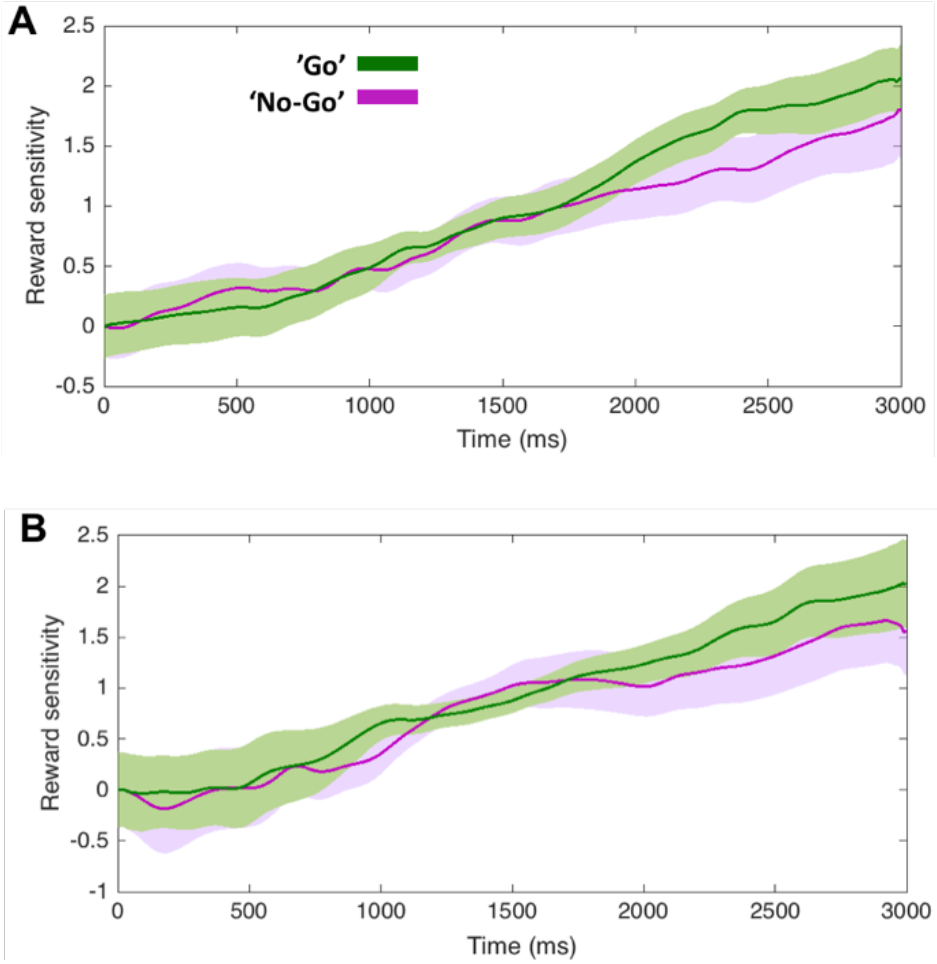

Figure 4.11A-B. PD pupil reward sensitivity during ON and OFF dopamine states.

A. *Average reward sensitivity over time in PD when ON in 'go' trials (green) compared to 'no-go' trials (violet) taken as the difference in the proportional pupil change between the 50p reward condition and the 0p reward. No significant difference was demonstrated between the two conditions over the course of the trial.*

B. *Average reward sensitivity over time in PD when OFF in 'go' trials (green) compared to 'no-go' trials (violet). No significant difference was present between the two conditions either.*

Comparison of reward sensitivity to apathy levels, in the 'go' and the 'no-go' condition produced some interesting results. When ON dopamine, the total LARS score obtained from patients correlated with pupil reward sensitivity. Remarkably however, this was only significant in the 'go' condition, r_s =-0.472, p=0.03. (**Fig, 4.12A** – Blue line). Further, the action initiation sub score of the LARS also only correlated with the 'go' condition, r_s=-0.476, p = 0.025. In the 'no-go' condition, where no initiation of action was needed, there was no significant correlation with the total LARS or the action initiation sub score (r_s=-0.116, p = 0.608 and r_s=-0.029, p = 0.897 respectively). There was also no significant correlation with either the 'go' or the 'no-go' pupil response in PD ON with other clinical assessments including

depression or cognitive function as measured by the BDI-II and the MoCA. When OFF dopamine these effects were abolished, LARS and 'go' pupil reward sensitivity no longer showed a significant correlation (r_s=-0.091, p = 0.686), (**Fig, 4.12B**). Again, there was also no correlation with cognitive function or depression when OFF dopamine.

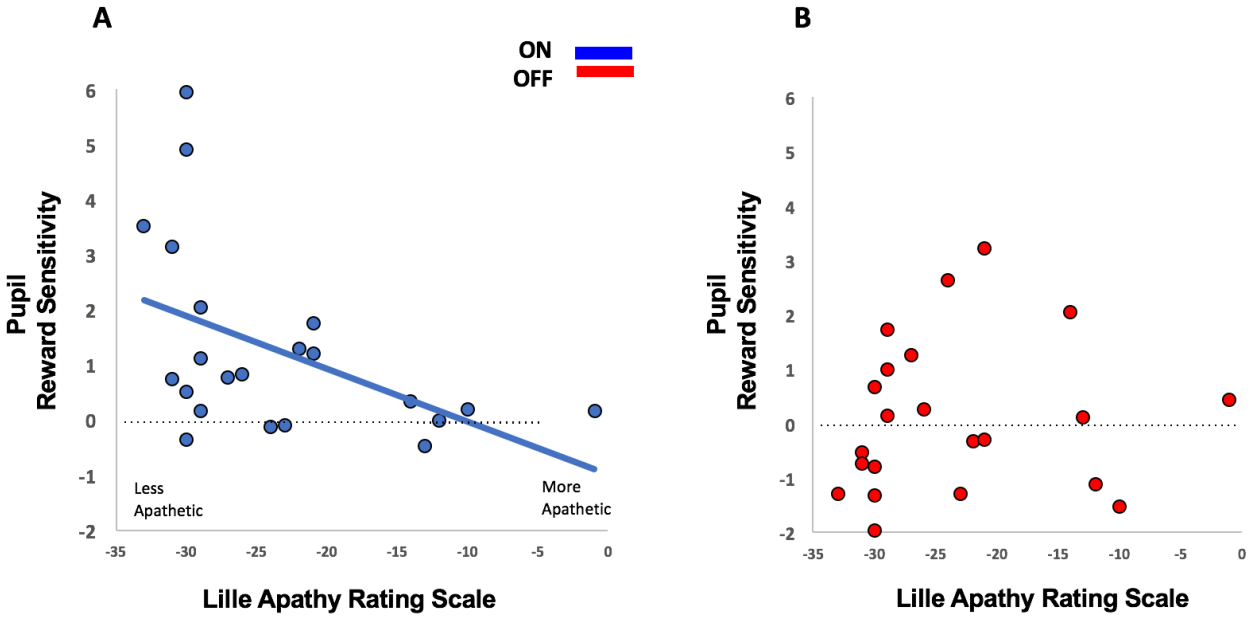

Figure 4.12A-B. Correlation with clinical apathy questionnaire scores (Lille Apathy Rating Scale) and pupil reward sensitivity in PD ON and OFF dopamine.

- ***A.*** *A significant correlation between average pupil reward sensitivity in Parkinson's disease patients ON (blue) and their clinical interview LARS score was present only in the 'go' trials. More apathetic patients exhibit less pupillary reward sensitivity compared to more motivated patients. No correlation was demonstrated in 'no-go' trials.*
- ***B.*** *The correlation between average pupil reward sensitivity in Parkinson's disease during 'go' trials and LARS score was abolished when OFF dopamine (red).*

4.3.5 Parkinson's disease versus elderly controls

4.3.5.1 Saccadic peak velocity

Performing a repeated measures ANOVA on the peak velocities for the 3 reward levels and a between groups comparison between elderly and PD patients ON revealed a main effect of reward ($F(2, 76) = 12.7$, $P < 0.0001$), and interestingly a trend towards a group by reward interaction ($F(2, 76) = 2.9$, $P = 0.06$). Exploring this trend suggests that PD patients ON dopamine have a greater response to reward in terms of saccadic peak velocity (steeper slope) compared to elderly controls. Although this did not reach statistical significance, potential explanations could include hyper-responsiveness to reward from being on dopamine therapy but interpretation needs to be cautious. (**Fig. 4.13A** – blue and black lines).

PD patients OFF compared to elderly controls also demonstrated a main effect of reward ($F(2, 76) = 11.5$, $P < 0.0001$), and a less strong trend towards a group by reward interaction ($F(2, 76) = 2.5$, $P = 0.091$), (**Fig. 4.13A** – red and black lines). There was no main effect of group between elderly controls and PD patients both ON or OFF.

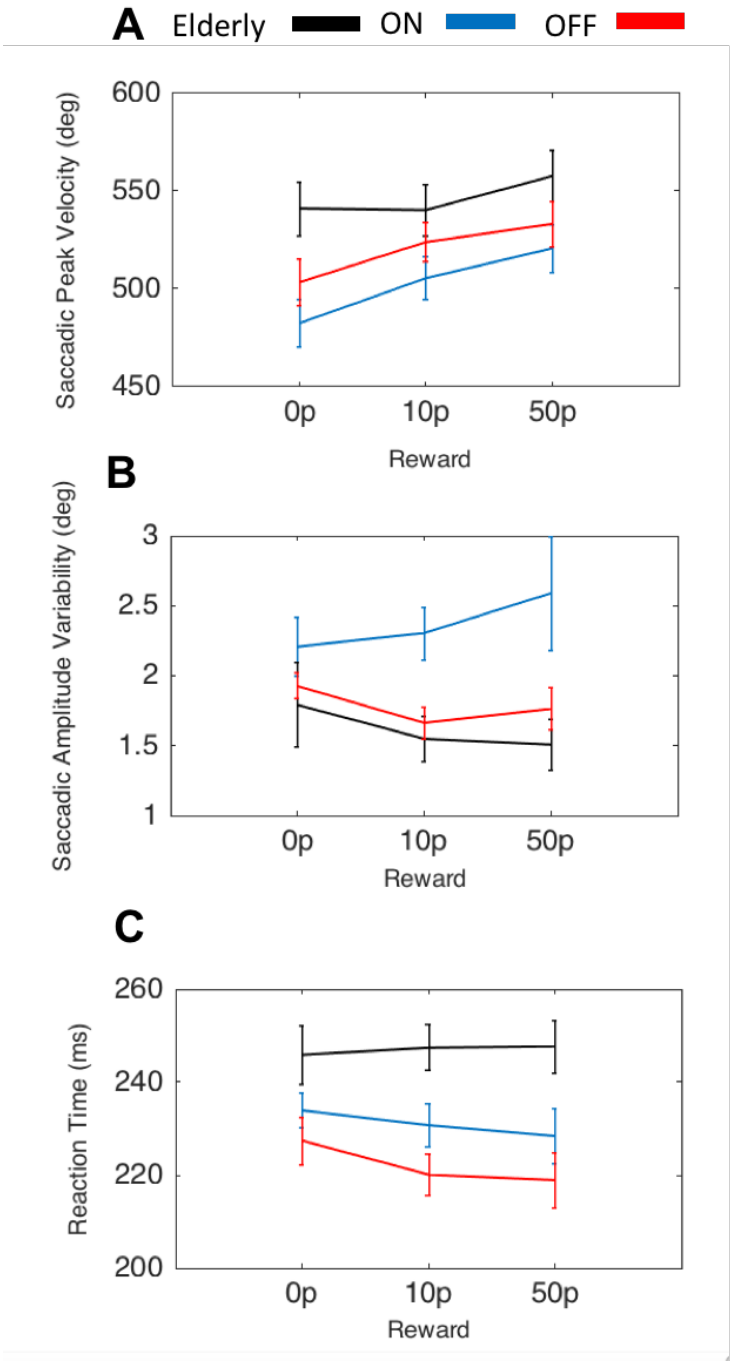

Figure 4.13A-C. Oculomotor properties in PD patients ON and OFF dopamine and elderly controls.

- ***A.*** *Faster saccades for larger rewards were present in PD ON, PD OFF and elderly controls, no significant group differences were present. A trend towards a steeper slope in PD patients ON dopamine compared to elderly controls was observed, p=0.06.*
- ***B.*** *PD patients ON had increased saccadic variability overall compared to elderly controls. When OFF dopamine there were no differences in saccadic accuracy between patients and elderly controls, although a trend towards more accurate saccades was seen in elderly participants and PD patients when OFF, p=0.08.*
- ***C.*** *No differences were present between PD patients ON dopamine and elderly controls in saccadic reaction time. When OFF dopamine, PD patients had faster reaction times in general compared to elderly controls, however no significant effect of reward was present.*

4.3.5.2 Saccadic accuracy

No main effects of reward or any interaction between elderly controls and PD patients when ON dopamine were present. There was however a worse overall effect of accuracy in PD patients ON dopamine with a main effect of group present ($F(1, 38) = 4.6$, $P = 0.038$), (**Fig. 4.13B**). This was abolished when OFF dopamine, with no main effect of group surviving comparison and no interaction present. A weak trend towards a significant main effect of reward ($F(2, 76) = 2.6$, $P = 0.08$) was seen when comparing PD OFF and elderly controls, with a trend towards more accurate and less variable saccades for larger rewards on offer.

4.3.5.3 Saccadic Reaction Time

There were no main effects of group, reward or any significant interaction when ON dopamine compared to elderly controls, (**Fig. 4.13C**). Perhaps surprisingly, when OFF dopamine, reaction times in PD were significantly faster in general compared to elderly controls with a main effect of group being present ($F(1, 38) = 4.5$, $P = 0.04$). There were no main effects of reward and no group by reward interaction between elderly controls and PD OFF.

4.3.5.4 Pupillary response to rewards is present in 'go' and 'no-go' trials

Pupil comparisons were made with PD patients ON dopamine, and elderly controls. Comparing the mean pupil change between the time period of interest (1400ms to 2400ms) in the 'go' condition between elderly controls and PD patients ON showed a main effect of reward only ($F(2, 76) = 7.0$, $P = 0.002$), (**Fig. 4.14A**). There were no main effects of group or interactions. Similarly, the 'no-go' condition comparison between PD ON and elderly controls demonstrated a main effect of reward only ($F(2, 76) = 16.1$, $P < 0.00001$) but no other significant differences, (**Fig. 4.14B**).

Pupil comparisons in PD patients when OFF dopamine and in the elderly also revealed a main effect of reward in both the 'go' (**Fig. 4.14A**) and the 'no-go' conditions (**Fig. 4.14B**),

(F(2, 76) = 8.6, *P* < 0.001) and (F(2, 76) = 3.9, *P* = 0.025) respectively. There were no other main effects or interactions.

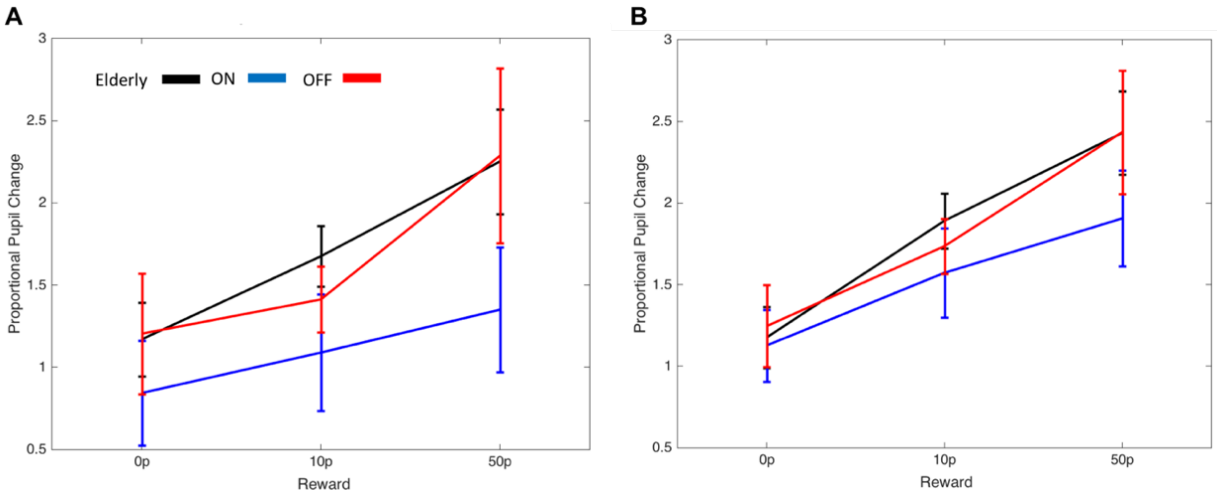

Figure 4.14A-B. PD and elderly pupil response to reward in the ON and OFF state during 'go' and 'no-go' trials.

A. *Using the average over the time epoch of interest in pupillary response (1400ms to 2400ms) all groups displayed an increase in pupil size for increasing reward level in 'go' trials. There were no differences in the average pupil change between ON (blue), OFF (red) or elderly controls (black).*

B. *No differences in the average pupil change between ON, OFF or elderly controls in 'no-go' trials were present either, however all groups demonstrated increases in pupil size for larger reward levels.*

4.4 Discussion

This study demonstrates that pupil responses to incentives increase both when initiation of an action is needed to obtain a reward ('go' trials) and when no action is necessary ('no-go' trials) (**Fig. 4.4A**). Furthermore, there were no robust associations with reaction time or saccadic peak velocity and subsequent pupil responses in our task. This adds to the evidence that pupillary response to reward reflects evaluation of cued incentives rather than motor preparation. As in the previous studies, pupil reward sensitivity also reduced with age (**Fig. 4.8**). Following on from findings in chapter 3, the link between pupillary reward sensitivity and clinical apathy in PD was replicated. Most notably however, the association with apathy in PD patients was only demonstrated in 'go' trials while ON dopamine (**Fig. 4.12**). This suggests that pupillary responses to reward may reflect the transition of incentive evaluation to initiation of action.

The links between initiation of actions and reward sensitivity is of particular interest in the context of apathy in PD. Patients with severe behavioural apathy, otherwise termed auto-activation deficit, have a significant reduction in goal directed behaviours (Muhammed and Husain, 2016), often describing a lack of drive to initiate and carry out tasks (Adam *et al.*, 2013). This study demonstrates that the relationship between the valuation of rewards and initiation of action is vital in this motivational process. Pupil dilation for reward cues in this study were only linked with apathy level when they are associated with an upcoming action (**Fig. 4.12**). This fits in well with work in rodents, where phasic dopamine spikes in the ventral striatum (NAcc specifically) only occur when motor action is also needed to receive a reward (Syed *et al.*, 2015). Therefore, there is substantial overlap between motivation and motor control (Salamone, 2010) with the NAcc potentially being the gateway between motivation and motoric action (Groenewegen, Wright and Beijer, 1996). In apathetic individuals, this connection may be dysfunctional shifting the balance between evaluation of rewards (reward insensitivity) and the motor output initiated in order to obtain them (Chong *et al.*, 2015). Much like cost benefit tradeoffs are narrowed for effort and reward in animal studies where dopamine is depleted in mesolimbic circuits and the NAcc (Walton *et al.*, 2006). In our study, when PD patients were dopamine deplete during the OFF state, the associations between reward sensitivity and apathy were abolished, possibly due to reduction of available dopamine within the striatum, impairing the link between limbic pathways involved in

motivation and motor pathways (Salamone and Correa, 2012). This suggests further that dopamine is the likely neurotransmitter involved in this motivation-motor process and possibly impaired in apathy (Salamone and Correa, 2012; Chong *et al.*, 2015; Salamone *et al.*, 2016).

Dopamine did not seem to change the pupil response to rewards when a saccade was made versus when it was not, demonstrating no link with motor preparation in the pupil (**Fig. 4.10**). In fact, irrespective of drug state, pupil responses, both when initiation of an action was needed to obtain a reward and when no action was necessary, increased with larger reward magnitudes. Although no statistical difference emerged between pupil responses in the 'go' and 'no-go' trials when ON or OFF dopamine, enough of a difference between drug states did take place to alter the association with apathy, which seems to be dopamine dependent (**Fig. 4.12**). No associations between pupil dilation and saccadic velocity or RT was present in PD regardless of dopamine state. In young controls, there was a link between velocity and pupil dilation in the 0p condition only. This is likely to have been a spurious result, as no other associations were found in any of the other groups tested. These results strengthen our findings that pupil response to available reward prior to the onset of an eye movement is not linked to saccadic velocity or action onset latency in this task. Reiterating that pupil dilation for rewards are linked to the evaluation of incentives and not motor preparation. Other oculomotor properties including saccadic velocity, reaction time and accuracy were not related to apathy, but as in the previous studies from chapters 2 and 3, rewards invigorated saccades while not impairing accuracy. Again, breaking speed accuracy tradeoffs and reproducing findings from others in the literature (Chen *et al.*, 2014; Manohar *et al.*, 2015, 2017; Muhammed *et al.*, 2016).

Due to the older age group of elderly participants in this study and the heterogeneous nature of PD, the distribution of scores varied from previous studies. Despite these differences, the overall effects of reward on pupil and saccadic properties did not change the results considerably in comparison to chapter 3. The main difference was most apparent when looking at the effect of dopamine on saccadic variability and latency – reaction time became slower and accuracy worsened while PD patients were ON dopamine. This worsening in performance is surprising and may reflect increased dopamine neuronal activity in the substantia nigra, which has been shown to impair attention and delay responsivity in rats

(Boekhoudt *et al.*, 2016). The patients on average in this cohort had larger levodopa equivalent doses compared to those from chapter 3. Therefore, this nigrostriatal pathway may be connected to attentional processes and stimulation of this pathway through dopaminergic medication in our study may have impaired behaviour.

A key characteristic of amotivational auto-activation disorders are lack of self-initiated actions (Laplane and Dubois, 2001). Importantly however, is the ability of external prompts to kick start a goal directed behaviour. One important consideration to make in this study and a potential limitation, is the difference in self-initiated versus cued actions. The onset of the targets in our study may be regarded as facilitating cued actions and therefore not representative of self-initiated movement. This makes our comparisons with apathy and reduced reward sensitivity when initiating actions harder to interpret as the actions in the task are not fully made under the participant's self-volition. Although participants are free to make a movement anytime they please after the target onset there is always an element of cuing present. Nevertheless, the association with apathy and cued actions for rewards in this study is still comparable to work that demonstrate striatal dopamine pathways as being important in goal directed behaviour (Syed *et al.*, 2015). This could be tested further by developing a task with non-cued targets to initiate an action and looking for similar comparisons.

The findings from this study add to the results from chapter 2 and 3 and give a more accurate interpretation of the mechanisms underlying apathy. Here we suggest that apathy is associated with a reduction in reward sensitivity but only when paired with the initiation of an action, and this link appears to be dopamine dependent.

5. Reward and Loss Sensitivity in Health and Parkinson's disease

5. Abstract

Dopamine plays a crucial role in reward related processes and goal directed behaviours. However, other forms of incentives, including the loss of wealth may also contribute. It is not fully understood how dopamine modulates oculomotor and pupillary responses to loss compared to reward. In this study, a novel eye tracking task was used to assess these properties. Young, elderly and PD patients ON and OFF dopamine were instructed to make saccades for three conditions: 1) to obtain money, 2) avoid losing money and 3) for a neutral baseline no loss and no win (0p) condition. The magnitude of reward and loss was matched and held constant across all participants while still being performance dependent.

Velocity of saccades was invigorated by *both* reward and loss in healthy people. However in PD patients OFF dopamine there was a blunting of peak velocity for the reward condition only, suggesting that *only* invigoration of motor actions for reward – and not loss – is dopamine dependent. The evaluation of rewards and loss as indexed by pupillary changes to the cues, revealed that pupils responded in the same manner within individuals, regardless of whether the trial was to obtain reward or avoid loss. Combining reward and loss to examine the effect of extrinsic incentivisation on pupillary responses revealed a significant dopamine-dependent effect in PD. Previous work has suggested that proxies for individual baseline dopamine levels in the motor and cognitive domains include UPDRS-III score (motor component of the Unified Parkinson's disease Rating Scale) and digit span respectively. An inverted U-shaped function relating these surrogate markers of baseline dopamine level and pupillary responses to extrinsic incentives might account for the variability observed across participants. Patients with a high level of baseline dopamine (as indexed by UPDRS-III or digit span) experienced reduced sensitivity to extrinsic incentives when ON dopaminergic therapy. By contrast, in patients with low baseline dopamine levels, enhanced sensitivity was observed when ON dopamine therapy. No significant correlation with apathy was found.

5.1 Introduction

Dysfunction of reward pathways in the brain have been associated with difficulties in goal directed behaviour and linked to motivational deficits (Mattox, Valle-Inclán and Hackley, 2006). However, inappropriate responses to punishment may be equally important to behavioural outcomes and might also contribute to clinical conditions including apathy. If patients are overly insensitive to loss, they might also engage less with the environment.

Motivation can be thought of as the performance of actions either to increase the likelihood of an outcome (as in reward sensitivity) or to reduce it if the outcome is unfavourable (loss sensitivity). Therefore reward could be defined as any event that energy is expended for to maximise an outcome and loss as a stimulus that leads to energy expenditure in order to reduce or avoid it (Seymour, Singer and Dolan, 2007).

Rewards and punishments may have differential effects on behaviour. For example, using serial reaction time tasks that require a button press in response to stimuli, Wachter *et al* (2009) demonstrated that punishments, but not rewards, significantly reduced reaction times (Wachter *et al.*, 2009). Learning rate, however, was greater for rewards even when accounting for faster RTs in the punishment group suggesting dissociable processes. Functional imaging in the two groups also revealed different patterns of brain activation. Dorsal striatum, NAcc and prefrontal cortex were associated with reward responses while bilateral insula activity was linked to punishment. In another imaging study, unexpected rewards also led to dorsal striatum responses whereas anterior ventral striatum activation was observed for both punishments and reward (Robinson *et al.*, 2010).

Incentives also influence motor actions. Too slow a response may lead to an opportunity cost of rewards, while too fast a response may require more energy which might not justify the return. Less, however, has been studied in terms of punishment. It is possible that some types of inaction are beneficial to an individual if they result in reduced likelihood of encountering a punishment (Griffiths and Beierholm, 2017). Though, if the action required was to go towards a reward or away from a punishment then invigoration of movement is likely to occur, indeed perhaps more so for punishment as it is considered to be disproportionally more salient compared to reward (Halbesleben *et al.*, 2014). These factors may have implications for PD, including in the understanding of the motor phenomenon

'Kinesia paradoxa' – the energisation of motor abilities linked to various types of external cues, more often those that pose an immediate threat (Schlesinger, Erikh and Yarnitsky, 2007). Thus, PD patients can run faster when there is an imminent danger such as fire for example.

Reward and loss also have independent effects on motor learning and retention. Using an arm movement motor adaptation paradigm, Galea *et al* (2015) showed that monetary punishments increased motor learning rates, provided outcomes were contingent on performance (Galea *et al.*, 2015). However, when performance feedback was removed there was a faster decay of motor memory for punishment, while rewards produced improved retention of learned motor adaptation. These findings again demonstrate the dissociable effects on behaviour between these two different types of incentive.

Physiological differences between reward and loss have been demonstrated in studies that have examined heart rate and skin conductance responses. One such investigation measured each of these autonomic variables in relation to cues that signalled potential monetary rewards or losses (Löw *et al.*, 2008). Three pictorial cue types, either neutral (e.g., a clock), rewarding (dollar bills) or aversive (a gun) appeared to progressively move closer to the viewer on each trial, while a change in background indicated a rapid key response was needed to either gain a reward or avoid a loss depending on the cue type. Skin conductance increased for both aversive and rewarding stimuli compared to the neutral condition and this became more prominent as the images moved closer to the viewer. Heart rate also initially accelerated equally for both incentive cue types compared to the neutral stimuli but a subsequent deceleration was later observed. The drop in heart rate was greater for loss than reward and was explained by the authors as 'fear bradycardia', again differentiating the autonomic effects between incentive types (Campbell, Wood and McBride, 1997). This autonomic response is also demonstrated in pupil modulation when anticipating rewards and punishment. When outcome is contingent on performance, pupil size on expectation of reward and loss leads to significant dilation but not when outcomes are random, suggesting that pupil autonomic arousal is performance dependent (Manohar *et al.*, 2017).

The interpretation of behavioural responses to incentives is an important factor in understanding potential brain mechanisms involved in the process. Responses to the

expectation of rewards and aversive outcomes may represent either the *value* of an outcome or instead may be related to its *salience*. For example, two stimuli may be equally salient, like an odor, however one may be associated with negative consequences such as the smell of a predator, while another linked with more appetitive consequences like the smell of food and therefore a positive value (Bissonette *et al.*, 2014). Differentiating (signed) value from (unsigned) salience is important to understand which populations of neurons encode expectation of outcomes.

If neurons encode value, rewarding stimuli should lead to the greatest activity, followed by neutral and then aversive stimuli the least. If however activity is influenced by the salience of rewarding and aversive stimuli then the direction of this activation should be the same for both when compared to a neutral stimuli (Bissonette *et al.*, 2014). A study by Cooper *et al* (2008) attempted to explore this difference using fMRI. They found BOLD responses in the NAcc were *equally* increased for both reward and loss only when outcome was linked to performance (indicative of salience encoding). Whereas when outcome was independent of performance, a graded pattern of activation was present, with least activation for loss, followed by the neutral condition and greatest activation for reward, suggestive of a valence response (Cooper and Knutson, 2008). Therefore, the context of the incentive is important, with motivational salience appearing to be performance dependent.

Dopamine influences how we behave towards extrinsic incentives (Aarts, van Holstein and Cools, 2011) and is important in reward related processes and motivation (Powell *et al.*, 1996; Adam *et al.*, 2013). However, there are diverse results from studies exploring its role related to both reward and punishment (Frank and O'Reilly, 2006). One suggestion for the differences between studies is that dopamine neurons support both motivational value (differentiating rewarding from aversive outcomes) and also motivational salience (exhibiting equal responses for reward and loss compared to motivationally neutral stimuli) (Bissonette *et al.*, 2014). This ability to encode both types of motivational response may be explained by differences in dopamine neurons themselves.

Midbrain dopamine neurons are known to respond to rewards. However, there has been increasing evidence that they also signal non-rewarding stimuli, which are equally salient in nature, such as aversive events. The work of Hikosaka and his colleagues has led to a new

hypothesis to explain multiple dopamine neuron types (Bromberg-Martin, Matsumoto and Hikosaka, 2010). Their findings support the existence of a distinct subgroup of dopamine neurons that encode motivational *value*, while another population encode motivational *salience*. The value encoding neurons are associated with evaluation and goal seeking behaviours and exhibit excitation by reward and inhibition by loss. By contrast, salience related dopamine neurons are activated for motivational drive and display the *same* direction of response for reward and loss. These distinct groups of dopamine neurons are excited or inhibited depending on the nature of the incentive. One study by Matsumoto *et al* (2009) revealed two populations of dopamine midbrain neurons in monkeys (Matsumoto and Hikosaka, 2009). One group of neurons was excited by rewards (fruit juice) while also being inhibited by aversive air puffs, while a second population displayed excitation for *both* rewards and punishment equally. Thus, different populations of dopamine neurons within the midbrain can encode both motivational value and salience and may explain the mixed findings in the literature regarding behavioural and brain responses to rewards and punishment.

Clinically, there is a large variability in how PD patients respond ON versus OFF dopamine therapies on different cognitive tasks. This may be due to different optimum dopamine levels needed for each activity, with the optimum varying between brain regions, tasks and the baseline dopamine level of an individual (Cools and D'Esposito, 2011). Previous findings in the literature, suggests that working memory capacity can reflect baseline dopamine levels, specifically in its ability to predict dopamine synthesis in the striatum (Cools *et al.*, 2008). Likewise, the level of the UPDRS-III motor score can index severity of disease burden in patients with Parkinson's disease (Martínez-Martín *et al.*, 2015) which is likely to reflect the extent of dopamine neuronal loss (Greffard *et al.*, 2006), and also indicative of baseline dopamine. In patients with PD, these proxies for dopamine level are likely to be important factors when trying to explore behavioural and physiological responses to reward and loss and their interplay with dopamine therapy.

How loss and reward modulate oculomotor properties may help distinguish differences and similarities in behavioural and physiological responses to incentives, shedding light on common or separate neural pathways. PD is a potentially useful model of dopamine depletion which can increase our understanding of the neurotransmitters involved in goal directed behaviour, giving us further insight into potential mechanisms when this behaviour

becomes dysfunctional in conditions like apathy. Having demonstrated the association between rewards and various oculomotor properties in previous chapters, the question arises if this is exclusive to predicted rewards or generally associated with how attractive *or* how aversive an incentive is. Therefore, valence was deconstructed into reward (win) and punishment (loss) and the effects of anticipated outcomes were assessed on a novel oculomotor task. The motor output and pupil response associated with *minimising loss* versus *maximizing reward* was studied in groups of young and elderly healthy participants, as well as a cohort of patients with idiopathic PD tested both ON and OFF their dopaminergic mediation.

5.2 Methods

5.2.1 Participants

Young, elderly and patients with idiopathic PD were recruited from the same sources as chapter 4, however the individuals tested were a new group of participants. All were screened for any significant comorbidities.

5.2.2 Young and Elderly control demographics

Twenty young healthy adults with no history of neurological or psychiatric history were tested, average age 25.1 years (SD $\pm$ 5.2 years), 18 right handed and 10 male.
Twenty-three elderly age-matched participants were also tested and comparisons made to the young group and those with idiopathic PD, their demographics are expanded further in **Table 5.1**.

5.2.3 Parkinson's disease demographics

Twenty-six patients with idiopathic PD were assessed in two counterbalanced sessions, once ON their normal dopaminergic medication and once OFF after an overnight fast from their medication. Apathy was assessed using the LARS, cognitive function by the MoCA and motor function was assessed using the UPDRS-III. Further demographic details are found in **Table 5.1**.

	Healthy Young Controls	Healthy Elderly Controls	Parkinson's Disease (Medicated)	Controls vs Parkinson's Disease p-value
n	20	20	26	
Age (years)	25.1(±5.2)	65.9 (±6.4)	66.2 (±5.4)	0.9
Apathy (LARS)	n/a	-25.9 (±4.3)	-22.3 (±8.1)	0.08
Depression Score (BDI)	n/a	3.7 (±3.9)	7.3 (±6.3)	0.03*
MoCA	n/a	28.9 (±1.5)	27.9 (±1.8)	0.05
UPDRS I	n/a	n/a	6.4 (±3.7)	n/a
UPDRS III ON	n/a	n/a	26.4 (±11.6)	n/a
Hoehn & Yahr Stage	n/a	n/a	1.6 (±0.5)	n/a
Hours since last dose ON vs OFF	n/a	n/a	3 (±1.4) vs 15.4 (±5.5)	n/a
Levodopa equivalent dose (mg/24 hours)	n/a	n/a	845.5 (±139.9)	n/a

Table 5.1. Demographics of healthy controls and total PD patients.

*Numbers in brackets represent standard deviations and standard error of the mean where appropriate. * Indicates a significant result. LARS = Lille Apathy Rating Scale; MoCA = Montreal Cognitive Assessment; BDI = Beck Depression Inventory II. UPDRS = Unified Parkinson's disease rating scale. 9 PD patients and 5 controls scored worse than the -22 apathy threshold on the LARS (therefore regarded as apathetic).*

5.2.4 Experimental paradigm

An infrared eye tracker (Eyelink 1000, SR Research) was used to monitor pupil diameter and eye position. Participants were instructed that the task involved making quick eye movements, the faster they looked at a target, the greater the proportion of incentive on offer they would either obtain or not lose depending on the trial type. Three trial types (reward, loss and a neutral 0p condition) were randomly performed in equal proportion throughout the experiment. All trials commenced with the onset of a disc at screen centre.

After participants had fixated this for 500ms, they heard a recorded voice that informed them of the trial type and the maximum reward available for that particular trial, there were three different trial conditions: Either 'Win 50p maximum', 'Lose 50p maximum' and the neutral ‘0p maximum’. Subsequently, after a randomly variable period of 1700, 1800 or 1900ms the central fixation disc disappeared and concurrently a new target disc appeared randomly either to the left or right of the screen. The targets measured 4° in diameter and appeared at 11° eccentricity, (**Fig. 5.2A**).

In the *"Win" condition*, participants received a proportion of the maximum reward on offer dependent on performance. In the *"Loss" condition* the amount of money lost was also dependent on performance: the faster the reaction time *the less money was lost* in the trial. The absolute amount of reward earned varied with reaction time, but crucially was dynamically adjusted using the average distribution of reaction times based on the preceding 20 trials. This allowed difficulty level to be maintained consistently over the experiment and factored in different individual fatigue rates and baseline reaction times and importantly provided *equal reward and loss amounts to all participants*. Therefore, unbeknown to the participant, the cumulative total earned at the end of the entire experiment was always 0p, (**Fig. 5.2B**).

To ensure participants were equally extrinsically motivated by the amount of reward they earned or lost, feedback was displayed within the target disc in pence at the end of each trial. Disc luminance was matched across all trials so as not to affect pupil dilation. In ‘win’ trials, if the reward obtained was 30p or greater, an audible 'cash register' sound was played after reward delivery at the end of the trial; in 'loss' trials if the amount lost was more than 30p and audible beep was played; no sound was heard for the neutral ‘0p maximum’ condition.

Young participants performed eight blocks of 54 trials each, with a 5-minute break between the first four and last four blocks, resulting in 432 trials in total with 144 trials for each condition. Behavioural indicators of task performance included saccadic velocity and saccadic variability. Elderly participants and patients with Parkinson’s disease performed five blocks of 54 trials each, with a 5-minute break between the first three and last two blocks.

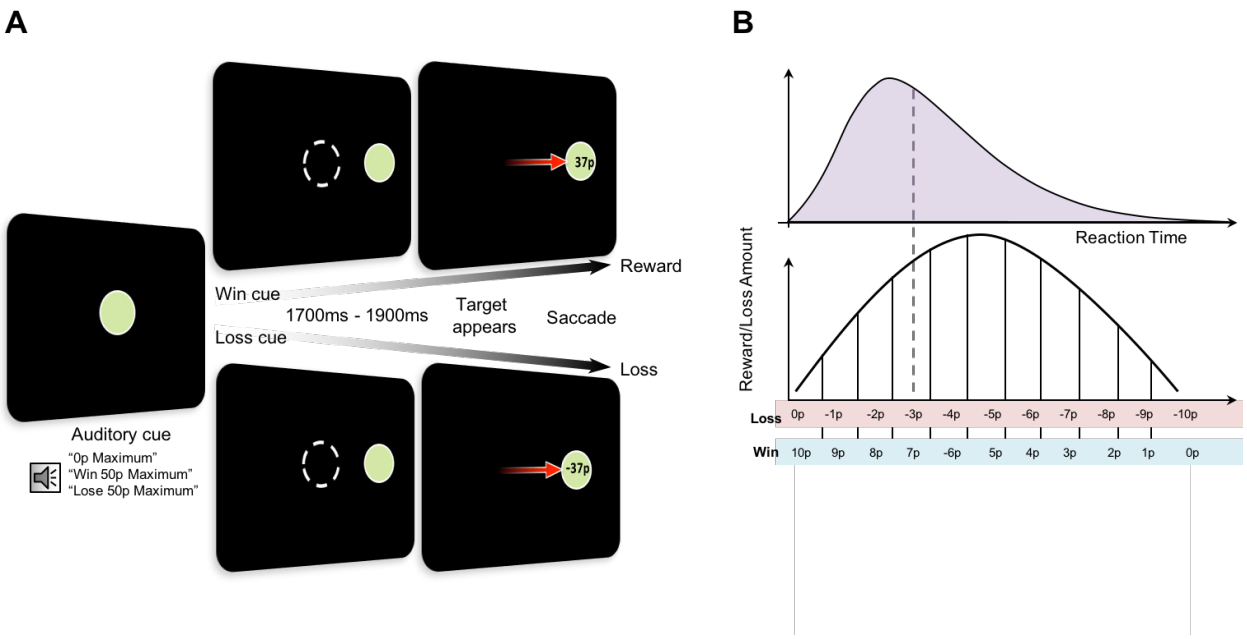

Figure 5.2A-B. The experimental paradigm.

A. *Participants were presented with one of three cues in each trial, 'win 50p maximum', 'lose 50p maximum' or '0p maximum'. They were instructed that the faster they moved their eyes to a target that appeared the more money they would obtain in 'win' trials, the less money they would lose in 'loss' trials and for the 'neutral' trials they would always receive 0p regardless of their reaction time. Performance feedback was provided at the end of each trial.*

B. *The amount won and lost was dependent on the normal distribution of the preceding 20 trials, this allowed the rate and total amount of reward and loss to be matched across the whole experiment. At the end of each experiment the total cumulative amount obtained was 0p, this however was not known to participants.*

5.2.5 Eye tracking recording and analysis

Eye tracking procedures were the same as in chapter 2, 3 and 4. Pupillometry analysis was performed using a 2x3 repeated measures ANOVA, with within-subjects factors of Drug (ON, OFF) and Valence (neutral, win, loss). This was also examined collapsed across win and loss conditions, to provide one set of data referred to as 'extrinsic incentivisation', and compared to the baseline (0p neutral condition). In this comparison a 2x2 repeated measures ANOVA was performed.

Pupillary modulation by reward or loss was taken as mean proportional change in pupil size from baseline pupil diameter at the beginning of each trial time, locked to the onset of the auditory cue. Mean pupillary size from 1700 – 2500ms after the auditory cue was used as the epoch of interest. This was delayed by 300ms compared to our previous studies as the target onsets in this task were also shifted 300ms to 1700ms, 1800ms and 1900ms. This change in foreperiod was to allow more time to measure pupil anticipatory responses following the auditory cue in each trial. An individual's *pupillary reward or loss sensitivity* was defined as the difference in mean proportional pupil change between the maximal 50p (win or loss) condition and the neutral 0p condition, over the time epoch defined above. A larger difference indicated greater reward or loss sensitivity.

5.3 Results

5.3.1 Young controls

5.3.1.1 Saccadic peak velocity

Conditions were divided into valence (0p, *win* 50p and *lose* 50p). There was a main effect of valence in saccadic peak velocity ($F(2, 38) = 18.55$, $P < 0.00001$). Bonferroni corrected pairwise comparisons showed that this was driven by significant differences between the 0p condition and 50p reward (p=0.001) and 0p and 50p loss (p=0.0001) but no difference in peak velocity between reward and loss (p=0.12; **Fig. 5.3A**).

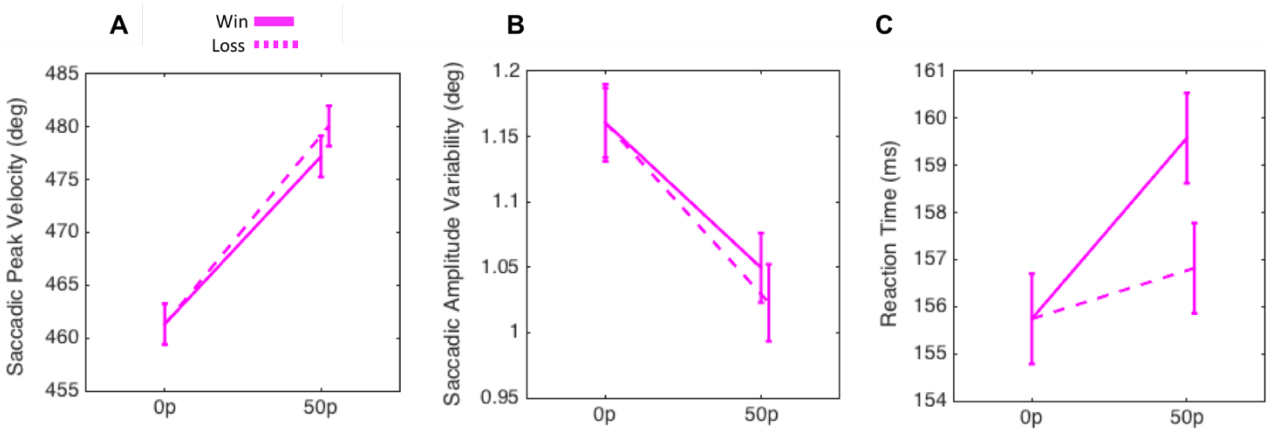

Figure 5.3A-C, Young Participants saccadic velocity, accuracy and reaction times.

A. *Saccadic peak velocity in young participants increased for win and loss 50p conditions compared to 0p. There were no differences in speed however, between win and loss trials.*

B. *Saccadic variability in young participants improved by the same amount for win and loss conditions compared to the neutral 0p outcome.*

C. *Saccadic reaction time in young participants was trending towards slower responses in the win and loss conditions compared to the neutral 0p cue, this was driven by slower latencies in the win trials however none of the overall effects reached statistical significance.*

5.3.1.2 Saccadic accuracy

A main effect of valence on saccadic accuracy – indexed by standard deviation of saccade amplitude – was observed, with general improvement of accuracy with incentive (F(2,

38) = 4.25, *P* = 0.02). Pairwise comparisons show a near significant difference between the 0p condition and 50p reward (p=0.051) whereas a significant difference was seen between 0p and 50p loss (p=0.031). There was no difference in accuracy between reward and loss however (p=0.45; **Fig. 5.3B**).

5.3.1.3 Saccadic Reaction Time

There was no significant main effect of reaction time in young controls, however a trend towards a difference was seen with slower RT for incentives compared to 0p ($F(2, 38) = 2.76$, $P = 0.076$). This was driven by a significantly slower RT for win compared to loss (p=0.018) and a trend towards slower RT for reward compared to the 0p baseline (p=0.06; **Fig. 5.3C**).

5.3.1.4 Pupil response to reward and loss in the young

The time epoch between 1700ms to 2500ms (highlighted by dashed area in **Fig. 5.4**), was used to calculate the average pupil change. It was apparent that over the time course from cue onset at 0ms, win and loss conditions caused greater pupil dilation compared to the neutral 0p condition. This average change over the epoch of interest is shown in **Fig. 5.5A.** Comparisons were performed on this epoch for each condition. There was a main effect of valence in pupillary response $F(2, 38) = 10.1$, $P < 0.001$. Pairwise comparisons revealed that this was driven by significant differences between the 0p condition and win (p=0.008) and 0p and loss (p=0.002) and a trend towards a difference between reward and loss (p=0.06). (**Fig. 5.5A**). Therefore, the smallest pupil dilation was detected for anticipation of 0p, followed by win 50p and the most dilation was for loss of 50p.

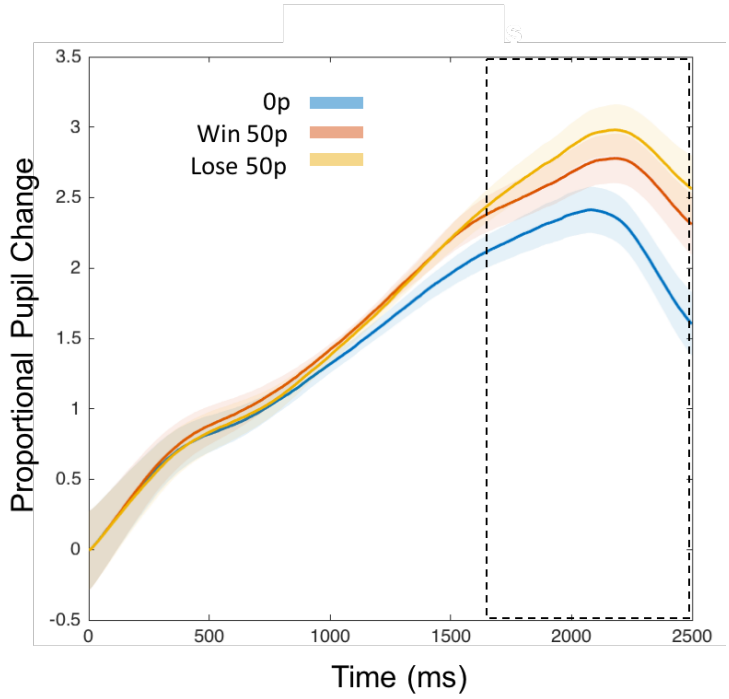

Figure 5.4. Pupil trace in young controls over time.

Pupil change over the course of the trial in response to the 0p, win 50p and lose 50p conditions in young healthy participants. This was calculated as a proportional change from the start of the trial. At time 0ms was the onset of the auditory cue. Pupils dilated in response to all cue types.

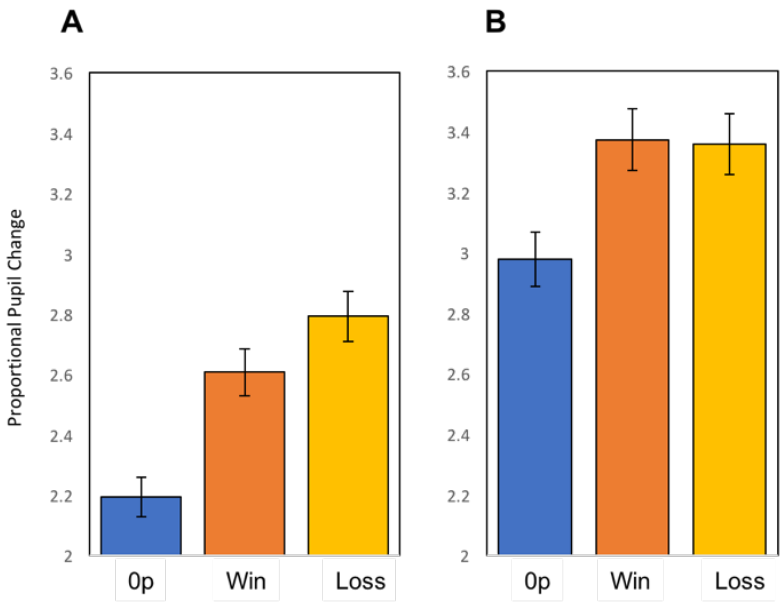

Figure 5.5A-B. Pupillary responses to incentives in young and elderly controls.

A. *Average proportional pupil size change over the epoch of interest (1700ms to 2500ms) in young participants. Significant differences were present between the 0p and win 50p condition (p<0.001), the 0p and lose 50p condition (p<0.01), and a trend towards a larger response in the loss compared to the win condition was also present (p=0.06).*

B. *Average proportional pupil size change over the epoch of interest in elderly participants. Larger pupil responses as a proportion of pupil baseline starting size was apparent in the elderly compared to young. There were significantly larger responses for both win and loss compared to the neutral 0p condition (p<0.05). No differences were present in the elderly between the pupil response for reward and the pupil response to loss, (p=0.85).*

5.3.2 Elderly Controls

5.3.2.1 Saccadic peak velocity

Like in the young control group, there was a main effect of valence in saccadic peak velocity $F(2, 38) = 10.1$, $P < 0.001$. Pairwise comparisons show that this was driven by significant differences between the 0p condition and 50p reward (p=0.01) and 0p and 50p loss (p=0.001) but no difference in peak velocity between reward and loss (p=0.15), (**Fig. 5.6A**).

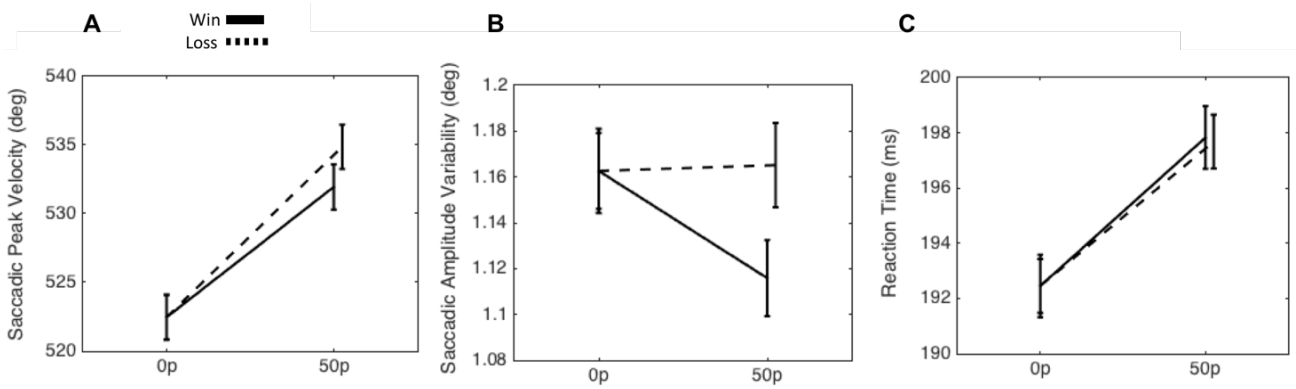

Figure 5.6A-C, Elderly participant saccadic properties.

A. *Saccadic peak velocity in elderly participants increased for win and loss 50p conditions compared to 0p. There were no differences in speed between win and loss trials.*

B. *Saccadic variability in elderly participants did not show any statistically significant differences between 0p conditions and win or loss. The variability of the win trials seems to have improved accuracy compared to loss and 0p, however this was not close to statistical significance despite its appearance when plotted, as the y-axis difference was only 0.04 of a degree.*

C. *Saccadic latency in elderly participants significantly increased for both win and loss compared to 0p. There were no differences in latency between win and loss.*

5.3.2.2 Saccadic accuracy

Unlike young controls, there was no main effect of valence for saccadic accuracy, $F(2, 38) = 1.12$, $P = 0.34$. Winning or losing did not seem to change the variability of saccades made in the elderly despite motor speeds being faster when rewarded or penalized. Although the accuracy for rewards appears to improve when plotted, this was not close to being significantly different from the loss response or the 0p baseline, (**Fig. 5.6B**).

5.3.2.3 Saccadic Reaction Time

In elderly controls, there was a main effect of valence in reaction time $F(2, 38) = 4.65$, $P = 0.02$. With the win 50p and lose 50p condition both having slower RT compared to the 0p neutral condition. Pairwise comparisons showed a slower RT between 50p win and the 0p baseline (p=0.028) and likewise for the 50p loss and 0p condition (p=0.015) but no difference in RT was demonstrated between win and loss (p=0.935), (**Fig. 5.6C**).

5.3.2.4 Pupil response to reward and loss in the elderly

There was a main effect of valence in pupillary responses in the elderly control group ($F(2, 38) = 5.58$, $P = 0.017$). Pairwise comparisons confirmed that this was driven by significant differences between the 0p condition and reward (p=0.031) and 0p and loss (p=0.043) but no difference between reward and loss pupil responses (p=0.85). This was unlike the results in the young controls where a trend was seen to emerge, potentially indicating that the differences in responses of the pupil to reward and loss are reduced with age, (**Fig. 5.5B**).

5.3.3 Young and elderly pupil response comparisons

5.3.3.1 Reward and loss pupil sensitivity

As discussed above, there were no differences in pupil responses to reward or loss in elderly participants, and only a trend towards greater pupil change for loss in the young. Taking the difference between the pupil response to 50p (both in the win and loss condition) and the neutral 0p condition, reward and loss sensitivity can be obtained. Reward sensitivity

(win 50p – 0p) is plotted over time for elderly (black trace) and young participants (violet trace; **Fig. 5.7A**) with no significant differences seen between them. Likewise, loss sensitivity (lose 50p – 0p) plotted over time for young and elderly also did not show any areas of significant difference, (**Fig. 5.7B**). Overall, there were no differences in reward or loss sensitivity *between* or *within* young and elderly participants.

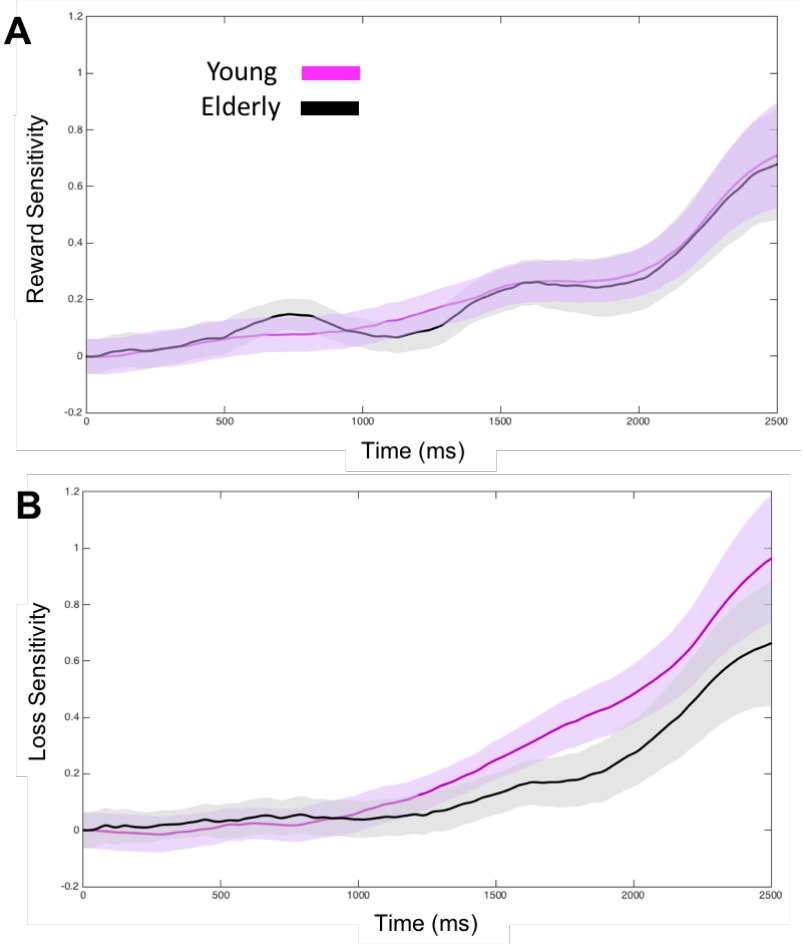

Figure 5.7A-B. Pupil sensitivity to rewards and loss in young and elderly control participants.

A. *Pupil reward sensitivity, taken as the difference between the win 50p and 0p condition plotted over time. Comparisons between reward sensitivity were made in young (violet line) and elderly (black line) controls, no areas of difference were found.*

B. *Pupil loss sensitivity, the difference between the lose 50p and 0p condition plotted over time. Comparisons between loss sensitivity in young (violet line) and elderly (black line) were made, again no areas of significant difference were found.*

5.3.3.2 Baseline Differences

Baseline pupil size, as measured in arbitrary Eyelink units, in young and elderly participants, was significantly different from each other ($p<0.0001$; **Fig 5.8A**). Collapsing across all conditions (reward, loss and neutral) young controls baseline pupil size was on average much larger than elderly control participants. This implies that young controls should have less scope to dilate to the cued conditions as they are already starting from a bigger baseline pupil. This difference is apparent when comparing the overall pupil proportional sizes in the young (**Fig 5.5A**) and in the elderly (**Fig 5.5B**), and explains why on average the maximal proportional dilation of the pupil in the young was less compared to the elderly. Of note, however, in our study we are interested in examining differences in responses between 0p and win/loss conditions and not overall pupil dilation.

5.3.3.3 Magnitude of reward and loss sensitivity by task

No differences emerged between the pupil sensitivities in young and elderly, whereas in previous chapters there was a difference. To investigate the cause for this, a comparison was made between the overall reward sensitivity response in the pupil from young controls in study 2 from chapter 2 *which was a pure reward task* and compare this to the reward sensitivity in the reward/loss task from this chapter. This would allow us to examine whether a difference in the task designs influenced the extent of the reward response elicited in the pupil. A significant reduction was found in the sensitivity to rewards in the reward/loss study compared to the pure reward task from chapter 2 ($p=0.012$; **Fig. 5.8B**). This reduction of reward sensitivity might have been secondary to the inclusion of *loss condition* trials intermixed with reward trials in the current task. This might have dampened the overall physiological response towards rewards.

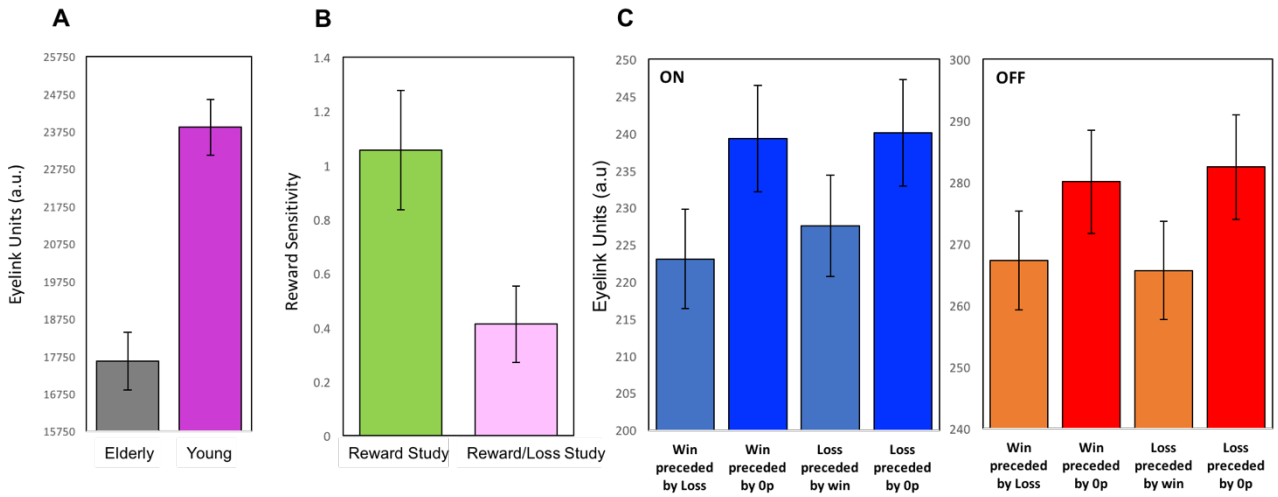

Figure 5.8A-C. Pupil characteristics across groups, studies, and trial to trial effects in PD.

A. *Baseline pupil size difference between young and elderly controls, measured in arbitrary Eyelink units. Young had a significantly larger baseline starting pupil size compared to elderly, which is why the overall amount of proportional changes to the cues was less than the elderly – see* ***Fig.5.5A-B****.*

B. *Comparison between the average pupil reward sensitivity in young controls from chapter 2, study 2 (green) and the reward sensitivity in young participants from the new reward/loss task developed in this chapter (pink). Reward sensitivity was significantly less in the new task. This is likely explained by the addition of the loss condition blunting the magnitude of the reward effects on pupil responses.*

C. *Comparison of preceding trials on win and loss pupil response both ON (blue) and OFF (red) dopamine. A significant reduction in pupil response was seen when a win or loss trial preceded another win or loss trial, this was true both ON and OFF ($p<0.05$) and could explain the reduced sensitivity levels in this task and why no difference was seen between pupil responses to both reward and loss. The inclusion of reward and loss conditions seems to dampen the overall effects of the incentives on the pupil.*

To confirm this dampening effect, the pupil response when ON and OFF dopamine in PD was examined when the preceding trial was either win during a loss trial or loss during a win trial. Results were compared to when the previous trial was the neutral 0p condition. There was a main effect of condition, with significantly smaller pupil responses to win and loss when the preceding trial was also a win or loss compared to when it was a 0p trial ($F(3, 75) = 4.41$, $P = 0.007$; **Fig. 5.8C**). This demonstrates that pupil responsiveness in this study was weaker through the inclusion of the two incentive types, and perhaps why no significant differences between reward and loss in the young and elderly were observed.

5.3.4 Parkinson's disease ON and OFF

5.3.4.1 Saccadic peak velocity

A 2x3 ANOVA was performed on saccadic peak velocity with drug (ON and OFF) and valence (0p baseline, win and loss). There was only a main effect of valence on PD saccadic peak velocity ($F(2, 50) = 3.7$, $P = 0.032$). Bonferroni pairwise comparisons of valence showed this was driven by significant differences between the 0p condition and loss condition (p=0.03) with no difference in peak velocity between 0p and reward (p=0.227) and a trend towards faster saccades for loss compared to reward (p=0.06). Although there was no overall effect of drug state, planned comparisons demonstrated a significant difference between rewards and loss. When OFF dopamine saccades were significantly slower for the 50p win condition versus the 50p loss condition, (t(25)=-2.51, p=0.019). This difference between 50p win and 50p loss was abolished when ON dopamine (t(25)=-0.66, p=0.52) suggesting that invigoration for rewards only and not loss is dopamine dependent (**Fig. 5.9A**).

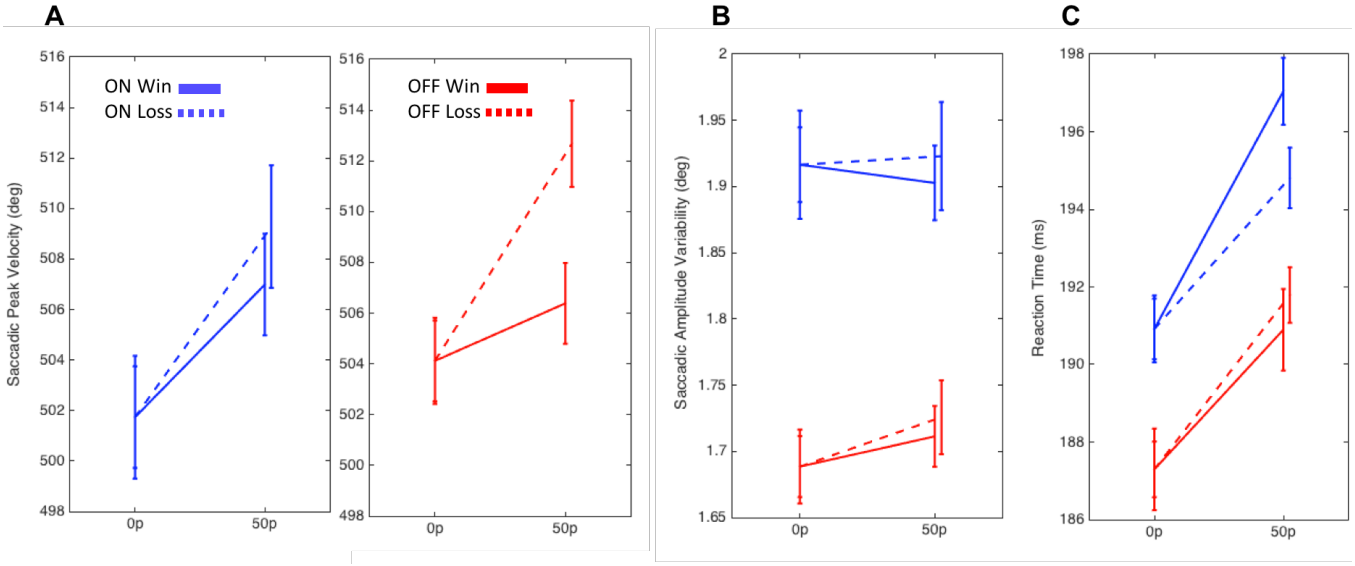

Figure 5.9A-C. Saccadic properties in Parkinson's disease, ON and OFF dopamine.

A. *Saccadic peak velocity in PD ON (blue) and OFF (red) show increases in speed for win and loss. When OFF dopamine, only the speed in the win condition is reduced, suggesting invigoration for rewards is dopamine dependent and loss is not.*

B. *Saccadic variability in PD ON (blue) and OFF (red) was worse when ON dopamine compared to when OFF. There were no differences between the reward, loss and 0p neutral conditions.*

C. *Saccadic latency (RT) in PD ON (blue) and OFF (red) was slower for reward and loss compared to 0p baseline. There were no significant differences in reaction time ON or OFF drug.*

5.3.4.2 Saccadic accuracy

Examination of saccadic accuracy in PD revealed a main effect of drug only ($F(2, 25) = 6.4$, $P = 0.018$), with worse accuracy (increased saccadic amplitude variability) ON dopamine (**Fig. 5.9B**).

5.3.4.3 Saccadic Reaction Time

There was a main effect of valence on RT in PD ($F(2, 50) = 8.6$, $P = 0.001$) with slower RTs for reward and loss conditions compared to 0p baseline. Pairwise comparisons showed this was the case for both reward and loss conditions, with significant differences between the 0p baseline and win ($p=0.004$) and 0p and loss ($p<0.001$) but no difference in RT between the win and loss conditions ($p=0.58$; **Fig. 5.9C**). There were no main effects of being ON or OFF dopaminergic therapy on RT.

5.3.4.4 Pupil reward and loss sensitivity

Analysis of mean pupil proportional change for reward, loss and neutral cues both in the ON and OFF state demonstrated pupil dilation for all stimuli, with greater proportional changes when OFF compared to ON (**Fig. 5.10**). The overall pupillary differences between ON and OFF, with OFF displaying larger pupil responses to the cues, can be explained by differences in the baseline pupil size as measured in Eyelink arbitrary units ($p=0.02$; **Fig. 5.11B**). When OFF dopamine, the pupil size starts off smaller, and therefore has more scope to dilate and produce overall larger proportional changes.

Performing a 2x3 ANOVA on the epoch of interest (1700ms – 2500ms) as indicated by the dashed line in **Fig. 5.10**, with drug and valence as factors, there was a main effect of drug ($F(1, 25) = 6.67$, $P = 0.016$), main effect of valence ($F(2, 50) = 9.77$, $P <0.001$) and a drug by valence interaction ($F(2, 50) = 3.26$, $P = 0.047$; **Fig. 5.11A**). Pairwise comparisons ON and OFF showed a significant increase in pupil response between the 0p condition and win ($p=0.002$) and the 0p and loss ($p=0.001$) but no difference between the win and loss conditions ($p=0.866$).

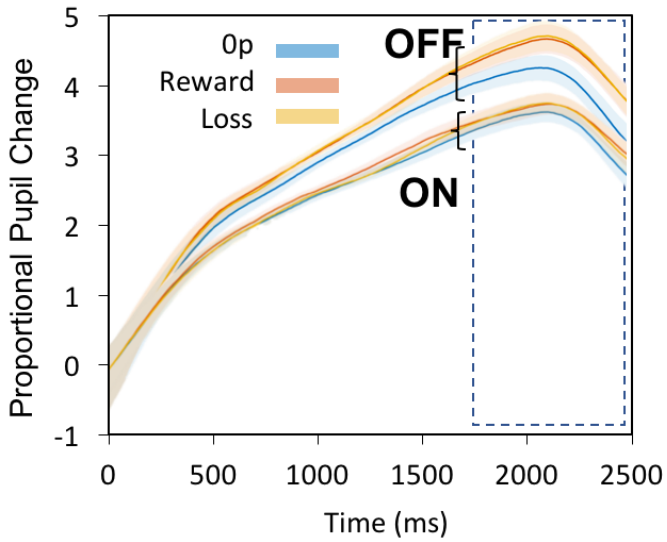

Figure 5.10. Pupil trace over time in Parkinson's disease ON and OFF dopamine.

Pupil change over the course of the trial calculated as proportional change, in response to the 0p, win 50p and lose 50p conditions in patients with PD tested ON dopamine (bottom 3 traces) and OFF dopamine (top 3 traces). Time epoch of interested that is used for subsequent ANOVA analysis is indicated by dashed black area from 1700ms to 2500ms.

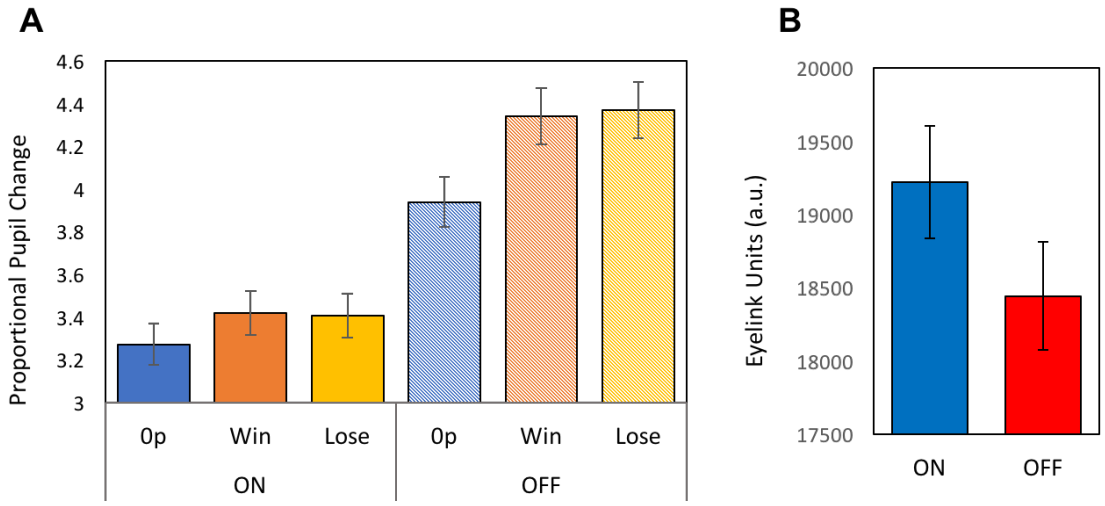

Figure 5.11A-B. Pupil responses to incentives in Parkinson's disease.

A. *Average proportional pupil size change over the epoch of interest (1700ms to 2500ms) in patients with PD, ON dopamine (solid bars) and OFF dopamine (light dashed bars). Significant differences were present between the 0p and win 50p condition and the 0p and lose 50p condition across both drug states. Responses overall were greater when OFF, due to baseline differences in starting pupil size – see* ***Fig. 5.11B.***

B. *Average pupillary differences measured in Eyelink arbitrary units, across all conditions when ON and OFF. Significantly larger baseline pupil size was present with OFF compared to ON (p=0.02).*

The pupil responses were broken down further into reward and loss sensitivity values (0p neutral condition subtracted from 50p win and 0p subtracted from 50p loss). Performing a 2x2 ANOVA on the sensitivities showed a main effect of drug was present ($F(1, 25) = 4.77$, $P = 0.038$), with pupil sensitivities conversely being greater when OFF dopamine. This occurred for both the reward and loss conditions and was demonstrated over the majority of each trial (**Fig. 5.12**). This effect could again be explained by the smaller starting pupil size when OFF (**Fig. 5.11B**) which allows for greater scope in dilation in response to rewards or punishment.

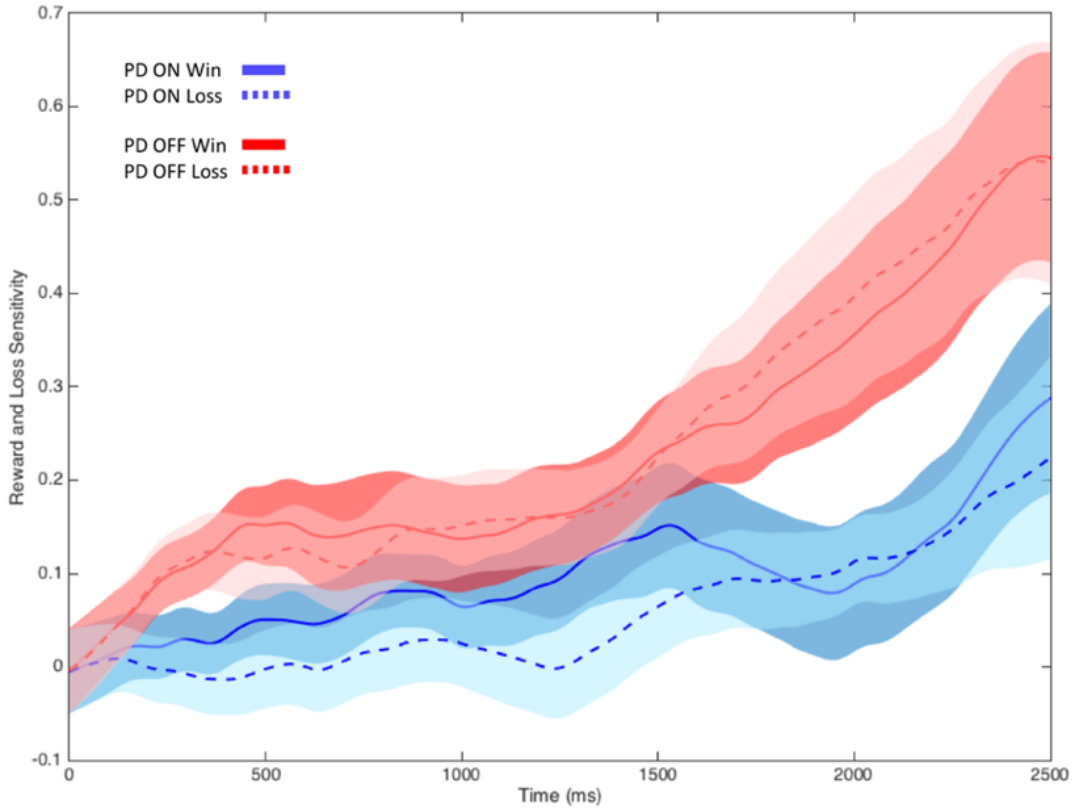

Figure 5.12. Pupil responses to incentives over time in Parkinson's disease

*Pupil reward and loss sensitivity, taken as the difference between the win 50p and 0p condition (solid line) and the difference between the lose 50p and 0p condition (dashed line). This is displayed when ON dopamine (blue traces) and when OFF dopamine (red traces). Pupil sensitives towards both reward and loss where greater in terms of proportional changes, when OFF dopamine. Differences in baseline pupil size (**Fig. 5.11B**) can account for this difference as pupil size OFF starts off smaller and therefore has more scope to dilate for reward and loss.*

Comparison of the effect of drug state on the win and loss conditions, showed dopamine had the same effect on pupil reward and loss sensitivity (**Fig. 5.13**). This is demonstrated by the strong correlation when examining the effect of dopamine on loss (pupil sensitivity to loss when ON minus pupil sensitivity to loss when OFF) against the effect of dopamine on reward (pupil sensitivity to win when ON minus pupil sensitivity to win when

OFF; r=0.72, p<0.0001). The strong correlation means that at an individual level, whatever effect dopamine has on sensitivities to reward, it has the same effect on sensitivities to loss within a person. However, in some individuals there was a dilating effect, while in others it was constrictive in nature.

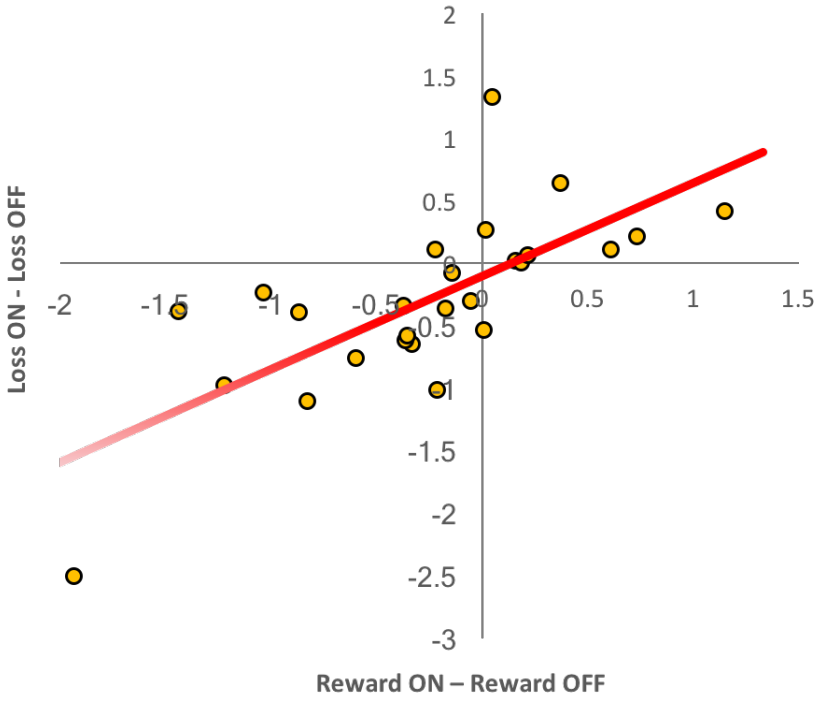

Figure 5.13. Modulatory effect of dopamine on reward and loss sensitivity in Parkinson's disease.

The correlation between the effect of dopamine on pupil loss sensitivity (pupil loss sensitivity in an individual when ON minus OFF) and its effect on pupil reward sensitivity (pupil reward sensitivity in an individual when ON minus OFF). This suggests that at an individual level, whichever direction dopamine modulates the pupil (dilate or constrict) it has the same effect on reward and loss sensitivity. In some individuals, it has a dilating effect on both, and in others it has a constrictive effect.

5.3.4.5 Pupil response to extrinsic incentivisation

As discussed, the effect of reward and loss was the same on pupil responses in all previous analyses. Therefore, it was deemed appropriate to collapse across both types of incentive and compare all extrinsic incentivisation responses (win and loss together) to the 0p baseline. A 2x2 ANOVA demonstrated a significant main effect of drug ($F(1, 25) = 6.22$, $P = 0.02$), a main effect of valence ($F(1, 25) = 14.12$, $P = 0.001$) and a drug by valence interaction ($F(1, 25) = 4.77$, $P = 0.038$), (**Fig. 5.14**). There was a significant difference between baseline and extrinsic incentives when ON (p=0.03) and this difference was increased further when OFF

dopamine (p=0.002). The deconstruction of the interaction is expanded on further in the next section.

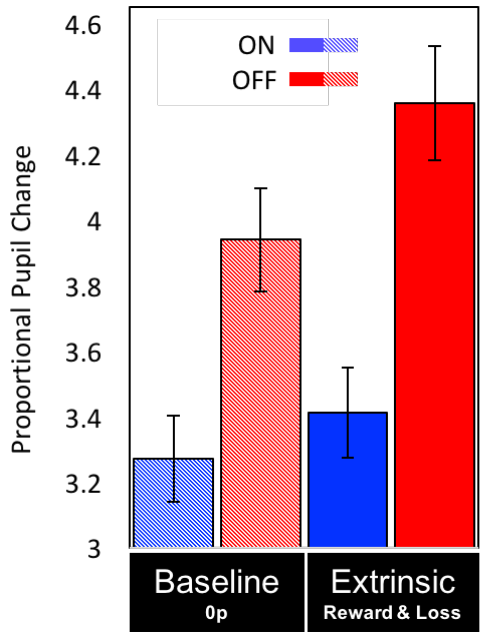

Figure 5.14. Pupil response to baseline neutral condition versus extrinsic incentivisation in Parkinson's disease, ON and OFF dopamine.

When reward and loss sensitivity are combined (extrinsic incentives) there is a significant difference between this and the 0p baseline response. This is true when ON and OFF dopamine. The overall size of proportional change appears greatest when OFF, again due to the differences in baseline pupil size between ON and OFF as discussed previously.

5.3.4.6 Baseline UPDRS-III and extrinsic incentivisation

Differences in pupil responses to extrinsic incentives may be influenced by the amount of *'baseline dopamine'* available to individuals. UPDRS-III OFF, which indicates motor severity in PD, may reflect this baseline dopamine level. It was therefore used as a co-variate in the analysis to investigate whether this influenced the pupil sensitivity results. A 2x2 ANOVA of pupil response to valence (0p baseline and extrinsic incentivisation) both ON and OFF dopamine while using z-scored UPDRS-III as a co-variate, resulted in a significant main effect of drug ($F(1, 24) = 5.97$, $P = 0.022$), a main effect of valence ($F(1, 24) = 14.88$, $P = 0.001$), a drug by valence interaction, ($F(1, 24) = 5.6$, $P = 0.026$), and a drug by valence by UPDRS-III three-way interaction ($F(1, 24) = 5.34$, $P = 0.03$).

To interpret this appropriately, the three-way interaction was deconstructed by taking the effect of drug on 0p baseline (ON baseline - OFF baseline) and the effect of drug on extrinsic response (ON extrinsic - OFF extrinsic) and calculating the double delta (the differences of the differences). The interaction was then plotted as a function of UPDRS-III, (r=0.427, p=0.03; **Fig 5.15**). The finding suggests that the better a patient's UPDRS-III (and therefore the inference being, the higher their baseline dopamine) the more being ON dopamine reduces their pupillary extrinsic sensitivity. Concomitantly, the worse the UPDRS-III score (therefore lower baseline dopamine) the more dopamine increases pupillary extrinsic sensitivity.

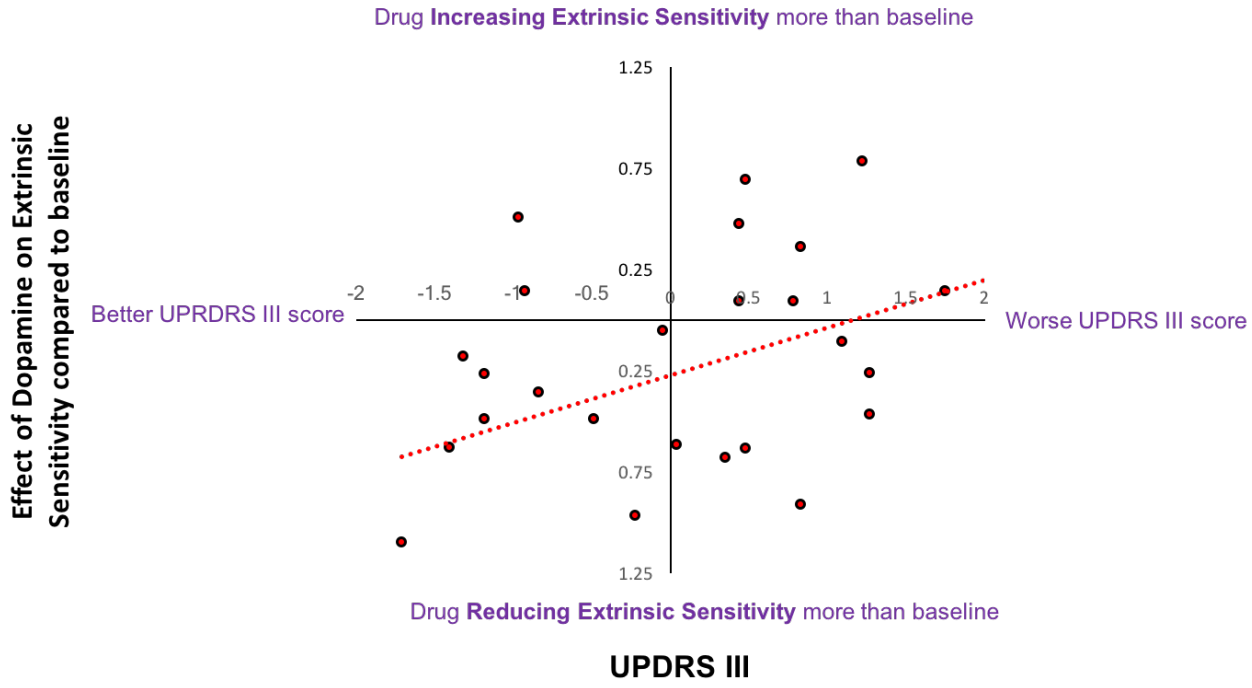

Figure 5.15. Three-way interaction between the effect of dopamine on pupil sensitivity for extrinsic incentives and UPDRS-III scores in Parkinson's disease.

The deconstruction of the three-way interaction between 0p baseline pupil responses compared to pupil sensitivity for extrinsic incentives, while taking into account the effect of dopamine and its relationship with UPDRS-III scores (used as a surrogate of individuals baseline dopamine). A significant correlation was present which is representative of the interaction. The worse an individual's UPDRS-III score, and therefore the lower their baseline dopamine level, the more dopamine increases pupil responses to extrinsic incentives, the opposite is true for smaller UPDRS-III scores. r=0.427, p=0.03.

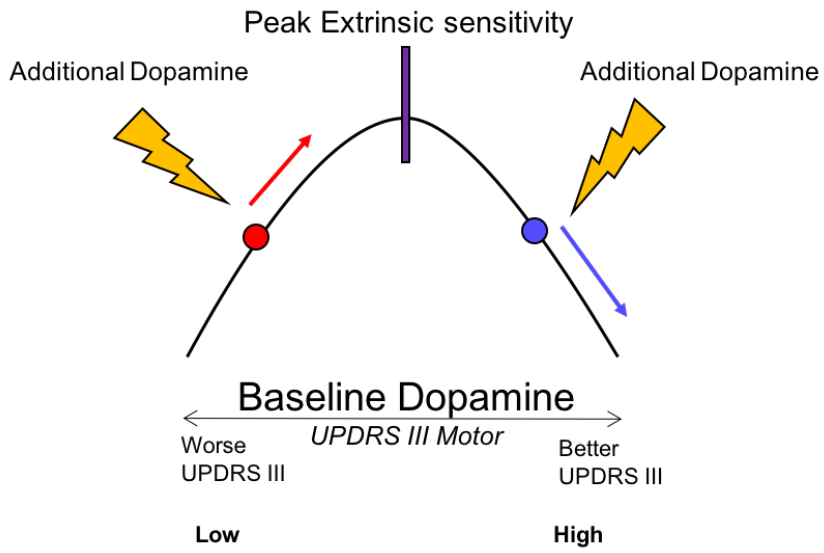

Figure 5.16. Illustration of the inverted U-shaped effect of dopamine on pupil sensitivity to extrinsic incentives using UPDRS-III as a surrogate for an individual's baseline dopamine level.

The x-axis represents an individual's 'baseline dopamine' level as assessed using UPDRS-III motor scores. The worse the UPDRS-III (indicated by a higher actual absolute score) the lower the 'baseline dopamine' level and visa-versa for better UPDRS-III scores. The lightning bolt indicates the addition of dopamine when in the ON state. Therefore, if baseline dopamine of an individual is low such that they lie on the left of the curve (red dot), when additional dopamine is given the pupil response to extrinsic incentives increases. The opposite is true if an individual has higher baseline dopamine to begin with (blue dot), the addition of dopamine in these patients actually worsens their sensitivity to extrinsic incentives and shifts them rightwards on the curve.

5.3.4.7 Baseline working memory and extrinsic incentivisation

There has been many reports that working memory as assessed using digit span may act as a surrogate for baseline dopamine within the striatum (Cools *et al.*, 2008; van der Schaaf *et al.*, 2012), specifically *backward* digit span which is thought to be more sensitive to deficits in working memory (Rosenthal *et al.*, 2006). This could potentially be used as an alternative to UPDRS-III as it will allow the study of other groups where UPDRS-III is not applicable – *see chapter 6*.

To confirm if digit span would also have a similar effect as UPDRS-III, a correlation analysis was performed (**Fig 5.17**). In this PD patient group both UPDRS-III and backward digit span were significantly correlated with each another (r=-0.527, p=0.007). These findings suggest that both scores might potentially index *baseline dopamine* level and can be used interchangeably as a co-variate in our analysis.

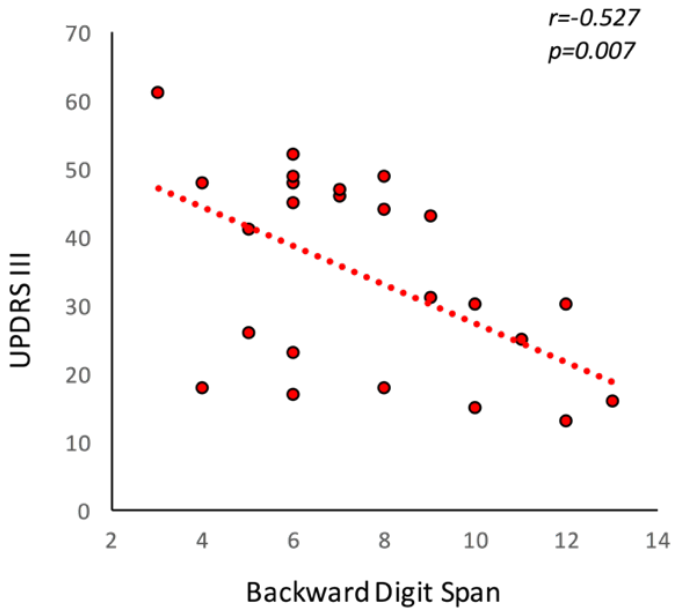

Figure 5.17. UPDRS-III score when OFF as a function of backward digit span in the PD patient group.

A significant correlation was present, the higher the UPDRS-III score (therefore the worse the motor function) the lower the backward digit span score. The lower the UPDRS-III score and therefore better motor function, was associated with a higher backward digit span score. This correlation suggests that the two scores may be assessing similar deficits and baseline dopamine level of an individual may be the common factor.

Performing the same co-variate ANOVA as demonstrated with UPDRS-III but now with z-scored backward digit span as the co-variate resulted in similar findings. There was a significant main effect of drug ($F(1, 24) = 6.25$, $P = 0.02$), a main effect of valence ($F(1, 24) = 16.02$, $P = 0.001$), a drug by valence interaction, ($F(1, 24) = 5.57$, $P = 0.027$), and a drug by valence by backward digit span three-way interaction, ($F(1, 24) = 5.17$, $P = 0.032$). This is deconstructed in **Fig 5.18**, with the interaction term (the effect of dopamine on extrinsic sensitivity compared to the 0p baseline score) on the y-axis, as a function of the digit span scores. A significant correlation was present (r=0.421, p=0.032). The worse a patient's memory (lower baseline dopamine) the more being ON dopamine medication increased patients pupillary extrinsic sensitivity. At the same time, the better the memory as assessed by greater digit span (therefore higher baseline dopamine) the more dopamine reduced patients' pupillary extrinsic sensitivity.

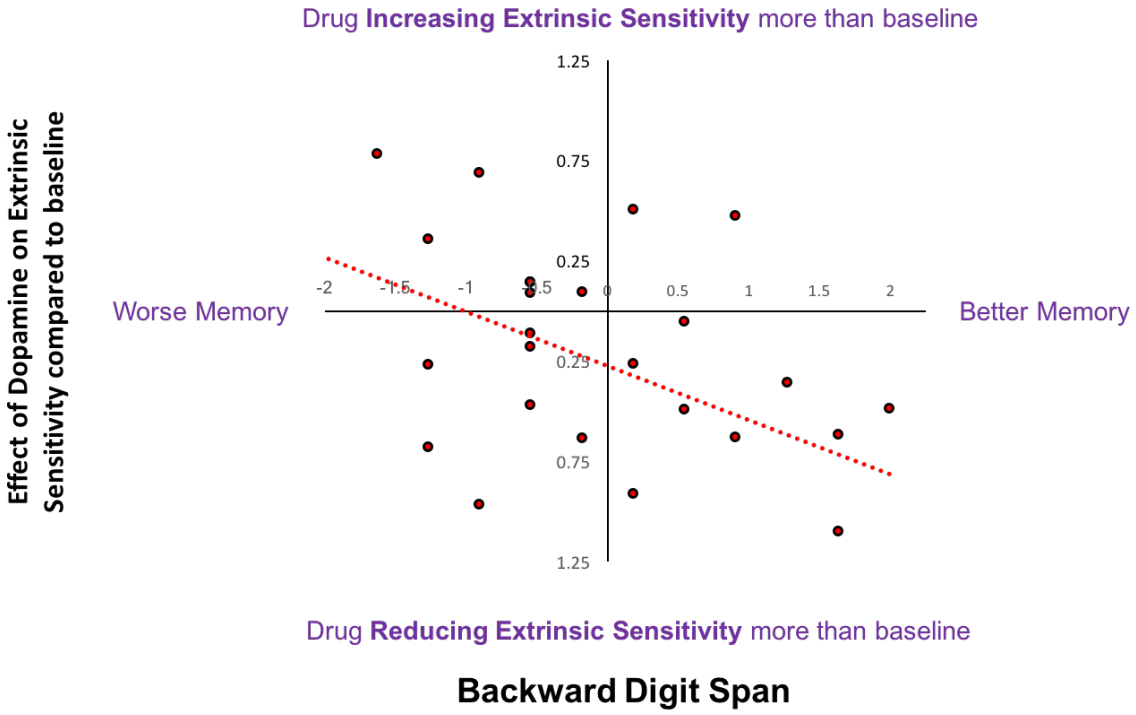

Figure 5.18. Three-way interaction between the effect of dopamine on pupil sensitivity for extrinsic incentives and working memory scores in Parkinson's disease.

The deconstruction of the three-way interaction between 0p baseline pupil responses compared to pupil sensitivity for extrinsic incentives, while taking in to account the effect of dopamine and its relationship with backwards digit span scores (used as a surrogate of individuals baseline dopamine). The smaller an individuals digit span, and therefore the lower their baseline dopamine level, the more dopamine increases pupil responses to extrinsic incentives. The reverse is true for larger backward digit span scores. r=0.421, p=0.032.

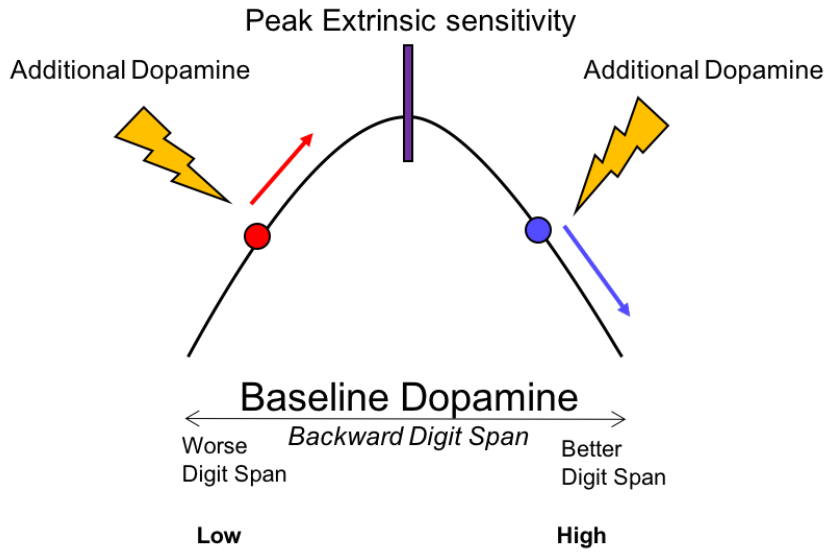

Figure 5.19. Illustration of the inverted U-shaped effect of dopamine on pupil sensitivity to extrinsic incentives using backward digit span as a surrogate for an individuals baseline dopamine level.

The worse the digit span the lower the 'baseline dopamine' level and visa-versa for better scores. The lightning bolt indicates the addition of dopamine when in the ON state. If baseline dopamine of an individual is low (left of the curve and represented by the red dot), when additional dopamine is given the pupil response to extrinsic incentives increases. The opposite is true, with worsening of sensitivity to extrinsic incentives if an individual has higher baseline dopamine to begin with (blue dot).

5.3.4.8 Parkinson's disease versus elderly controls

There were no significant differences in pupil response to baseline or extrinsic incentives when comparing PD patients both ON and OFF to elderly control participants. Main effects of valence were present in all comparisons ($p<0.05$), strengthening the result that pupil responses to extrinsic incentives are larger compared to the 0p baseline condition across all groups tested. No significant correlation with reward, loss and extrinsic pupil sensitivities were found with apathy (LARS scores) in any of the groups.

5.4 Discussion

This study demonstrated that in young participants, the vigour of movement was increased both for rewards and punishment (**Fig. 5.3A**). Crucially only movement invigoration for rewards – but not losses – was dopamine dependent in PD. This was evidenced by a significant reduction in speed for rewards when OFF dopamine, with no change in the loss condition (**Fig. 5.9A**). Like previous chapters, evaluation of incentives could be detected through pupillary responses to monetary cues. Both reward and loss modulated the pupil in the same direction and demonstrated significantly greater dilation compared to the neutral baseline condition (**Fig. 5.5**). Further, pupillary sensitivity collapsed across rewarding and aversive cues in PD was dopamine dependent, and was influenced by baseline dopamine levels as assessed by UPDRS-III motor scores (**Fig. 5.15**) and digit span (**Fig. 5.18**).

Invigoration of saccadic velocity in PD remained for loss cues even when OFF dopamine and may explain certain motor phenomena such as *kinesia paradoxa*. Also known as paradoxical kinesis, the term describes the significant motor improvement of akinetic PD patients when placed in situations of extreme motivation. This tends to be most apparent in situations where an activity prevents an aversive outcome such as escaping danger or falling from a bicycle (Schlesinger, Erikh and Yarnitsky, 2007; Snijders and Bloem, 2010). A similar phenomenon has also been demonstrated in rats when depleted of dopamine. In one such study, rats became akinetic after striatal brain lesions and pretreatment with dopamine antagonists. Yet, when placed in a deep-water bath the animals were able to swim effectively and escape (Keefe *et al.*, 1989). Hence, an incentive to avoid drowning improved motor output even when dopamine deficient. This effect may therefore be dopamine independent and instead rely on different brain pathways and neurotransmitters.

The expectation of aversive and not rewarding outcomes in our study resulted in faster saccades, particularly when OFF dopamine (**Fig. 5.9A** – Red lines). The exact mechanism behind this is unclear. However, our findings suggests that dopamine is less important for invigoration towards aversive outcomes, and perhaps alternative neurotransmitters may be involved (Schlesinger, Erikh and Yarnitsky, 2007) with outcome expectation being necessary (Distler *et al.*, 2016). Studies such as Frank *et al* (2004) that have looked at other end outcomes including learning rate response to incentives, have also shown differences

between PD patients ON and OFF dopamine. Learning rate was better to avoid choices that lead to negative outcomes in dopamine deplete states, while improved for rewarding outcomes when ON dopamine (Frank, 2004). These findings are similar to the saccadic velocity results seen in this study. Given the basal ganglia dysfunction in PD, our results suggest motor outputs for certain negative incentives may bypass the striatum, fitting with studies that have implicated the use of alternative pathways via the cerebellum for example, as a compensatory neural mechanism in PD (Goerendt, Lawrence and Brooks, 2004).

Unlike the invigoration of motor speeds, the evaluation of reward and loss as measured by pupillary responses in this task seemed to be influenced in the same manner. This suggests, at least in terms of pupil sensitivity, the way that the pupil responds to reward and losses are not distinguishable. Dopamine did not alter the evaluation between rewarding and aversive stimuli either. These findings do not mean reward and loss do not have separate brain processes with distinct neural substrates, but at a physiological level through pupillary responses, we could not dissociate them in this study. One explanation for this could be in the task design. Incentive outcomes in the study were performance dependent, potentially representing motivational *salience* (regardless of reward or loss) rather than value *per se,* leading to similar effects on the pupil (Bissonette *et al.*, 2014). Furthermore, in the current investigation total amounts won and lost over the course of the experiment were matched, despite still being dependent on performance. Other experiments in the literature that have found distinctions between incentive type often have variable cumulative and final totals or different probabilities of receiving reward versus loss (van der Schaaf *et al.*, 2012, 2013; Spronk *et al.*, 2016) which likely influenced their results. Similarly, those studies often explore differences in decisions or learning rates between reward and loss (Frank, 2004; Cools, Altamirano and D'Esposito, 2006), which is a very different outcome measure from physiological pupil response reported here.

When pupillary responses for reward and loss were collapsed to obtain an overall response to 'extrinsic incentive cues' there was an interesting relationship with surrogate measures of baseline dopamine level in PD. Responses to a number of cognitive processes are dependent on the baseline dopamine level of an individual (Cools and D'Esposito, 2011). Backward digit span was used in our study to assess baseline dopamine as it is most sensitive to deficits in working memory (Rosenthal *et al.*, 2006), a measure which is often used to index

dopamine level in the literature (Kimberg, D'Esposito and Farah, 1997; Frank and O'Reilly, 2006; Cools *et al.*, 2008; van der Schaaf *et al.*, 2012, 2013; Spronk *et al.*, 2016). Likewise, the UPDRS-III motor score when OFF was also assessed as it is likely reflective of dopamine neuronal loss (Greffard *et al.*, 2006). An inverted Yerkes-Dodson curve was representative of the pupillary findings in PD. This can be depicted graphically showing that the effect of dopamine varies depending on the amount of 'baseline dopamine' as derived by UPDRS-III score (**Fig. 5.16**) and backward digit span scores (**Fig. 5.19**). PD patients with lower starting levels of baseline dopamine benefited from dopaminergic medication (increased sensitivity to extrinsic incentives) while those with already high levels of baseline dopamine exhibited impaired sensitivity to extrinsic incentives through additional dopamine intake. These findings suggest that pupillary responses to reward and loss may index motivational salient incentives and that baseline dopamine plays a role in sensitivity to these incentives within individuals. This association suggests that an optimal level of dopamine exists for eliciting the maximal pupil response for incentives. Too little or too much dopamine can have disadvantageous effects (Cools and D'Esposito, 2011; Sinha, Manohar and Husain, 2013). This same pattern of results has been shown in studies where bromocriptine, a dopamine D2 agonist, was given to participants with low and high working memory (Kimberg, D'Esposito and Farah, 1997). Those with low memory scores gained a benefit in their executive function when given dopamine compared to impairment in those with high scores. Our findings are consistent with maximal sensitivity to incentives being represented by an inverted U-shaped curve, with optimal sensitivity being dependent on baseline dopamine level (Cools and D'Esposito, 2011).

The three-way interaction of drug by valence by baseline dopamine surrogate (UPDRS-III or digit span) was appropriately deconstructed so that it could be interpreted graphically. This was done by taking the effect of drug on 0p baseline (ON baseline - OFF baseline) and the effect of drug on extrinsic pupil response (ON extrinsic - OFF extrinsic) and calculating the double delta (the differences of the differences). This interaction was then plotted as a function of baseline dopamine surrogate. This correlation represents the full three-way interaction and allows correct interpretations to be made. (**Fig. 5.15 and 5.18)**

Surprisingly, pupillary reward and loss sensitivity was greater when OFF dopamine in this study (**Fig. 5.12**). This was inconsistent with previous chapters where dopamine increased sensitivity to reward. One explanation might be the task design and the chosen set of

conditions. There was a significantly reduced overall effect of pupil response to reward in this task compared to previous tasks from chapter 2 and 3 (**Fig. 5.8B**). This might have been due to the addition of a loss condition, which produced an interaction between condition types, dampening the pupil responses and possibly accounting for our findings (**Fig. 5.8C**). Furthermore, the absolute baseline differences between ON and OFF could also account for the increased response to reward and loss conditions when OFF (**Fig. 5.11B**). Baseline size of the pupil OFF was considerably smaller compared with ON, allowing for greater dilation in response to stimuli and may contribute to the results. These differences may also account for why no associations were found with pupil sensitivities and apathy in any of the tested groups. Finally, as demonstrated in chapter 4, saccadic variability was impaired when ON dopamine in this study. This may reflect increased dopamine neuronal activity within the basal ganglia, which has been shown to impair function in animal studies (Boekhoudt *et al.*, 2016).

Overall, the findings reported here show that pupillary responses to anticipated reward and loss outcomes are not dissociable. However, when the data from both types of incentive are collapsed together, responsiveness was shown to be modulated by dopamine and dependent on intrinsic baseline dopamine levels (using UPDRS-III and backward digit span scores as proxies). This suggests that the baseline dopamine of an individual may modulate processes that impact upon incentivized goal directed behaviour. Therefore, any potential dopamine therapy for motivational deficits might potentially be tailored in PD depending on baseline levels, so that optimal sensitivities can be achieved. From a motor perspective, studies using auditory and visual cues have already shown promising results in movement therapies for PD (Suteerawattananon *et al.*, 2004), however the findings presented here suggest that greater benefit might be derived from the valence and expectancy that these cues elicit, with cues that are aversive in nature increasing movement speed the most irrespective of dopamine state.

6. Reward and Loss sensitivity and dopaminergic associations

6. Abstract

Dopamine influences how we behave towards incentives and is important in motivated behaviour. However, there are mixed results from studies exploring its role related to both reward and punishment. A placebo controlled double-blinded drug study in young healthy participants was performed to examine the role of dopamine in behavioural and physiological responses towards rewarding and aversive outcomes, using the oculomotor task described in chapter 5. The D2 dopamine receptor antagonist haloperidol was the active drug of choice. Saccadic velocities in general were faster for incentives compared to a neutral condition, irrespective of whether they were aversive or rewarding in nature. Saccadic peak velocity and accuracy were modulated by haloperidol. When on placebo, velocity was fastest for losses compared to rewards but this difference was abolished on haloperidol. Accuracy in general was worsened when on haloperidol.

Participants' anticipatory responses for rewards and punishments were indexed by pupillary changes to monetary incentives. The anticipation of *both* rewards and losses produced significantly more pupil dilation compared to responses for the neutral outcome (0p). An association with motivation and pupil response to reward but not loss was found when participants where on haloperidol. Haloperidol also produced different degrees of pupil modulation between subjects. However, within a subject, the change was always in the same direction for reward and loss. As there were no dissociable effects of drug on pupillary responses between reward and loss, the conditions were combined to examine the effect of extrinsic insentivisation and its association with baseline dopamine levels. Previous work has suggested that working memory capacity may be a proxy for individual baseline dopamine level. Participants with higher digit span scores experienced reduced sensitivity to extrinsic incentives on haloperidol; by contrast people with low digit spans showed increased pupil sensitivity to incentives on drug. The results are discussed in the context of the small 2.5mg

dosage of haloperidol administered. At these small doses, presynaptic auto-receptor effects may boost – rather than antagonize – phasic dopaminergic transmission.

6.1 Introduction

Dopamine is a key player in the neural pathways that process reward (Wise, 2004; Schultz, 2006, 2007). Diseases that affect these systems, for example PD, can have a profound effect on behaviours that are influenced by incentives (Levy, 2006). Here we aim to investigate how dopamine modulates behaviour governed by both reward and loss. To do this, the D2 receptor antagonist haloperidol was administered to healthy individuals while behavioural and physiological observations were made using a novel oculomotor task. Haloperidol is a commonly prescribed antipsychotic medication used in disorders such as schizophrenia and psychosis. It acts on D2-*type* dopamine receptors, which include D2, D3, and D4 receptors located in subcortical areas such as the striatum (Honey *et al.*, 2003; Mehta *et al.*, 2003). Haloperidol was chosen as it has 25 times greater affinity for D2 over D1 (Frank and O'Reilly, 2006). These brain regions are particularly important in motivation and in incentivized behaviours (Thobois *et al.*, 2013). It was hoped that transiently manipulating the dopamine system using a D2 receptor antagonist may further our understanding of the neurochemical mechanisms that underlie motivational deficits.

Mixed results have been reported in previous dopamine drug and patient studies. These outcomes seem to be dependent on many factors including the dose of drugs used, the task design, the population studied and the end outcomes being measured (Frank and O'Reilly, 2006). For example, in patients with PD, when OFF dopamine medication, learning to avoid punishment was superior than learning from rewards, but when ON dopamine, this bias was reversed (Frank, 2004). Conversely, in a healthy population that was given the dopamine-enhancing drug methylphenidate, impaired reward versus punishment learning occured instead (van der Schaaf *et al.*, 2013). Dopamine therefore has a complex mechanism of action, via multiple neural pathways which may determine behavioural outcomes (Salamone and Correa, 2012).

This complexity, which includes how incentives are encoded, is likely to be contributory to the variety of results. Neural recordings in monkey studies for example, have

suggested distinct subgroups of dopamine neurons that encode both rewarding and aversive stimuli within the midbrain (Matsumoto and Hikosaka, 2009). When incentive outcomes are performance dependent, (i.e. dependent on an action and the exact outcome is initially uncertain) motivationally salient activation of dopamine neurons occurs, increasing responses to both reward and loss equally. If, however, incentives are not dependent on performance, the evaluation of an outcome and assessment of its utility is derived, termed motivational value. For motivational value, rewards rank higher than punishments and neutral conditions inbetween (Bromberg-Martin, Matsumoto and Hikosaka, 2010; Bissonette *et al.*, 2014). Therefore, exposure to incentives and the motivational context they are presented in also likely influences behavioural and physiological changes that may be dopamine dependent. Following the administration of a presumed post-synaptic D2 receptor antagonist like haloperidol, reduced motivational value and salience would be predicted, as synaptic dopamine levels should decrease.

The mechanisms of D2 receptor modulating drugs also needs to be considered in order to understand their effects. Normally, D2 modulators are thought to act post-synaptically, inhibiting the release of dopamine. However, this may be an overly simplistic interpretation. Animal studies have suggested that low doses can actually act pre-synaptically and modulate D2 auto receptors which produces opposite effects (Richfield, Penney and Young, 1989). Given reward and loss evaluation depend on phasic dopamine bursts within the D2 system (Schultz, 2006; Bromberg-Martin, Matsumoto and Hikosaka, 2010), the potential effects of haloperidol on pre-synaptic D2 receptors via inhibitory feedback may, paradoxically, increases phasic dopamine release in the striatum (Wu *et al.*, 2002), so caution is needed in interpretation of results.

Another factor of significant importance in understanding how dopamine agonists and antagonists modulate behaviour is the strong link with working memory (Kimberg, D'Esposito and Farah, 1997; Frank and O'Reilly, 2006; van der Schaaf *et al.*, 2012, 2013; Spronk *et al.*, 2016). In working memory tasks, the extent to which these drugs influence outcomes seem to depend upon baseline working memory performance. For example, administration of the D2 agonist bromocriptine demonstrated improvement in executive performance in people with low digit spans, but impaired performance in participants with high spans (Kimberg, D'Esposito and Farah, 1997). These type of findings support the concept that individual

differences in working memory may be determined by differences in the dopaminergic system, specifically with respect to D2 receptors (Frank and O'Reilly, 2006). Indeed, this has been advocated further through the use of PET imaging, where working memory capacity has been shown to predict dopamine synthesis within the striatum (Cools *et al.*, 2008). The relationship between dopamine level and behavioural responses also appears to be represented by an inverted-U shaped function, where both insufficient and excessive levels of dopamine can hinder performance (Cools and D'Esposito, 2011).

Most research on the role of dopamine in incentivisation has concentrated on its influence on decision-making or learning behaviour (Czernecki *et al.*, 2002; Cools *et al.*, 2003; Schmidt *et al.*, 2008; Torta and Castelli, 2008; Torta *et al.*, 2009; Chong *et al.*, 2015). However, this does not address the potential lower-level effect of dopamine modulation on the motor system. Dopamine's influence on basic parameters of motor control such as accuracy, velocity and reaction time, as well as physiological changes in response to incentives may be important in helping understand the mechanisms of goal directed behaviour. Therefore, in young healthy participants, a within subject's double blinded placebo controlled drug study was performed to assess the effect of the D2 receptor antagonist, haloperidol, on physiological and behavioural responses to reward and loss. In addition, working memory capacity was used as a covariate (potential proxy for baseline dopamine levels across individuals) to investigate whether behavioural outcomes and interactions with haloperidol were systematically affected by this measure.

6.2 Methods

6.2.1 Participants

Thirty healthy volunteers were recruited from local adverts placed in the Oxford area. Inclusion criteria included age between 18 and 45 years and a BMI within 18.5 to 29.5. Informed consent was obtained and all participants were given written study information. Participants were also informed they could leave the study at any point if they wished and were provided with a named point of contact. All participants were screened for any medical illnesses and excluded if any of the following conditions applied:

- A history of cardiovascular disease, specifically; hypotension, arrhythmias or valvular disease.
- Neurological conditions including stroke, epilepsy or psychiatric conditions.
- Kidney or liver disease.
- Anybody taking medication with contraindications to the drug haloperidol.
- Any regular medication - excluding the oral contraceptive pill.
- Recent recreational drugs use within the last 2 weeks or alcohol and drug dependency.
- Known allergies to any medication.
- Pregnant or breastfeeding.
- Inherited blood conditions such as porphyria.
- Lactose hypersensitivity due to the placebo contents.

All participants also had a single lead ECG examination to exclude prolonged Q-Tc interval. This was performed as haloperidol can result in arrhythmias in those with abnormally prolonged QT. On the day of testing, all volunteers were rescreened for any contraindications in person. Participants were also asked not drive after the study until the following morning due to the possibility that they might experience drowsiness.

6.2.2 Drug dose and formulation

The choice of drug was haloperidol, a predominantly D2 receptor dopamine antagonist. Haloperidol is a licenced prescription only medicine in the UK. It is used in the management of acute and chronic psychosis and delirium, including in schizophrenia. The normal therapeutic dose is 2 to 20 mg divided throughout the day. Haloperidol is normally administered orally but can be given through other routes and is often prescribed for prolonged periods of time. It is a relatively safe drug, well tolerated and taken by a significant number of people worldwide. The dose of haloperidol administered in the study was a single 2.5mg tablet that was over-encapsulated in an opaque gelatin coating to ensure blinding. This dose was selected based on previous studies to minimize the chances of side effects whilst also modulating behaviour (Norbury *et al.*, 2015). The placebo was a 200mg lactose tablet also over-encapsulated for blinding.

6.2.3 Study design

In order to blind both the researcher and participant to the drug and placebo, before the start of the study each tablet type was over-encapsulated so that they were of the same appearance. They were then placed into numbered containers, with a unique code known only to a second researcher not actively running the sessions in the study. Participants were assigned to one condition (drug or placebo) by a random number generator, the order of sessions were counterbalanced by drug and placebo and also by gender. A third researcher not actively running the sessions independently verified this whole process. Neither the researcher carrying out the sessions nor the participant was able to determine whether active drug or placebo was being taken.

All participants attended two study sessions, both beginning at 8:30am. They were re-screened for suitability and an ECG was performed. Participants then completed a number of questionnaires including: The Addenbrookes Cognitive Examination-III (ACE-III) (Hsieh *et al.*, 2013), The Apathy Motivation Index (AMI) (Ang *et al.*, 2017) and digit span. Following this the capsule for the session was administered. Testing began exactly 2 hours post dose to allow blood concentration to peak if active drug had been administered (Midha *et al.*, 1989). The

second session was always carried out a minimum of 1 week apart at the exact same time of day.

6.2.4 Demographics

Thirty healthy volunteers that met the eligibility criteria were recruited and tested, 16 males and 14 females. Mean age was 25.1 years, (SD +/- 4.2), with normal or corrected to normal vision and average BMI of 22.3, (SD +/- 2.6). Scores on ACE-III were within normal range for cognitive function in all participants, 96, (SD +/- 3).

6.2.5 Experimental paradigm and eye tracking recording and analysis

Eye tracking procedures and the experimental paradigm were identical to those described in chapter 5, (**Fig. 5.2**). Pupillometry analysis was performed using a 2x3 repeated measures ANOVA, with within-subject factors of Drug (haloperidol, placebo) and Valence (neutral, reward, loss). This was also examined collapsed across reward and loss, which was classified as 'extrinsic incentivisation' versus the baseline (0p neutral condition). In this comparison a 2x2 repeated measures ANOVA was performed. Pupillary modulation by reward or loss was taken as mean proportional change in pupil size from baseline pupil diameter at the beginning of each trial time, locked to the onset of the auditory cue. Mean pupillary size from 1700 – 2500ms after the auditory cue was used as the epoch of interest. An individual's *pupillary reward or loss sensitivity* was defined as the difference in mean proportional pupil change between the maximal 50p (win or loss) condition and the neutral 0p condition, over the time epoch defined above. A larger difference indicated greater reward or loss sensitivity.

6.3 Results

6.3.1 Saccadic peak velocity

A 2x3 ANOVA was performed on saccadic peak velocity with drug (haloperidol and placebo) and valence (0p baseline, win and loss) as the factors of interest. There was a main effect of valence on saccadic peak velocity only ($F(2, 58) = 13.38$, $P < 0.0001$). Pairwise

comparisons showed that reward and loss led to higher peak velocities and were significant between all conditions (0p baseline vs. reward, p=0.002, 0p baseline vs. loss, p=0.0003 and faster saccades for loss compared to reward, p=0.016; **Fig. 6.1A**). Comparisons between the win and loss condition revealed saccades were fastest for loss while on placebo (p=0.009), but no different from each other when haloperidol was administered (p=0.15).

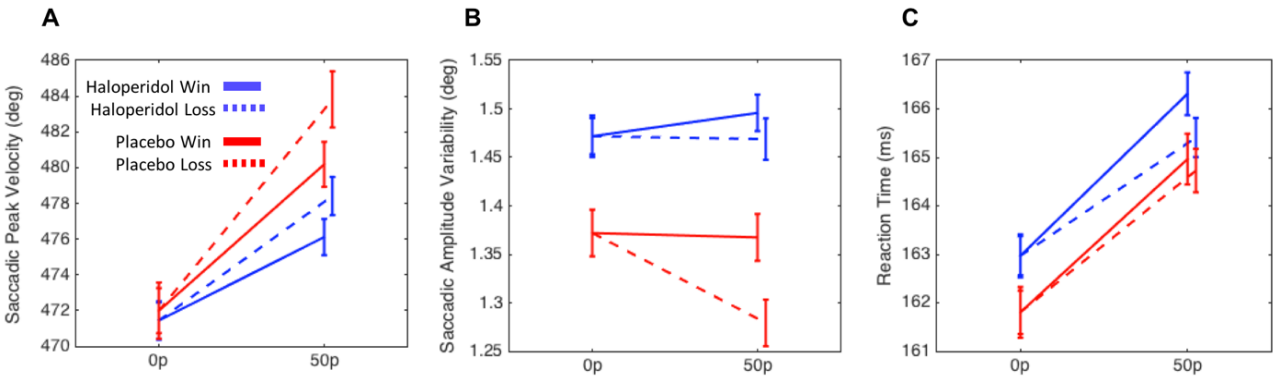

Figure 6.1A-C. Saccadic properties when on placebo versus haloperidol.

A. *Saccadic peak velocity on haloperidol (blue) and placebo (red). Saccades were faster for reward (solid line) and loss (dashed line) both on placebo and haloperidol compared to 0p. Saccades were fastest for loss compared to win when on placebo but this difference was abolished on haloperidol.*

B. *There was a trend for saccadic accuracy to be more variable on haloperidol (blue) compared to placebo (red), p=0.056.*

C. *Saccadic latency (RT) on haloperidol (blue) and placebo (red) were both slower for the win and the loss condition compared to the 0p baseline.*

6.3.2 Saccadic accuracy

Accuracy was assessed by recording variability in saccadic amplitude. There was a trend to a main effect of drug only ($F(1, 29) = 3.96$, $P = 0.056$), and as might have been expected, worsening accuracy in general when participants were on haloperidol. The variability in the loss condition was increased the most by haloperidol with a significant difference between loss when on placebo compared to on haloperidol (p=0.01). There was no statistical difference between accuracy for rewards when on haloperidol and when on placebo (**Fig. 6.1B**).

6.3.3 Saccadic reaction time

There was a main effect of valence on saccadic RT ($F(2, 58) = 15.66$, $P < 0.00001$) with slower RT for reward and loss conditions compared to the 0p baseline (**Fig. 6.1C**). Pairwise comparisons revealed this was true for both reward and loss conditions with significant differences between the 0p baseline and reward ($p<0.001$) and 0p and loss ($p<0.0001$) but no difference in RT between the reward and loss conditions themselves ($p=0.21$).

6.3.4 Pupil reward and loss responses

A 2x3 ANOVA with drug state (haloperidol / placebo) and valence (0p baseline / reward / loss) as factors demonstrated a main effect of valence only ($F(2, 58) = 4.02$, $P = 0.023$). Group pairwise comparisons revealed a significant increase in pupil response between the 0p condition and reward only ($p=0.017$) and a trend between the 0p and loss condition ($p=0.065$) but no difference between reward and loss conditions ($p=0.565$; **Fig. 6.2A**). There was a significant difference in the baseline size of the pupil between haloperidol vs. placebo, when collapsed across all reward levels ($p=0.042$; **Fig. 6.2B**). On haloperidol the absolute baseline pupil size was smaller than when on placebo. Therefore there was more scope for dilation in response to incentives when on haloperidol compared to placebo, which should be considered when interpreting results.

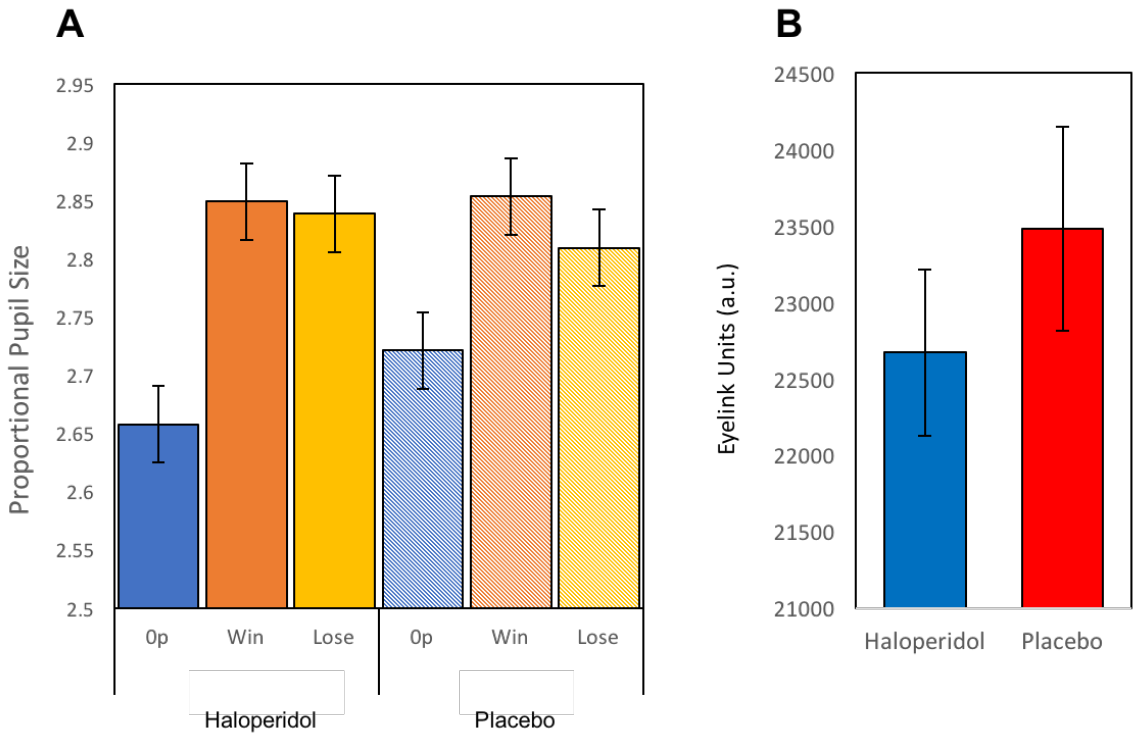

Figure 6.2A-B. Pupil responses to incentives when taking placebo and haloperidol.

A. *Average proportional pupil size change over the epoch of interest (1700ms to 2500ms) in participants on haloperidol (solid bars) and on placebo (light dashed bars). Significant differences were present between the 0p and win 50p condition and trended towards a difference in the 0p and lose 50p condition across both haloperidol and placebo conditions.*

B. *Average pupillary differences measured in Eyelink arbitrary units, across all conditions when on haloperidol and on placebo. A slightly larger baseline pupil size was present when on placebo compared to haloperidol (p=0.042)*

Comparison of differences in reward and loss sensitivity (win 50p minus 0p baseline and lose 50p minus 0p baseline respectively) revealed no main effects over the time period of interest (average pupil trace between 1700- 2500ms) or across the entire course of the trial (**Fig.6.3** – solid versus dashed lines). Similarly, there were no statistical differences in pupil sensitivities when on haloperidol compared to placebo (**Fig.6.3** – blue versus red lines respectively).

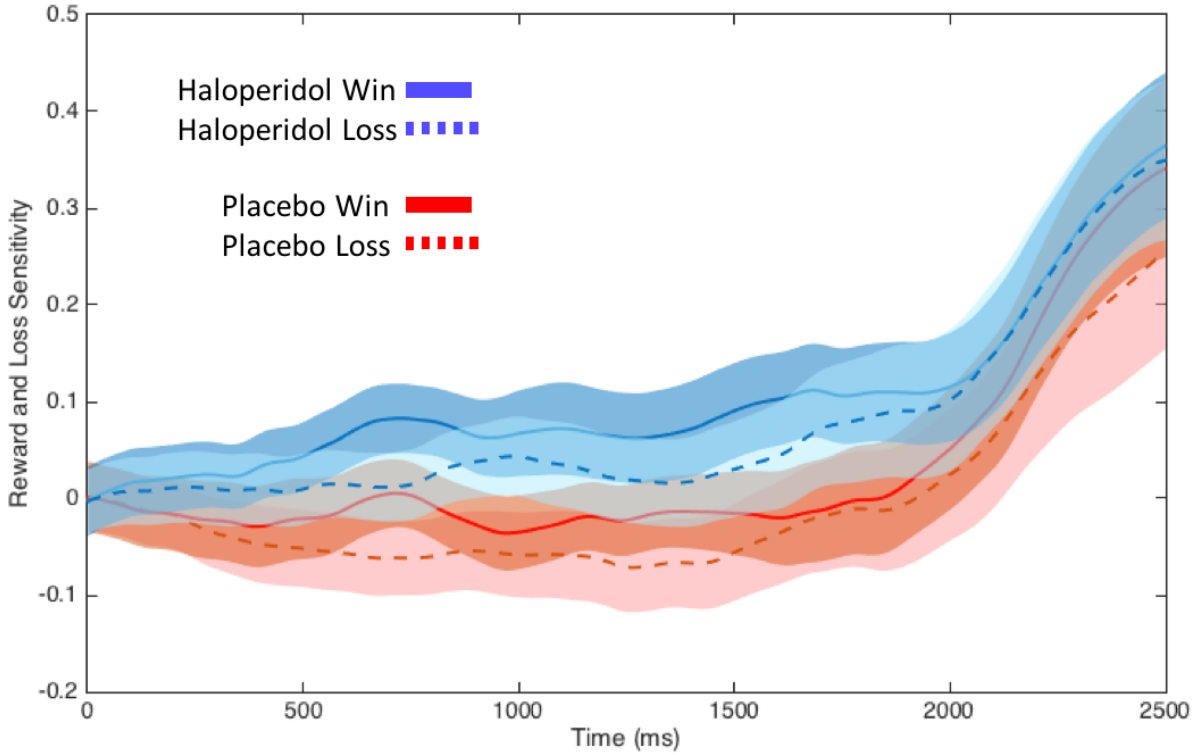

Figure 6.3. Pupil responses to incentives over time when taking placebo and haloperidol.

Pupil reward and loss sensitivity, taken as the difference between the win 50p and 0p condition (solid line) and the difference between the lose 50p and 0p condition (dashed line). This is both when on haloperidol (blue traces) and when on placebo (red traces). There were no time periods of significant difference between any of the incentive conditions either on haloperidol or placebo.

Examination of the effect of haloperidol on pupil reward and loss sensitivity at an individual level revealed that any effect that haloperidol had was in the same direction for both reward and loss. This is demonstrated by a significant positive correlation between the drug effect on loss sensitivity and the drug effect on reward sensitivity, ($r=0.624$, $p<0.001$; **Fig. 6.4**).

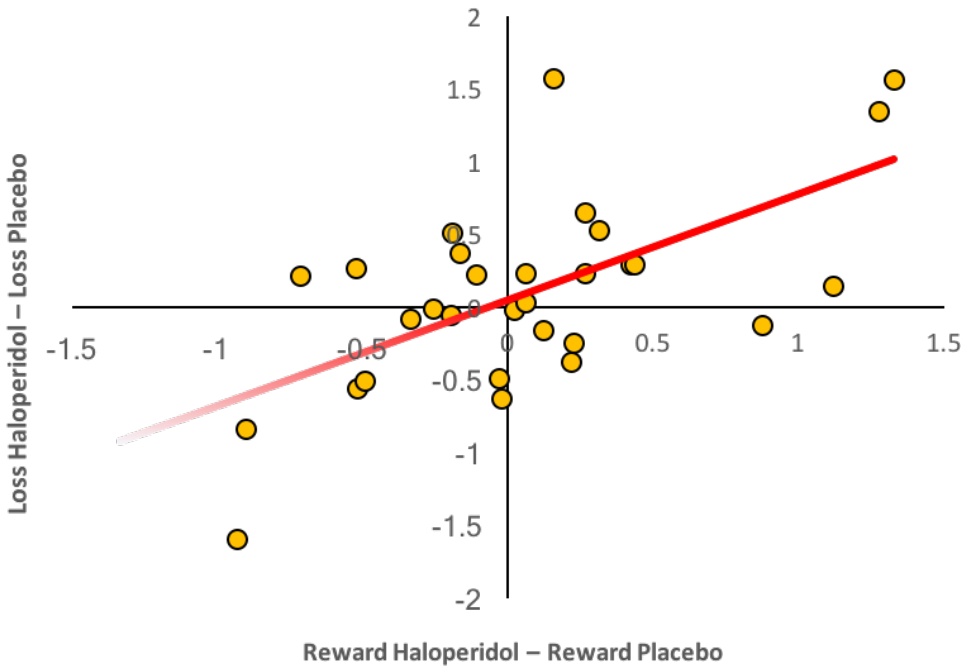

Figure 6.4. The effect of haloperidol on pupil reward and loss sensitivity.

A correlation analysis between the effect of haloperidol on pupil loss sensitivity (individual pupil loss sensitivity when on haloperidol minus when on placebo) and the effect of haloperidol on pupil reward sensitivity (pupil reward sensitivity in an individual when on haloperidol minus when on placebo). This suggests that at an individual level, whichever direction haloperidol modulates the pupil (i.e. the extent of dilation relative to the 0p baseline) it has the same effect on reward and loss sensitivity. r=0.624, p<0.001.

6.3.5 Extrinsic incentivisation

As the overall effects of reward and loss were the same on pupil responses in all previous analyses, a new analysis was performed collapsed across incentive: responses to win and loss were combined and subsequently compared to the 0p baseline. A 2x2 ANOVA on these data demonstrated a main effect of valence only ($F(1, 29) = 5.59$, $P = 0.025$) with pupil responses to extrinsic incentives being significantly higher than the 0p baseline (**Fig. 6.5**).

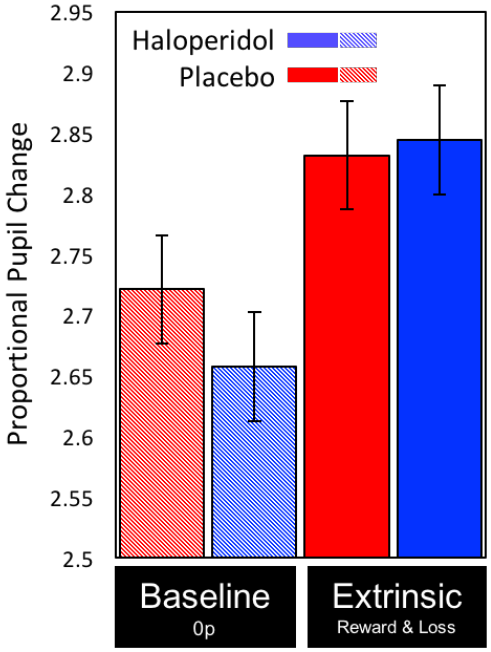

Figure 6.5. Pupil response to baseline neutral condition versus extrinsic incentivisation when on placebo versus haloperidol.

When reward and loss sensitivity are combined (extrinsic incentives) there is a significant difference between the 0p baseline and the extrinsic incentive response. This is true when on haloperidol and when on placebo.

6.3.6 Baseline working memory and extrinsic incentivisation

Differences in pupil responses to extrinsic incentives may be influenced by the amount of *'baseline dopamine'* available to individuals. Taking the findings from chapter 5 and from other studies that suggest baseline dopamine is related to working memory capacity (Cools *et al.*, 2008), z-scored backward digit span was used as a measure of working memory performance and included into the analysis as a covariate. Backward digit span was used as it is most sensitive to deficits in working memory (Rosenthal *et al.*, 2006). This revealed interesting results that were analogous with the findings reported in chapter 5.

When working memory was used as a covariate, the main effect of valence remained ($F(1, 28) = 5.41$, $P = 0.028$) and a main effect of drug was still not present. However, most significantly, a Drug X Valence X Backwards digit span three-way interaction emerged ($F(1, 28) = 7.72$, $P = 0.01$). This three-way interaction was deconstructed further by correlating the two-way interaction between haloperidol and its effect on extrinsic sensitivity as a function of backward digit span score, r= -0.561, p= 0.001. Haloperidol modulated the effect that extrinsic incentives (reward and loss combined) have on pupil response, dependent on baseline

working performance (**Fig. 6.6**). The worse an individual's working memory (proxy for low baseline dopamine) the more haloperidol increased pupillary extrinsic sensitivity. The better the working memory performance (higher baseline dopamine) the more haloperidol reduced extrinsic sensitivity.

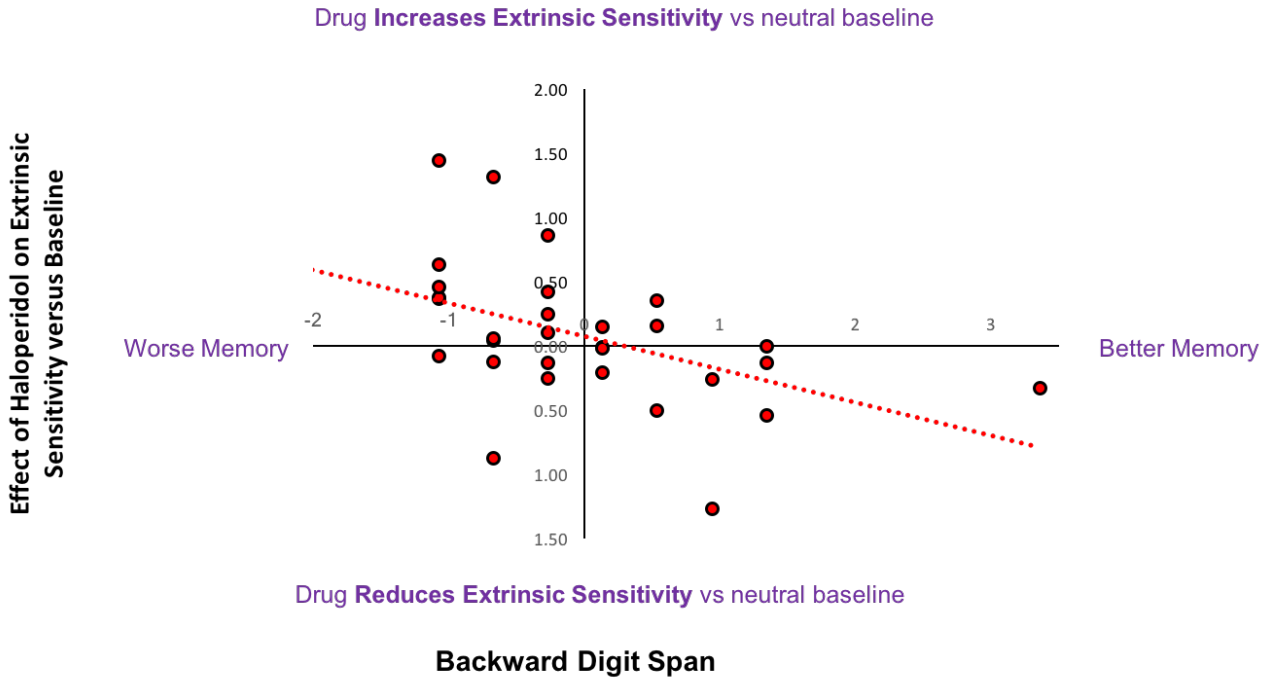

Figure 6.6. Three-way interaction between the effect of haloperidol on pupil sensitivity for extrinsic incentives and working memory scores.

The deconstruction of the three-way interaction between 0p baseline pupil responses compared to pupil sensitivity for extrinsic incentives, while taking into account the effect of haloperidol and its relationship with backward digit span scores (surrogate for baseline dopamine levels). The smaller the backward digit span score, and therefore the lower the baseline dopamine level, the more haloperidol increased pupil responses to extrinsic incentives. The reverse was true for higher backward digit span scores. r= -0.561, p= 0.001.

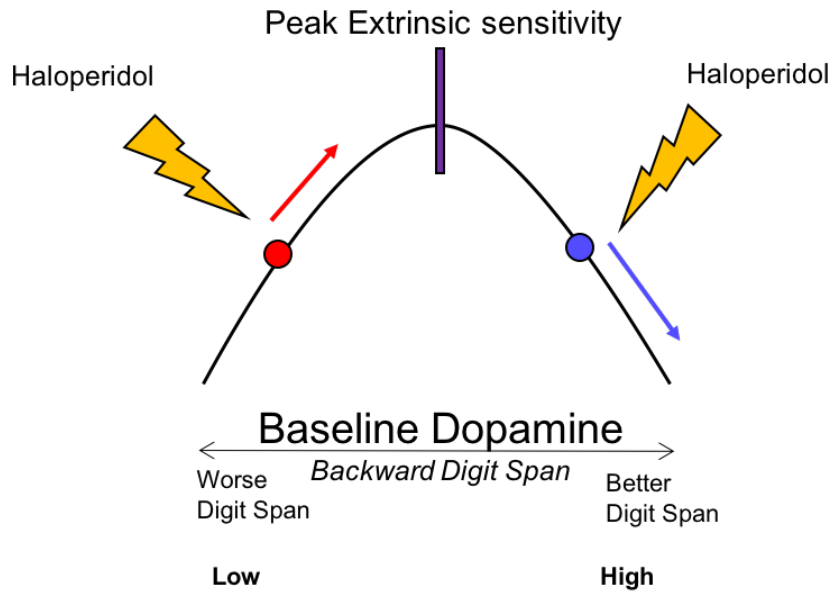

Figure 6.7. Illustration of the inverted U-shaped effect of haloperidol on pupil sensitivity to extrinsic incentives using backward digit span as a surrogate for baseline dopamine.

If baseline dopamine of an individual is low (left of the curve and represented by the red dot), when haloperidol is given (indicated by lightning bolt) the pupil response to extrinsic incentives increases. The opposite is true if an individual has higher baseline dopamine as represented by the blue dot.

6.3.7 Extrinsic incentivisation and motivation

Associations with an individual's self-reported motivation level and pupil response to extrinsic incentives were also investigated. The Apathy Motivation Index score (AMI) for each participant was correlated with pupil sensitivities (reward, loss and extrinsic responses all subtracted from the 0p baseline condition). There was a significant relationship between AMI-Emotional Sensitivity (AMI-ES) and reward sensitivity when on haloperidol (**Fig. 6.8)**. The greater the pupil reward sensitivity when on haloperidol, the more emotionally sensitive individuals were on the AMI-ES, r_s= 0.389, p= 0.033.

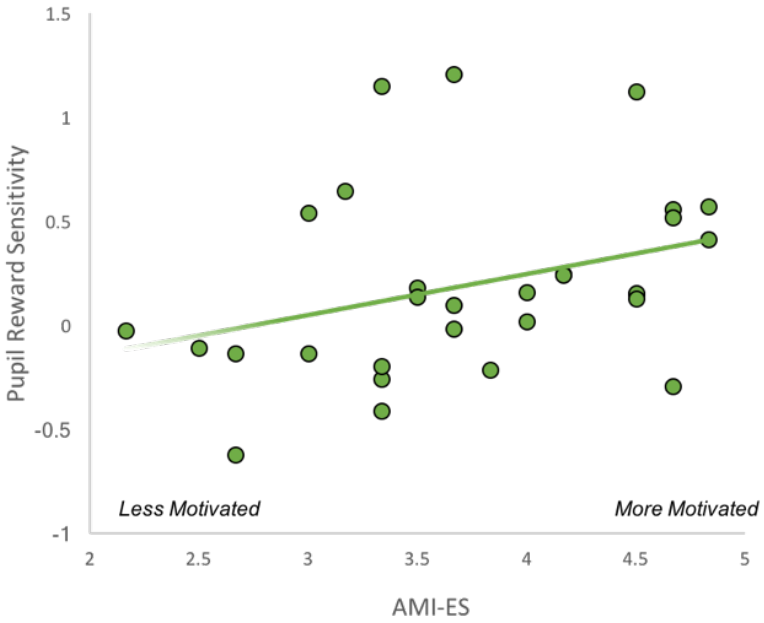

Figure 6.8. Correlation between pupil reward sensitivity and motivation scores.

A significant correlation was present between pupil reward sensitivity and the Apathy Motivation Index Emotional Sub score (AMI-ES) when subjects were on haloperidol. The more motivated an individual on the questionnaire, the greater their pupillary reward sensitivity.

6.4 Discussion

This study investigated the modulatory effects of the D2 antagonist haloperidol on oculomotor and physiological responses to incentives in young healthy participants. Surprisingly, the pupillometry results bare resemblance to those from chapter 5, replicating many of the findings in PD. We had predicted that haloperidol would antagonize dopamine release and therefore produce opposite effects to when PD patients were ON treatment. Unexpectedly, this was not the case, instead haloperidol modulated pupil responses to incentives in the same manner as dopamine therapy in PD. Crucially, this study was in a young healthy population, eliminating many factors that may influence results when investigating patient groups. This includes possible non-dopamine effects in PD which may have occurred secondary to unaccounted neurodegeneration, co-morbidities and concurrent medication use. The findings confirmed that saccadic motor properties are affected by the expectation of incentives and modulated by dopamine targeting drugs even in young healthy participants. Saccadic velocities became invigorated for incentives irrespective of whether they were aversive or rewarding in nature. When on placebo, velocity was fastest for losses compared to rewards but haloperidol abolished this difference (**Fig. 6.1A**) and worsened accuracy (**Fig. 6.1B**). Similarly, pupil dilation was also modulated by the expectation of incentives. This effect was equal in direction and magnitude for both rewarding and aversive outcomes (**Fig. 6.2A**). The modulation of pupil responses to extrinsic incentives by haloperidol was dependent on baseline dopamine levels as assessed by measures of working memory capacity (**Fig. 6.6**).

Previous D2 antagonist studies in healthy individuals have yielded mixed results (see Frank et al, 2006 for a summary). The findings from this study revealed several important points of discussion. Firstly, haloperidol, a D2 antagonist, produced the same effect on pupil sensitivity towards incentives as dopamine and its agonists did when given to patients with PD in chapter 5. When factoring in low backward digit span scores (a proxy for lower baseline dopamine) there was an increase in sensitivity to incentives (both reward and loss combined) when on haloperidol compared to placebo, and the opposite occurred when baseline dopamine was high. This was a similar result to when PD patients were ON dopamine compared to OFF in chapter 5 (**Fig 5.18**). The effect of haloperidol on pupil sensitivity in this study can be depicted graphically using a U-shaped response curve as a function of baseline

dopamine level (**Fig. 6.7**). Basal levels were indexed by backward digit span, an assessment which is sensitive for working memory deficits (Rosenthal *et al.*, 2006) and used by a number of studies (Kimberg, D'Esposito and Farah, 1997; Frank and O'Reilly, 2006; van der Schaaf *et al.*, 2012, 2013; Spronk *et al.*, 2016). Individual differences in working memory appeared to alter sensitivities to extrinsic rewards when a fixed dose of haloperidol was administered, demonstrating the potential interplay between striatal dopamine synthesis (Cools *et al.*, 2008), pupil sensitivity to incentives and dopamine drug therapy. This emphasizes the importance of inter- and intra-individual differences when considering potential dopaminergic therapies for patients and during drug research (van der Schaaf *et al.*, 2013).

As discussed, we had predicted that the haloperidol group would display opposite pupil effects to those seen in PD when ON dopamine, but instead the results were similar. This may be explained by the mechanism of action of haloperidol on dopaminergic neuron receptors, with presynaptic, rather than post synaptic D2 receptor binding. Although we do not have direct evidence for the affinity of haloperidol to presynaptic receptors in this study, low dose haloperidol (which a single dose of 2.5mg could be regarded) may affect presynaptic dopamine auto-receptors and has been demonstrated experimentally in animal studies to *increase* dopamine (Richfield, Penney and Young, 1989). Further, evidence that low dose haloperidol may enhance dopaminergic transmission also comes from a small clinical study that claimed very low doses of haloperidol (40 micrograms a day) alongside normal dopamine therapy in PD, actually increased dopamine receptor sensitivity and improved clinical response in patients (Hudson, Seeman and Seeman, 2014). Pre-synaptic D2 receptors modulate inhibitory feedback of dopamine release and subsequently increase phasic dopamine bursts within the basal ganglia (Frank and O'Reilly, 2006). If this were the case, then it would explain our findings and suggests that a higher dose of haloperidol may be needed to show opposing antagonist effects.

A link between apathy and pupil reward sensitivity was also uncovered (**Fig.6.8**). The emotional subdomain of the Apathy Motivation Index (AMI), a validated questionnaire for apathy in younger healthy populations (Ang *et al.*, 2017), significantly correlated with individuals pupillary *reward* sensitivity but not with loss sensitivity or the combination of the two. This was demonstrated only when participants were ON haloperidol, potentially due to an increased dopamine state rather than dopamine depletion (see discussion above for

explanation). If this is true, then the results compliment findings from previous chapters that revealed pupillary reward sensitivity is associated with apathy in PD only when ON dopamine. Therefore, in the context of pupil modulation within this study design, *reward* sensitivity appears to be specifically associated with apathy, rather than other incentive types like punishment, and this link may be dopamine dependent.

As this study was in healthy young control participants, The Apathy Motivation Index (AMI) (Ang *et al.*, 2017) was used. The AMI is a recently validated and reliable measure of individual differences in apathy and was designed to provide a useful means of probing different mechanisms underlying sub-clinical lack of motivation in healthy populations. It was developed and validated in healthy control populations based on the LARS and LARS-e. Given that all the questionnaires used in the study are derived and validated from the original patient LARS (Sockeel, 2006), they are comparable to some extent, however no direct comparison across studies with apathy have been made in the thesis.

Finally, the similar pupil responses for incentives and the inability of haloperidol to dissociate between pupil reward and loss sensitivity raises potential questions about the task design and the neurotransmitter systems involved. As discussed in chapter 5, the lack of dissociation may be related to the context of the incentives. Motivational *salience,* which refers to the significance of a stimulus rather than its value, is associated with contexts where outcomes are uncertain and dependent on performance (Knutson and Cooper, 2005). The outcomes in this study were performance dependent, which may increase motivationally salient responses rather than lead to encoding of incentive value (Bissonette *et al.*, 2014). If this is the case, it may explain the similar pupil responses for reward and loss (**Fig. 6.4**), but it does not explain why haloperidol also modulated them in the same direction. This finding may suggest that other neurotransmitter systems are also involved in the process and may be more selective for incentive types. One potential candidate is the serotonergic system. Unlike dopaminergic neurons, which can be associated with both rewarding and aversive contexts (Matsumoto and Hikosaka, 2009), serotonergic pathways have commonly been implicated in anticipation of *negative* outcomes and prediction errors for future punishment (Daw, Kakade and Dayan, 2002; Guitart-Masip *et al.*, 2014; Hu, 2016). However, this opposing view of dopamine and serotonin is likely to be too simplistic (Boureau and Dayan, 2011). There is now emerging evidence that suggests serotonin may also be involved in reward encoding.

Examples of such studies include optogenetic stimulation of midbrain serotonin (5-HT) neurons in mice. Following stimulation, enhanced patience for expected rewards was observed. Mice waited significantly longer to receive a food pellet when stimulated, suggesting a role for serotonin specifically in reward anticipatory behaviour (Miyazaki *et al.*, 2014). Likewise, fibre photometry and single-unit recording from midbrain dorsal raphe 5-HT neurons in mice also demonstrated activation when rewards such as sucrose or food were sought, however, aversive stimuli such as quinine and foot shocks did not lead to neuronal firing (Li *et al.*, 2016). These findings add to the complexity and potential overlap between the dopamine and serotonin systems particularly in relation to motivational disorders.

7. General Discussion

7.1 Overview of findings

Over the past few decades it has become increasingly recognized that apathy poses a significant health burden to both patients and caregivers producing large socioeconomic costs (van Reekum, Stuss and Ostrander, 2005; Muhammed and Husain, 2016). However, less is known about the mechanism of apathy and potential therapeutic interventions. PD provides an ideal model to investigate mechanisms underlying apathy, which is common in the condition (den Brok *et al.*, 2015), and explore the potential modulation by dopamine.

In chapter 2, two novel eye tracking tasks were developed to explore if oculomotor properties including saccadic peak velocity and saccadic variability could be modulated by monetary rewards (**Fig 2.1 and Fig 2.6**). This was performed in young and elderly healthy participants. The results demonstrated that rewards can invigorate eye movement velocity without hindering accuracy (**Fig 2.2B-C and Fig 2.8A-C**). Crucially, sensitivity to rewards could also be indexed by pupillary responses to anticipated incentives (**Fig 2.3**), and this sensitivity seemed to decline with age (**Fig 2.9D**). With respect to apathy, reward sensitivity as indexed by pupil responses to rewards significantly correlated with questionnaire measures of motivation level in healthy participants (**Fig 2.5**).

To explore if pupil reward sensitivity could be used to index apathy clinically, a simplified version of the eye tracking study developed in chapter 2 was administered to 40 people with idiopathic PD. 30 of the patients were assessed while ON and OFF their dopamine medication in two counterbalanced sessions and a further 10 were drug naïve. Oculomotor properties were examined and saccadic peak velocity was again invigorated by reward but less in PD patients OFF dopamine compared to ON (**Fig 3.8**). Accuracy in PD also improved for larger rewards but to a smaller degree compared to healthy participants (**Fig 3.9**). The key findings from chapter 3 were that pupillary dilation to increasing levels of monetary reward on offer provided a quantifiable metric of motivation in patients **(Fig 3.5)**. PD patients had

reduced pupillary modulation by incentives, and this was predictive specifically of apathy severity **(Fig 3.6A-B)** and not depression **(Fig 3.11)**. Reward sensitivity was modulated by dopaminergic state, with blunted sensitivity when patients were OFF dopaminergic drugs **(Fig 3.4)** suggesting that reward insensitivity may be a contributory mechanism to apathy in PD. This was independent of motor impairment, dopamine dose and autonomic dysfunction as assessed using overnight heart rate variability monitoring **(Table 3.7)**. Statistical age correction was performed in chapter 3 to establish that no effects of age difference within the PD group was present. No differences in experimental findings were found when age was factored into the analysis, therefore this was only performed in chapter 3 as the subsequent chapters follow a similar design and analysis criteria.

Following on from these results, two further questions were explored in chapter 4. Firstly, are pupil responses to rewards secondary to motor preparation signals? Secondly, are reward sensitivity deficits in clinical apathy associated with impairments in the evaluation of rewards only or, based on evidence from rodent studies (Syed *et al.*, 2015), are they also action dependent? Using an adapted 'go/no-go' eye tracking paradigm (**Fig 4.2**), the link between motivation and motor output was explored in humans and the effect of dopamine assessed in PD patients tested ON and OFF dopamine. Pupil dilation for reward was present regardless of whether actions to obtain a reward were required or not (**Fig 4.4A** in young**, Fig 4.6A** in elderly and **Fig 4.10 in** PD ON and OFF). Thus, pupil reward sensitivity results in previous chapters were not exclusively due to motor preparation and did indeed signal reward evaluation. Dopamine did not change the pupil response to rewards when a saccade was made versus when it was not. However, a significant correlation between apathy and reward sensitivity was demonstrated only when ON in 'go' trials, when an action was initiated to obtain a reward (**Fig 4.12A**). This strengthens the findings from chapter 3, replicating the link between apathy and reward insensitivity, but also suggests that action initiation and dopamine might be crucial.

Although rewards modulate physiological and behavioural responses and appear to have a strong association with motivation, other forms of incentives, including loss, may also contribute to this process. A new eye tracking task was developed to compare how dopamine modulates oculomotor and pupillary responses to loss versus reward (**Fig 5.2**). This was

performed in a young, elderly and a PD patient group ON and OFF dopamine. In young healthy participants, saccadic velocity and accuracy was modulated by *both* incentive types in a similar manner. Reward and loss *both* increased velocity while also improving accuracy (**Fig 5.3A-B**). In elderly participants, peak velocity also increased with reward and loss but accuracy did not significantly change (**Fig 5.6A and B**). In PD patients ON dopamine, saccadic velocities were greater for both rewards and loss. However, when OFF, saccades for rewards were less invigorated compared to saccades to avoid loss, suggesting a dopamine invigorating effect on *rewards only* (**Fig 5.9A**).

Greater pupil dilation was present for both types of incentive (win or loss) compared to a neutral condition (**Fig. 5.5**). However, this modulation was equal for rewards and losses in all groups tested. In PD, dopamine also had the same effect on pupil responses for incentives. Therefore data were collapsed across win and loss trials to investigate the effects of (unsigned) extrinsic incentivisation. On the basis of previous work, motor UPDRS-III scores and backward digit span were used as surrogates for baseline dopamine levels (Cools *et al.*, 2008). PD patients with lower baseline dopamine demonstrated greater increases in pupillary extrinsic sensitivity when ON dopamine compared to OFF. Concomitantly, when basal dopamine level was high, additional dopamine when ON reduced pupillary extrinsic sensitivity (**Fig. 5.15 and Fig. 5.18**). This effect might be accounted for by an inverted U-shaped response to dopamine (Cools and D'Esposito, 2011), with too much dopamine reducing sensitivity to reward and visa versa (**Fig. 5.16 and Fig. 5.19**).

To further assess the neurochemical mechanism of incentivisation on goal directed behaviour, a double blinded placebo controlled drug study in young healthy participants was performed. The D2 dopamine receptor antagonist haloperidol was the active drug of choice and the modulation of behaviour and physiological response to reward and punishment was examined using the same oculomotor task from chapter 5. In this study, saccadic peak velocity increased for both reward and loss with fastest saccades observed for loss on placebo. Haloperidol reduced this effect by bringing velocities for loss down to the same level of reward (**Fig 6.1A**). Accuracy in general was worsened when on haloperidol with the greatest increase in saccadic variability being between the loss condition when on haloperidol compared to placebo (**Fig 6.1B**).

There was an increase in pupil response towards rewards and loss. This was present in both the placebo and the haloperidol groups (**Fig 6.2A**). Reward and loss had similar effects on pupil responses regardless of whether a participant was on haloperidol or placebo. Therefore results were collapsed across incentive and compared to a neutral condition (**Fig 6.5**). The effect of baseline dopamine was again taken into consideration by using working memory scores as a surrogate. Haloperidol increased and decreased the effect that extrinsic incentives had on the pupil depending on baseline dopamine levels (**Fig 6.6**). The lower a participant's basal dopamine, the more haloperidol increased pupillary extrinsic sensitivity; whereas the higher baseline dopamine, the more haloperidol reduced extrinsic sensitivity (**Fig 6.7**). These findings may be explained by presynaptic D2 auto-receptors activation by haloperidol, leading to subsequent *increases* rather than decrease of dopamine within the basal ganglia (Frank and O'Reilly, 2006).

Interestingly, an association with motivation and pupil response to reward and not loss was found in participants while on haloperidol. Similar to findings in previous chapters, the more motivated an individual the more reward sensitive their pupil response was (**Fig. 6.8**).

7.2 Interpretation and future work

7.2.1 Movement and Saccades

Throughout the experiments presented in this thesis, saccades have been used as a model for the motor system. Incentives in each experimental paradigm and across multiple populations, reliably improved *both* saccadic velocity and accuracy. The findings presented here are consistent with saccadic tasks in humans and monkeys which show that reward improves performance (Chen *et al.*, 2013, 2014) breaking established laws of speed and accuracy. The speed-accuracy tradeoff is thought to arise because faster movements are subject to greater neural motor noise (Harris and Wolpert, 1998). From my findings, incentives – including rewards and avoidance of losses – are able to break this tradeoff. Dopamine also appears to increase this effect further by potentially reducing the cost of control through its neuro-modulatory effects on synaptic noise (Kroener *et al.*, 2009).

This is in line with more recent models of motor control which propose that reward might effectively "pay the cost" for neural noise reduction, with dopamine acting as the currency (Manohar *et al.*, 2015). Saccades provide simple and reliable responses to examine motor responses. However, it would be important to test whether the same results would also be observed for limb movements. For example, do limb movements display invigoration of motor action for incentives? This has been explored with regards to risk sensitivity showing variation in limb velocity depending on how risk-aversive or risk-seeking an individual is but not in the context of apathy (Nagengast, Braun and Wolpert, 2011). Although I did not find clear links with saccadic invigoration and apathy in any of the studies in the thesis, it may not be the case for limb movements. Limb movements are more effortful compared to saccades and may potentiate effort-reward tradeoff effects which have been linked to apathy in other decision making tasks (Valerie Bonnelle, Kai-Riin Veromann, Stephanie Burnett Heyes, Elena Lo Sterzo, Sanjay Manohar, no date; Bonnelle, Manohar, *et al.*, 2015; Bonnelle, Veromann, *et al.*, 2015; Chong *et al.*, 2015).

Using devices that are driven by participants' arm movements, such as force generating robotic manipulandums (vBOT), force levels to make a movement can be consistently manipulated. Therefore the effort required to make an action may become more meaningful to participants and more closely reflect real life motivation to act. These methods have already been used to examine risk sensitivity and the association with speed accuracy tradeoff as well as learning rates from rewards and punishments (Nagengast, Braun and Wolpert, 2011; Galea *et al.*, 2015), but little work to my knowledge has explored associations with limb movement invigoration for rewards and potential links with apathy. In PD such an approach might be particularly useful to aid understanding of the phenomenon of paradoxical kinesia – the improvement of motor abilities under certain environmental factors (Schlesinger, Erikh and Yarnitsky, 2007). As discussed in chapter 5, the prospect of losses or punishments may be able to elicit paradoxical kinesia. Therefore the use of aversive stimuli to incentivise limb movements might provide a means to test this effect as force generation can be measured and compared to controls. In addition, this approach might provide insights into how motivational incentives are able to 'bypass' dysfunctional basal ganglia pathways, and perhaps access intact routes such as cerebellar circuits or ventral tegmental limbic projections for motor control (Glickstein and Stein, 1991).

7.2.2 Pupillometry and incentives

The most robust and clinically useful finding to emerge from this body of work was the association of pupillary responses to rewards and the link with clinical apathy in PD. This not only sheds light on the possible mechanisms of the disorder but also potentially provides an inexpensive and portable methodology for aiding in the diagnosis and monitoring of patient groups with motivation deficits. The responses were consistent in PD and replication of apathy and pupil reward sensitivity associations were evident across several studies including in healthy controls. Furthermore, the importance of action initiation on pupil reward sensitivity and its association with apathy was also demonstrated, highlighting that the context of producing a goal directed behaviour is crucial in assessing motivation when using pupillary responses to rewards.

The exploration of other incentives, such as the avoidance of loss, was also performed. Unexpectedly, pupil responsiveness to both rewards and losses were the same. I had initially predicted that motivational value of incentives would be represented by pupillary responses. Motivational value occurs during evaluation of goals, it represents encoding of outcome values, thus rewarding cues should lead to the greatest response, followed by neutral and then aversive stimuli the least (Bissonette *et al.*, 2014). Instead, in our task, pupil modulation responded equally for both rewards and losses. One explanation may be that the pupil is unable to differentiate between incentive types and instead represents the general motivational salience of an outcome, i.e. how arousing it is, rather than the actual value. At a neuronal level, whether valence or salience are encoded appears to be dependent on the context of the experiment (Bissonette *et al.*, 2014) so this may also be a factor to consider. Some studies, for example, have shown that when incentives are expected motivational value is encoded by neuronal activation and when outcomes are performance dependent and not certain, general salience responses occur (Cooper and Knutson, 2008). The oculomotor task I used was performance dependent and outcomes were not known to participants until after their action. Therefore, this may explain why we were unable to differentiate between rewards and loss in terms of pupillary sensitivity. It may be interesting for future experiments to adapt the task so that outcomes are certain, and not performance dependent. This may allow motivational value signals to be reflected in the pupillary response, rather than salience.

It may also help explain why no associations with apathy and pupil changes were found in PD when reward and loss were presented.

Moving forward from task design, although the technology is still in its infancy, in the future it might be useful to try different eye tracking platforms in order to develop more portable and less expensive arrangements. This would allow the tasks to be translated into more clinically useful environments – such as homes or directly in the clinic – permitting greater patient access, recruitment and testing. The larger power from bigger sample sizes might also help draw stronger inferences. One potential methodology is the use of virtual reality (VR) headsets with integrated eye tracking capabilities. VR technology is portable and has become relatively cheap in recent years. Examples such as Fove inc. (California/Tokyo) have incorporated eye tracking into VR headsets, making testing such a system more realistic and accessible.

Taking the concept of VR one step further, the use of augmented reality (AR) to enhance motivation as a rehabilitation or therapeutic intervention might also be plausible. AR superimposes virtual images onto the user's real environment and has the potential to boost perceived rewards available to patients in their own surroundings. Making everyday objects more attractive to interact with could potentially increase goal directed behaviours. For example, if it is more appealing to perform normal day-to-day tasks by increasing salience or gamifying chores, the behavioural manifestation of apathy might be reduced, perhaps improving quality of life and reducing caregiver burden. Although hypothetical, based on this concept, motor symptoms in PD might also show improvement with the use of AR. As discussed earlier, kinesia paridoxia in PD can occur in response to aversive or rewarding environments. With the use of AR, patient's perceptions could be altered to become more sensitive to certain stimuli, potentially causing faster responses and improving motor output. This would be an interesting field to explore further, with potential for useful practical implications in PD.

7.2.3 Neurochemistry of motivation

Through the findings in this thesis and complementary studies in the literature (Chong *et al.*, 2015; Manohar and Husain, 2015; Chong and Husain, 2016), the neurotransmitter

dopamine has been strongly implicated in reward sensitivity and apathy. However, differences in some of the experimental findings suggest that it may not be the only neuromodulator involved in evaluation of incentives. Indeed, it may be one of a variety of neurotransmitters that interact to modulate motivation, particularly in the context of oculomotor and pupillary responses to incentives.

The involvement of several different neurotransmitters might explain why pupil responses for reward and loss were modulated in a similar manner when PD patients were tested ON and OFF dopamine, and also when haloperidol was administered. Serotonin, for example, is another potential candidate for incentive encoding (Boureau and Dayan, 2011). Animal studies have implicated it in both reward expectation (Miyazaki *et al.*, 2014; Li *et al.*, 2016), loss anticipation (Daw, Kakade and Dayan, 2002; Guitart-Masip *et al.*, 2014; Hu, 2016) and clinically serotonergic pathway degeneration has also been shown to be associated with apathy severity in PD (Maillet *et al.*, 2016). This suggests that the neurochemical basis of incentive processing and of apathy is multifactorial in nature. Therefore, testing the effects of serotonergic drugs, like serotonin reuptake inhibitors (SSRI), would be a useful additional approach. This might allow the differentiation between pupil responses to anticipated rewards and loss and, in complement to dopamine, perhaps also modulate sensitivity to incentives in apathy.

Further evidence to support non-dopaminergic neurotransmitter involvement in motivation and incentive processing comes from the findings reported in chapter 3. The addition of dopamine in PD patients with clinical apathy did not fully normalize levels of pupillary reward sensitivity. This might have been dose related, but the result also suggests that other neurotransmitters are involved alongside dopamine. As well as serotonin, acetylcholine might play a role in modulating motivation. Cholinergic pathways are often disrupted in PD, and cognitive dysfunction has been increasingly treated by cholinergic medication (Bohnen and Albin, 2011). In particular, the clinical use of acetylcholinesterase inhibitors like rivastigmine in PD has been reported to reduce symptoms of apathy (Devos *et al.*, 2014). In view of this, it would be informative to perform similar ON/OFF study designs in PD patients who are already taking rivastigmine for other cognitive symptoms and assessing the effect on incentive sensitivity, rather than just manipulating dopamine dose. This may help establish if acetylcholine is also involved in the mechanisms of apathy.

Finally, in chapters 5 and 6, when pupillary responses for reward or loss were collapsed to yield an overall response to 'extrinsic incentives' there was an interaction with surrogate measures of baseline dopamine – both in PD and in healthy controls taking the D2 receptor antagonist haloperidol. Working memory performance is often used to index basal dopamine levels (Kimberg, D'Esposito and Farah, 1997; Frank and O'Reilly, 2006; Cools *et al.*, 2008; van der Schaaf *et al.*, 2012, 2013; Spronk *et al.*, 2016), and is thought to be related to dopamine synthesis capacity in the striatum (Cools *et al.*, 2008). However, it may not necessarily be a very accurate assessment and is not likely specific for actual baseline dopamine of an individual. If future studies were to be attempted, it would be beneficial and more precise to obtain actual blood levels of peak dopamine response to any administered drug. This is also useful because dopamine modulating drugs such as haloperidol are not entirely specific for D2 receptors; they also act with various affinities to D1 targets, histamine and serotonin receptors (Midha *et al.*, 1989; Kudo and Ishizaki, 1999; Kroeze *et al.*, 2003; Frank and O'Reilly, 2006) and depending on dosage can target pre or post synaptic receptors (Frank and O'Reilly, 2006) making precise mechanisms of action harder to identify. Having blood markers of dopamine level would remove some uncertainty and allow stronger interpretations of results to be made. In addition, PET imaging could also be used to assess striatal dopamine synthesis within individuals, giving a far more accurate index of baseline dopamine (van der Schaaf *et al.*, 2013) rather than working memory digit span as a proxy.

7.2.4 Neural correlates

Fronto-striatal circuits have often been implicated in reward processing and goal directed behaviours (Levy, 2006). Therefore, it would also be important to demonstrate that the physiological pupillary measures of reward sensitivity developed in this body of work are also associated with motivation related brain areas. For example, white matter connectivity in the cingulum bundle has been associated with apathy questionnaire scores in young healthy participants (Bonnelle, Manohar, *et al.*, 2015) and clinically when the area is damaged post stroke (Barris and Schuman, 1953). Therefore, using diffusion tensor anisotropy (DTI) to assess the potential strength of connectivity between fronto-striatal areas such as the cingulum, and pupillary reward sensitivity indices would be a useful association to make. If similar pathways were found for both it would strengthen the relationship between physiological measures of reward sensitivity and brain areas known to be linked to motivation.

Anatomical and functional imaging of smaller subcortical structures that are involved in reward processing and motivation, such as the NAcc, would also be interesting to explore. Levels of atrophy in the NAcc of PD patients have already been associated with apathy scores (Carriere *et al.*, 2014). Therefore, attempting to link structural change with objective assessment of reward evaluation, such as pupil reward sensitivity, may provide additional evidence for the anatomical origins of these reward signals. Furthermore, bridging the results from previous animal work and the findings in chapter 4 might be possible. Rodent studies have demonstrated that phasic spikes of dopamine occur specifically in the NAcc only when motor action is needed to obtain a reward (Syed *et al.*, 2015). Attempting to translate this result into humans using functional imaging and the oculomotor 'go/no-go' task would be useful in linking striatal phasic dopamine with physiological responses. Like in other imaging studies, fMRI may be able to demonstrate BOLD signal change in the NAcc in response to reward (Cooper and Knutson, 2008), if this response occurred only when a motor action for reward was made, then it may strengthen the likelihood that phasic dopamine signaling also occurs during initiation of action for rewards in humans during goal directed behaviours.

7.2.5 Other disorders and potential implications

Apathy is known to be highly prevalent in a host of neurological disorders including PD (Chaudhuri and Schapira, 2009; den Brok *et al.*, 2015), Alzheimer's disease (Landes *et al.*, 2001a), vascular dementia, small vessel disease (Hollocks *et al.*, 2015), frontotemporal dementia, amyotrophic lateral sclerosis (Lillo *et al.*, 2012; Mioshi *et al.*, 2014; Bertoux *et al.*, 2015) and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) (Santangelo *et al.*, 2013). Therefore, it is also important to use a trans-diagnostic approach to assess groups of patients with different underlying pathology in order to determine if the same mechanisms that produce apathy in each condition share common processes, e.g., insensitivity to reward. This would be relatively easy to explore and would allow a system-based approach to understand an important neuropsychiatric disorder across a range of different diseases.

The work throughout this thesis suggests pupillary sensitivity is indicative of reward processing potentially linked to fronto-striatal motivation circuits within the brain. In PD patients, degeneration of these areas have also been linked to apathy (Reijnders *et al.*, 2010;

Carriere *et al.*, 2014; Baggio *et al.*, 2015). Therefore, blunting of pupillary responses might also relate to this degeneration and potentially be used as markers of disease onset or progression. For example, the pupil response to reward might potentially be useful to detect prodromal PD. One approach would be to study a sample of patients with rapid eye movement (REM) sleep behaviour disorder (RBD) a parasomnia where individuals appear to act out their dreams (Boeve, 2010). Up to 80% of these patients go on to develop PD, making it one of the biggest risk factors for the neurodegenerative disease (Boeve and Mahowald, 2013). Furthermore, apathy is also very common in RBD (Molano, Boeve and Roberts, 2008; Barber *et al.*, 2017). Therefore, there may be early differences in reward sensitivity in this cohort detectable through pupillary responses, which may help in stratification of disease progression.

Lastly, pupillary responses to incentives might also be helpful in the prediction of other dopamine related neuropsychiatric disorders, not just apathy. Findings throughout this thesis link dopamine to the modulation of pupil reward sensitivity. This association may provide a useful predictor of various dopamine related behavioural changes that can also occur in PD. Impulse control disorder (ICD) and dopamine dysregulation syndromes can develop after the introduction of dopaminergic medication in some patients with PD (Marechal *et al.*, 2015). Dopamine agonists seem to be the largest risk factor for the disorder, and it results in significant quality of life impairments due to excessive risk taking behaviours such as gambling, promiscuity and inappropriate social interactions (Marechal *et al.*, 2015). Currently there are no clear methods for predicting ICD, but perhaps assessing pupil reward sensitivity pre and post dopamine therapy initiation can help stratify those more likely to develop the disorder. Those with greater risk may have larger reward sensitivity responses ON dopamine compared to less susceptible individuals, providing early warning indicators for clinicians and increasing vigilance in therapy monitoring.

7.3 Conclusions

In conclusion, this thesis examined the potential mechanisms of apathy using Parkinson's disease as a model to study the condition. Novel oculomotor tasks that used eye movement and pupillary responses were developed to help assess if insensitivity to incentives could be an underlying component of apathy. Insensitivity to rewards modulated by dopamine was found to be a contributory mechanism of apathy in Parkinson's disease, specifically associated with the initiation of actions. Aversive incentives also played a role in motivating behaviour and modulating physiological responses but no association with apathy was found. The findings in general are applicable to mechanisms of motivation in healthy populations and have potential for future clinical assessments and therapeutic monitoring in patient groups such as PD.

8. Bibliography

Aarsland, D. *et al.* (1999) 'Range of neuropsychiatric disturbances in patients with Parkinson's disease.', *Journal of neurology, neurosurgery, and psychiatry*, 67(4), pp. 492–6.

Aarsland, D., Marsh, L. and Schrag, A. (2009) 'Neuropsychiatric symptoms in Parkinson's disease.', *Movement disorders : official journal of the Movement Disorder Society*, 24(15), pp. 2175–86. doi: 10.1002/mds.22589.

Aarts, E., van Holstein, M. and Cools, R. (2011) 'Striatal Dopamine and the Interface between Motivation and Cognition', *Frontiers in Psychology*, 2. doi: 10.3389/fpsyg.2011.00163.

Abrantes, A. M. *et al.* (2012) 'Physical activity and neuropsychiatric symptoms of Parkinson disease.', *Journal of geriatric psychiatry and neurology*, 25(3), pp. 138–45. doi: 10.1177/0891988712455237.

Adam, R. *et al.* (2013) 'Dopamine reverses reward insensitivity in apathy following globus pallidus lesions', *Cortex*. Elsevier Srl, 49(5), pp. 1292–1303. doi: 10.1016/j.cortex.2012.04.013.

Ahearn, D. J. *et al.* (2012) 'An exploration of apathy and impulsivity in Parkinson disease.', *Current gerontology and geriatrics research*, 2012(1), p. 390701. doi: 10.1155/2012/390701.

American Psychiatric Association (2013) *DSM-V*, *American Journal of Psychiatry*. doi: 10.1176/appi.books.9780890425596.744053.

Anderson, T. J. and MacAskill, M. R. (2013) 'Eye movements in patients with neurodegenerative disorders.', *Nature reviews. Neurology*, 9(2), pp. 74–85. doi: 10.1038/nrneurol.2012.273.

Ang, Y.-S. *et al.* (2017) 'Distinct Subtypes of Apathy Revealed by the Apathy Motivation Index', *Plos One*, 12(1), p. e0169938. doi: 10.1371/journal.pone.0169938.

Assogna, F. *et al.* (2011) 'Anhedonia in Parkinson's disease: a systematic review of the literature.', *Movement disorders : official journal of the Movement Disorder Society*, 26(10), pp. 1825–34. doi: 10.1002/mds.23815.

Aston-Jones, G. and Cohen, J. D. (2005) 'An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance.', *Annual Review of Neuroscience*, 28(1), pp. 403–450. doi: 10.1146/annurev.neuro.28.061604.135709.

Baggio, H. C. *et al.* (2015) 'Resting-state frontostriatal functional connectivity in Parkinson's disease-related apathy.', *Movement disorders : official journal of the Movement Disorder Society*, 30(5), pp. 671–9. doi: 10.1002/mds.26137.

Bahill, A., Clark, M. and Stark, L. (1975) 'The main sequence, a tool for studying human eye movements', *Mathematical Biosciences*, 204, pp. 191–204.

Barber, T. R. *et al.* (2017) 'Apathy in rapid eye movement sleep behaviour disorder is common and under-recognised', *European Journal of Neurology*. doi: 10.1111/ene.13515.

Barnhart, W. J., Makela, E. H. and Latocha, M. J. (2004) 'SSRI-induced apathy syndrome: a clinical review.', *Journal of psychiatric practice*, 10(3), pp. 196–9.

Baron, J. (2008) *Thinking and Deciding*. Cambridge University Press.

Barone, P. *et al.* (2009) 'The PRIAMO study: A multicenter assessment of nonmotor

symptoms and their impact on quality of life in Parkinson's disease', *Movement Disorders*, 24(11), pp. 1641–1649. doi: 10.1002/mds.22643.
Barris, R. W. and Schuman, H. R. (1953) 'Bilateral anterior cingulate gyrus lesions; syndrome of the anterior cingulate gyri', *Neurology*, 3(1), pp. 44–52.
Beck, A. T., Steer, R. A. and Brown, G. K. (1996) 'Manual for the Beck depression inventory-II', *San Antonio, TX: Psychological Corporation*, pp. 1–82.
Benito-León, J., Cubo, E. and Coronell, C. (2012) 'Impact of apathy on health-related quality of life in recently diagnosed Parkinson's disease: The ANIMO study', *Movement Disorders*, 27(2), pp. 211–218. doi: 10.1002/mds.23872.
Berridge, K. C. (2004) 'Motivation concepts in behavioral neuroscience.', *Physiology & behavior*, 81(2), pp. 179–209. doi: 10.1016/j.physbeh.2004.02.004.
Berridge, K. C. (2007) 'The debate over dopamine's role in reward: the case for incentive salience', *Psychopharmacology*, 191(3), pp. 391–431. doi: 10.1007/s00213-006-0578-x.
Berridge, K. C. and Kringelbach, M. L. (2008) 'Affective neuroscience of pleasure: reward in humans and animals.', *Psychopharmacology*, 199(3), pp. 457–80. doi: 10.1007/s00213-008-1099-6.
Bertoux, M. *et al.* (2015) 'Fronto-Striatal Atrophy in Behavioral Variant Frontotemporal Dementia and Alzheimer's Disease', *Frontiers in Neurology*, 6(July), pp. 1–9. doi: 10.3389/fneur.2015.00147.
Bhatia, K. P. and Marsden, C. D. (1994) 'The behavioural and motor consequences of focal lesions of the basal ganglia in man', *Brain*, 117(4), pp. 859–876. doi: 10.1093/brain/117.4.859.
Bissonette, G. B. *et al.* (2014) 'Impact of appetitive and aversive outcomes on brain responses: linking the animal and human literatures.', *Frontiers in systems neuroscience*, 8(March), p. 24. doi: 10.3389/fnsys.2014.00024.
Boekhoudt, L. *et al.* (2016) 'Chemogenetic Activation of Midbrain Dopamine Neurons Affects Attention, but not Impulsivity, in the Five-Choice Serial Reaction Time-Task in Rats.', *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. Nature Publishing Group, 42(6), pp. 1–11. doi: 10.1038/npp.2016.235.
Boeve, B. F. (2010) 'REM Sleep Behavior Disorder: Updated Review of the Core Features, the RBD-Neurodegenerative Disease Association, Evolving Concepts, Controversies, and Future Directions', *Annals of the New York Academy of Sciences*, (1184), pp. 15–54. doi: 10.1111/j.1749-6632.2009.05115.x.REM.
Boeve, B. F. and Mahowald, M. W. (2013) 'Delayed emergence of a parkinsonian disorder or dementia in 81% of older men initially diagnosed with idiopathic rapid eye movement sleep behavior disorder: a 16-year update on a previously reported series', *Sleep Medicine*, 14(8), pp. 744–748. doi: 10.1016/j.sleep.2012.10.009.
Bogart, K. R. (2011) 'Is apathy a valid and meaningful symptom or syndrome in Parkinson's disease? A critical review.', *Health Psychology*, 30(4), pp. 386–400. doi: 10.1037/a0022851.
Bohnen, N. I. and Albin, R. L. (2011) 'The cholinergic system and Parkinson disease', *Behavioural Brain Research*. Elsevier B.V., 221(2), pp. 564–573. doi: 10.1016/j.bbr.2009.12.048.
Bonnelle, V., Veromann, K.-R., *et al.* (2015) 'Characterization of reward and effort mechanisms in apathy', *Journal of Physiology-Paris*. Elsevier Ltd, 109(1–3), pp. 16–26. doi: 10.1016/j.jphysparis.2014.04.002.

Bonnelle, V., Manohar, S., *et al.* (2015) 'Individual Differences in Premotor Brain Systems Underlie Behavioral Apathy', *Cerebral Cortex*, pp. 1–13. doi: 10.1093/cercor/bhv247.

Botvinick Matthew, Huffstetler S, M. J. (2009) 'Effort discounting in human nucleus accumbens', *Cogn Affect Behav Neurosci*, 9(1), pp. 16–27. doi: 10.3758/CABN.9.1.16.

Boureau, Y.-L. and Dayan, P. (2011) 'Opponency revisited: competition and cooperation between dopamine and serotonin.', *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. Nature Publishing Group, 36(1), pp. 74–97. doi: 10.1038/npp.2010.151.

Bouret, S. and Richmond, B. J. (2010) 'Ventromedial and orbital prefrontal neurons differentially encode internally and externally driven motivational values in monkeys.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 30(25), pp. 8591–601. doi: 10.1523/JNEUROSCI.0049-10.2010.

Bradley, M. M. *et al.* (2008) 'The pupil as a measure of emotional arousal and autonomic activation', *Psychophysiology*, 45(4), pp. 602–607. doi: 10.1111/j.1469-8986.2008.00654.x.

den Brok, M. G. H. E. *et al.* (2015) 'Apathy in Parkinson's disease: A systematic review and meta-analysis', *Movement Disorders*, 30(6), pp. 759–769. doi: 10.1002/mds.26208.

Bromberg-Martin, E. S., Matsumoto, M. and Hikosaka, O. (2010) 'Dopamine in motivational control: rewarding, aversive, and alerting.', *Neuron*. Elsevier Inc., 68(5), pp. 815–34. doi: 10.1016/j.neuron.2010.11.022.

Brown, C. A. *et al.* (2012) 'Dopamine pathway loss in nucleus accumbens and ventral tegmental area predicts apathetic behavior in MPTP-lesioned monkeys', *Experimental Neurology*. Elsevier Inc., 236(1), pp. 190–197. doi: 10.1016/j.expneurol.2012.04.025.

Brown, R. G. and Pluck, G. (2000) 'Negative symptoms: The "pathology" of motivation and goal-directed behaviour', *Trends in Neurosciences*, 23(9), pp. 412–417. doi: 10.1016/S0166-2236(00)01626-X.

Campbell, B. A., Wood, G. and McBride, T. (1997) 'Origins of orienting and defensive responses: An evolutionary perspective.', in *Attention and Orienting: sensory and motivational processes.*, pp. 41–67.

Carnicella, S. *et al.* (2014) 'Implication of dopamine D3 receptor activation in the reversion of Parkinson's disease-related motivational deficits.', *Translational psychiatry*. Nature Publishing Group, 4(6), p. e401. doi: 10.1038/tp.2014.43.

Carpenter, R. (2004) 'The saccadic system: a neurological microcosm', *Adv Clin Neurosci Rehabil*, 4(1), pp. 2–3.

Carpenter, R. H. S. (2004) 'Contrast, Probability, and Saccadic Latency', *Current Biology*, 14(17), pp. 1576–1580. doi: 10.1016/j.cub.2004.08.058.

Carriere, N. *et al.* (2014) 'Apathy in Parkinson's disease is associated with nucleus accumbens atrophy: a magnetic resonance imaging shape analysis.', *Movement disorders : official journal of the Movement Disorder Society*, 29(7), pp. 897–903. doi: 10.1002/mds.25904.

Chase, T. N. (2011) 'Apathy in neuropsychiatric disease: diagnosis, pathophysiology, and treatment.', *Neurotoxicity research*, 19(2), pp. 266–78. doi: 10.1007/s12640-010-9196-9.

Chatterjee, A. and Fahn, S. (2002) 'Methylphenidate treats apathy in Parkinson's disease.', *The Journal of neuropsychiatry and clinical neurosciences*, 14(4), pp. 461–2.

doi: 10.1176/jnp.14.4.461.
Chaudhuri, K. R. *et al.* (2006) 'Non-motor symptoms of Parkinson's disease: diagnosis and management.', *The Lancet. Neurology*, 5(3), pp. 235–45. doi: 10.1016/S1474-4422(06)70373-8.
Chaudhuri, K. R. and Schapira, A. H. V (2009) 'Non-motor symptoms of Parkinson's disease: dopaminergic pathophysiology and treatment.', *The Lancet. Neurology*. Elsevier Ltd, 8(5), pp. 464–74. doi: 10.1016/S1474-4422(09)70068-7.
Chen, L. L. *et al.* (2013) 'Cognitive regulation of saccadic velocity by reward prospect.', *The European journal of neuroscience*, 38(3), pp. 2434–44. doi: 10.1111/ejn.12247.
Chen, L. L. *et al.* (2014) 'Monetary reward speeds up voluntary saccades.', *Frontiers in integrative neuroscience*. Frontiers in Integrative Neuroscience, 8(June), p. 48. doi: 10.3389/fnint.2014.00048.
Chong, T. T.-J. *et al.* (2015) 'Dopamine enhances willingness to exert effort for reward in Parkinson's disease', *Cortex*. Elsevier Ltd, 69, pp. 40–46. doi: 10.1016/j.cortex.2015.04.003.
Chong, T. T.-J. *et al.* (2017) 'Neurocomputational mechanisms underlying subjective valuation of effort costs', *PLOS Biology*. Edited by B. Seymour, 15(2), p. e1002598. doi: 10.1371/journal.pbio.1002598.
Chong, T. T.-J. and Husain, M. (2016) 'The role of dopamine in the pathophysiology and treatment of apathy', in *Handbook of Motivation and Cognition Across Cultures*, pp. 389–426. doi: 10.1016/bs.pbr.2016.05.007.
Civelli, O. *et al.* (1991) 'Molecular biology of the dopamine receptors', *European Journal of Pharmacology: Molecular Pharmacology*, 207(4), pp. 277–286. doi: 10.1016/0922-4106(91)90001-X.
Cools, R. *et al.* (2003) 'l-Dopa medication remediates cognitive inflexibility, but increases impulsivity in patients with Parkinson's disease', *Neuropsychologia*, 41(11), pp. 1431–1441. doi: 10.1016/S0028-3932(03)00117-9.
Cools, R. *et al.* (2008) 'Working memory capacity predicts dopamine synthesis capacity in the human striatum.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 28(5), pp. 1208–1212. doi: 10.1523/JNEUROSCI.4475-07.2008.
Cools, R., Altamirano, L. and D'Esposito, M. (2006) 'Reversal learning in Parkinson's disease depends on medication status and outcome valence', *Neuropsychologia*, 44(10), pp. 1663–1673. doi: 10.1016/j.neuropsychologia.2006.03.030.
Cools, R. and D'Esposito, M. (2011) 'Inverted-U-shaped dopamine actions on human working memory and cognitive control', *Biological Psychiatry*. Elsevier Inc., 69(12), pp. e113–e125. doi: 10.1016/j.biopsych.2011.03.028.
Cooper, J. C. and Knutson, B. (2008) 'Valence and salience contribute to nucleus accumbens activation', *NeuroImage*, 39(1), pp. 538–547. doi: 10.1016/j.neuroimage.2007.08.009.
Corcoran, C., Wong, M. L. and O'Keane, V. (2004) 'Bupropion in the management of apathy.', *Journal of psychopharmacology (Oxford, England)*, 18(1), pp. 133–5. doi: 10.1177/0269881104043284.
Crawford, T. J., Henderson, L. and Kennard, C. (1989) 'Abnormalities of nonvisually-guided eye movements in Parkinson's disease.', *Brain : a journal of neurology*, 112 (Pt 6, pp. 1573–86.
Croxson, P. L. *et al.* (2009) 'Effort-based cost-benefit valuation and the human brain.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*,

29(14), pp. 4531–41. doi: 10.1523/JNEUROSCI.4515-08.2009.
Cubo, E. *et al.* (2012) 'Clinical correlates of apathy in patients recently diagnosed with Parkinson's disease: the ANIMO study.', *Neuroepidemiology*, 38(1), pp. 48–55. doi: 10.1159/000334314.
Cummings, J. *et al.* (2015) 'Apathy in Neurodegenerative Diseases: Recommendations on the Design of Clinical Trials', *Journal of Geriatric Psychiatry and Neurology*, 28(3), pp. 159–173. doi: 10.1177/0891988715573534.
Cummings, J. L. *et al.* (1994) 'The Neuropsychiatric Inventory: comprehensive assessment of psychopathology in dementia.', *Neurology*, 44(12), pp. 2308–14. doi: 10.1212/WNL.44.12.2308.
Czernecki, V. *et al.* (2002) 'Motivation, reward, and Parkinson's disease: influence of dopatherapy.', *Neuropsychologia*, 40(13), pp. 2257–67.
Czernecki, V. (2005) 'Does bilateral stimulation of the subthalamic nucleus aggravate apathy in Parkinson's disease?', *Journal of Neurology, Neurosurgery & Psychiatry*, 76(6), pp. 775–779. doi: 10.1136/jnnp.2003.033258.
Czernecki, V. *et al.* (2008) 'Apathy following subthalamic stimulation in Parkinson disease: a dopamine responsive symptom.', *Movement disorders : official journal of the Movement Disorder Society*, 23(7), pp. 964–9. doi: 10.1002/mds.21949.
Daw, N. D., Kakade, S. and Dayan, P. (2002) 'Opponent interactions between serotonin and dopamine', *Neural Networks*, 15(4–6), pp. 603–616. doi: 10.1016/S0893-6080(02)00052-7.
Delaville, C., Deurwaerdère, P. De and Benazzouz, A. (2011) 'Noradrenaline and Parkinson's disease.', *Frontiers in systems neuroscience*, 5(May), p. 31. doi: 10.3389/fnsys.2011.00031.
Denk, F. *et al.* (2005) 'Differential involvement of serotonin and dopamine systems in cost-benefit decisions about delay or effort', *Psychopharmacology*, 179(3), pp. 587–596. doi: 10.1007/s00213-004-2059-4.
Devos, D. *et al.* (2014) 'Rivastigmine in apathetic but dementia and depression-free patients with Parkinson's disease: a double-blind, placebo-controlled, randomised clinical trial', *Journal of Neurology, Neurosurgery & Psychiatry*, 85(6), pp. 668–674. doi: 10.1136/jnnp-2013-306439.
Dirnberger, G. and Jahanshahi, M. (2013) 'Executive dysfunction in Parkinson's disease: A review', *Journal of Neuropsychology*, 7(2), pp. 193–224. doi: 10.1111/jnp.12028.
Distler, M. *et al.* (2016) 'Paradoxical kinesia in Parkinson's disease revisited: Anticipation of temporal constraints is critical', *Neuropsychologia*. Elsevier, 86, pp. 38–44. doi: 10.1016/j.neuropsychologia.2016.04.012.
Drapier, D. *et al.* (2006) 'Does subthalamic nucleus stimulation induce apathy in Parkinson's disease?', *Journal of neurology*, 253(8), pp. 1083–91. doi: 10.1007/s00415-006-0177-0.
Drijgers, R. L. *et al.* (2009) 'Pharmacological Treatment of Apathy in Neurodegenerative Diseases: A Systematic Review', *Dementia and Geriatric Cognitive Disorders*, 28(1), pp. 13–22. doi: 10.1159/000228840.
Drui, G. *et al.* (2013) 'Loss of dopaminergic nigrostriatal neurons accounts for the motivational and affective deficits in Parkinson's disease.', *Molecular psychiatry*. Nature Publishing Group, (December 2012), pp. 1–10. doi: 10.1038/mp.2013.3.
Duffy, J. (2000) 'Apathy in neurologic disorders.', *Current psychiatry reports*, 2(5), pp. 434–9.
Dujardin, K. *et al.* (2007) 'Characteristics of apathy in Parkinson's disease', *Movement*

Disorders, 22(6), pp. 778–784. doi: 10.1002/mds.21316.
Dujardin, K. *et al.* (2008) 'The Lille Apathy Rating Scale: Validation of a caregiver-based version', *Movement Disorders*, 23(6), pp. 845–849. doi: 10.1002/mds.21968.
Dujardin, K. *et al.* (2009) 'Apathy may herald cognitive decline and dementia in Parkinson's disease.', *Movement disorders : official journal of the Movement Disorder Society*, 24(16), pp. 2391–7. doi: 10.1002/mds.22843.
Dujardin, K. *et al.* (2013) 'Assessing apathy in everyday clinical practice with the short-form Lille Apathy Rating Scale.', *Movement disorders : official journal of the Movement Disorder Society*, 28(14), pp. 2014–9. doi: 10.1002/mds.25584.
Dujardin, K. and Defebvre, L. (2012) 'Apathy in Parkinson disease: What are the underlying mechanisms?', *Neurology*, 79(11), pp. 1082–1083. doi: 10.1212/WNL.0b013e3182698dd4.
Duncan, G. W. *et al.* (2014) 'Health-related quality of life in early Parkinson's disease: The impact of nonmotor symptoms', *Movement Disorders*, 29(2), pp. 195–202. doi: 10.1002/mds.25664.
Elliott, R., Friston, K. J. and Dolan, R. J. (2000) 'Dissociable neural responses in human reward systems.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 20, pp. 6159–6165. doi: 20/16/6159 [pii].
Everling, S. *et al.* (1998) 'Comparison of the discharge characteristics of brain stem omnipause neurons and superior colliculus fixation neurons in monkey: implications for control of fixation and saccade behavior.', *Journal of neurophysiology*, 79(2), pp. 511–528.
Favier, M. *et al.* (2014) 'Pramipexole reverses Parkinson's disease-related motivational deficits in rats', *Movement Disorders*, 29(7), pp. 912–920. doi: 10.1002/mds.25837.
Fawcett, J. (1983) 'Assessing Anhedonia in Psychiatric Patients', *Archives of General Psychiatry*, 40(1), p. 79. doi: 10.1001/archpsyc.1983.01790010081010.
Fellows, L. K. (2004) 'The cognitive neuroscience of human decision making: a review and conceptual framework.', *Behavioral and cognitive neuroscience reviews*, 3(3), pp. 159–72. doi: 10.1177/1534582304273251.
Flowers, K. A. and Downing, A. C. (1978) 'Predictive control of eye movements in parkinsons disease', *Annals of Neurology*, 4(1), pp. 63–66. doi: 10.1002/ana.410040112.
Frank, M. J. (2004) 'By Carrot or by Stick: Cognitive Reinforcement Learning in Parkinsonism', *Science*, 306(5703), pp. 1940–1943. doi: 10.1126/science.1102941.
Frank, M. J. and O'Reilly, R. C. (2006) 'A Mechanistic Account of Striatal Dopamine Function in Human Cognition: Psychopharmacological Studies With Cabergoline and Haloperidol', *Behavioral Neuroscience*, 120(3), pp. 497–517. doi: 10.1037/0735-7044.120.3.497.
Galea, J. M. *et al.* (2015) 'The dissociable effects of punishment and reward on motor learning', *Nature Neuroscience*. Nature Publishing Group, 18(4), pp. 597–602. doi: 10.1038/nn.3956.
Garcia-Ruiz, P. J. *et al.* (2014) 'Impulse control disorder in patients with Parkinson's disease under dopamine agonist therapy: a multicentre study.', *Journal of neurology, neurosurgery, and psychiatry*, 85(8), pp. 840–844. doi: 10.1136/jnnp-2013-306787.
Gerardin, E. *et al.* (2004) 'Distinct striatal regions support movement selection, preparation and execution.', *Neuroreport*, 15(15), pp. 2327–2331. doi: 10.1097/00001756-200410250-00005.
Glickstein, M. and Stein, J. (1991) 'Perspectives on disease: Paradoxical movement in

Parkinson's disease', *TINS*, 14(11), pp. 480–482.
Goerendt, I. K., Lawrence, A. D. and Brooks, D. J. (2004) 'Reward Processing in Health and Parkinson's Disease: Neural Organization and Reorganization', *Cerebral Cortex*, 14(1), pp. 73–80. doi: 10.1093/cercor/bhg105.
Goetz, C. G. *et al.* (2008) 'Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): scale presentation and clinimetric testing results.', *Movement disorders : official journal of the Movement Disorder Society*, 23(15), pp. 2129–70. doi: 10.1002/mds.22340.
Gold, J. I. and Shadlen, M. N. (2000) 'Representation of a perceptual decsion in developing oculomotor commands', *Nature*, 404, pp. 390–394. doi: 10.1038/35006062.
Gold, J. I. and Shadlen, M. N. (2002) 'Banburismus and the brain: Decoding the relationship between sensory stimuli, decisions, and reward', *Neuron*, 36(2), pp. 299–308. doi: 10.1016/S0896-6273(02)00971-6.
Gratwicke, J., Jahanshahi, M. and Foltynie, T. (2015) 'Parkinson's disease dementia: a neural networks perspective', *Brain*, 138(6), pp. 1454–1476. doi: 10.1093/brain/awv104.
Gray, M. J. *et al.* (2014) 'Adaptation of Naturally Paced Saccades.', *Journal of neurophysiology*. doi: 10.1152/jn.00905.2013.
Greffard, S. *et al.* (2006) 'Motor score of the Unified Parkinson Disease Rating Scale as a good predictor of Lewy body-associated neuronal loss in the substantia nigra.', *Archives of neurology*, 63(4), pp. 584–588. doi: 10.1001/archneur.63.4.584.
Griffiths, B. and Beierholm, U. R. (2017) 'Opposing effects of reward and punishment on human vigor', *Scientific Reports*. Nature Publishing Group, 7(September 2016), p. 42287. doi: 10.1038/srep42287.
Groenewegen, H. J., Wright, C. I. and Beijer, A. V (1996) 'The nucleus accumbens: gateway for limbic structures to reach the motor system?', *Progress in brain research*, 107, pp. 485–511.
Guiard, B. P., El Mansari, M. and Blier, P. (2008) 'Cross-talk between dopaminergic and noradrenergic systems in the rat ventral tegmental area, locus ceruleus, and dorsal hippocampus.', *Molecular pharmacology*, 74(5), pp. 1463–75. doi: 10.1124/mol.108.048033.
Guitart-Masip, M. *et al.* (2011) 'Action dominates valence in anticipatory representations in the human striatum and dopaminergic midbrain', *J Neurosci*, 31(21), pp. 7867–7875. doi: 10.1523/JNEUROSCI.6376-10.2011.
Guitart-Masip, M. *et al.* (2014) 'Action versus valence in decision making', *Trends in Cognitive Sciences*. Elsevier Ltd, pp. 1–9. doi: 10.1016/j.tics.2014.01.003.
Guttman, M. and Jaskolka, J. (2001) 'The use of pramipexole in Parkinson's disease: are its actions D(3) mediated?', *Parkinsonism & related disorders*, 7(3), pp. 231–234. doi: 10.1016/S1353-8020(00)00062-6.
Haapaniemi, T. H. *et al.* (2001) 'Ambulatory ECG and analysis of heart rate variability in Parkinson's disease.', *Journal of neurology, neurosurgery, and psychiatry*, 70(3), pp. 305–10. doi: 10.1136/jnnp.70.3.305.
Halbesleben, J. R. B. *et al.* (2014) 'Getting to the "COR"', *Journal of Management*, 40(5), pp. 1334–1364. doi: 10.1177/0149206314527130.
Hanes, D. P. and Schall, J. D. (1996) 'Neural control of voluntary movement initiation.', *Science (New York, N.Y.)*, 274(5286), pp. 427–30. doi: 10.1126/science.274.5286.427.
Harris, C. M. and Wolpert, D. M. (1998) 'Signal-dependent noise determines motor

planning.', *Nature*, 394(6695), pp. 780–4. doi: 10.1038/29528.
Harris, C. M. and Wolpert, D. M. (2006) 'The main sequence of saccades optimizes speed-accuracy trade-off.', *Biological cybernetics*, 95(1), pp. 21–9. doi: 10.1007/s00422-006-0064-x.
Le Heron, C., Apps., M. A. J. and Husain, M. (2017) 'The anatomy of apathy: a neurocognitive framework for amotivated behavior', *Neuropsychologia*. Elsevier Ltd, (July), pp. 0–1. doi: 10.1016/j.neuropsychologia.2017.07.003.
Herrmann, N. *et al.* (2008) 'Methylphenidate for the Treatment of Apathy in Alzheimer Disease', *Journal of Clinical Psychopharmacology*, 28(3), pp. 296–301. doi: 10.1097/JCP.0b013e318172b479.
Hikosaka, O. (2005) 'Basal Ganglia Orient Eyes to Reward', *Journal of Neurophysiology*, 95(2), pp. 567–584. doi: 10.1152/jn.00458.2005.
Hikosaka, O. (2007) 'Basal ganglia mechanisms of reward-oriented eye movement.', *Annals of the New York Academy of Sciences*, 1104, pp. 229–49. doi: 10.1196/annals.1390.012.
du Hoffmann, J. and Nicola, S. M. (2014) 'Dopamine Invigorates Reward Seeking by Promoting Cue-Evoked Excitation in the Nucleus Accumbens', *Journal of Neuroscience*, 34(43), pp. 14349–14364. doi: 10.1523/JNEUROSCI.3492-14.2014.
Hollocks, M. J. *et al.* (2015) 'Differential relationships between apathy and depression with white matter microstructural changes and functional outcomes', *Brain*, 138(12), pp. 3803–3815. doi: 10.1093/brain/awv304.
Honey, G. D. *et al.* (2003) 'Dopaminergic drug effects on physiological connectivity in a human cortico-striato-thalamic system', *Brain*, 126(8), pp. 1767–1781. doi: 10.1093/brain/awg184.
Hong, S. and Hikosaka, O. (2008) 'The globus pallidus sends reward-related signals to the lateral habenula.', *Neuron*, 60(4), pp. 720–9. doi: 10.1016/j.neuron.2008.09.035.
Howe, M. W. *et al.* (2013) 'Prolonged dopamine signalling in striatum signals proximity and value of distant rewards.', *Nature*. Nature Publishing Group, 500(7464), pp. 575–9. doi: 10.1038/nature12475.
Hsieh, S. *et al.* (2013) 'Validation of the Addenbrooke's Cognitive Examination III in Frontotemporal Dementia and Alzheimer's Disease', *Dementia and Geriatric Cognitive Disorders*, 36(3–4), pp. 242–250. doi: 10.1159/000351671.
Hu, H. (2016) 'Reward and Aversion', *Annual Review of Neuroscience*, 39(1), pp. 297–324. doi: 10.1146/annurev-neuro-070815-014106.
Hudson, C. J., Seeman, P. and Seeman, M. V. (2014) 'Parkinson's disease: Low-dose haloperidol increases dopamine receptor sensitivity and clinical response', *Parkinson's Disease*, 2014. doi: 10.1155/2014/684973.
Hughes, T. A. *et al.* (2004) 'Mortality in Parkinson's disease and its association with dementia and depression', *Acta Neurologica Scandinavica*, 110(2), pp. 118–123. doi: 10.1111/j.1600-0404.2004.00292.x.
Irwin, D. J. *et al.* (2016) 'Deep clinical and neuropathological phenotyping of Pick disease', *Annals of Neurology*, 79(2), pp. 272–287. doi: 10.1002/ana.24559.
Jainta, S. *et al.* (2011) 'The pupil reflects motor preparation for saccades – even before the eye starts to move', *Frontiers in Human Neuroscience*, 5(97), pp. 1–10. doi: 10.3389/fnhum.2011.00097.
Jones-Gotman, M. and Milner, B. (1977) 'Design fluency: the invention of nonsense drawings after focal cortical lesions', *Neuropsychologia*, 15(4–5), pp. 653–674.
Jordan, L. L. *et al.* (2013) 'Hedonic and behavioral deficits associated with apathy in

Parkinson's disease: Potential treatment implications', *Movement Disorders*, 28(9), pp. 1301–1304. doi: 10.1002/mds.25496.
Joshi, S. *et al.* (2016) 'Relationships between Pupil Diameter and Neuronal Activity in the Locus Coeruleus, Colliculi, and Cingulate Cortex', *Neuron*. Elsevier Inc., 89(1), pp. 221–234. doi: 10.1016/j.neuron.2015.11.028.
Kahneman, D. and Tversky, A. (1983) 'Choices, Values and Frames', *American Psychologist*, pp. 341–350.
Kaji, Y. and Hirata, K. (2011) 'Apathy and Anhedonia in Parkinson's Disease', *ISRN Neurology*, 2011, pp. 1–9. doi: 10.5402/2011/219427.
Kalesnykas, R. P. and Hallett, P. E. (1994) 'Retinal Eccentricity and the Latency of Eye Saccades', *Vision research*, 34(4), pp. 517–531.
Kawagoe, R., Takikawa, Y. and Hikosaka, O. (1998) 'Expectation of reward modulates cognitive signals in the basal ganglia.', *Nature neuroscience*, 1(5), pp. 411–6. doi: 10.1038/1625.
Keefe, K. a *et al.* (1989) 'Paradoxical kinesia in parkinsonism is not caused by dopamine release. Studies in an animal model.', *Archives of neurology*, 46(10), pp. 1070–5. doi: 10.1001/archneur.1989.00520460046012.
Kimberg, D. Y., D'Esposito, M. and Farah, M. J. (1997) 'Effects of bromocriptine on human subjects depend on working memory capacity', *Neuroreport*, 8(16), pp. 3581–3585. doi: 10.1097/00001756-199711100-00032.
Kirsch-Darrow, L. *et al.* (2006) 'Dissociating apathy and depression in Parkinson disease', *Neurology*, 67(1), pp. 33–38. doi: 10.1212/01.wnl.0000230572.07791.22.
Kirsch-Darrow, L. *et al.* (2011) 'Apathy and Depression: Separate Factors in Parkinson's Disease', *Journal of the International Neuropsychological Society*, 17(6), pp. 1058–1066. doi: 10.1017/S1355617711001068.
Kirsch-Darrow, L. *et al.* (2011) 'The trajectory of apathy after deep brain stimulation: From pre-surgery to 6 months post-surgery in Parkinson's disease', *Parkinsonism & Related Disorders*. Elsevier Ltd, 17(3), pp. 182–188. doi: 10.1016/j.parkreldis.2010.12.011.
Kish, S. J., Shannak, K. and Hornykiewicz, O. (1988) 'Uneven pattern of dopamine loss in the striatum of patients with idiopathic Parkinson's disease. Pathophysiologic and clinical implications.', *The New England journal of medicine*, 318(14), pp. 876–80. doi: 10.1056/NEJM198804073181402.
Knutson, B. *et al.* (2001) 'Dissociation of reward anticipation and outcome with event-related fMRI.', *Neuroreport*, 12(17), pp. 3683–3687. doi: 10.1097/00001756-200112040-00016.
Knutson, B. and Cooper, J. C. (2005) 'Functional magnetic resonance imaging of reward prediction', *Current Opinion in Neurology*, 18(4), pp. 411–417. doi: 10.1097/01.wco.0000173463.24758.f6.
Kohno, N. *et al.* (2010) 'Successful treatment of post-stroke apathy by the dopamine receptor agonist ropinirole', *Journal of Clinical Neuroscience*. Elsevier Ltd, 17(6), pp. 804–806. doi: 10.1016/j.jocn.2009.09.043.
van der Kolk, N. M. and King, L. a. (2013) 'Effects of exercise on mobility in people with Parkinson's disease', *Movement Disorders*, 28(11), pp. 1587–1596. doi: 10.1002/mds.25658.
Kool, W., Mcguire, J. T. and Botvinick, M. M. (2010) 'Decision Making and the Avoidance of Cognitive Demand', 139(4), pp. 665–682. doi: 10.1037/a0020198.
Korczyn, A. D. and Keren, O. (1980) 'The effect of dopamine on the pupillary diameter in

mice', *Life Sciences*, 26(10), pp. 757–763. doi: 10.1016/0024-3205(80)90280-5.
Kringelbach, M. L. and Berridge, K. C. (2012) 'The Joyful Mind', *Scientific American*. Nature Publishing Group, 307(2), pp. 40–45. doi: 10.1038/scientificamerican0812-40.
Kroener, S. *et al.* (2009) 'Dopamine modulates persistent synaptic activity and enhances the signal-to-noise ratio in the prefrontal cortex', *PLoS ONE*, 4(8). doi: 10.1371/journal.pone.0006507.
Kroeze, W. K. *et al.* (2003) 'H1-Histamine Receptor Affinity Predicts Short-Term Weight Gain for Typical and Atypical Antipsychotic Drugs', *Neuropsychopharmacology*, 28(3), pp. 519–526. doi: 10.1038/sj.npp.1300027.
Kudo, S. and Ishizaki, T. (1999) 'Pharmacokinetics of haloperidol - An update', *Clinical Pharmacokinetics*, 37(6), pp. 435–456. doi: 10.2165/00003088-199937060-00001.
Kurniawan, I. T. *et al.* (2010) 'Choosing to make an effort: the role of striatum in signaling physical effort of a chosen action.', *Journal of neurophysiology*, 104(1), pp. 313–21. doi: 10.1152/jn.00027.2010.
Kurniawan, I. T., Guitart-Masip, M. and Dolan, R. J. (2011) 'Dopamine and effort-based decision making.', *Frontiers in neuroscience*, 5(June), p. 81. doi: 10.3389/fnins.2011.00081.
Laeng, B., Sirois, S. and Gredebäck, G. (2012) 'Pupillometry', *Perspectives on Psychological Science*, 7(1), pp. 18–27. doi: 10.1177/1745691611427305.
Landes, A. M. *et al.* (2001a) 'Apathy in Alzheimer's disease', *Journal of the American Geriatrics Society*, 49(12), pp. 1700–1707. doi: 10.1046/j.1532-5415.2001.49282.x.
Landes, A. M. *et al.* (2001b) 'Apathy in Alzheimer's Disease', *Journal of the American Geriatrics Society*, 49(12), pp. 1700–1707. doi: 10.1046/j.1532-5415.2001.49282.x.
Lansdall, C. J. *et al.* (2017) 'Apathy and impulsivity in frontotemporal lobar degeneration syndromes', *Brain*, 140(6), pp. 1792–1807. doi: 10.1093/brain/awx101.
Laplane, D. *et al.* (1989) 'Obsessive-compulsive and other behavioural changes with bilateral basal ganglia lesions. A neuropsychological, magnetic resonance imaging and positron tomography study.', *Brain : a journal of neurology*, 112 (Pt 3, pp. 699–725.
Laplane, D. and Dubois, B. (2001) 'Auto-activation deficit: A basal ganglia related syndrome', *Movement Disorders*, 16(5), pp. 810–814. doi: 10.1002/mds.1185.
Lawrence, A. D., Goerendt, I. K. and Brooks, D. J. (2011) 'Apathy blunts neural response to money in Parkinson's disease.', *Social neuroscience*, 6(5–6), pp. 653–62. doi: 10.1080/17470919.2011.556821.
Leentjens, A. F. G. *et al.* (2008) 'Apathy and anhedonia rating scales in Parkinson's disease: Critique and recommendations', *Movement Disorders*, 23(14), pp. 2004–2014. doi: 10.1002/mds.22229.
Lehmann, S. J. and Corneil, B. D. (2016) 'Transient Pupil Dilation after Subsaccadic Microstimulation of Primate Frontal Eye Fields', *Journal of Neuroscience*, 36(13), pp. 3765–3776. doi: 10.1523/JNEUROSCI.4264-15.2016.
Leigh, R. J. and Kennard, C. (2004) 'Using saccades as a research tool in the clinical neurosciences.', *Brain : a journal of neurology*, 127(Pt 3), pp. 460–77. doi: 10.1093/brain/awh035.
Leiknes, I. *et al.* (2010) 'Caregiver distress associated with neuropsychiatric problems in patients with early Parkinson's disease: the Norwegian ParkWest study.', *Acta neurologica Scandinavica*, 122(6), pp. 418–24. doi: 10.1111/j.1600-0404.2010.01332.x.
Lemke, M. R. *et al.* (2005) 'Anhedonia, depression, and motor functioning in Parkinson's

disease during treatment with pramipexole.', *The Journal of neuropsychiatry and clinical neurosciences*, 17(2), pp. 214–20. doi: 10.1176/jnp.17.2.214.
Leon, M. I. and Shadlen, M. N. (1999) 'Effect of Expected Reward Magnitude on the Response of Neurons in the Dorsolateral Prefrontal Cortex of the Macaque', 24, pp. 415–425.
Leroi, I. *et al.* (2012) 'Apathy and impulse control disorders in Parkinson's disease: A direct comparison', *Parkinsonism & Related Disorders*. Elsevier Ltd, 18(2), pp. 198–203. doi: 10.1016/j.parkreldis.2011.10.005.
Levy, M. L. *et al.* (1998) 'Apathy Is Not Depression', *The Journal of Neuropsychiatry and Clinical Neurosciences*, 10(3), pp. 314–319. doi: 10.1176/jnp.10.3.314.
Levy, R. (2006) 'Apathy and the Functional Anatomy of the Prefrontal Cortex-Basal Ganglia Circuits', *Cerebral Cortex*, 16(7), pp. 916–928. doi: 10.1093/cercor/bhj043.
Li, Y. *et al.* (2016) 'Serotonin neurons in the dorsal raphe nucleus encode reward signals', *Nature Communications*, 7, p. 10503. doi: 10.1038/ncomms10503.
Lieberman, a (2006) 'Depression in Parkinson's disease - A review', *Acta Neurologica Scandinavica*, 113(1), pp. 1–8. doi: 10.1111/j.1600-0404.2006.00536.x.
Lillo, P. *et al.* (2012) 'Amyotrophic lateral sclerosis and frontotemporal dementia: A behavioural and cognitive continuum', *Amyotrophic Lateral Sclerosis*, 13(1), pp. 102–109. doi: 10.3109/17482968.2011.639376.
Loas, G., Krystkowiak, P. and Godefroy, O. (2012) 'Anhedonia in Parkinson's Disease: An Overview', *The Journal of Neuropsychiatry and Clinical Neurosciences*. American Psychiatric Association, 24(4), pp. 444–451. doi: 10.1176/appi.neuropsych.11110332.
Loued-Khenissi, L. and Preuschoff, K. (2015) 'Apathy and noradrenaline: silent partners to mild cognitive impairment in Parkinson's disease?', *Current Opinion in Neurology*, 28(4), pp. 344–350. doi: 10.1097/WCO.0000000000000218.
Löw, A. *et al.* (2008) 'Both Predator and Prey', *Psychological Science*, 19(9), pp. 865–873. doi: 10.1111/j.1467-9280.2008.02170.x.
Maetzler, W. *et al.* (2015) 'Time and frequency domain parameters of heart rate variability and sympathetic skin response in Parkinson's disease', *Journal of Neural Transmission*, 122(3), pp. 419–425. doi: 10.1007/s00702-014-1276-1.
Magnard, R. *et al.* (2016) 'What can rodent models tell us about apathy and associated neuropsychiatric symptoms in Parkinson's disease?', *Translational Psychiatry*. Nature Publishing Group, 6(3), p. e753. doi: 10.1038/tp.2016.17.
Maillet, A. *et al.* (2016) 'The prominent role of serotonergic degeneration in apathy, anxiety and depression in de novo Parkinson's disease', *Brain*, 139(9), pp. 2486–2502. doi: 10.1093/brain/aww162.
Manohar, S. G. *et al.* (2015) 'Reward Pays the Cost of Noise Reduction in Motor and Cognitive Control', *Current Biology*. Elsevier Ltd, 25(13), pp. 1707–1716. doi: 10.1016/j.cub.2015.05.038.
Manohar, S. G. *et al.* (2017) 'Distinct Motivational Effects of Contingent and Noncontingent Rewards', *Psychological Science*, p. 95679761769332. doi: 10.1177/0956797617693326.
Manohar, S. G. and Husain, M. (2015) 'Reduced pupillary reward sensitivity in Parkinson's disease', *npj Parkinson's Disease*, 1(November), p. 15026. doi: 10.1038/npjparkd.2015.26.
Manohar, S. and Husain, M. (2016) 'Human ventromedial prefrontal lesions alter incentivisation by reward', *Cortex*. Elsevier Ltd, 76, pp. 104–120. doi:

10.1016/j.cortex.2016.01.005.
Marechal, E. *et al.* (2015) 'Impulse control disorders in Parkinson's disease: an overview from neurobiology to treatment', *Journal of Neurology*, 262(1), pp. 7–20. doi: 10.1007/s00415-014-7361-4.
Marianna Amboni, Gabriella Santangelo, P. B. (2015) *Neuropsychiatric Symptoms of Movement Disorders*. Edited by H. Reichmann. Cham: Springer International Publishing (Neuropsychiatric Symptoms of Neurological Disease). doi: 10.1007/978-3-319-09537-0.
Marin, R. (1996) 'Apathy: Concept, Syndrome, Neural Mechanisms, and Treatment.', *Seminars in clinical neuropsychiatry*, 1(4), pp. 304–314. doi: 10.1053/SCNP00100304.
Marin, R. S. (1991) 'Apathy: a neuropsychiatric syndrome.', *The Journal of neuropsychiatry and clinical neurosciences*, 3(3), pp. 243–54. doi: 10.1176/jnp.3.3.243.
Marin, R. S., Biedrzycki, R. C. and Firinciogullari, S. (1991) 'Reliability and validity of the Apathy Evaluation Scale.', *Psychiatry research*, 38(2), pp. 143–62. doi: 10.1016/0165-1781(91)90040-V.
Martinez-Horta, S. *et al.* (2014) 'Apathy in Parkinson's Disease: Neurophysiological Evidence of Impaired Incentive Processing', *Journal of Neuroscience*, 34(17), pp. 5918–5926. doi: 10.1523/JNEUROSCI.0251-14.2014.
Martinez-Martin, P. *et al.* (2011) 'The impact of non-motor symptoms on health-related quality of life of patients with Parkinson's disease', *Movement Disorders*, 26(3), pp. 399–406. doi: 10.1002/mds.23462.
Martínez-Martín, P. *et al.* (2015) 'Parkinson's disease severity levels and MDS-Unified Parkinson's Disease Rating Scale.', *Parkinsonism & related disorders*, 21(1), pp. 50–4. doi: 10.1016/j.parkreldis.2014.10.026.
Matsumoto, M. and Hikosaka, O. (2009) 'Two types of dopamine neuron distinctly convey positive and negative motivational signals', *Nature*, 459(7248), pp. 837–841. doi: 10.1038/nature08028.
Mattox, S. T., Valle-Inclán, F. and Hackley, S. A. (2006) 'Psychophysiological evidence for impaired reward anticipation in Parkinson's disease', *Clinical Neurophysiology*, 117(10), pp. 2144–2153. doi: 10.1016/j.clinph.2006.05.026.
Mazzoni, P. (2007) 'Why Don't We Move Faster? Parkinson's Disease, Movement Vigor, and Implicit Motivation'.
McKinlay, A. *et al.* (2008) 'A profile of neuropsychiatric problems and their relationship to quality of life for Parkinson's disease patients without dementia', *Parkinsonism & Related Disorders*, 14(1), pp. 37–42. doi: 10.1016/j.parkreldis.2007.05.009.
Mega, M. S. *et al.* (1999) 'The spectrum of behavioral responses to cholinesterase inhibitor therapy in Alzheimer disease.', *Archives of neurology*, 56(11), pp. 1388–93. doi: 10.1001/archneur.56.11.1388.
Mehta, M. A. *et al.* (2003) 'Systemic sulpiride modulates striatal blood flow: Relationships to spatial working memory and planning', *NeuroImage*, 20(4), pp. 1982–1994. doi: 10.1016/j.neuroimage.2003.08.007.
Meyniel, F. *et al.* (2016) 'A specific role for serotonin in overcoming effort cost', *eLife*, 5(NOVEMBER2016), pp. 1–18. doi: 10.7554/eLife.17282.
Middleton, F. A. and Strick, P. L. (2000) 'Basal Ganglia Output and Cognition: Evidence from Anatomical, Behavioral, and Clinical Studies', *Brain and Cognition*, 42(2), pp. 183–200. doi: 10.1006/brcg.1999.1099.
Midha, K. K. *et al.* (1989) 'Intersubject variation in the pharmacokinetics of haloperidol

and reduced haloperidol.', *Journal of clinical psychopharmacology*, p. 98.
Mioshi, E. *et al.* (2014) 'Neuropsychiatric changes precede classic motor symptoms in ALS and do not affect survival.', *Neurology*, 82(2), pp. 149–55. doi: 10.1212/WNL.0000000000000023.
Miyazaki, K. W. *et al.* (2014) 'Optogenetic activation of dorsal raphe serotonin neurons enhances patience for future rewards', *Current Biology*. Elsevier Ltd, 24(17), pp. 2033–2040. doi: 10.1016/j.cub.2014.07.041.
Mogenson, G. J., Jones, D. L. and Yim, C. Y. (1980) 'From motivation to action: Functional interface between the limbic system and the motor system', *Progress in Neurobiology*, 14(2–3), pp. 69–97. doi: 10.1016/0301-0082(80)90018-0.
Molano, J., Boeve, B. and Roberts, R. (2008) 'Anxiety and apathy are associated with probable REM sleep behavior disorder among cognitively normal elderly subjects: The Mayo clinic study off aging', *Neurology (suppl 1):A146*.
Moreau, C. *et al.* (2012) 'Methylphenidate for gait hypokinesia and freezing in patients with Parkinson's disease undergoing subthalamic stimulation: a multicentre, parallel, randomised, placebo-controlled trial', *The Lancet Neurology*. Elsevier Ltd, 11(7), pp. 589–596. doi: 10.1016/S1474-4422(12)70106-0.
Morel, P., Ulbrich, P. and Gail, A. (2017) 'What makes a reach movement effortful? Physical effort discounting supports common minimization principles in decision making and motor control', *PLOS Biology*. Edited by M. Rushworth, 15(6), p. e2001323. doi: 10.1371/journal.pbio.2001323.
Moresi, S. *et al.* (2008) 'Pupil dilation in response preparation.', *International journal of psychophysiology : official journal of the International Organization of Psychophysiology*, 67(2), pp. 124–30. doi: 10.1016/j.ijpsycho.2007.10.011.
Moretti, R. *et al.* (2012) 'Apathy: A Complex Symptom Specific to the Clinical Pattern of Presentation of Parkinson's Disease?', *American Journal of Alzheimer's Disease and Other Dementias*, 27(3), pp. 196–201. doi: 10.1177/1533317512445502.
Muhammed, K. *et al.* (2016) 'Reward sensitivity deficits modulated by dopamine are associated with apathy in Parkinson's disease', *Brain*, 139(10), pp. 2706–2721. doi: 10.1093/brain/aww188.
Muhammed, K. and Husain, M. (2016) 'Clinical Significance of Apathy in Parkinson's Disease', *EMJ Neurology*, (4), pp. 56–63.
Mulin, E. *et al.* (2011) 'Diagnostic criteria for apathy in clinical practice', *International Journal of Geriatric Psychiatry*, 26(2), pp. 158–165. doi: 10.1002/gps.2508.
Munoz, D. P. and Everling, S. (2004) 'Look away: the anti-saccade task and the voluntary control of eye movement.', *Nature reviews. Neuroscience*, 5(3), pp. 218–28. doi: 10.1038/nrn1345.
Nachev, P., Kennard, C. and Husain, M. (2008) 'Functional role of the supplementary and pre-supplementary motor areas.', *Nature reviews. Neuroscience*, 9(11), pp. 856–69. doi: 10.1038/nrn2478.
Nagengast, A. J., Braun, D. a and Wolpert, D. M. (2011) 'Risk sensitivity in a motor task with speed-accuracy trade-off.', *Journal of neurophysiology*, 105(6), pp. 2668–2674. doi: 10.1152/jn.00804.2010.
Nasreddine, Z. S. *et al.* (2005) 'The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment.', *Journal of the American Geriatrics Society*, 53(4), pp. 695–9. doi: 10.1111/j.1532-5415.2005.53221.x.
Nieuwenhuis, S., Aston-Jones, G. and Cohen, J. D. (2005) 'Decision making, the P3, and the locus coeruleus-norepinephrine system.', *Psychological bulletin*, 131(4), pp. 510–

32. doi: 10.1037/0033-2909.131.4.510.
Niv, Y. *et al.* (2007) 'Tonic dopamine: opportunity costs and the control of response vigor.', *Psychopharmacology*, 191(3), pp. 507–20. doi: 10.1007/s00213-006-0502-4.
Niv, Y. (2013) 'Neuroscience: Dopamine ramps up', *Nature*, 500(7464), pp. 533–535. doi: 10.1038/500533a.
Norbury, A. *et al.* (2015) 'Dopamine regulates approach-avoidance in human sensation-seeking', *International Journal of Neuropsychopharmacology*, 18(10), pp. 1–10. doi: 10.1093/ijnp/pyv041.
O'Callaghan, C., Bertoux, M. and Hornberger, M. (2014) 'Beyond and below the cortex: the contribution of striatal dysfunction to cognition and behaviour in neurodegeneration.', *Journal of neurology, neurosurgery, and psychiatry*, 85(4), pp. 371–8. doi: 10.1136/jnnp-2012-304558.
Obeso, J. a *et al.* (2008) 'Functional organization of the basal ganglia: therapeutic implications for Parkinson's disease.', *Movement disorders : official journal of the Movement Disorder Society*, 23 Suppl 3, pp. S548-59. doi: 10.1002/mds.22062.
Oguru, M. *et al.* (2010) 'Apathy and Depression in Parkinson Disease', *Journal of Geriatric Psychiatry and Neurology*, 23(1), pp. 35–41. doi: 10.1177/0891988709351834.
Opris, I., Lebedev, M. A. and Nelson, R. J. (2016) 'Neostriatal neuronal activity correlates better with movement kinematics under certain rewards', *Frontiers in Neuroscience*, 10(AUG). doi: 10.3389/fnins.2016.00336.
Pagonabarraga, J. *et al.* (2015) 'Apathy in Parkinson's disease: clinical features, neural substrates, diagnosis, and treatment', *The Lancet Neurology*. Elsevier Ltd, 14(5), pp. 518–531. doi: 10.1016/S1474-4422(15)00019-8.
Paré, M. and Munoz, D. P. (1996) 'Saccadic reaction time in the monkey: advanced preparation of oculomotor programs is primarily responsible for express saccade occurrence.', *Journal of neurophysiology*, 76(6), pp. 3666–3681.
Pedersen, K. F. *et al.* (2009) 'Occurrence and risk factors for apathy in Parkinson disease: a 4-year prospective longitudinal study', *Journal of Neurology, Neurosurgery & Psychiatry*, 80(11), pp. 1279–1282. doi: 10.1136/jnnp.2008.170043.
Pedersen, K. F. *et al.* (2010) 'Apathy in drug-naïve patients with incident Parkinson's disease: the Norwegian ParkWest study', *Journal of Neurology*, 257(2), pp. 217–223. doi: 10.1007/s00415-009-5297-x.
Peterson, D. A. *et al.* (2009) 'Probabilistic reversal learning is impaired in Parkinson's disease.', *Neuroscience*, 163(4), pp. 1092–101. doi: 10.1016/j.neuroscience.2009.07.033.
Pluck, G. C. (2002) 'Apathy in Parkinson's disease', *Journal of Neurology, Neurosurgery & Psychiatry*, 73(6), pp. 636–642. doi: 10.1136/jnnp.73.6.636.
Pont-Sunyer, C. *et al.* (2015) 'The Onset of Nonmotor Symptoms in Parkinson's disease (The ONSET PDStudy)', *Movement Disorders*, 30(2), pp. 229–237. doi: 10.1002/mds.26077.
Pospíšil, P. *et al.* (2008) 'Autonomic Dysfunction and Progression of Parkinson's Disease', *Scripta Medica*, 81(December), pp. 239–248.
Powell, J. H. *et al.* (1996) 'Motivational deficits after brain injury: effects of bromocriptine in 11 patients.', *Journal of Neurology, Neurosurgery & Psychiatry*, 60(4), pp. 416–421. doi: 10.1136/jnnp.60.4.416.
Preuschoff, K., T Hart, B. M. and Einhäuser, W. (2011) 'Pupil Dilation Signals Surprise: Evidence for Noradrenaline's Role in Decision Making.', *Frontiers in neuroscience*,

5(September), p. 115. doi: 10.3389/fnins.2011.00115.
Pujara, M. S. *et al.* (2016) 'Ventromedial Prefrontal Cortex Damage Is Associated with Decreased Ventral Striatum Volume and Response to Reward', *Journal of Neuroscience*, 36(18), pp. 5047–5054. doi: 10.1523/JNEUROSCI.4236-15.2016.
Rajendra Acharya, U. *et al.* (2006) 'Heart rate variability: a review.', *Medical & biological engineering & computing*, 44(12), pp. 1031–51. doi: 10.1007/s11517-006-0119-0.
Rajkowski, J., Kubiak, P. and Aston-Jones, G. (1994) 'Locus coeruleus activity in monkey: phasic and tonic changes are associated with altered vigilance', *Brain Res Bull*, 35(5–6), pp. 607–616.
Rangel, A., Camerer, C. and Montague, P. R. (2008) 'A framework for studying the neurobiology of value-based decision making.', *Nature reviews. Neuroscience*, 9(7), pp. 545–56. doi: 10.1038/nrn2357.
van Reekum, R., Stuss, D. T. and Ostrander, L. (2005) 'Apathy: Why Care?', *The Journal of Neuropsychiatry and Clinical Neurosciences*, 17(1), pp. 7–19. doi: 10.1176/jnp.17.1.7.
Reijnders, J. S. A. M. *et al.* (2010) 'Neuroanatomical correlates of apathy in Parkinson's disease: A magnetic resonance imaging study using voxel-based morphometry', *Movement Disorders*, 25(14), pp. 2318–2325. doi: 10.1002/mds.23268.
Remy, P. *et al.* (2005) 'Depression in Parkinson's disease: Loss of dopamine and noradrenaline innervation in the limbic system', *Brain*, 128(6), pp. 1314–1322. doi: 10.1093/brain/awh445.
Renfroe, J. B. *et al.* (2016) 'Motivational engagement in Parkinson's disease: Preparation for motivated action', *International Journal of Psychophysiology*, 99(1), pp. 24–32. doi: 10.1016/j.ijpsycho.2015.11.014.
Reppert, T. R. *et al.* (2012) 'Changes in saccade kinematics associated with the value and novelty of a stimulus', 46, pp. 1–5.
Resnick, B. *et al.* (1998) 'Use of the Apathy Evaluation Scale as a measure of motivation in elderly people.', *Rehabilitation nursing : the official journal of the Association of Rehabilitation Nurses*, 23(3), pp. 141–7. doi: 10.1002/j.2048-7940.1998.tb01766.x.
Ressler, N. (2004) 'Rewards and punishments, goal-directed behavior and consciousness.', *Neuroscience and biobehavioral reviews*, 28(1), pp. 27–39. doi: 10.1016/j.neubiorev.2003.10.003.
Richfield, E. K., Penney, J. B. and Young, A. B. (1989) 'Anatomical and affinity state comparisons between dopamine D1 and D2 receptors in the rat central nervous system', *Neuroscience*, 30(3), pp. 767–777. doi: 10.1016/0306-4522(89)90168-1.
Robbins, T. W. and Everitt, B. J. (1996) 'Neurobehavioural mechanisms of reward and motivation.', *Current opinion in neurobiology*, 6(2), pp. 228–36. doi: 10.1016/S0959-4388(96)80077-8.
Robert, G. *et al.* (2012) 'Apathy in patients with parkinson disease without dementia or depression; A PET study', *Neurology*, 79(11), pp. 1155–1160. doi: 10.1212/WNL.0b013e3182698c75.
Robert, G. H. *et al.* (2014) 'Preoperative factors of apathy in subthalamic stimulated Parkinson disease: A PET study', *Neurology*, 83(18), pp. 1620–1626. doi: 10.1212/WNL.0000000000000941.
Robert, P. *et al.* (2009) 'Proposed diagnostic criteria for apathy in Alzheimer's disease and other neuropsychiatric disorders', *European Psychiatry*. Elsevier Masson SAS, 24(2), pp. 98–104. doi: 10.1016/j.eurpsy.2008.09.001.
Robert, P. H. *et al.* (2002) 'The Apathy Inventory: assessment of apathy and awareness

in Alzheimer's disease, Parkinson's disease and mild cognitive impairment', *International Journal of Geriatric Psychiatry*, 17(12), pp. 1099–1105. doi: 10.1002/gps.755.
Robinson, O. J. *et al.* (2010) 'Dissociable responses to punishment in distinct striatal regions during reversal learning', *NeuroImage*. Elsevier B.V., 51(4), pp. 1459–1467. doi: 10.1016/j.neuroimage.2010.03.036.
Rochat, L. *et al.* (2013) 'Poor reward sensitivity and apathy after stroke Implication of basal ganglia', pp. 1674–1681.
Rolfs, M. and Vitu, F. (2007) 'On the limited role of target onset in the gap task: support for the motor-preparation hypothesis.', *Journal of vision*, 7(10), p. 7.1-20. doi: 10.1167/7.10.7.
Rommelfanger, K. S. and Weinshenker, D. (2007) 'Norepinephrine: The redheaded stepchild of Parkinson's disease', *Biochemical Pharmacology*, 74(2), pp. 177–190. doi: 10.1016/j.bcp.2007.01.036.
Rosenberg, P. B. *et al.* (2013) 'Safety and efficacy of methylphenidate for apathy in Alzheimer's disease: a randomized, placebo-controlled trial.', *The Journal of clinical psychiatry*, 74(8), pp. 810–6. doi: 10.4088/JCP.12m08099.
Rosenthal, E. N. *et al.* (2006) 'Digit Span components as predictors of attention problems and executive functioning in children', *Archives of Clinical Neuropsychology*, 21(2), pp. 131–139. doi: 10.1016/j.acn.2005.08.004.
Rothkirch, M. *et al.* (2013) 'The influence of motivational salience on saccade latencies', *Experimental Brain Research*, 224(1), pp. 35–47. doi: 10.1007/s00221-012-3284-4.
Rowe, J. B., Hughes, L. and Nimmo-Smith, I. (2010) 'Action selection: A race model for selected and non-selected actions distinguishes the contribution of premotor and prefrontal areas', *NeuroImage*. Elsevier Inc., 51(2), pp. 888–896. doi: 10.1016/j.neuroimage.2010.02.045.
Rudebeck, P. H. *et al.* (2014) 'A role for primate subgenual cingulate cortex in sustaining autonomic arousal', *Proceedings of the National Academy of Sciences*, 111(14), pp. 5391–5396. doi: 10.1073/pnas.1317695111.
Saga, Y. *et al.* (2011) 'Origins of multisynaptic projections from the basal ganglia to rostrocaudally distinct sectors of the dorsal premotor area in macaques', *European Journal of Neuroscience*, 33(2), pp. 285–297. doi: 10.1111/j.1460-9568.2010.07492.x.
Salamone, J. and Correa, M. (2012) 'The Mysterious Motivational Functions of Mesolimbic Dopamine', *Neuron*, 76(3), pp. 470–485. doi: 10.1016/j.neuron.2012.10.021.
Salamone, J. D. *et al.* (1991) 'Haloperidol and nucleus accumbens dopamine depletion suppress lever pressing for food but increase free food consumption in a novel food choice procedure', *Psychopharmacology*, 104(4), pp. 515–521. doi: 10.1007/BF02245659.
Salamone, J. D. *et al.* (2007) 'Effort-related functions of nucleus accumbens dopamine and associated forebrain circuits.', *Psychopharmacology*, 191(3), pp. 461–82. doi: 10.1007/s00213-006-0668-9.
Salamone, J. D. (2010) 'Motor Function and Motivation', in *Neuroscience and Biobehavioral Psychology*, pp. 267–276. doi: 10.1016/B978-0-12-809324-5.00365-5.
Salamone, J. D. *et al.* (2016) 'Activational and effort-related aspects of motivation: neural mechanisms and implications for psychopathology', *Brain*, 139(5), pp. 1325–1347. doi: 10.1093/brain/aww050.
Salamone, J. D., Cousins, M. S. and Bucher, S. (1994) 'Anhedonia or anergia? Effects of

haloperidol and nucleus accumbens dopamine depletion on instrumental response selection in a T-maze cost/benefit procedure.', *Behavioural brain research*, 65(2), pp. 221–229. doi: 10.1016/0166-4328(94)90108-2.
Santangelo, G. *et al.* (2013) 'Apathy in Parkinson's disease: diagnosis, neuropsychological correlates, pathophysiology and treatment.', *Behavioural neurology*, 27(4), pp. 501–13. doi: 10.3233/BEN-129025.
Satterthwaite, T., Green, L. and Myerson, J. (2007) 'Dissociable but inter-related systems of cognitive control and reward during decision making: evidence from pupillometry and event-related fMRI', *Neuroimage*, 37(3), pp. 1017–1031. doi: 10.1016/j.neuroimage.2007.04.066.
Scangos, K. W. and Stuphorn, V. (2010) 'Medial frontal cortex motivates but does not control movement initiation in the countermanding task.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 30(5), pp. 1968–82. doi: 10.1523/JNEUROSCI.4509-09.2010.
van der Schaaf, M. E. *et al.* (2012) 'Establishing the Dopamine Dependency of Human Striatal Signals During Reward and Punishment Reversal Learning', *Cerebral Cortex*, 24(3), pp. 633–642. doi: 10.1093/cercor/bhs344.
van der Schaaf, M. E. *et al.* (2013) 'Working memory capacity predicts effects of methylphenidate on reversal learning.', *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. Nature Publishing Group, 38(10), pp. 2011–8. doi: 10.1038/npp.2013.100.
Schlesinger, I., Erikh, I. and Yarnitsky, D. (2007) 'Paradoxical kinesia at war', *Movement Disorders*, 22(16), pp. 2394–2397. doi: 10.1002/mds.21739.
Schmidt, L. *et al.* (2008) 'Disconnecting force from money: effects of basal ganglia damage on incentive motivation.', *Brain : a journal of neurology*, 131(Pt 5), pp. 1303–10. doi: 10.1093/brain/awn045.
Schmidt, L. *et al.* (2014) 'Mind matters: placebo enhances reward learning in Parkinson's disease.', *Nature neuroscience*. Nature Publishing Group, (October), pp. 1–10. doi: 10.1038/nn.3842.
Schultz, W. (1999) 'The Primate Basal Ganglia and the Voluntary Control of Behaviour', (8), pp. 31–45.
Schultz, W. (2006) 'Behavioral theories and the neurophysiology of reward.', *Annual review of psychology*, 57, pp. 87–115. doi: 10.1146/annurev.psych.56.091103.070229.
Schultz, W. (2007) 'Behavioral dopamine signals.', *Trends in neurosciences*, 30(5), pp. 203–10. doi: 10.1016/j.tins.2007.03.007.
Schultz, W. (2010) 'Dopamine signals for reward value and risk: basic and recent data', *Behavioral and Brain Functions*, 6(1), p. 24. doi: 10.1186/1744-9081-6-24.
Schultz, W. (2016) 'Dopamine reward prediction error coding', *Dialogues Clin Neurosci*, 18(1), pp. 23–32.
Schultz, W., Dayan, P. and Montague, P. R. (1997) 'A neural substrate of prediction and reward.', *Science*, 275(June 1994), pp. 1593–1599. doi: 10.1126/science.275.5306.1593.
SE Starkstein, SE Mayberg, TJ Preziosi, et al (1992) 'Reliability, validity, and clinical correlates of apathy in Parkinson's disease', *The Journal of Neuropsychiatry and Clinical Neurosciences*. American Psychiatric Publishing, 4(2), pp. 134–139. doi: 10.1176/jnp.4.2.134.
Seymour, B., Singer, T. and Dolan, R. (2007) 'The neurobiology of punishment', *Nature*

Reviews Neuroscience, 8(4), pp. 300–311. doi: 10.1038/nrn2119.
Sharp, M. E. *et al.* (2016) 'Dopamine selectively remediates "model-based" reward learning: a computational approach', *Brain*, 139(2), pp. 355–364. doi: 10.1093/brain/awv347.
Shulman, L. M. (2000) 'Apathy in patients with Parkinsons disease', *International Review of Psychiatry*, 12(4), pp. 298–306. doi: 10.1080/09540260020002523.
Sinha, N., Manohar, S. and Husain, M. (2013) 'Impulsivity and apathy in Parkinson's disease.', *Journal of neuropsychology*, 7(2), pp. 255–83. doi: 10.1111/jnp.12013.
Skorvanek, M. *et al.* (2013) 'Apathy in Elderly Nondemented Patients With Parkinson's Disease: Clinical Determinants and Relationship to Quality of Life', *Journal of Geriatric Psychiatry and Neurology*, 26(4), pp. 237–243. doi: 10.1177/0891988713500587.
Skorvanek, M. *et al.* (2015) 'The associations between fatigue, apathy, and depression in Parkinson's disease', *Acta Neurologica Scandinavica*, 131(2), pp. 80–87. doi: 10.1111/ane.12282.
Snijders, A. H. and Bloem, B. R. (2010) 'Cycling for Freezing of Gait', *New England Journal of Medicine*, 362(13), p. e46. doi: 10.1056/NEJMicm0810287.
Sockeel, P. (2006) 'The Lille apathy rating scale (LARS), a new instrument for detecting and quantifying apathy: validation in Parkinson's disease', *Journal of Neurology, Neurosurgery & Psychiatry*, 77(5), pp. 579–584. doi: 10.1136/jnnp.2005.075929.
Sparks, D. L. (2002) 'The brainstem control of saccadic eye movements', *Nature Reviews Neuroscience*, pp. 952–964.
Spronk, D. B. *et al.* (2016) 'Acute effects of cocaine and cannabis on reversal learning as a function of COMT and DRD2 genotype', *Psychopharmacology*, 233(2), pp. 199–211. doi: 10.1007/s00213-015-4141-5.
Stanton, B. R. and Carson, A. (2015) 'Apathy: A practical guide for neurologists', *Practical Neurology*, p. practneurol-2015-001232. doi: 10.1136/practneurol-2015-001232.
Starkstein, S. E. *et al.* (1992) 'Reliability, validity, and clinical correlates of apathy in Parkinson's disease.', *The Journal of neuropsychiatry and clinical neurosciences*, 4(2), pp. 134–9. doi: 10.1176/jnp.4.2.134.
Starkstein, S. E. *et al.* (2001) 'Syndromic validity of apathy in Alzheimer's disease', *American Journal of Psychiatry*, 158(6), pp. 872–877. doi: 10.1176/appi.ajp.158.6.872.
Starkstein, S. E. and Leentjens, a F. G. (2008) 'The nosological position of apathy in clinical practice', *Journal of Neurology, Neurosurgery & Psychiatry*, 79(10), pp. 1088–1092. doi: 10.1136/jnnp.2007.136895.
Stevenson, S. A., Elsley, J. K. and Corneil, B. D. (2008) 'A "Gap Effect" on Stop Signal Reaction Times in a Human Saccadic Countermanding Task', *Journal of Neurophysiology*, 101(2), pp. 580–590. doi: 10.1152/jn.90891.2008.
Stuss, D., Van Reekum, R. and Murphy, K. (2000) 'Differentiation of states and causes of apathy', in *The neuropsychology of emotion*, pp. 340–363.
Stuss, D. T. (2011) 'Functions of the Frontal Lobes: Relation to Executive Functions', *Journal of the International Neuropsychological Society*, 17(5), pp. 759–765. doi: 10.1017/S1355617711000695.
Sugrue, L. P., Corrado, G. S. and Newsome, W. T. (2005) 'Choosing the greater of two goods: neural currencies for valuation and decision making.', *Nature reviews. Neuroscience*, 6, pp. 363–375. doi: 10.1038/nrn1666.

Suteerawattananon, M. *et al.* (2004) 'Effects of visual and auditory cues on gait in individuals with Parkinson's disease', *Journal of the Neurological Sciences*, 219(1–2), pp. 63–69. doi: 10.1016/j.jns.2003.12.007.
Swainson, R. *et al.* (2000) 'Probabilistic learning and reversal deficits in patients with Parkinson's disease or frontal or temporal lobe lesions: possible adverse effects of dopaminergic medication.', *Neuropsychologia*, 38(5), pp. 596–612. doi: 10.1016/S0028-3932(99)00103-7.
Syed, E. C. J. *et al.* (2015) 'Action initiation shapes mesolimbic dopamine encoding of future rewards', *Nature Neuroscience*, 19(1), pp. 34–36. doi: 10.1038/nn.4187.
Tachibana, Y. and Hikosaka, O. (2012) 'The primate ventral pallidum encodes expected reward value and regulates motor action.', *Neuron*. Elsevier Inc., 76(4), pp. 826–37. doi: 10.1016/j.neuron.2012.09.030.
Takikawa, Y. *et al.* (2002) 'Modulation of saccadic eye movements by predicted reward outcome.', *Experimental brain research. Experimentelle Hirnforschung. Expérimentation cérébrale*, 142(2), pp. 284–91. doi: 10.1007/s00221-001-0928-1.
Talmi, D., Atkinson, R. and El-Deredy, W. (2013) 'The Feedback-Related Negativity Signals Salience Prediction Errors, Not Reward Prediction Errors', *Journal of Neuroscience*, 33(19), pp. 8264–8269. doi: 10.1523/JNEUROSCI.5695-12.2013.
Terao, Y. *et al.* (2013) 'New perspectives on the pathophysiology of Parkinson's disease as assessed by saccade performance: A clinical review', *Clinical Neurophysiology*, pp. 1491–1506. doi: 10.1016/j.clinph.2013.01.021.
Thobois, S. *et al.* (2010) 'Non-motor dopamine withdrawal syndrome after surgery for Parkinson's disease: predictors and underlying mesolimbic denervation.', *Brain : a journal of neurology*, 133(Pt 4), pp. 1111–27. doi: 10.1093/brain/awq032.
Thobois, S. *et al.* (2013) 'Parkinsonian apathy responds to dopaminergic stimulation of D2/D3 receptors with piribedil.', *Brain : a journal of neurology*, 136, pp. 1568–77. doi: 10.1093/brain/awt067.
Thomsen, K. R., Whybrow, P. C. and Kringelbach, M. L. (2015) 'Reconceptualizing anhedonia : novel perspectives on balancing the pleasure networks in the human brain', 9(March), pp. 1–52. doi: 10.3389/fnbeh.2015.00049.
Thurstone, L. L. (1938) *Primary mental abilities.* Chicago: University of Chicago Press.
Tian, L. *et al.* (2015) 'Neuroimaging Analysis of the Dopamine Basis for Apathetic Behaviors in an MPTP-Lesioned Primate Model.', *PloS one*, 10(7), p. e0132064. doi: 10.1371/journal.pone.0132064.
Tom, S. M. *et al.* (2007) 'The neural basis of loss aversion in decision-making under risk.', *Science (New York, N.Y.)*, 315(5811), pp. 515–518. doi: 10.1126/science.1134239.
Torta, D. M. E. *et al.* (2009) 'On the role of dopamine replacement therapy in decision-making, working memory, and reward in Parkinson's disease: Does the therapy-dose matter?', *Brain and Cognition*. Elsevier Inc., 71(2), pp. 84–91. doi: 10.1016/j.bandc.2009.04.003.
Torta, D. M. E. and Castelli, L. (2008) 'Reward pathways in Parkinson's disease: clinical and theoretical implications.', *Psychiatry and clinical neurosciences*, 62(2), pp. 203–13. doi: 10.1111/j.1440-1819.2008.01756.x.
Tremblay, L. *et al.* (2015) 'Selective dysfunction of basal ganglia subterritories: From movement to behavioral disorders', *Movement Disorders*, 30(9), pp. 1155–1170. doi: 10.1002/mds.26199.
Turner, R. S. and Desmurget, M. (2010) 'Basal ganglia contributions to motor control: a

vigorous tutor', *Current Opinion in Neurobiology*. Elsevier Ltd, 20(6), pp. 704–716. doi: 10.1016/j.conb.2010.08.022.
Valerie Bonnelle, Kai-Riin Veromann, Stephanie Burnett Heyes, Elena Lo Sterzo, Sanjay Manohar, M. H. (no date) 'Characterization of reward and effort mechanisms in apathy'.
Vallerand, R. J., O'Connor, B. P. and Hamel, M. (1995) 'Motivation in later life: theory and assessment.', *International journal of aging & human development*, 41(3), pp. 221–38. doi: 10.2190/YLFM-DGUE-HRL2-VWLG.
Varazzani, C. *et al.* (2015) 'Noradrenaline and Dopamine Neurons in the Reward/Effort Trade-Off: A Direct Electrophysiological Comparison in Behaving Monkeys', *Journal of Neuroscience*, 35(20), pp. 7866–7877. doi: 10.1523/JNEUROSCI.0454-15.2015.
Wachter, T. *et al.* (2009) 'Differential Effect of Reward and Punishment on Procedural Learning', *Journal of Neuroscience*, 29(2), pp. 436–443. doi: 10.1523/JNEUROSCI.4132-08.2009.
Walton, M. E. *et al.* (2006) 'Weighing up the benefits of work: behavioral and neural analyses of effort-related decision making.', *Neural networks : the official journal of the International Neural Network Society*, 19(8), pp. 1302–14. doi: 10.1016/j.neunet.2006.03.005.
Walton, M. E., Bannerman, D. M. and Rushworth, M. F. S. (2002) 'The role of rat medial frontal cortex in effort-based decision making.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 22(24), pp. 10996–1003.
Wang, C.-A., Brien, D. C. and Munoz, D. P. (2015) 'Pupil size reveals preparatory processes in the generation of pro-saccades and anti-saccades', *European Journal of Neuroscience*, 41(February), p. n/a-n/a. doi: 10.1111/ejn.12883.
Wang, C.-A. and Munoz, D. P. (2015) 'A circuit for pupil orienting responses: implications for cognitive modulation of pupil size', *Current Opinion in Neurobiology*. Elsevier Ltd, 33(Figure 1), pp. 134–140. doi: 10.1016/j.conb.2015.03.018.
Wang, C. A. *et al.* (2016) 'Disruption of pupil size modulation correlates with voluntary motor preparation deficits in Parkinson's disease', *Neuropsychologia*. Elsevier, 80, pp. 176–184. doi: 10.1016/j.neuropsychologia.2015.11.019.
Wise, R. A. (2004) 'Dopamine, learning and motivation', *Nature Reviews Neuroscience*, 5(6), pp. 483–494. doi: 10.1038/nrn1406.
Wongpakaran, N. *et al.* (2007) 'Selective serotonin reuptake inhibitor use associates with apathy among depressed elderly: a case-control study.', *Annals of general psychiatry*, 6(7), pp. 1–6. doi: 10.1186/1744-859X-6-7.
Wu, Q. *et al.* (2002) 'Concurrent autoreceptor-mediated control of dopamine release and uptake during neurotransmission: an in vivo voltammetric study', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 22(14), pp. 6272–6281. doi: 20026630.
Xu-Wilson, M., Zee, D. S. and Shadmehr, R. (2009) 'The intrinsic value of visual information affects saccade velocities.', *Experimental brain research. Experimentelle Hirnforschung. Expérimentation cérébrale*, 196(4), pp. 475–81. doi: 10.1007/s00221-009-1879-1.
Yager, L. M. *et al.* (2015) 'The ins and outs of the striatum: role in drug addiction.', *Neuroscience*, 301(June), pp. 529–41. doi: 10.1016/j.neuroscience.2015.06.033.
Ziropadja, L. *et al.* (2012) 'Apathy and depression in Parkinson's disease: the Belgrade PD study report.', *Parkinsonism & related disorders*. Elsevier Ltd, 18(4), pp. 339–42. doi: 10.1016/j.parkreldis.2011.11.020.

9. Appendix

Lille Apathy Rating Scale – LARS

- Instructions for administration of the Lille Apathy Rating Scale –

The Lille Apathy Rating Scale (LARS) comprises 33 queries belonging to nine domains, each corresponding to a clinical manifestation of apathy.

The interview is structured and the questions should to be posed exactly as stated. To obtain the best validity, it is not advisable to change the vocabulary or to add additional comments to the questions. Before beginning the interview, the patient has to be instructed as follows:

"*I am going to ask you some questions about your daily life. It is important that you base your answers on your life over the last four weeks*"

If the patient evokes general events or any that predate the last month, he or she must be reminded that only the current situation must be referred to: "*Please try to answer according to your current way of life, by referring to the last four weeks*"

A precise scoring mode is proposed for each reply and should be followed as closely as possible. When an item does not apply to the patient, it is scored "0", for non-applicable (NA). When the reply is not clear at all and cannot be classified, it is also scored "0" for a non-classifiable reply. The scale's overall score ranges from –36 to +36

- Lille Apathy Rating Scale –

1. Everyday productivity

- What do you do during the day? Tell me about your day-to-day life.

Time taken to reply[1]

no reply	2
reply after prompting	1
spontaneous reply but only after some time	0
immediate reply, one activity mentioned without hesitation	-1
immediate reply, several activities mentioned without hesitation	-2

Number and variety of activities mentioned

none	2
one activity but prompting needed to obtain another	1
several activities mentioned	0
detailed organisation of a typical day but every day follows the same schedule.	-1
detailed organisation of a typical day but the reply shows that the activities change according to the day of the week or the time of year (for example housework, going to the cinema, watching TV, gardening, visiting friends, etc.)	-2

Interests

• What are you interested in? What do you like doing to keep yourself occupied?

[1] The delay must reflect a deficit in or absence of reactivity from the subject. Delays due to speaking or word-finding difficulties should not be considered when scoring these items

Time taken to reply

no reply	2
reply after prompting	1
spontaneous reply but only after some time	0
immediate reply, one activity mentioned without hesitation	-1
immediate reply, several activities mentioned without hesitation	-2

Number of activities mentioned

none or only one	1
several	0
regrets having to choose between so many activities	-1

- How many times a week do you …(*do the first hobby or pastime mentioned above*)?

Less than once a week	1
Once or several times a week	0
Regrets not being able to devote more time to the activity	-1

3. Taking the initiative

- In general, do you decide to do things or does someone have to push you a little?

I have to be pushed		1
N.A. ☐	Non-classifiable reply ☐	0
I decide to do things myself		-1

- When you have to go to an appointment, a meeting or a formal occasion, do you have to be told to get yourself ready?

I need to be told		1
N.A. ☐	Non-classifiable reply ☐	0
I get ready spontaneously		-1

- When you have to make an appointment (for example with the doctor or dentist), do you do it yourself or do you wait for someone to do it for you?

I wait for someone to do it for me		1
N.A. ☐	Non-classifiable reply ☐	0
I do it myself		-1

- Do you take part spontaneously in daily living activities or do you need to be asked?

I have to be asked		1
N.A. ☐	Non-classifiable reply ☐	0
I take part spontaneously		-1

4. Novelty seeking

- Do you like finding out about something new (a new TV programme or a new book)?

No, that doesn't interest me		1
N.A. ☐	Non-classifiable reply ☐	0
Yes, that interests me		-1

- Do you like trying out new products, tools or recipes that you're not familiar with?

No, that doesn't interest me		1
N.A. ☐	Non-classifiable reply ☐	0
Yes, I like trying things I'm not familiar with		-1

- Do you like visiting places you've never been to before?

No, that doesn't interest me		1
N.A. ☐	Non-classifiable reply ☐	0
Yes, I like visiting places I've never been to before		-1

- When you go out for a drive or when you're travelling by train or bus, do you enjoy looking at the countryside, the houses?

No, that doesn't interest me	1
N.A. ☐ Non-classifiable reply ☐	0
Yes, I like to see if anything has changed	-1

5. Motivation - Voluntary actions

- When you decide to do something, are you easily able to make an effort or is it difficult?

I find it difficult to make an effort	1
N.A. ☐ Non-classifiable reply ☐	0
I can easily make an effort	-1

- When you don't manage to do something, do you try to find other solutions?

No, I give up	1
N.A. ☐ Non-classifiable reply ☐	0
Yes, I try again	-1

- When you decide to do something, do you see it through to the end or do you tend to give up?

I tend to give up (I am easily discouraged)	1
N.A. ☐ Non-classifiable reply ☐	0
I see it through to the end	-1

- When you can't find something (for example a document or an object), do you go to a lot of trouble looking for it?

No, if I don't find it quickly, I stop looking	1
N.A. ☐ Non-classifiable reply ☐	0
Yes, I keep looking until I find it	-1

6. Emotional responses

- When you watch a film, do you easily become emotional or moved?

No, I don't experience any particular emotion	1
N.A. ☐ Non-classifiable reply ☐	0
Yes, I am easily moved	-1

- When someone tells you a joke or when you watch a comedy sketch on TV, do you laugh easily?

No, I don't experience any particular emotion	1
N.A. ☐ Non-classifiable reply ☐	0
Yes, it makes me laugh	-1

- Do you feel happy when you hear some good news?

No, I don't experience any particular emotion	1
N.A. ☐ Non-classifiable reply ☐	0
Yes, I'm happy	-1

- Do you feel sad when you hear some bad news?

No, I don't experience any particular emotion	1
N.A. ☐ Non-classifiable reply ☐	0
Yes, I'm sad, it worries me	-1

7. Concern

- When you have a problem (for example when your TV set breaks down), does it worry you?

No		1
N.A. ☐	Non-classifiable reply ☐	0
Yes, I worry easily		-1

- When something's not working or when something unexpected happens, do you think about finding a solution?

No, I give up		1
N.A. ☐	Non-classifiable reply ☐	0
Yes, I look for a solution		-1

- When your partner or children have a minor problem (when they're ill, for example), does that concern you, do you worry about them?

No, I don't feel very concerned about that		1
N.A. ☐	Non-classifiable reply ☐	0
Yes, I worry.		-1

- Do you like to ask how your family and friends are on a regular basis?

No, often I wait until someone tells me how they are		1
N.A. ☐	Non-classifiable reply ☐	0
Yes, I often ask them how they are (I phone them, etc).		-1

8. Social life

- Do you have friends?

No, not many or I don't see them any more		1
N.A. ☐	Non-classifiable reply ☐	0
Yes, and having friends matters a lot to me		-1

- When you meet friends, do you enjoy spending time with them or it is a chore?

It's a chore		1
N.A. ☐	Non-classifiable reply ☐	0
I enjoy it		-1

- In conversation, do you start talking or do the others tend to speak to you first?

I only talk if someone starts talking to me		1
N.A. ☐	Non-classifiable reply ☐	0
I start talking with no prompting		-1

- During a discussion, do you give your own opinion spontaneously or do you fall into line with someone else's opinion?

I tend to fall into line with someone else's opinion		1
N.A. ☐	Non-classifiable reply ☐	0
I give my own opinion spontaneously		-1

9. Self-awareness

- When you've finished doing something, do you take stock of the situation and think about what is going well and what's not?

No, I don't think about the end result	1
N.A. ☐ Non-classifiable reply ☐	0
Yes, I take stock of the situation	-1

- After having taken a decision, do you sometimes think that you've made the wrong choice?

No, I'm happy with the choice I make	1
N.A. ☐ Non-classifiable reply ☐	0
Yes, I sometimes regret having made certain choices	-1

- When you've been unpleasant to someone, do you sometimes feel guilty afterwards?

No, I don't care	1
N.A. ☐ Non-classifiable reply ☐	0
Yes, I'm ashamed of myself	-1

- If, during a discussion, you realize that you're in the wrong, are you able to admit it - at least to yourself?

No, I don't admit that I'm in the wrong	1
N.A. ☐ Non-classifiable reply ☐	0
Yes, I admit it.	-1

Total score	/36

Sub-scales		Scores
Everyday productivity	EP	-4 -3 -2 -1 0 1 2 3 4
Interests	INT	-4 -3 -2 -1 0 1 2 3 4
Taking the initiative	INI	-4 -3 -2 -1 0 1 2 3 4
Novelty seeking	NS	-4 -3 -2 -1 0 1 2 3 4
Motivation - Voluntary actions	M	-4 -3 -2 -1 0 1 2 3 4
Emotional responses	ER	-4 -3 -2 -1 0 1 2 3 4
Concern	C	-4 -3 -2 -1 0 1 2 3 4
Social life	SL	-4 -3 -2 -1 0 1 2 3 4
Self-awareness	SA	-4 -3 -2 -1 0 1 2 3 4

Factorial sub-scores are calculated from sub-scale scores using the formulas given below.

Factorial sub-scores			Scores
Intellectual curiosity	(INT+NS+M+SL)/4	IC	-4 -3 -2 -1 0 1 2 3 4
Emotion	(ER+C)/2	E	-4 -3 -2 -1 0 1 2 3 4
Action initiation	(EP+INI)/2	AI	-4 -3 -2 -1 0 1 2 3 4
Self-awareness	(SA)	SA	-4 -3 -2 -1 0 1 2 3 4

Extended Lille Apathy Rating Scale - LARS-E

Below are a number of statements. Each statement asks you to think about your life over the last 2 weeks.

For each statement, circle a number 1-5 to indicate how true the statement is of you over the last 2 weeks, i.e. how appropriately it describes your life right now.

Circle “1” if the statement does not describe you at all over the last 2 weeks, “5” if the statement describes you perfectly, and use the numbers in between accordingly. Circle “NA” if the statement is not applicable to you over the last 2 weeks.

1 = Completely UNTRUE: The statement is exactly the opposite of me over the last 2 weeks
2 = Mostly untrue of me over the last 2 weeks
3 = Neither untrue nor true of me over the last 2 weeks
4 = Quite true of me over the last 2 weeks
5 = Completely TRUE: The statement exactly describes me over the last 2 weeks

	In the last 2 weeks…	UN-TRUE = TRUE					
1 E-ER	I felt bad when I heard an acquaintance had had an accident or illness.	1	2	3	4	5	NA
2 AI-EP	I didn’t like to laze around.	1	2	3	4	5	NA
3 IC-N	If a new restaurant opened locally, I'd want to try it out.	1	2	3	4	5	NA
4 SA	If I realised I'd been unpleasant to someone, I felt terribly guilty afterwards.	1	2	3	4	5	NA
5 AI-EP	I liked to have a busy schedule.	1	2	3	4	5	NA
6 IC-S	I started conversations with random people.	1	2	3	4	5	NA
7 AI-EP	I usually got up in the morning even if I didn't have to go to work.	1	2	3	4	5	NA
8 IC-M	When I didn’t manage to do something, I tried to find other solutions.	1	2	3	4	5	NA
9 IC-S	I enjoyed spending time with my friends.	1	2	3	4	5	NA
10 SA	If I couldn't do something, I accepted help from others.	1	2	3	4	5	NA
11 AI-I	I got things done when they need to be done, without being reminded by others.	1	2	3	4	5	NA
12 AI-I	I made decisions firmly and without hesitation.	1	2	3	4	5	NA
13 IC-N	If someone had asked me “Would you like to visit every continent in the world?” I’d have said yes.	1	2	3	4	5	NA
14 AI-I	When I had something I needed to do, I did it straightaway so it was out of the way.	1	2	3	4	5	NA

	In the last 2 weeks…	UN-TRUE = TRUE					
15 SA	I changed my mind during an argument when I realised the other person might be right.	1	2	3	4	5	NA
16 AI-I	I liked being in charge.	1	2	3	4	5	NA
17 IC-M	If I had made a list of my attributes, my ability to push myself to achieve would have been one of them.	1	2	3	4	5	NA
18 E-ER	I laughed out loud when listening to a comedy show.	1	2	3	4	5	NA
19 IC-N	I enjoyed visiting new places.	1	2	3	4	5	NA
20 IC-M	When I couldn't find something (e.g. a document), I went to a lot of trouble looking for it.	1	2	3	4	5	NA
21 SA	Based on the last 2 weeks, I'd say I care deeply how my loved ones think of me.	1	2	3	4	5	NA
22 AI-EP	If I didn't achieve my goals for the day, I got annoyed.	1	2	3	4	5	NA
23 E-ER	I felt sad or upset when I heard bad news.	1	2	3	4	5	NA
24 AI-I	If I had to RSVP to an invitation, I did it promptly, without needing a reminder.	1	2	3	4	5	NA
25 IC-S	I suggested activities for me and my friends to do.	1	2	3	4	5	NA
26 IC-I	I enjoyed choosing what to do from a range of activities.	1	2	3	4	5	NA
27 IC-N	I enjoyed doing things with people I'd just met.	1	2	3	4	5	NA
28 AI-I	I was easily able to decide to do things by myself, without needing someone to push or encourage me.	1	2	3	4	5	NA
29 IC-S	I started conversations without being prompted.	1	2	3	4	5	NA
30 E-ER	When I listened to music, it could affect me deeply.	1	2	3	4	5	NA
31 IC-N	I enjoyed trying out products, tools or recipes that I was unfamiliar with.	1	2	3	4	5	NA
32 SA	After having taken a decision, I wondered if I ‘d made the wrong choice.	1	2	3	4	5	NA
33 IC-M	When I decided to do something, I was motivated to see it through to the end.	1	2	3	4	5	NA
34 IC-S	If I hadn't seen a close friend for a while, I got in touch by phone/internet.	1	2	3	4	5	NA
35 SA	When I finished doing something, I took stock of the situation to identify what was going well and what wasn't.	1	2	3	4	5	NA
36 IC-N	I enjoyed learning new things at work.	1	2	3	4	5	NA
37 IC-S	I was pleased when I received unexpected invitations from friends.	1	2	3	4	5	NA
38 IC-I	I would say I've been quite interested in life.	1	2	3	4	5	NA

	In the last 2 weeks…	UN-TRUE = TRUE					
39 IC-M	When I decided to do something, I was easily able to make an effort.	1	2	3	4	5	NA
40 SA	I felt awful if I said something insensitive.	1	2	3	4	5	NA
41 IC-I	I've been feeling there is so much to do in life and I want to try everything.	1	2	3	4	5	NA
42 E-ER	When someone told me a joke I laughed easily.	1	2	3	4	5	NA
43 AI-EP	Getting something done by 9am gave me a sense of satisfaction.	1	2	3	4	5	NA
44 SA	If, during a discussion, I realised I was in the wrong, I admitted it – at least to myself.	1	2	3	4	5	NA
45 IC-S	I've been going out with friends on a weekly basis.	1	2	3	4	5	NA
46 IC-M	I found it annoying when I was in the middle of doing a task at work and I had to stop to do something else.	1	2	3	4	5	NA
47 IC-I	I wished I had more spare time for hobbies and leisure activities.	1	2	3	4	5	NA
48 IC-M	If I thought of something I needed to do, I wrote it down so I wouldn't forget.	1	2	3	4	5	NA
49 IC-M	I tried out new ways of doing things at work or home.	1	2	3	4	5	NA
50 E-ER	I felt anxious when I was waiting to hear important news (e.g. exam result or job interview).	1	2	3	4	5	NA
51 E-ER	I was easily 'moved' by films/TV programs.	1	2	3	4	5	NA

Apathy Motivation Index – AMI

Below are a number of statements. Each statement asks you to think about your life over the last 2 weeks. For each statement, select how appropriately it describes your life right now. Select "Completely true" if the statement describes you perfectly, "Completely untrue" if the statement does not describe you at all over the last 2 weeks, and use the answers in between accordingly.

		Completely UNTRUE	Mostly untrue	Neither true nor untrue	Quite true	Completely TRUE
1	I feel sad or upset when I hear bad news.	☐	☐	☐	☐	☐
2	I start conversations with random people.	☐	☐	☐	☐	☐
3	I enjoy doing things with people I have just met.	☐	☐	☐	☐	☐
4	I suggest activities for me and my friends to do.	☐	☐	☐	☐	☐
5	I make decisions firmly and without hesitation.	☐	☐	☐	☐	☐
6	After making a decision, I will wonder if I have made the wrong choice.	☐	☐	☐	☐	☐
7	Based on the last two weeks, I will say I care deeply about how my loved ones think of me.	☐	☐	☐	☐	☐
8	I go out with friends on a weekly basis.	☐	☐	☐	☐	☐
9	When I decide to do something, I am able to make an effort easily.	☐	☐	☐	☐	☐
10	I don't like to laze around.	☐	☐	☐	☐	☐
11	I get things done when they need to be done, without requiring reminders from others.	☐	☐	☐	☐	☐
12	When I decide to do something, I am motivated to see it through to the end.	☐	☐	☐	☐	☐
13	I feel awful if I say something insensitive.	☐	☐	☐	☐	☐
14	I start conversations without being prompted.	☐	☐	☐	☐	☐
15	When I have something I need to do, I do it straightaway so it is out of the way.	☐	☐	☐	☐	☐
16	I feel bad when I hear an acquaintance has an accident or illness	☐	☐	☐	☐	☐
17	I enjoy choosing what to do from a range of activities.	☐	☐	☐	☐	☐
18	If I realise I have been unpleasant to someone, I will feel terribly guilty afterwards.	☐	☐	☐	☐	☐

Scoring Instructions

Each item is negatively scored i.e. you will need to REVERSE ALL ITEMS:
Completely TRUE = 0
Quite true = 1
Neither true nor untrue = 2
Mostly untrue = 3
Completely UNTRUE = 4

Three domains of apathy-motivation are assessed with the mean score, which ranges from 0-4 (with 0 being motivated and 4 being apathetic).

(1) Behavioural: Q5, 9, 10, 11, 12, 15
(2) Social: Q2, 3, 4, 8, 14, 17
(3) Emotional: Q1, 6, 7, 13, 16, 18