The use of Real-Time fMRI Neurofeedback to Enhance Post-Stroke Motor Recovery



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Abstract

Real-time fMRI neurofeedback is a tool that allows individuals to self-modulate their ongoing brain activity. This may have clinical potential in disorders which are associated with aberrant brain activity, for example in stroke-induced hemiparesis.

In this thesis two studies are presented, which aim to explore the use of fMRI neurofeedback in chronic stroke patients suffering from hemiparesis. The first study examined the effect of having multiple training sessions and whether this boosted neurofeedback learning in healthy individuals. This was examined across two different conditions in which ipsilateral motor cortex activity was either up or down-regulated. Whilst cumulative effects over multiple training sessions could be observed when up-regulating ipsilateral activity, there was no apparent cumulative effect in the condition where ipsilateral activity was down-regulated.

The second study presented in this thesis is a double-blind, sham-controlled randomised trial in chronic stroke patients, in which patients were instructed to use neurofeedback to increase lateralisation of motor cortex activity. Patients were able to use neurofeedback to increase lateralisation of brain activity within training days. However, there was not any evidence that patients could continue to modulate brain activity after feedback was removed, although there were indications that this is related to how well patients performed during the neurofeedback training.

Additionally, improvements in gross motor performance of the stroke-affected limb were observed in the group receiving Real neurofeedback, compared to the Sham group. However, there was not any evidence of improvements on clinical measures of upper limb functioning. There was also evidence of changes in brain function and structure. This included increased activity in neurofeedback-related brain regions during a visuomotor squeeze task, as well as changes in the white-matter microstructure of the corticospinal tracts.

Finally, variability in patients' neurofeedback learning success was explored with respect to baseline characteristics of the patients. No relationship could be found with baseline upper limb functioning, however there were some indications of relationships with baseline brain activity and brain structure which could help inform future studies aiming to predict neurofeedback outcomes.

Together, this work provides novel insight into the potential of real-time fMRI neurofeedback as a tool for stroke rehabilitation and opens up opportunities for future exploration of flexible and personalised treatment approaches using neurofeedback.

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1
General Introduction

1.1 Background

Stroke is a common and debilitating neurological condition that affects more than 100,000 people in the UK each year (Stroke Association 2018). Improvements in medical treatment mean that more people than ever are surviving strokes (Feigin et al. 2014), with approximately 1.2 million stroke survivors living in the UK (Stroke Association 2018). However, stroke remains one of the leading causes of complex adult disability, with a majority of patients reporting severe disability (Adamson et al. 2004). The most common disability to occur after stroke is motor impairments, with ~75% of patients suffering from upper or lower limb impairment. This can have a big impact on patients' independence, making it difficult to carry out daily tasks such as dressing or washing (Veerbeek et al. 2011), and can have a negative impact on patients' quality of life (Franceschini et al. 2010).

1.1.1 Timeline of stroke recovery

A stroke occurs when the blood supply to a region of the brain is interrupted due to a blockage (ischaemic stroke) or a burst blood vessel (haemorrhagic stroke), which leads to brain tissue being deprived of oxygen and nutrients. Immediately after a stroke occurs there is a period of cell death, the lack of blood supply leads to a collapse in the maintenance of neuronal resting membrane potential, triggering increased depolarisation of neurons and ultimately causing excitotoxicity. This results in an infarct core of dead cell tissue, surrounded by perilesional tissue in which partial neuronal loss can also occur (Carmichael

2016). Additionally, focal stroke can lead to a disconnection of distant but related brain regions, and disrupt the functioning of brain networks (Carrera & Tononi 2014). For example, a stroke in the brain white matter can lead to thinning of connected cortical areas (Duering et al. 2015). However, not long after the initial insult, a period of endogenous spontaneous recovery is observed, during which a substantial, albeit highly variable, amount of recovery occurs, which typically plateaus around 3 months post-stroke (Duncan et al. 1992; Hankey et al. 2007). Mechanisms underlying this initial recovery include increased neuro- and gliogenesis, axonal sprouting and glial progenitor responses (Carmichael 2016).

Some studies have tried to take advantage of this limited period of heightened recovery by attempting to intervene with intensive rehabilitation in this early phase post-stroke. For example, Ng and colleagues (2015) used a mouse stroke model to explore whether responsiveness to intensive training differed depending on how early after a stroke it was initiated. They found that the mice made a remarkable recovery if training was initiated on the first day after the stroke, in contrast, if the training start was delayed by one week the mice were unable to fully regain functioning on the experimental task. This has led to the suggestion that as time progresses after stroke, there is a diminishing response to training, and that an early phase of heightened plasticity could allow full functional recovery to occur. The authors argue this may in part also be due to temporary shifts in the cortical excitatory-inhibitory balance, which may allow cortical remapping to occur (Carmichael 2012; Schiene et al. 1996). Therefore, it is possible that earlier interventions in humans may also lead to heightened recovery, but it has been highlighted that in the early weeks after experiencing a stroke, patients often experience little rehabilitation and stimulation on the whole (Bernhardt et al. 2004). Nevertheless, currently there is little evidence of a

beneficial effect of early interventions in humans (Zeiler 2019), with some very early intervention trials instead finding a potentially negative effect of early intense rehabilitation (Dromerick et al. 2009; Langhorne et al. 2017).

Beyond this initial period of recovery, many patients reach a plateau in their recovery after the first months, and are often left with debilitating, long-term impairments in their motor functioning. Additional intensive rehabilitation in the chronic phase post-stroke can further improve motor functioning (for example: Park et al., 2016; Wu et al., 2016), however providing one-to-one training is often time-consuming and expensive. Therefore, there has been increasing interest in developing strategies and techniques to further boost motor recovery after stroke.

1.1.2 Theories of motor recovery after stroke

Recovery after stroke is thought to occur via two different mechanisms. True recovery or restitution is used to refer to a return to pre-morbid functioning using the same rudimentary motor patterns. Alternatively, recovery can occur through compensation which refers to the use of alternative strategies or motor patterns to achieve a task; for example, if a patient uses their unaffected hand in order to perform a task. On a neural level, restitution may occur through the restoration of functioning of initially damaged tissue, whereas compensation may involve recruitment of alternative brain regions not previously involved in a function (Levin et al. 2009).

Interhemispheric competition model

A prominent theory in the stroke literature, and the basis of many previous neuromodulation studies (see below), is the idea of an imbalance in inhibition between the

two hemispheres following a stroke (Ward & Cohen 2004). In healthy individuals the two hemispheres mutually inhibit each other via the corpus callosum. However, after a stroke there is decreased activity in the ipsilesional hemisphere due to the stroke damage, which in turn leads to disinhibition of the contralesional hemisphere. Disinhibition of the contralesional hemisphere is thought to be maladaptive as this can lead to increased and unopposed inhibition of the ipsilesional hemisphere, thereby potentially hindering motor recovery. Evidence for this comes from studies showing that more bilateral activity patterns (Figure 1.1A; Ward et al., 2003), and a failure to release interhemispheric inhibition before movement onset (Murase et al. 2004), are correlated with worse motor functioning. Therefore, this theory would suggest that down-regulating 'maladaptive' contralesional motor cortex activity, or increasing ipsilesional motor cortex activity may be beneficial, by (either directly or indirectly) reducing inhibition to the ipsilesional motor cortex and *'re-normalising'* brain activity patterns (Figure 1.1B).

Conversely, there have also been some suggestions that contralesional activity could in fact be adaptive in some cases (Buetefisch 2015). In healthy individuals, increased activity can be observed in ipsilateral motor cortex during complex motor tasks (Barany et al. 2020; Buetefisch et al. 2014; Verstynen et al. 2005), and activity in ipsilateral motor cortex appears to increase with normal ageing (Ward & Frackowiak 2003; Ward et al. 2008), indicating that ipsilateral motor cortex may play a role in normal motor functioning. This could suggest that bilateral brain activity in stroke patients might be due to stroke patients finding motor tasks more challenging, due to their impaired hand functioning. In line with this, there have been some imaging studies demonstrating bilateral activity patterns in stroke patients with good or excellent motor recovery (Bütefisch et al. 2005; Schaechter & Perdue 2008). Additionally, it has been found that temporary 'silencing' of contralesional

motor cortex using Transcranial Magnetic Stimulation (TMS) during motor sequence production impairs performance in patients (Lotze et al. 2006). On the other hand, studies have also found beneficial effects of silencing contralesional motor cortex (Grefkes et al. 2010; Takeuchi et al. 2005). These inconsistencies have been hypothesised to be somewhat related to the extent of damage to the cortico-spinal tract (CST; Biernaskie et al., 2005; Di Pino et al., 2014; Lotze et al., 2012), how much time has passed since the stroke (Grefkes & Ward 2014) and on the specific task demands (Volz et al. 2017). Together this suggests that in some cases it could be beneficial to attempt to upregulate *'compensatory'* activity in contralesional motor cortex (Figure 1.1B).

However, there has been some recent evidence challenging the interhemispheric competition model. Mcdonnell and Stinear (2017) recently carried out a meta-analysis of over 100 previous TMS studies investigating interhemispheric interactions and motor cortex excitability. They found that while there was evidence of reduced excitability of the stroke-affected hemisphere across the studies, no clear evidence could be found for increased excitability of the stroke-unaffected hemisphere, or imbalances in interhemispheric inhibition. The authors concluded that therapeutic interventions should focus directly on increasing excitability of the stroke-affected hemisphere. Additionally, a recent longitudinal study found that interhemispheric interactions appear to be normal in the acute stage post-stroke, when patients are most impaired, and only becomes abnormal in the chronic stage, although emergence of abnormal interhemispheric inhibition was associated with poor recovery of finger individuation (Xu et al. 2019). The authors suggest that abnormal interhemispheric inhibition is a marker for the state of recovery, rather than a cause of poor recovery. However, as pointed out by the authors, these results cannot be generalised to patients from which it is not possible to obtain motor-evoked potentials,

which is typically the case in the more severely affected patients. Therefore, whilst the interhemispheric competition model has informed and inspired a large amount of stroke research, there appears to be some discrepancy regarding which approach is the best to use and this seems, in part, to be due to the large heterogeneity of the stroke population (see also **predictors and variability in response** below).

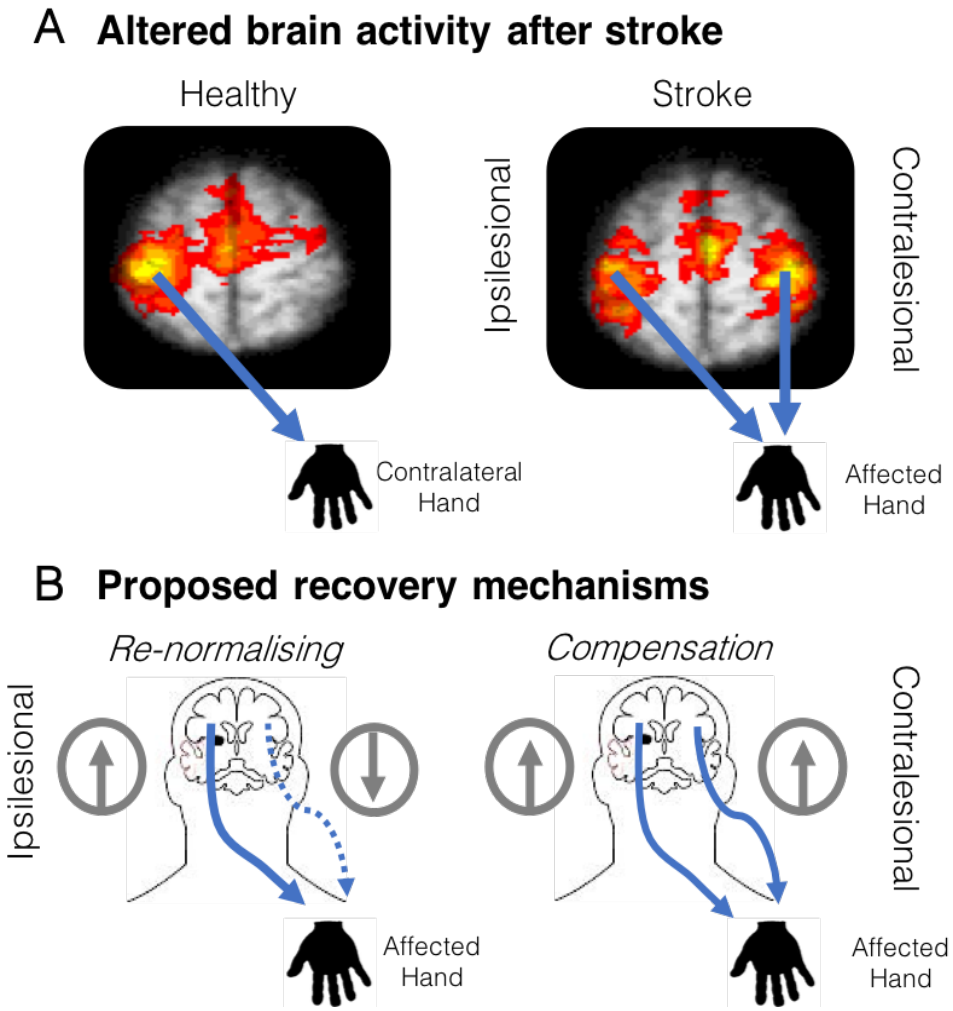


Figure 1.1 Aberrant brain activity is observed when stroke patients move their stroke affected hand. A) In comparison to healthy individuals (left), a more bilateral activity pattern is observed in stroke patients (right), with increased activity in ipsilateral/contralesional sensorimotor cortex. Adapted from Johansen-Berg et al (2002). **1B**: Some patients may benefit from rehabilitation techniques aimed at *re-normalising* brain activity by decreasing contralesional activity and increasing ipsilesional activity. Alternatively, other patients, especially those with greater impairments, may benefit from increasing *compensatory* activity in the contralesional sensorimotor cortex.

Descending fibres

The CST is the major descending white matter tract connecting motor areas in the brain with the spinal cord, which in primates, includes mono-synaptic connections terminating directly on motor neurons and interneurons in the spinal cord. This direct connection to spinal motoneurons is thought to play an important role in dextrous, fine-grained control of the hand (Lemon 2008), allowing primates to interact with the environment in a complex and distinctive manner. Damage to the CST, for example due to a stroke, leads to paralysis in the contralateral limb, and evidence suggests that the extent of damage to the CST is related to the severity of motor impairment and the likelihood of recovery (Stinear et al. 2007; Zhu et al. 2010). Whilst primarily projecting contralaterally, with fibres decussating in the medulla, some CST fibres do not decussate and instead project ipsilaterally (Kuypers 1981). Additionally, some CST fibres that initially decussate in the medulla, later cross back within the spinal cord by generating intraspinal collaterals (Figure 1.2A; Rosenzweig et al., 2009). This has been hypothesised to potentially underlie why some stroke patients display additional weakness in their stroke-unaffected arm/hand (Noskin et al. 2008). Ipsilaterally projecting CST fibres from the healthy hemisphere have been suggested to potentially play a role in recovery of function after a unilateral stroke (Schaechter et al. 2009). However, this idea has been challenged by research showing that in healthy primates there are no direct inputs from the ipsilateral CST to forearm or hand muscle motor neurons (Soteropoulos et al. 2011), and this does not appear to change after a stroke (Zaaimi et al. 2012), making it unlikely that these fibres play a role in recovery of distal upper limb movements after stroke.

Alternatively, some restoration of function has been suggested to occur via the recruitment of alternative descending pathways, such as the reticulospinal tract (RST; Baker, 2011;

McPherson et al., 2018). The RST originates in the ponto-medullary reticular formation and receives input from ipsilateral motor cortices, projecting bilaterally onto the spinal cord. It is hypothesised to play a role in more gross motor functioning of the hand due to its divergent output, making it unable to selectively activate individual muscles needed for dextrous hand functioning (Baker 2011; Herbert et al. 2015). Zaaimi and colleagues (2012) provided evidence for a role of the RST in motor recovery in macaque monkeys. After creating CST lesions and allowing monkeys to recover for 6 months, the authors found that excitatory post-synaptic potentials in motor neurons were significantly increased when stimulating the medial brainstem pathways (consisting primarily of RST fibres, Figure 1.2B), whereas there was no change when stimulating ipsilateral CST fibres. However, this was only the case for motor neurons innervating forearm flexor and intrinsic hand muscles, with no strengthening of input to extensor muscles observed. This may explain the common stroke phenotype consisting of extensor weakness and flexor spasms. Indeed flexor synergies (McPherson et al. 2018), as well as mirror movements (Ejaz et al. 2018) after stroke have been hypothesised to be due to increased recruitment of RST, at the cost of fine-motor control. This has led to the suggestions that the chronic stroke behavioural phenotype may be due in part to an imbalance in the interaction between the RST and the remaining CST (Krakauer & Carmichael 2017).

Imaging studies have also provided some evidence for plasticity of contralesional RST in humans and relationships with motor functioning. For example, a recent brainstem and spinal cord imaging study investigated white matter changes in chronic stroke patients compared to age-matched healthy controls (Karbasforoushan et al. 2019). In this study, increased white matter integrity of the contralesional RST was found in stroke patients, and this was correlated with motor impairment. This suggests an increased reliance on this

tract after stroke, especially in the most severely affected patients. Therefore, there appears to be some evidence for a role of the RST in motor recovery after stroke, although this seems to come at the cost of fine motor skills which appear to rely heavily on the CST.

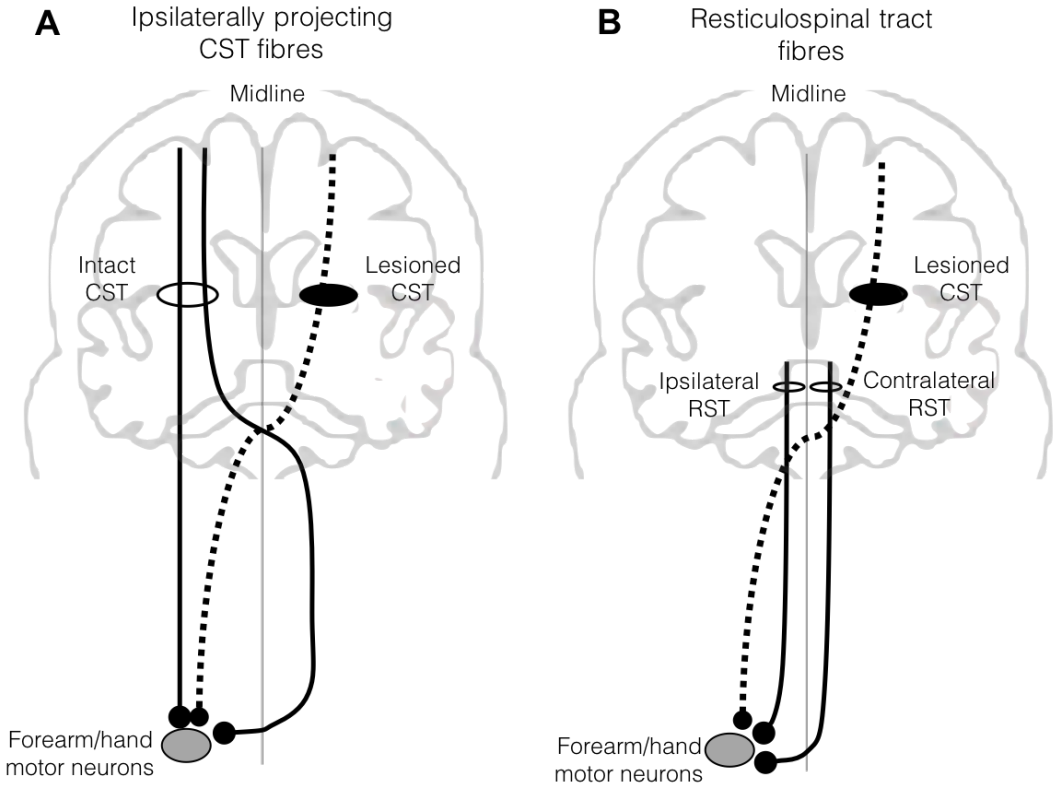


Figure 1.2 Potential alternative descending pathways which may play a role in motor recovery after stroke. Lesioned CST is shown in dashed line. **A)** Hypothetical pathways descending via ipsilateral (intact) CST that may contribute novel connections after stroke are shown in black. **B)** Reticulospinal tract shown in black. Adapted from Zaaimi et al., 2012.

Role of secondary motor areas

As mentioned above, stroke patients, in comparison to healthy adults, often have altered brain activity patterns when moving their stroke affected limb. Increased activity has been observed in multiple secondary motor areas, as well as in the cerebellum (Grefkes et al. 2008; Ward et al. 2003a; Weiller et al. 1992). This could be due to stroke patients' need to recruit additional neural substrates in order to perform movements with their stroke-affected limb. Evidence that some of these brain regions may play a role in motor functioning after stroke comes from studies in which functioning of brain regions is

disrupted. For example, disrupting the function of the Supplementary Motor Area (SMA) in recovered rhesus monkeys with motor cortical strokes, led to a re-introduction of motor impairment suggesting that the SMA supported recovery of motor functioning (McNeal et al. 2010). Similarly, evidence has shown that disrupting premotor cortex (PMC) function can also lead to worsening of motor impairments (Fridman et al. 2004; Johansen-Berg et al. 2002b). In a review it was proposed that PMC may exert an effect on motor functioning by either strengthening its connectivity with residual ipsilesional M1, or by increasing its inputs directly to CST (Kantak et al. 2012). Furthermore, it was proposed that whether ipsi- or contralesional PMC is recruited to aid recovery may depend on lesion size, with ipsilesional PMC reorganisation occurring with smaller lesions, whereas contralesional PMC may play a role when larger lesions occur by increasing facilitatory input to ipsilesional M1 via transcallosal pathways (Bestmann et al. 2010).

Structural imaging studies have also provided some evidence of secondary motor areas playing a role in motor recovery after stroke. For example, a previous study using diffusion-weighted imaging and tractography has suggested that the microstructural integrity of the CST originating from PMC is related to motor recovery (Schulz et al. 2012). A further study from the same group suggested that reliance on ipsilesional PMC-M1 connectivity differed depending on the extent of damage to the CST (Schulz et al. 2017), suggesting that with greater damage to the CST there is increased dependency on the PMC-M1 connectivity for motor function. Therefore, the increased activity seen in the PMC in chronic stroke patients may reflect increased reliance on alternative structural connections in order to complete motor tasks.

1.1.3 Neuromodulation for stroke rehabilitation

Rehabilitation after stroke typically focuses on the stroke affected limb, however, there has been increased interest in interventions that target the brain, in the hope that this may have a positive effect on behaviour. These interventions have typically been based on, and informed some of, the theories of stroke recovery described above.

Non-invasive brain stimulation

Brain stimulation techniques, such as repetitive TMS and transcranial direct current stimulation (TDCS), have been proposed as potentially useful adjunctive therapy to facilitate motor recovery by enhancing neuroplastic processes. For example, transcranial direct current stimulation (TDCS) has been shown to boost motor learning in healthy individuals (Reis et al. 2009; Stagg et al. 2011), and therefore may also boost effects of stroke rehabilitation, which is thought to rely on motor learning principles (Krakauer 2006). Most of the previous brain stimulation work in stroke patients has targeted the primary motor cortices, attempting to either increase ipsilesional activity, or decrease contralesional activity, in line with the interhemispheric competition model. Using these approaches there have been some promising indications of improvements in hand functioning. For example, Allman and colleagues (2016) carried out repeated sessions of anodal (excitatory) ipsilesional TDCS, on top of a motor training programme in chronic stroke patients, and found that TDCS boosted the effects of motor training up to 3 months post treatment, in comparison to a sham group.

However, there have also been studies which have reported no effect of brain stimulation on motor functioning (e.g. Hesse et al., 2011; Pomeroy et al., 2007). Several meta-analyses have been carried out, with some reporting indications of positive effects (Chhatbar et al.

2016; Kang et al. 2016; O'Brien et al. 2018) and others reporting no effect of brain stimulation on motor functioning (Hao et al. 2013; Tedesco Triccas et al. 2016). Notably a recent Cochrane systematic review on the use of TDCS in stroke found no effect of TDCS on arm function, although there was slight indication of a positive effect on Activities of Daily Living (Elsner et al. 2020). Within the brain stimulation literature there is a lot of variability in terms of the stimulation parameters of techniques applied, whether they are applied in combination with physical training, outcome measures used and the number of sessions given. In addition stroke patients are a highly heterogenous group of individuals both in terms of lesion characteristics (size, location etc), as well as other factors which may have an impact on the success of an intervention, such as time since stroke (Grefkes & Fink 2016). Therefore, the need for more high-quality research and larger randomised controlled trials (RCTs) has been highlighted in order to clarify the potential of brain stimulation techniques in post-stroke motor recovery.

Neurofeedback as a tool for neuromodulation

Neurofeedback (NF) is a form of biofeedback, in which brain activity is measured and presented to participants in real-time in the hope that they can use this to learn to self-regulate their brain activity. Through this, participants may learn how to regulate brain activity in a way that is beneficial to them and has a positive effect on their behaviour. Whilst NF was originally carried out using electroencephalogram (EEG), there has recently been a rise in NF studies using functional Magnetic Resonance Imaging (fMRI; Thibault et al., 2018) which allows greater spatial specificity, as well as targeting of subcortical structures. Additionally, in comparison to other neuromodulation approaches such as brain stimulation, changes in brain activity are achieved endogenously which may ultimately allow participants to continue to regulate brain activity outside of the NF setting.

Furthermore, NF is a fairly flexible approach, which can be used to target multiple brain regions at the same time, connectivity between different brain regions or to target multivoxel activity patterns, therefore allowing the training of fine-grained patterns of brain activity. In healthy individuals, real-time fMRI NF has been used to alter brain activity in a wide range of different brain regions. Moreover, some studies have been able to show exciting behavioural effects such as enhanced visual perception (Scharnowski et al. 2012; Shibata et al. 2011), increased attention (DeBettencourt et al. 2015) and altered emotional valence (Caria et al. 2010).

NF may also be a useful tool in different clinical disorders which are associated with aberrant brain activity. In these cases, NF could be used to target aberrant activity in the hope of alleviating associated symptoms. Indeed, several attempts have been made to use NF to alter brain activity in a variety of different patient groups, ranging from mood disorder such as anxiety and depression disorders (Young et al. 2017; Zilverstand et al. 2015), to neurological conditions such as Parkinson's disease (Subramanian et al. 2011). Studies typically target activity in brain regions associated with the symptoms, for example the Anterior Cingulate Cortex in an effort to control pain perception (DeCharms et al. 2005) or the amygdala for depression (Young et al. 2017). Others have aimed to unconsciously mimic exposure therapy in phobic patients by targeting the neural activity pattern for a feared object (such as a spider; Koizumi et al., 2016; Taschereau-Dumouchel et al., 2018).

However, results from studies have often been mixed, and the need to apply more rigorous methodology and reporting standards has been highlighted (Ros et al. 2020; Thibault et al. 2018). In a recent review examining data from real-time fMRI NF studies to date, there

were, however, positive indications that a majority of studies (approximately two thirds) show that participants were able to control brain activity, although studies differed substantially in how this was measured (Thibault et al. 2018). In contrast, fewer studies were able to find that participants could retain the ability to modulate brain activity after feedback is removed (also called transfer effect), or that NF had an effect on behaviour.

More recently, some studies have started to explore brain changes that occur with NF training. For example, previous work has shown post-NF changes in white matter microstructure in tracts related to the NF targets (Marins et al. 2019; Sampaio-Baptista et al. 2020), and changes in functional connectivity (Marins et al. 2019; Rubia et al. 2019; Scheinost et al. 2013). Exploring brain changes occurring with NF may help to shed light on mechanisms involved in NF learning, and mechanisms underlying any observed behavioural effect.

Neurofeedback in Stroke rehabilitation

Real-time fMRI NF has previously been proposed as a potentially useful therapeutic tool in stroke rehabilitation (Linden & Turner 2016; Wang et al. 2018). One of the advantages of NF is the flexibility of the approach. Whilst most previous studies using brain stimulation have targeted the primary motor cortices, NF can be used to target regions that may be difficult to access using brain stimulation techniques, such as the SMA which extends to the medial wall of the cerebral cortex, or subcortical structures. Indeed, studies in healthy individuals have shown that participants appear to be able to control brain activity in areas that may be important for motor recovery such as the primary motor cortices (Berman et al. 2012; Neyedli et al. 2018; Sampaio-Baptista et al. 2020), as well as secondary motor areas such as the SMA (Hampson et al. 2011; Mehler et al. 2019) or the PMC (Marins et al. 2015; Xie et al. 2015). However, there are some additional challenges

when it comes to translating this approach to the stroke population as their ability to use NF may be compromised due to a number of reasons. For example, stroke may lead to damage in brain regions or networks implicated in NF learning (such as the reward system), or patients may have an altered hemodynamic response (Bonakdarpour et al. 2007; Pineiro et al. 2002) making the feedback more difficult to control. Additionally, patients may find it more difficult to lie in the scanner for long periods or may suffer from more general stroke-induced deficits in learning or cognition.

There have been some initial proof-of-principle studies in stroke patients using real-time fMRI NF. These studies typically only include a handful of patients, but have targeted a variety of areas, including SMA (Mehler et al. 2020), ventral PMC (Sitaram et al. 2012) and connectivity between ipsilesional primary motor cortex and the thalamus (Liew et al. 2016). These previous pilot studies have typically shown that at least some, although usually not all, of the patients were able to use NF to modulate brain activity. However, they often do not include a control group, making it difficult to determine whether these changes are due to the NF training and not other factors. Additionally, on top of patients' ability to use NF, it also remains to be established whether there are any lasting effects on patients' behaviour or their brain function and structure. Therefore, although there are promising indications from studies in healthy individuals and from pilot studies, a lot of open questions remain about the use of NF in stroke patients.

1.1.4 Predictors and variability in response

It has been highlighted both in the brain stimulation literature (López-Alonso et al. 2014; Wiethoff et al. 2014), as well as in the NF literature (Haugg et al. 2020), that there is usually a large amount of variability in participants response to these interventions. In the brain

stimulation literature, some of this variation may be explained through variations in the electric field caused by factors such as skull thickness or anatomy of underlying gyri and sulci (Laakso et al. 2015, 2019). This has led to an increase in studies attempting to model current flows in the hope of explaining some of the variability in response. In the NF literature, there have been some initial attempts at finding predictive factors for NF learning success such as baseline activity in the target brain regions (Haugg et al. 2020) or psychological factors (Kadosh and Staunton, 2019; see Chapter 6 for more on this), however it is still an early area of investigation. Ideally, there would be a simple measure that could be carried out before NF training that could predict a participant's likelihood at succeeding, allowing targeting of participants who are most likely to benefit from the treatment. Additionally, more work could be carried out to identify ways of improving NF success in non-responders, for example through adding monetary reward for additional motivation and reward (see also Chapter 2).

In addition to variability in participants' response to interventions, there is also a lot of variability in the participants themselves, especially in a group such as stroke patients. This could potentially impact what the best approach for recovery may be in individual patients and therefore what activity pattern to target. A prominent example is the work by Stinear and colleagues, who developed an algorithm to predict stroke recovery based on initial impairment and on the functional status of the CST (Stinear et al. 2012). The authors also suggest that treatment focus may differ depending on these factors, so that in patients with severe damage to the CST and low functioning, the aim of therapy may involve compensatory mechanisms, such as increasing contralesional brain activity, whereas in patients where less damage has occurred to the CST, recovery may be possible by increasing activity in the ipsilesional motor cortex.

This variability in the patients and their response to certain treatments suggests that a one-size-fits-all solution seems unlikely to succeed and rather that a more personalised approach to rehabilitation may be beneficial. The Stroke Recovery and Rehabilitation Roundtable consensus group recently recommended several biomarkers, including CST integrity, which could be used to stratify patients into sub-groups which may benefit from different treatment approaches (Boyd et al. 2017). Stratification would decrease variability in samples and reduce the sample size required to detect effects of interventions (Cramer 2010; Stinear et al. 2018). As mentioned above, one of the benefits of fMRI NF is that it is a very flexible approach in terms of which brain areas and functions can be targeted, making this a potential approach that could allow for patients to receive individualised treatment.

1.2 Aims of this thesis

The aim of this thesis is to explore the utility of real-time fMRI NF to aid motor rehabilitation in chronic stroke patients. Although fMRI NF has been suggested as a potentially promising tool for stroke rehabilitation, relatively few studies have explored this approach in stroke patients to date.

First, some methodological considerations for NF experiments will be discussed in Chapter 2. As fMRI-NF is a relatively new intervention, there are several open questions regarding the best ways to implement NF training. Some of these questions are discussed with reference to the studies presented in this thesis.

In Chapter 3, a feasibility study in healthy participants is described, in which the effect of having multiple training sessions was explored on healthy individuals' ability to bi-directionally modulate brain activity in the ipsilateral sensorimotor cortex when performing movements with their non-dominant hand.

In Chapter 4, a randomised, double-blind, sham-controlled trial of real-time fMRI NF in chronic stroke patients is introduced. In this chapter, the patients' ability to use NF to increase laterality of brain activity, and whether they can continue to successfully regulate brain activity without NF during a transfer run, is explored. Behavioural and brain structural and functional data were additionally acquired before and after NF training, and the effect of NF on these measures is investigated in Chapter 5.

Variability in response to NF training, which is commonly observed in NF studies, is explored in Chapter 6, as well as relationships between baseline measures and patients' ability to use NF. Finally, Chapter 7 provides a summary of the work presented in this thesis and outlines possible directions for future studies based on the findings presented here.

2
Methodological considerations for Neurofeedback experiments

Neurofeedback involves presenting participants with their ongoing brain activity in the hope that they can learn to self-regulate this activity. In a typical NF experiment, participants experience regulation blocks, in which they are instructed to try different strategies to control the feedback presented to them, which are interspersed with Rest blocks. The feedback presented to participants typically represents the activity of a particular brain region, and the participants may be asked to attempt to up-regulate or down-regulate this activity. An important decision to make when planning a NF experiment is which brain region or region-of-interest (ROI) to target in the NF experiment. This decision usually relies on indications from previous literature highlighting brain regions where activity is thought to be strongly related to a certain behaviour or disease symptom. However, it is not always clear in patient populations whether it is best to try and 'normalise' brain activity, by pushing activity towards the typical pattern seen in healthy adults, or whether targeting compensatory activity or networks may be a better approach (Paret et al. 2019).

Additionally, the flexibility of NF allows multiple ROIs to be targeted, in which activity can be either increased or decreased, or the functional connectivity between regions can be increased or decreased. Recent developments also allow multivariate activity patterns to be targeted in decoded NF experiments (also called DecNef), which allows increased spatial sensitivity (Watanabe et al. 2017). As well as deciding on what brain area and

function to target with NF training, there are many additional decisions that need to be considered when planning a NF experiment, some of which will be discussed further in this chapter.

2.1 Neurofeedback modality

Multiple neuroimaging modalities have been investigated as potential tools for NF experiments, with the most commonly used modalities being EEG and fMRI (for review see: Thibault et al., 2016). The first NF experiments in the 1960's were conducted using EEG-NF, which involves measuring electrical activity from pyramidal cell populations on the scalp of the participants with high temporal resolution. In NF experiments, due to the need to analyse data in real-time in order to extract the feature of interest, there is typically a delay between the measurement of the neural activity and when this information is shown to the participant. However, due to the speed of EEG acquisition this delay is usually minimal. In contrast to the high temporal resolution afforded by EEG, the spatial resolution is fairly limited and restricted to superficial brain regions, therefore limiting its application with regards to subcortical brain regions. One of the main advantages of EEG-NF is the low costs of EEG and the transportability of the set-up, making it an attractive option for clinical applications. However, a lack of evidence of clinical effectiveness of EEG-NF has been highlighted in several reviews (Thibault et al. 2015, 2016). Additionally, it seems that participants often need to undergo many training sessions in order to be able to self-modulate their brain activity using EEG-NF, and a significant proportion of participants fail to do so even after 30-40 sessions (Alkoby et al. 2018).

More recently, there has been a rise in studies utilising fMRI to provide NF to participants (Watanabe, 2017). In contrast to EEG-NF, fMRI relies on indirect indices of neuronal

activity by measuring changes in the brain's blood oxygenation level dependent (BOLD) signal. Due to the sluggishness of the brain hemodynamic response, and the lower temporal resolution of fMRI acquisitions, there is a greater feedback delay with fMRI-NF and typically participants are informed that there will be a 5-6 second delay between their current strategy and the feedback. However, despite this it has been shown that participants appear to be able to learn to modulate brain activity using fMRI-NF, and that they appear to be able to learn self-regulation faster than using EEG-NF (Thibault et al. 2016). In contrast to EEG, fMRI has excellent spatial resolution and allows targeting both cortical as well as deep sub-cortical structures. Additionally, the whole brain coverage achieved with fMRI allows monitoring of NF-learning related changes in brain activity in remote brain regions, and may shed light on neural mechanisms underlying NF learning. As the work presented in this thesis is based on fMRI-NF, the following discussion will focus primarily on this modality, although many of the points discussed are also relevant for NF using different modalities.

2.2 Feedback display

An important decision to make when designing NF studies, is how and when to present feedback to the participants. Typically (although not exclusively), feedback is presented using a visual format, and previous studies have used a wide range of feedback displays, ranging from relatively simple, such as bars, thermometers or circles, to more complex feedback displays. Whilst more complex feedback displays may be more engaging and motivating to participants, they may also be more distracting, require additional multi-tasking and higher cognitive demands (Paret et al. 2019).

Dual-task demands can be mitigated by providing intermittent rather than continuous NF to the participants. Intermittent NF involves only showing the participants feedback of their brain activity after a discrete amount of time, whereas with continuous NF, feedback is constantly shown to the participant and typically updated with every new volume acquired. Showing feedback to the participant continuously means there is less of a time delay between the participant trying a strategy and receiving feedback. This also means that participants can change their strategies more quickly if they notice a particular strategy is not working, and this could facilitate faster NF learning. In contrast, with the use of intermittent NF, participants are usually instructed to use the same strategy throughout one whole regulation block, with feedback shown to them at the end of the block. This means participants receive less feedback overall, and they may therefore learn more slowly. However, intermittent NF is less prone to noise, as information from several timepoints is averaged. Additionally, brain activity during the regulation blocks is not confounded by activity in brain regions which are processing the feedback and may be involved in reward processing.

Previous studies have found mixed results with regards to which feedback schedule is superior. Emmert and colleagues (2017) compared intermittent and continuous NF in patients with tinnitus when attempting to down-regulate activity in primary auditory cortex. The authors found slight indications that continuous NF may lead to stronger deactivations, although the two conditions were not significantly different from each other. A more robust study comparing regulation of Amygdala activity found an advantage of intermittent NF over continuous in terms of overall BOLD signal change, although the two groups did not differ when examining learning slopes or transfer effects (Hellrung et al. 2018). The authors argued this may be due to the higher cognitive demands with

continuous feedback presentation, and show greater activity in the insula and inferior frontal gyrus for the continuous group versus the intermittent group on the last run of NF training. Nonetheless, more work is needed to assess which type of feedback schedule is superior, though it may be possible that the ideal timing of feedback is dependent on the brain area being targeted with NF, as well as the population being investigated. Especially older participants, children or patient groups may benefit from having intermittent NF with less distractions and dual-task demands. In contrast, young healthy participants may learn more quickly and efficiently with a continuous NF schedule.

Another consideration with regards to the feedback display is whether or not to provide participants with monetary reward that is contingent on their NF performance. Providing monetary reward could incentivise participants performance, as well as potentially being a more explicit form of feedback. This may become more important in studies where participants are not provided with explicit instructions on how to regulate their brain activity, or in cases where participants may have low motivation. The use of monetary reward is particularly common in decoded (multivariate) NF studies, in which the subjects are often not provided with explicit strategies (Watanabe et al. 2017). A previous study compared some of these factors and how they are related to participants' ability to use NF, by comparing participants' self-regulation ability of SMA activity with or without monetary reward and explicit instructions (Sepulveda et al. 2016). The authors found that the highest BOLD signal change occurred when monetary reward was given, and found some indication that learning effects (measured as the difference in SMA activity on the first and second training day) were stronger when no strategy was provided. This may however be due to a ceiling effect, with participants in the group receiving explicit instructions finding successful strategies sooner and not improving further. Recent findings

that self-regulation success is correlated with participants' reward sensitivity (Hellrung et al. 2020) also suggests that reinforcement learning may play an important role in NF learning (Sitaram et al. 2017), and may further support the use of monetary reward to optimise learning.

2.3 Control conditions

Previous research in both healthy participants and patient groups have provided some promising indications that individuals appear to be able to use fMRI-NF to self-regulate brain activity, however the lack of adequate control groups in many studies makes it difficult to attribute changes in brain function or behaviour to the NF intervention (Thibault et al. 2018). Indeed, the recently developed NF reporting consensus checklist (Ros et al. 2020), which provides guidelines for robust NF experimental designs and reporting, lists the inclusion of control groups or conditions as an essential item.

Previous research has utilised a variety of different control conditions, but it has proven difficult to find a control condition that can account for the wide variety of different factors that could play a role in NF training effects, outside of the NF training itself. Sorger and colleagues (2019) recently reviewed the different control conditions that have been used in previous NF experiments, and highlighted the strengths and weaknesses of the different approaches. Additionally the authors highlighted a number of confounding factors that need to be controlled for in order to establish a causal link between NF training and changes in brain function or behaviour. These include factors such as the motivation and perceived success of participants, neurophysiological specificity, placebo effects and factors such as arousal and perceptual input.

Two types of control conditions were employed in the work presented in this thesis and will be discussed in greater detail. The first is the use of *bi-directional regulation control,* which involves asking participants to regulate brain activity in opposite directions. For example, participants may have one session in which they are instructed to increase activity in a ROI, and another in which they have to decrease activity in the same ROI. Sorger and colleagues (2019) highlight that this type of control condition can control for placebo effects and motivation of the participant, as well as other factors such as arousal and perceptual input. Additionally, if behavioural changes are observed in opposing directions this also provides evidence of the neurophysiological specificity of the effect. However, bi-directional regulation of a specific brain region may not always be ethical, especially in patient groups where regulating activity in a certain direction may be associated with worsening symptoms.

The second type of control condition used in this thesis is *placebo control,* in which participants in the control group are shown an alternative feedback signal. For example, in the studies presented in this thesis, participants in the control (sham) group were shown yoked feedback, recorded from a previous participant who had been in the real group and that is unrelated to the ongoing brain activity of the sham control participant. Using yoked feedback allows for closely matching participants' experience in terms of perceived success and frustration across the groups, as the feedback displayed to the sham participants matches exactly that from a participant in the real group. In contrast, other forms of alternative feedback signals, such as feedback from non-brain signals or artificially generated feedback will be less well matched across conditions and may be associated with differences in perceived success or frustration if these signals are perceived as more difficult to control. However, in order for yoked feedback to be an effective control

condition, it is important that participants do not become unblinded by detecting non-contingencies between the NF signal and their own behaviour and strategies. Therefore, it is important to assess whether blinding has been maintained. One disadvantage of placebo controls is that they do not typically allow demonstration of neurophysiological specificity of the trained ROI, with the exception being when placebo feedback consists of activity of an alternative brain region. However, the alternative brain region should be fully independent from the region of interest in the real group, but should additionally be relatively well matched in terms of how easily controllable the region is. These requirements can make it quite difficult to choose suitable alternative brain regions for placebo feedback.

2.4 Hardware and Software requirements

Most fMRI-NF experiments use Echo-Planar imaging (EPI), which allows the relatively fast acquisition of whole brain volumes, and has become a fairly standard acquisition protocol for collecting fMRI data. However, there are additionally decisions that can be made with regards to both temporal and spatial resolution. Developments in MRI physics (such as parallel imaging), and increasing field strengths (ultra-high field scanners) have allowed fMRI to be acquired at increasingly higher spatial resolution. Whilst high spatial resolution allows targeting of smaller brain structures, including the possibility to target individual cortical-layers (for example in visual cortex: Kok et al., 2016, or in motor cortex: Huber et al., 2017), this typically comes at the cost of lower Signal-to-Noise Ratio (SNR) and a lower temporal resolution. This decrease in temporal resolution can be counter-acted by decreasing the field of view (FOV) and not recording signals from the whole brain, although this limits later exploration of the data in terms of brain regions that may be involved in NF learning but were not captured with the reduced FOV.

In contrast, and of particular interest for studies that are interested in functional connectivity NF, pushing the temporal resolution of fMRI acquisition to better capture the timeseries of brain activity is possible by using parallel imaging or multi-band sequences, which can allow sub-second repetition times (TR). The use of MR-encephalography, which permits TR's below 100ms, has recently been suggested as a potentially promising fMRI acquisition technique for real-time NF applications (Lührs et al. 2019), both by increasing sensitivity to dynamic changes in functional connectivity, but also by allowing accurate detection of respiratory and cardiac noise components without additional equipment such as respiratory bellows. Therefore, decisions regarding the fMRI acquisition protocol for NF studies should weigh up the ever-increasing options available in order to optimise data acquisition.

In order to deliver rapid neurofeedback, the acquired fMRI data needs to be reconstructed and sent from the scanner console to a computer that will carry out the real-time analysis (see figure 2.1). This can be achieved through a direct TCP/IP network link and a Server Message Block (SMB) communication protocol which allows mapping of a drive to a remote file share. Once the data has been received on the real-time analysis computer, pre-processing of the data can begin. Several different software packages are available to conduct online analysis and pre-processing. Examples include freeware such as OpenNFT (Koush et al. 2017), FRIEND (Sato et al. 2013) and BART (Hellrung et al. 2015). Commercially available and fairly widely used is TurboBrainVoyager (TBV; Brain Innovation, Maastricht, Netherlands), which was the software used in the studies presented in the current thesis. Data analysis and pre-processing carried out using these software packages are similar to offline analyses steps and include motion correction, spatial

smoothing and linear trend removal. Due to the requirement to perform analysis quickly, ideally within the time it takes to acquire each volume, pre-processing and analysis steps are often adapted and simplified (see Heunis et al., 2020 for an extensive overview).

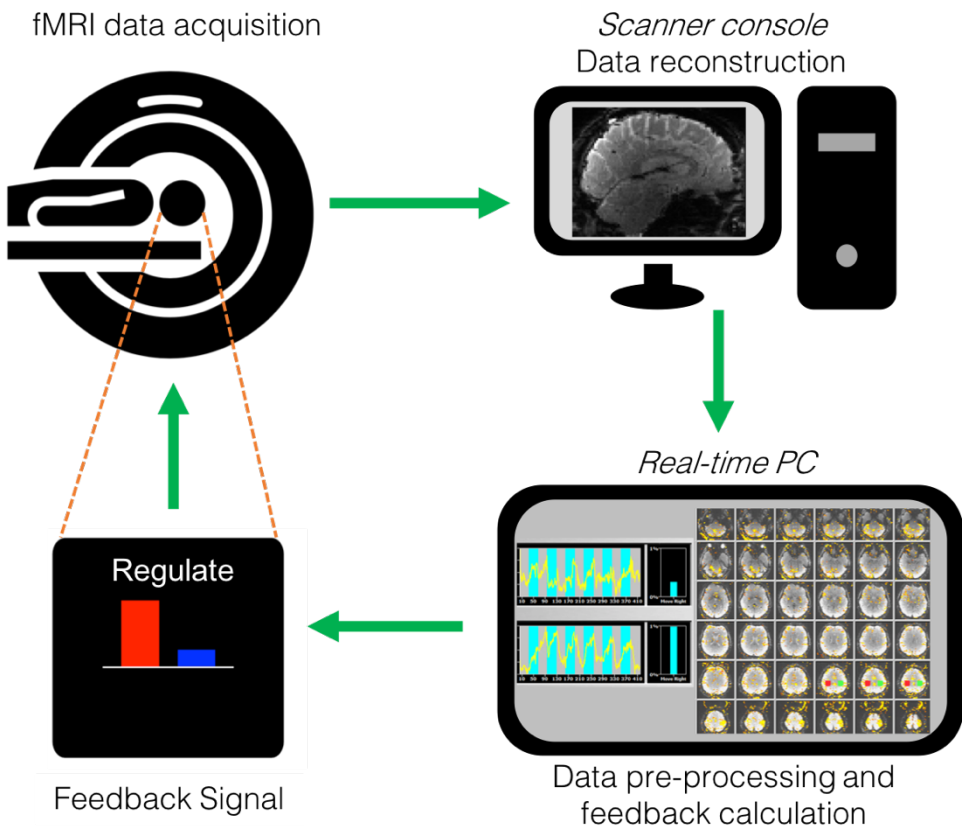


Figure 2.1 Graphical overview of a typical fMRI-NF experiment.

Additionally, it has been recommended to record physiological measures, such as respiration and heart rate, which may influence the NF signal of interest. These measures would ideally be transferred into the real-time analysis software in order to correct for physiological noise in real-time. This would reduce the possibility for participants to inadvertently learn to control these confound measures rather than actual brain activity. For example, a recent functional connectivity NF paper found strong associations between functional connectivity estimates and respiratory measures, and strongly recommended correcting for physiological confounds online (Weiss et al. 2020).

2.5 Multimodal imaging measures

As mentioned above, one of the benefits of fMRI-NF is that it is possible to acquire whole-brain data during NF training, which allows offline exploration of NF-related activity changes throughout the brain. However, studies have also gone further and employed different imaging modalities in order to explore NF effects on brain structure, connectivity and function. Most commonly investigated are changes in functional connectivity after NF training, with previous studies reporting altered functional connectivity in a variety of different networks (e.g. Haller et al., 2013; MacInnes et al., 2016; Marins et al., 2019). NF-related changes in functional connectivity have also been demonstrated in a variety of different clinical disorders, with some studies finding relationships between changes in patients' symptoms and functional connectivity changes, for example in depression (Young et al. 2018), ADHD (Rubia et al. 2019) or Huntingdon's disease (Papoutsi et al. 2018). This could suggest that NF training may lead to on-going changes in the brain's functional connectivity network, which may in some cases also lead to improvements in clinical symptoms.

Less frequently studied have been changes in brain structure after NF training. A couple of recent studies used diffusion weighted imaging to demonstrate changes in white matter microstructure following NF training (Marins et al. 2019; Sampaio-Baptista et al. 2020). These results are in line with recent research demonstrating experience-dependent white matter changes in adults which may play a role in learning (Sampaio-Baptista & Johansen-Berg 2017). One recent study in patients with Huntington's disease also demonstrated that improved clinical scores after NF training of the SMA was correlated with increases in grey matter volume in pre-SMA (Papoutsi et al. 2018). Therefore, there have been some

initial indications of structural brain changes after NF training, however this remains a fairly unexplored area of NF research.

More recently, studies have also begun to explore relationships between baseline brain measures and NF learning ability. Whilst a large-scale meta-analysis combining data across multiple studies has focused on fMRI data from functional localisers or transfer runs (Haugg et al. 2020), individual studies have also begun to probe relationships between other brain measures and NF success, such as brain structure at baseline (Sampaio-Baptista et al. 2020; Zhao et al. 2021) and functional connectivity (Scheinost et al. 2014).

In summary, there have been some indications in the previous literature that NF may lead to more widespread changes (beyond the trained ROI) in the brain. The inclusion of additional follow-up multimodal imaging measures can shed light on what brain changes are produced by NF, and through what mechanisms NF training may have an effect on behaviour. Additionally, the inclusion of multimodal imaging measures allows exploration of further NF-related questions, such as what factors may be related to how well a participant will be able to perform during NF training, which may allow future studies to predict NF success from baseline imaging measures.

2.6 Conclusion

In this chapter, several methodological issues were discussed that need to be considered when designing a NF study. Initially, decisions regarding NF modality and related acquisition parameters should consider what region is being targeted and how to optimise the spatial or temporal resolution. More research is required to establish the best way to

display NF to participants, however it may be important to consider the population being studied. In order to demonstrate NF-specific learning effects it is important to include appropriate control conditions, and additional multi-modal follow-up measures may shed light on mechanisms involved in NF learning and/or in behavioural improvements.

3
Bi-directional modulation of ipsilateral motor cortex activity over multiple training sessions

3.1 Introduction

Previous studies have demonstrated the utility of NF to alter activity in different parts of the brain, suggesting that this may be a useful tool in clinical populations with aberrant brain activity (Thibault et al. 2018). For example, following a stroke aberrant brain activity has been demonstrated in the ipsilateral (contralesional) primary motor cortex (M1) when patients move their stroke-affected limb (Johansen-Berg et al. 2002a; Ward et al. 2003b). This aberrant brain activity has been suggested to be maladaptive in some patients (Ward et al. 2003a), whereas in other patients, in particular those with more extensive damage to the CST or to M1, it may play a compensatory role (Biernaskie et al., 2005; Buetefisch, 2015; Lotze et al., 2012; see also: **General Introduction**). This suggests that therapeutic interventions targeting brain activity to improve motor rehabilitation in stroke patients, may benefit from taking a more personalised approach.

However, most of the previous work both in the brain stimulation (e.g. Hesse et al., 2011; O'Shea et al., 2014; Stagg et al., 2012), as well as in the NF literature (e.g. Auer et al., 2015; Neyedli et al., 2017) have targeted decreasing ipsilateral M1 activity. In contrast, Sampaio-Baptista and colleagues (2020) recently showed that healthy participants were able to use NF to bi-directionally alter activity in ipsilateral M1 when carrying out motor movements, and that this was associated with opposing white matter changes in the corpus

callosum. This provided some initial promising evidence that NF can be used to both up- and down-regulate activity in ipsilateral M1.

Additionally, previous work has frequently used motor imagery rather than executed movements to drive NF. Using motor imagery may allow patients who cannot produce overt movements to use NF to modulate M1, however, results of studies using motor imagery have provided inconsistent findings. Several studies have reported that participants are unable to increase activity in M1 using motor imagery guided NF (Berman et al. 2012; Chiew et al. 2012; Mehler et al. 2019), a result that is in line with work suggesting that motor imagery primarily recruits supplementary motor area (SMA; Hanakawa, 2016). Whilst there have been some studies that do show up-regulation of M1 activity with motor imagery NF (e.g. Blefari et al., 2015; Perronnet et al., 2017; Yoo et al., 2008), not all of these studies control for muscle contractions. In the current study we opted to use motor execution to modulate activity in M1 as the utility of motor imagery in motor rehabilitation has previously been questioned (Ietswaart et al. 2011), and rehabilitation programs encouraging active movements have gained increasing support (Sunderland et al. 1992; Wu et al. 2016). Fewer studies have investigated M1 modulation with executed movements, however there have been some promising results showing that participants can increase M1 activity (Berman et al. 2012), increase laterality of brain activity (Neyedli et al. 2018) or bi-directionally modulate ipsilateral M1 activity (Sampaio-Baptista et al. 2020).

An additional unanswered question in the NF literature is how much NF training is needed and whether there are benefits of training over multiple days. Previous NF studies have varied widely in the amount of training given, from single sessions to training over multiple

days. Due to the costs associated with fMRI NF, it is important to establish how many NF training sessions are necessary in order to achieve self-modulation. Ideally, NF could be used in order to identify strategies to alter brain activity that could potentially be practised further outside of the scanner or in conjunction with rehabilitation. In the current study we examined the progression of NF learning over multiple days of training to establish whether cumulative improvements in participants' ability to modulate brain activity are seen.

3.2 Aims

The aim of this chapter was to explore NF learning over multiple training days, in order to investigate whether having multiple training sessions improved NF learning and how this evolves over time. Additionally, a secondary aim was to replicate the previous findings of Sampaio-Baptista and colleagues (2020) showing that healthy participants are able to bi-directionally alter brain activity in the ipsilateral M1 with a single session of NF.

3.3 Methods

3.3.1 Participants

Thirty-two right-handed participants were initially recruited to take part in this study and informed consent was obtained in line with the Declaration of Helsinki and the ethics committee of the University of Oxford approved protocol (MSD-IDREC-C1-2012-151). Unbeknownst to the participants, they were assigned to either the Sham or the Real NF group, with sixteen participants in each group. Seven participants withdrew from the study, four withdrew after feeling unwell or uncomfortable in the 7T scanner environment, one was excluded due to large movement artefacts, one was lost to follow-up and one participant was excluded after the scanner crashed during their first scanning session. This

resulted in a final sample size of 15 participants in the real group (5 females, 21-30 years old), and 10 participants in the sham group (7 females, 21-31 years old).

3.3.2 Study design:

The study included a cross-over design within the real NF group, whereby participants experienced two different NF conditions over repeated sessions. Participants in the real group were invited to attend six sessions of real-time fMRI NF training. During these sessions participants experienced two different NF conditions (described below), with each condition being conducted over three training days, followed by a one-month wash-out period before participants returned to complete the second condition (see Figure 3.1 bottom). For each condition, the first two training days were separated by 24 hours, whereas the second and third session were separated by 48 hours.

The study also included a between-group comparison to a sham control group for the first two days of NF training for each condition only. Participants in the sham group were invited to attend four sessions of real-time fMRI neurofeedback training, with each NF condition conducted over two days separated by 24 hours. This was followed by a one month wash out period before participants returned for the second condition, also conducted over two consecutive days.

During all scans for both groups a functional localiser was always conducted at the beginning of the scan. During the functional localiser participants saw the instructions “Rest”, “Tap Right” or “Tap Left” on the screen. During the tapping blocks they were instructed to consecutively tap the individual fingers of the given hand, from index finger to little finger at a rate of ~1 tap per second. Participants experienced three blocks of

tapping for each hand (12secs each), interspersed with rest blocks (24secs). Peak activity in each of the hand knob regions of the primary sensorimotor cortex were identified using a real-time general linear model (GLM) by setting contrasts to Left or Right hand movement > Rest. Two Regions-of-interest (ROI; 18 x 18 x 10mm) were centred on the peak activity and were used to extract values for the NF display.

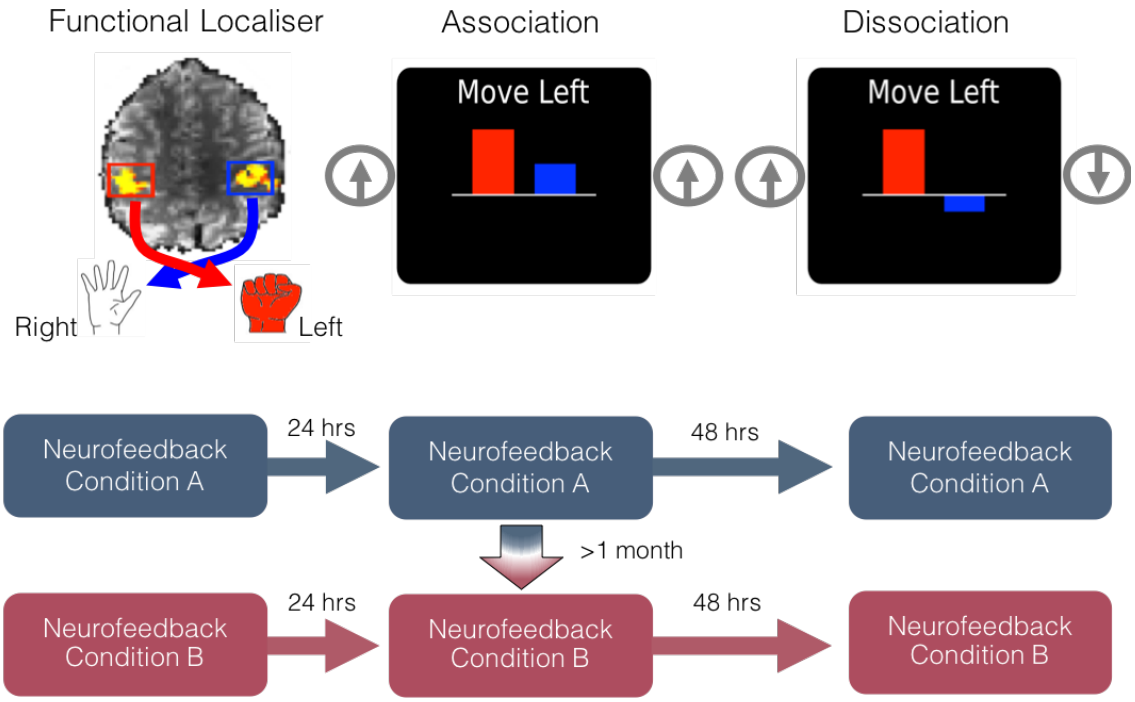


Figure 3.1 Study design. Top: ROIs for the NF training are centred on the peak voxels identified during a functional localiser. In both NF conditions participants see two bars on the screen and are instructed to move their left hand only to manipulate the bar height. The red bar reflects activity in the contralateral M1, whereas the blue bar reflects activity in the ipsilateral M1. During the *Association* condition, participants are instructed to try and increase the size of both of the bars. In the *Dissociation* condition participants are instructed to increase the size of the red bar while decreasing the size of the blue bar. **Bottom**: Participant training sessions. Participants first experienced one of the conditions over three training days with the first two sessions separated by 24 hours and the second and third session separated by 48 hours. After a one month wash-out period, participants were invited back for the second condition over the same three training days.

During all the NF runs participants saw two bars on the screen (see Figure 3.1 top), with a red bar on the left side reflecting activity in right M1, and a blue bar on the right side of the screen reflecting activity in left M1. Above the bars, participants saw the instructions "Rest" or "Move Left". During the Move Left condition, participants were instructed to move their left hand only, to alter the size of bars that they saw on the screen.

Electromyography (EMG) was acquired to ensure that participants did not move their right hand. The participants experienced two different conditions; during the *Association* condition participants were instructed to try and increase the size of both the bars. For the Real NF group, this corresponded to increasing activity in both the contralateral and the ipsilateral M1. In contrast, during the *Dissociation* condition, participants were instructed to increase the size of the red bar whilst decreasing the size of the blue bar. For the Real NF group, this corresponded to increasing activity in the contralateral M1, whilst decreasing activity in the ipsilateral M1. During each NF training day, participants experienced three NF runs during which six 30-second Movement blocks were interspersed with six 30-second Rest blocks. The order in which participants experienced the two conditions was counter-balanced across the participants. Participants in the Sham group received identical instructions and tasks as the participants in the Real group, however instead of the bars during the NF runs reflecting their own brain activity, they were played a video of the bars from a previous participant in the Real group.

3.3.3 MRI acquisition

All fMRI data was acquired on a Siemens 7 Tesla Magnetom scanner (Siemens AG, Erlangen, Germany) with a 32-channel head coil. All fMRI data was acquired using echo planar imaging (EPI; 16 slices, TR=2000ms, TE=25ms, flip angle=90°, slice thickness=2mm, in-plane resolution=2x2mm, GRAPPA factor=2) with the limited field-of-view centred over the hand knob area of the primary sensorimotor cortices. Additionally, a single whole brain EPI image was acquired for registration purposes (80 slices, TR=7370ms, TE=25ms, flip angle=90°, slice thickness=2mm, in-plane resolution=2x2mm, GRAPPA factor=2). A whole-brain T1-weighted (MPRAGE) image

was also acquired with a 1mm isotropic resolution (TR=2200ms, TE=2.82ms, flip angle=7°, TI=1050ms, slices=176, GRAPPA=4).

3.3.4 Online data analysis

Turbo-BrainVoyager 3.2 (TBV; Brain Innovation. Maastricht, Netherlands) was used to process fMRI data in real-time. Dicom images were first transferred from the scanner console to the real-time computer using a direct TCP/IP network link. Following this, images were motion corrected, spatially smoothed with a gaussian kernel of 3mm full-width-half-maximum (FWHM) and linear temporal drifts were removed from the data. A recursive GLM was used to compute the feedback signal and the bars were updated with each repetition time (TR; 2secs). The bar height was calculated as the difference in activity between the current BOLD signal and the mean BOLD signal during the previous rest blocks. Positive values were displayed above a centre line and negative values below a centre line. Bar height was determined as follows:

$([ROI_{curr} - ROI_{Rest}] / ROI_{Rest}) / MaxBarHeight$

Where ROI_{curr} is the current BOLD signal in the ROI, ROI_{Rest} is the mean BOLD signal during the previous rest blocks and MaxBarHeight is the maximum level of the bar. The maximum level of the bar was set to double the Percent Signal Change (PSC) measured in the ROI during the functional localiser.

3.3.5 Offline data analysis

The fMRI data was analysed using the FMRIB Software Library (FSL, FMRIB, Oxford) version 6.0. FEAT (FMRIB's Expert Analysis Tool) was used to carry out standard pre-processing steps, including motion correction using MCFLIRT (Jenkinson 2002), brain extraction using BET (Smith, 2002), spatial smoothing using a 5mm FWHM Gaussian kernel and high pass temporal filtering with a cut-off of 90 seconds. Co-registration was achieved by first using linear registration (FLIRT; Jenkinson et al 2002; Jenkinson & Smith 2001) to register the limited FOV functional data to the single whole brain EPI image. Next, the whole brain EPI image was linearly registered to the T1-weighted image using boundary-based registration. Structural T1-images were processed using the FSL anatomical processing script (fsl_anat) which carries out bias-field correction and non-linearly registers the structural image to standard MNI space using FNIRT.

A first-level GLM analysis was carried out for each fMRI run. A double-gamma HRF convolved boxcar regressor and its temporal derivative, was used to model the Move Left and Rest blocks for each run, and contrasts were set to Move Left > Rest. Intermediate fixed effects analysis was carried out to average runs within (average of all runs per day) and across (average of each run over days) days and to calculate difference maps between days and runs (e.g. Day1 > Day3; Run 1 > Run 3). FMRIB's Local Analysis of Mixed Effects (FLAME) was used to carry out higher level analyses with group z-statistic images being thresholded using clusters determined by $Z > 3.1$ and a family-wise corrected cluster significance threshold of $p<0.05$.

Additionally, a ROI based analysis was carried out by extracting the average PSC from the ROIs used for the NF training using featquery. This resulted in PSC values for Move Left

vs Rest for both the contra- and the ipsilateral M1 for each NF run on each training day. These values were statistically compared between and within groups using mixed design or repeated-measures ANOVA respectively.

3.4 Results

3.4.1 Multi-Day learning effects

The first aim of this study was to investigate the effect of having multiple training days on NF learning. In order to assess this, learning in the Real group was assessed over three training days in both of the NF conditions. The PSC values (for Move Left vs Rest) in each ROI were extracted across all of the runs and training days. A repeated measures ANOVA was carried out for both the ipsilateral and the contralateral hemisphere with the factors of Condition (Association, Dissociation), Day (1,2,3) and Run (1,2,3). One participant was removed due to being an outlier (PSC >2.5 SDs above mean).

In the ipsilateral ROI (Figure 3.2A) there was a main effect of Condition ($F(1,13)=10.782$, $p=0.006$, $\eta_p^2=0.453$) which reflected higher average activity in the Association condition (estimated marginal mean (EMM)=0.189) in comparison to the Dissociation condition (EMM=-0.142). The three-way interaction Condition x Day x Run was not significant ($F(4,52)=0.646$, $p=0.632$, $\eta_p^2=0.047$). However, there was a significant Condition x Day interaction ($F(2,26)=5.066$, $p=0.014$, $\eta_p^2=0.280$). Follow-up tests examining the effect of Day in each Condition separately (Figure 3.2B) found a significant main effect and linear trend in the Association Condition ($F(2,26)=6.833$, $p=0.004$, $\eta_p^2=0.345$), with average activity in the ipsilateral hemisphere increasing over training days (EMM: Day1=0.032 ,

Day2=0.170, Day3=0.366). However, there was no significant effect of Day in the Dissociation Condition (F(2,26)=0.239, p=0.789, η_p^2=0.018).

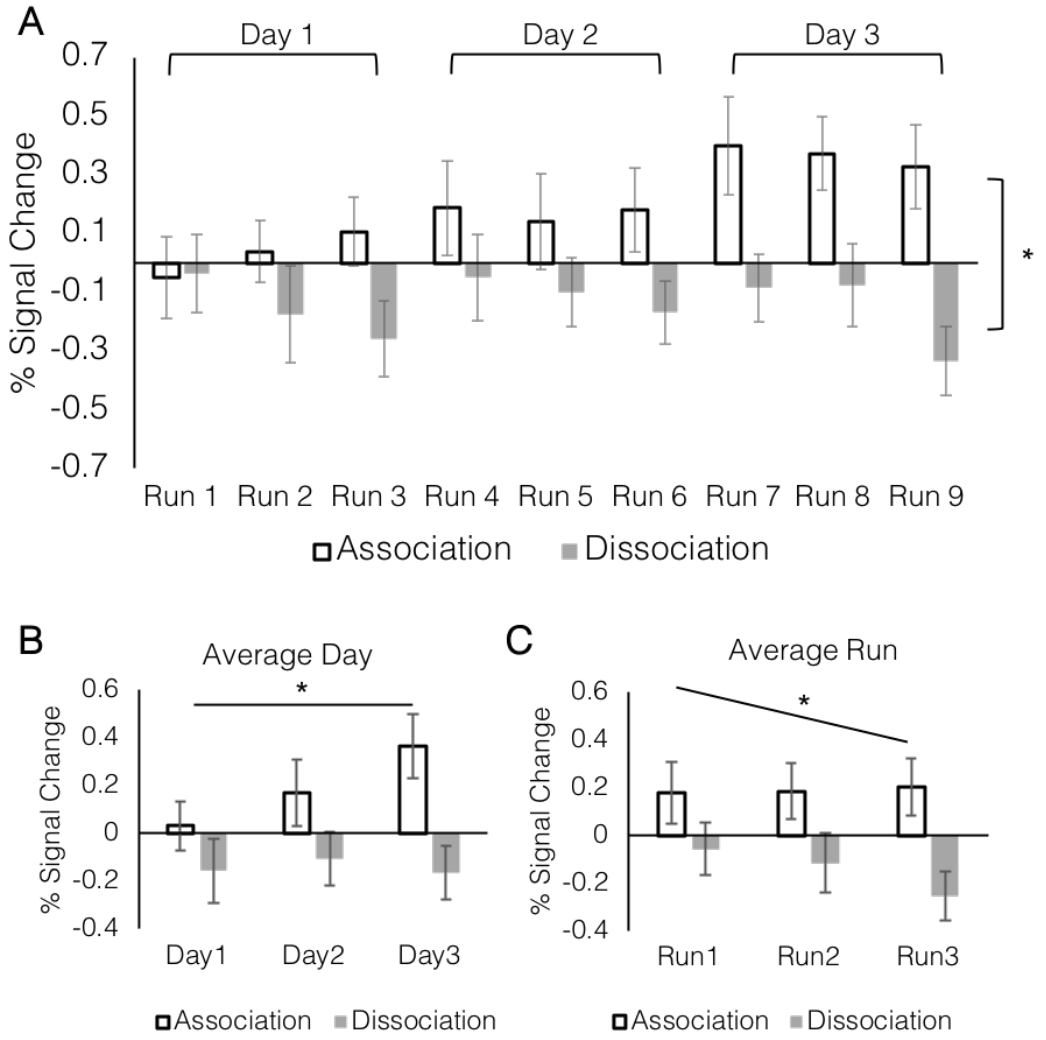


Figure 3.2: Ipsilateral ROI results A) Mean percent signal change (PSC) over all the runs and training days for the *Association* (white) and *Dissociation* (grey) condition for the Real group. There was significantly higher ipsilateral activity in the Association condition than the Dissociation. **B)** Average PSC over all the runs on each training day. A significant interaction was found between average Day PSC and Condition **C)** Average PSC for each run averaged across training days. A significant (negative) linear trend was found for the main effect of Run. Error bars represent s.e.m. * indicates a significant effect ($p < 0.05$) # indicates a trend ($p < 0.08$)

In contrast, the Condition x Run interaction failed to reach significance (F(2,52)=2.181, p=0.133, η_p^2=0.144). The main effect of Day was also not significant (F(2,26)=2.533, p=0.099, η_p^2=0.163), however there was a significant main effect of Run (F(2,26)=5.635, p=0.009, η_p^2=0.302). The main effect of Run was driven by a significant linear contrast over Runs (F(1,13)=11.836, p=0.004, η_p^2=0.477), with activity linearly decreasing over

Runs when averaged over conditions (EMM: Run1=0.061, Run2=0.034, Run3=-0.024). However, when visually inspecting the effect of run per condition (Figure 3.2C), this effect appears to be primarily driven by decreasing activity in the Dissociation condition, with little change seen in the Association condition.

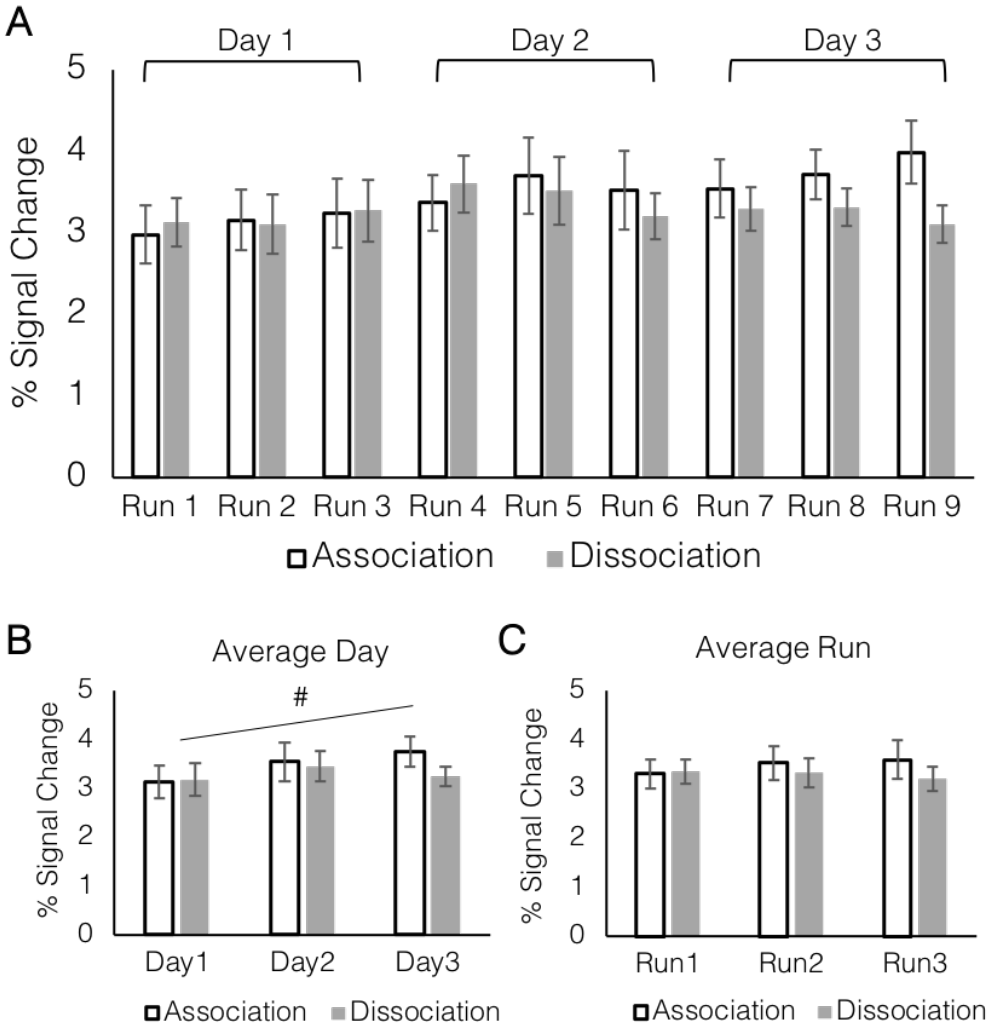


Figure 3.3 Contralesional ROI results A) Mean percent signal change (PSC) over all the runs and training days for the *Association* (white) and *Dissociation* (grey) condition for the Real group. **B)** Average PSC over all the runs on each training day. A trend towards a linear increase was found for the main effect of Day. **C)** Average PSC for each run averaged across training days. Error bars represent s.e.m. * indicates a significant effect ($p < 0.05$) # indicates a trend ($p < 0.08$)

When carrying out the same repeated measures ANOVA on the data from the contralateral ROI (Figure 3.3), there were no significant main effects or interactions between any of the factors (all F's<2.5, all p's > 0.1), however there was a trend towards a significant linear

effect of Day (F(1,13)=3.872, p=0.071, η_p^2=0.230), with average activity increasing marginally over training days (Figure 3.3B; EMMs: Day1=3.148, Day2=3.488, Day3=3.493).

Voxel-wise analysis was additionally carried out. First, the average activity for both the conditions was compared by averaging activity over all the runs and all the training days to test for main effects of Condition. For the contrast Association > Dissociation, two significant clusters were found (Figure 3.4A), one in the ipsilateral sensorimotor cortex (p<0.001, 243 voxels, MNI coordinate of max T-stat = -38, -20, 56) and a second cluster in the supplementary motor area (SMA; p<0.001, 392 voxels, MNI coordinate of max T-stat = 4, -14, 60), indicating higher activity on average in these areas in the Association condition than the Dissociation. No significant clusters were found for the contrast Dissociation > Association.

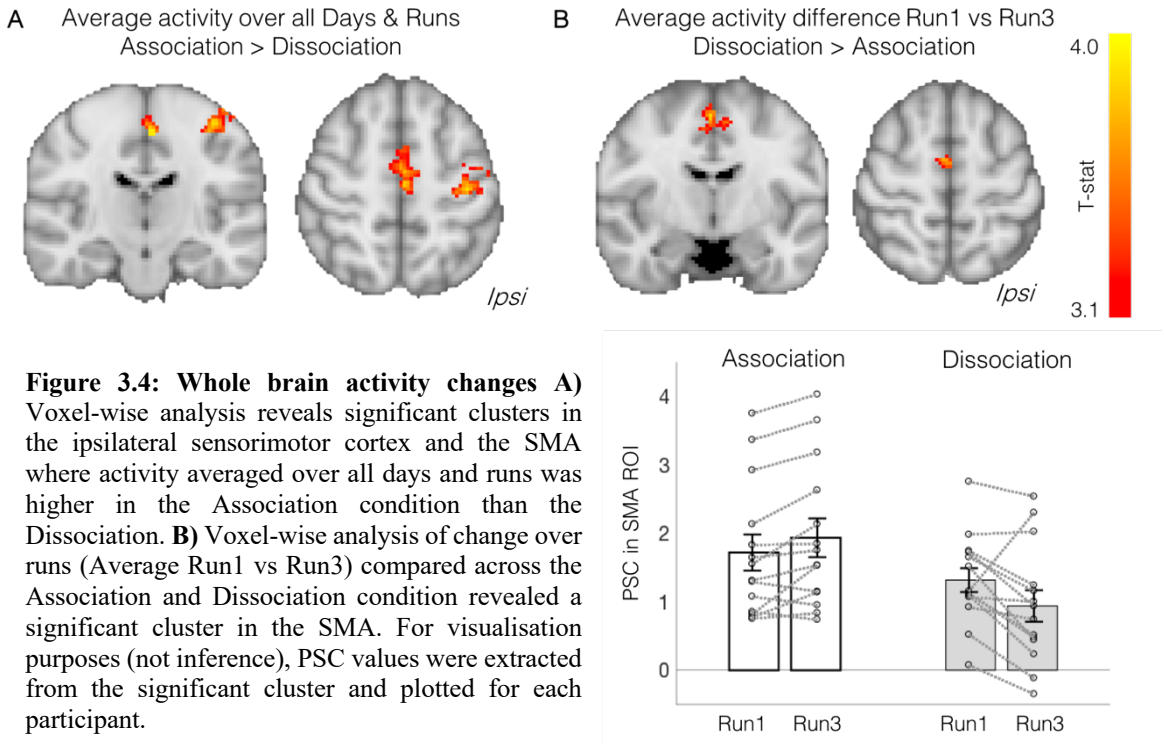


Figure 3.4: Whole brain activity changes A) Voxel-wise analysis reveals significant clusters in the ipsilateral sensorimotor cortex and the SMA where activity averaged over all days and runs was higher in the Association condition than the Dissociation. **B)** Voxel-wise analysis of change over runs (Average Run1 vs Run3) compared across the Association and Dissociation condition revealed a significant cluster in the SMA. For visualisation purposes (not inference), PSC values were extracted from the significant cluster and plotted for each participant.

How brain activity changes over Days and Runs, across the two conditions, was additionally examined by first averaging either across runs (mean of each run across days) or across days (mean of all runs within each day). Next, difference maps were calculated for each run or day comparison (e.g. Day1 vs Day3; Run 1 vs Run 3), which were then compared between the Association and Dissociation condition. When comparing changes across Days, no significant differences were found between the two conditions. When comparing changes across Runs, a significant cluster was found in the SMA (figure 3.4B; p=0.005, 118 voxels, MNI coordinate of max T-stat = 2, -8, 56) when comparing activity changes between Run1 and Run3 between the two conditions (Dissociation > Association). This suggests there is a greater change in activity in this region in the Dissociation condition than the Association condition between these runs. In order to visualise this effect, the PSC was extracted from the significant voxels and is displayed in figure 3.4B. It can be seen that while activity slightly increases over runs in the Association condition, activity in the Dissociation condition, decreases over Runs. No other significant effects were found.

3.4.2 No replication of first day learning effects

The second aim of this study was to replicate the previous findings from Sampaio-Baptista and colleagues (2020), in which it was demonstrated in a single training day that participants in the Real NF group could bi-directionally modulate ipsilateral brain activity in comparison to a Sham group. In order to assess this, only the data from the first training day was considered (figure 3.5). A ROI analysis was carried out considering PSC values (for Move Left vs Rest) using a mixed ANOVA with between-subjects factors of Group (Real, Sham) and within-subject factors of Run (1,2,3) and Condition (Association, Dissociation). However, despite closely matching the previous study design, in the current

study we failed to replicate the significant three-way interaction in the Ipsilateral ROI (Figure 3.5A) Group x Condition x Run (F(2,44)=1.39, p =0.260, η_p^2=0.059). There was also no main effect of Condition (F(1,22)=0.839, p=0.370, η_p^2=0.037), or a Group x Condition interaction (F(1,22)=1.841, p=0.189, η_p^2=0.077).

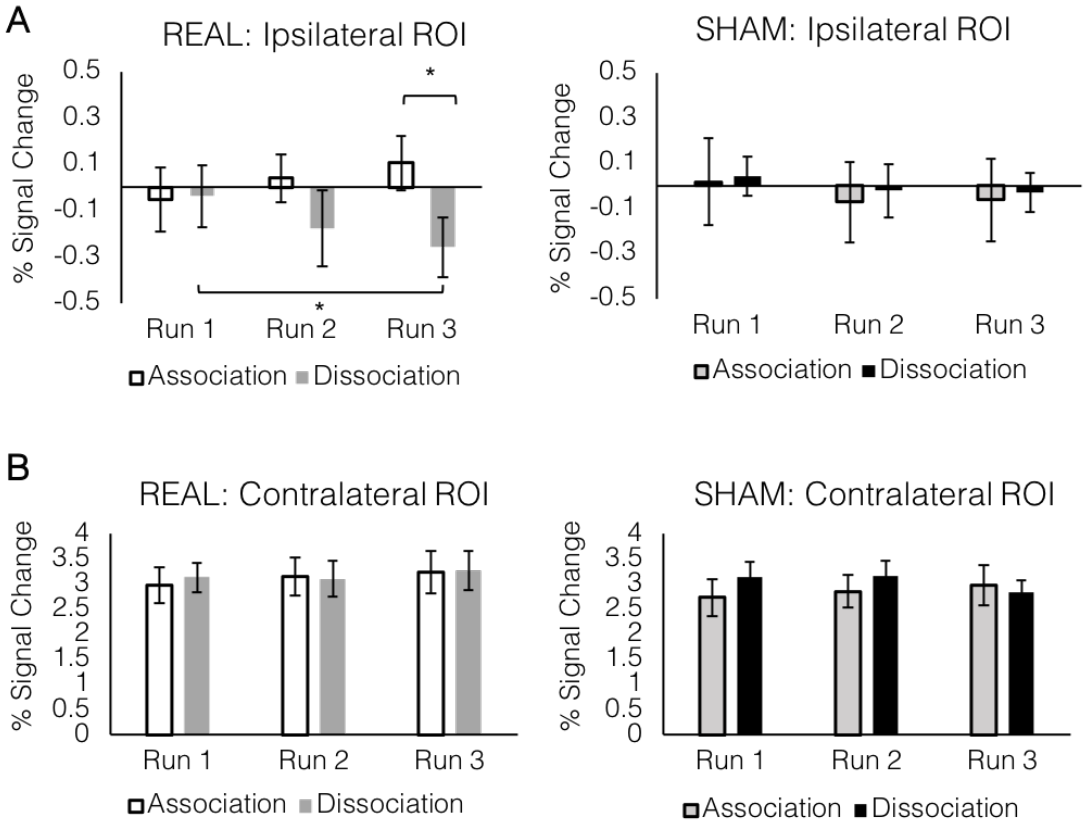


Figure 3.5: No single training session effect. A) Mean PSC from the ipsilateral ROI in the Real (left) and Sham (right) group for the two NF conditions after a single NF training session. **B)** Mean PSC from the contralateral ROI in the Real (left) and Sham (right) group for the two NF conditions after a single NF training session. Error bars represent standard error of the mean. * indicates a significant effect ($p < 0.05$) in the exploratory post-hoc analyses.

As this was an attempt to replicate previous findings, the predicted post-hoc comparisons were also explored for completeness. When comparing activity across the conditions for each run within the ipsilateral ROI, there was a significant difference between conditions in the Real group on Run 3 (t(13)=4.114 p=0.001, *d*=1.1; Bonferroni corrected p-value for 3 comparisons = 0.016), no significant differences were found in the Sham group (all t < 1, all p >0.5). Additionally, when exploring changes across runs within conditions, there

was a significant difference between Run1 and Run3 in the Dissociation condition in the Real group ($t(14)=3.249$, $p=0.006$, $d=0.839$). None of the comparisons across runs in the Association condition or in the Sham group were significant (all $t < 1.5$, all $p > 0.15$).

When comparing values in the contralateral ROI (Figure 3.5B), in line with the previous work there were no significant interactions or main effects (all F's < 1, all p's $>.4$), with this ROI being strongly activated in both conditions and both groups.

Additionally, a voxel-wise analysis was carried out to compare differences in the average activity of the two conditions (Association vs. Dissociation) across the two groups (Real vs. Sham) as in the previous study. This was achieved by first averaging the runs within the first training day for each condition and each group, then difference maps were calculated between the Association and Dissociation condition average activity for the two groups. Higher level analysis then compared the difference maps between the two groups. No significant clusters were found in this comparison.

3.5 Discussion

In this chapter, I explored the effects of having multiple training days on participants' NF performance when attempting to bi-directionally modulate brain activity in ipsilateral M1. Additionally, I attempted to replicate previous results demonstrating bi-directional modulation of M1 after a single training session. The main results of this study are discussed below:

3.5.1 Multi-day learning effects

The first aim of this study was to investigate the effect of having multiple training days on NF performance. In order to assess this, participants attended three training days for each of the conditions. We found that, for the Association condition, there was clear evidence of a cumulative multi-day learning effect, with the largest increase in ipsilateral activity occurring on the third training day (Figure 3.2A, white bars). In contrast, in the Dissociation condition, learning appeared to occur primarily within training days (Figure 3.2A, grey bars). Whilst the Condition*Run interaction failed to reach significance, there was a significant main effect of Run, with activity on average decreasing over runs. When visually inspecting the data, activity appears to primarily decrease in the Dissociation condition and remain relatively stable in the Association condition across runs. The reason why there was no cumulative multi-day learning effect when attempting to decrease ipsilateral activity is unclear. There was additionally a trend towards a significant linear increase in activity in the contralateral hemisphere over all the runs, irrespective of condition (Figure 3.3B). Both groups were instructed to try and boost activity in the contralateral hemisphere and there seems to be some indication that having multiple training sessions helps participants to achieve this. This could have important implications in clinical populations such as stroke patients who have decreased activity in the contralateral (or stroke affected) hemisphere and therefore have more scope for increasing activity in this hemisphere.

The optimal dosage of NF training is an important unanswered question in the NF literature (Sulzer et al. 2013). Especially when using a fairly expensive tool such as fMRI to provide NF training, it is important to establish the cost-effectiveness of the intervention and how many sessions are necessary to observe a change in behaviour or symptoms. A previous

study, which examined dosage effects of a brain-computer interface on chronic stroke patients motor recovery, found that more frequent and intense practice led to greater gains in the strength component of the Stroke Impact Scale questionnaire, although no changes were found on the Action Research Arm Test (ARAT; Young et al., 2015). This result is in line with other results in the rehabilitation literature suggesting that more training sessions typically lead to better outcomes (Kwakkel et al. 1999; Lang et al. 2015; Lohse et al. 2014). Beyond rehabilitation, dose-response relationships have also been found in other skilled activities such as sports and playing a musical instrument (Ericsson 2004). However, whether NF learning exhibits the same dose-response relationship as these skilled activities remains unclear. Furthermore, with NF training, participants may reach a point where they are able to successfully modulate their brain activity without NF, indicating transfer of learning. At this point participants may not require further NF training per se, but could continue to modulate brain activity outside of the scanner, for example during rehabilitation sessions. Finally, the optimal number of NF training sessions may also depend on the brain region being targeted, as well as the population being studied. Some brain regions may be more difficult to modulate, for example due to a delayed hemodynamic response or having lower SNR.

3.5.2 Differences in ipsilateral sensorimotor and supplementary motor area activity

When carrying out the voxel-wise analysis comparing the average activity over all the runs of the two NF conditions, it could be seen that there was greater activity in the ipsilateral sensorimotor cortex and the SMA in the Association condition, compared to the Dissociation condition (Figure 3.4A). Increased activity in the ipsilateral sensorimotor cortex is in line with the ROI results showing a main effect of condition in the ipsilateral

NF ROI, and these regions were also identified in the previous study by Sampaio-Baptista and colleagues (2020).

When examining changes in brain activity over average NF runs, it could be seen that activity in the SMA decreased from Run 1 to Run 3 in the Dissociation condition (Figure 3.4B). The SMA has been implicated in playing a role in internal movement generation, motor sequence production, and bimanual coordination (Nachev et al. 2008), and is densely inter-connected with the hand area of the sensorimotor cortex (Johansen-Berg et al. 2004; Luppino et al. 1993). Brain stimulation studies have demonstrated a modulatory effect of SMA on M1 activity (Arai et al. 2012; Côté et al. 2020). Therefore, it is a possibility that one way through which participants could influence ipsilateral sensorimotor activity is through alterations in SMA activity, which may in turn modulate sensorimotor activity. Alternatively, decreased activity in SMA could also be a by-product of decreasing ipsilateral sensorimotor cortex activity. Increased activity in both the ipsilateral sensorimotor cortex and the SMA has previously been linked to increased task complexity (Boenstrup et al. 2014; Shibasaki et al. 1993; Winstein et al. 1997), therefore, a potentially useful strategy for participants in the Dissociation condition may be to make less complex movements, which could have reduced activity in both these areas.

3.5.3 No clear replication of findings after a single training session

In the current study we failed to replicate the interaction previously reported by Sampaio-Baptista and colleagues (2020), despite very closely matching the design and set-up of the previous study. Exploratory post-hoc tests did confirm that changes in activity with feedback were occurring in the predicted direction (i.e. increasing activity over runs in ipsilateral ROI for the association condition, and a difference between conditions by the

end of training), but the PSC achieved by the participants in the current study was smaller than in the previous study. It has been pointed out in previous NF literature that participants' ability to use NF is fairly variable, with recent research attempting to address factors associated with NF performance (see also Chapter 6). Therefore, it is possible that the current study had a greater number of non-responders in comparison to the previous study.

Future work identifying factors associated with successful NF learning could help to identify responders from non-responders a-priori. Work has begun to explore factors such as baseline brain activity (Haugg et al. 2020) and psychological factors (Kadosh & Staunton 2019) which may be related to NF learning success. Sampaio-Baptista and colleagues (2020) also explored relationships between NF success and brain structure at baseline, finding indications that the strength of the motor network at baseline may impact NF learning. Baseline brain structure has also been implicated in several types of motor learning (Kahn et al. 2017; Sampaio-Baptista et al. 2014). Additionally, participants' ability to learn could be improved by optimising the NF intervention itself, for example by adding monetary reward or making the NF display more engaging (see Chapter 2). This may help participants to boost activity further and learn more quickly, which would be beneficial in participants who may be able to modulate brain activity but are not reaching their full potential.

3.5.4 Limitations

There were several limitations of the current study. Firstly, as the Sham group did not receive training over the same number of sessions as the Real group, it was not possible to compare the multi-day learning effects to the Sham group. However, bi-directional control

in itself is considered an appropriate form of control group for NF studies (Sorger et al., 2019; see further discussion Chapter 2), and therefore could be used to examine the multi-day learning effect across the two conditions. Secondly, as the fMRI data was acquired using a limited FOV, it was not possible in the current study to assess changes in brain activity that may be occurring elsewhere in the brain. Measuring whole brain activity during NF can allow identification of areas that may contribute to NF learning, but are not the regions directly targeted by the NF training. This can shed light on potential mechanisms underlying NF control and learning (for example see Shibata et al., 2019). Finally, the current study did not include measures of transfer of NF learning. These measures are often included in studies to assess whether participants can continue to modulate brain activity when NF is removed. In terms of identifying the appropriate dose of NF, it may be important to additionally consider offline effects such as these as they may give indications of when participants can successfully modulate brain activity without the need of further NF training.

3.5.5 Future work

The potential of fMRI NF to bi-directionally modulate ipsilateral M1 activity could be harnessed in future work targeting motor rehabilitation in stroke patients. For example, the remainder of this thesis focuses on using the Dissociation NF condition trialled here, as a means to increase laterality of motor cortical activity in a study of chronic stroke patients. Further future work could attempt to utilise the two conditions presented in this chapter to provide a more personalised rehabilitation approach, with previous literature suggesting that some patients may benefit from decreasing aberrant activity in ipsilateral M1, whilst others may benefit from enhancing activity. Potential predictors have been identified in previous work, which would allow stratification of patients based on baseline

characteristics into groups that are more likely to benefit from up- versus down-regulation (Stinear et al. 2012). These predictive markers could be applied in future NF work targeting stroke patients.

3.6 Conclusion

In this chapter I explored the effect of having multiple training days on NF learning when participants attempted to bi-directionally modulate activity in ipsilateral M1. Although it was not possible to replicate previous findings of bi-directional modulation on the first training day, there was evidence of beneficial effects of multiple training days. In particular, there appears to be a benefit of multiple training days when trying to increase ipsilateral activity, and additionally some indication of a benefit when increasing contralateral activity. This could have important implications for future studies suggesting that additional training sessions may be beneficial to boost NF learning.

4

Neurofeedback to Increase Laterality of Brain Activity in Chronic Stroke Patients

Parts of this Chapter were adapted from: Sanders, Z. B., Fleming, M. K., Smejka, T., Marzolla, M. C., Zich, C., Rieger, S. W., ... & Johansen-Berg, H. (2021). Self-modulation of motor cortex activity after stroke alters behavior and corticospinal tract structure: a randomized controlled trial. medRxiv.

4.1 Introduction

Modulation of primary motor cortex (M1) activity has previously been suggested as a potential therapeutic approach in chronic stroke patients suffering with hemiparesis (Di Pino et al. 2014; Hummel & Cohen 2006). Targets for modulation include up-regulation of activity in the stroke-affected M1, and up- or down-regulation of activity in the intact M1 (see review: Grefkes and Fink, 2016). Indeed, previous work using brain stimulation techniques, such as transcranial direct current stimulation (TDCS), to modulate activity, have shown some promising improvements in hand functioning in chronic stroke patients. For example, long lasting improvements on clinical outcomes were recently shown in stroke patients following ipsilesional excitatory stimulation (Allman et al. 2016). FMRI Neurofeedback (NF) has also been proposed to be a potentially promising tool for modulation of brain activity (Sitaram et al. 2017; Thibault et al. 2018). In contrast to brain stimulation techniques, NF allows endogenous self-modulation of brain activity, greater spatial specificity and the possibility to target a wider range of brain structures and functions, without relying on external stimulation. Therefore, there may be some benefits in the use of NF over and above the use of external brain stimulation techniques.

Previous work looking at modulation of motor cortex activity using fMRI-NF, has typically focused on healthy younger individuals (e.g. Auer et al., 2015; Blefari et al., 2015; Chiew et al., 2012), although some work has also been conducted in older individuals (Neyedli et al. 2018). Additionally, some pilot studies have been conducted in stroke patients (Liew et al. 2016; Lioi et al. 2020; Mehler et al. 2020), although these typically only included a hand-full of patients and no control group, making it difficult to attribute any modulation achieved due to NF, rather than just due to task practice (either physical or using motor imagery). Therefore, it remains relatively unknown whether stroke patients with hemiparesis are able to use NF to modulate motor cortex activity. The ability to use NF in this sample may be hampered due to a number of factors, such as the actual damage to the brain, altered hemodynamic response or due to more diverse effects of the stroke on cognition or learning in patients. It is therefore important to establish whether stroke patients are able to use NF in a sham-controlled experiment. It also remains unknown how much training is needed in order to be able to self-modulate brain activity. In particular, stroke patients may require more training in order to successfully modulate brain activity in comparison to young healthy participants, however this needs to be weighed against the comfort of the patients who may not tolerate long scanning sessions, as well as the cost of having multiple MRI-scans.

An additional unanswered question in the NF literature is whether participants are able to maintain learning achieved during NF training, when feedback is removed. This is a key consideration in a clinical context and may be one way in which NF could have lasting effects, by allowing participants to learn suitable strategies to modulate brain activity during the NF training, and then continuing to do so in their daily life when required

(Sulzer et al. 2013). A recent systematic review of fMRI-NF studies, found that only approximately one third of studies include a transfer test (Thibault et al. 2018). Of the studies that included a transfer test, approximately two thirds of the studies showed that participants were able to modulate their brain activity in the absence of NF, with the remaining third showing no transfer effect. Therefore, more work is required to determine whether participants can continue to regulate brain activity after feedback is removed, and which factors may be associated with successful transfer of learning.

4.1.1 Aims

A double-blind, sham-controlled randomised control trial (RCT) was carried out (ClinicalTrials.gov NCT03775915) to investigate the efficacy of three sessions of real-time fMRI NF in chronic stroke survivors with ongoing upper-limb impairments. The primary outcome measures of this trial were 1) changes in *lateralization of motor cortex activity* during movements of the stroke affected hand, and 2) changes in *hand function* as assessed using the Jebsen Taylor Test (JTT; Jebsen et al. 1969). Additionally, secondary outcomes included measures of short-term and long-term NF learning transfer, where participants' ability to maintain changes in brain activity was assessed, as well as changes in clinical scores and brain structure.

The current chapter will assess whether chronic stroke patients are able to use real-time fMRI NF to increase lateralisation of brain activity in M1 over multiple training days (the first primary outcome measure). Additionally, it was examined whether any learning achieved during the NF training was maintained when feedback was removed (secondary outcome measure).

4.2 Methods

4.2.1 Recruitment

One-hundred and seventy-three chronic (>6 months post-stroke) stroke patients were contacted to take part in this registered clinical trial (NCT03775915) between January 2018 and March 2020. Of these, 29 patients were lost to follow-up after initial contact, 18 declined to take part and 11 were pending screening when the study was halted (see recruitment flow diagram Appendix B figure S4.1). The remaining 115 patients were screened to assess whether they met inclusion criteria. Initial inclusion criteria included that patients aged 18-85 needed to have had a symptomatic stroke with ongoing effects on upper limb movements on one side of their body. Additionally, patients needed to have some residual movement in their stroke-affected limb. Exclusion criteria included MRI scanning counter-indications (including claustrophobia), previous history of neurological or psychiatric illness and limited communication or inadequate understanding of instructions. In June 2018 criteria were expanded to allow more participants to be included: the upper age limit was removed and participants with more than one stroke could be included provided only one side of the body was affected. Eighty-eight patients were excluded after screening with the most common reasons for exclusion being either not having sufficient movement (34 patients), or not showing any impairments in movement ability of the stroke affected limb (27 patients). Twenty-seven patients were excluded due to other reasons, including MRI contraindications and severe aphasia.

	Patient	Age (years)	Sex	Time post-stroke (months)	Baseline ARAT	Baseline FM	Lesion side / size (mm^3)
Real	1	62	M	120	29	32	R / 52,217
	2	74	F	34	39	60	R / 95,761
	3	54	F	230	8	35.5	R / 2,599
	7	84	M	125	40	55.5	R / 364
	9	64	M	25	19	34.5	R / 6,686
	12	50	M	35	18	28.5	L / 132,460
	13	64	M	52	35	39.5	R / 5,189
	17	57	F	182	25	45.5	R / 23,909
	18	74	M	11	54	52.5	R / 34,917
	20	51	M	57	40	54	R / 12,486
	23	72	M	15	38	42.5	L / 9
	26	59	F	66	38	50.5	R / 4,132
		63.8 (10.5)	**4F/8M**	**79.3(70.2)**	**31.9(12.6)**	**44.2(10.3)**	**2L/10R**
Sham	4	86	M	44	55	60	R / 10,366
	6	41	M	99	17	27	L / 70,517
	8	60	M	9	53	56	L / 1,266
	10	57	M	104	40	47.5	L / 2,006
	11	61	M	70	34	36	R / 3,449
	14	54	F	237	37	52	L / -
	15	59	M	96	20	18.5	R / 11,769
	16	75	M	67	53	63	R / 27,762*
	21	36	M	29	35	60	L / 548
	22	59	M	23	35	41.5	R / 168,702
	24	64	M	7	12	31	R / 1,664
	25	59	M	58	28	38.5	R / 3,220
		59.3 (13.1)	**1F/11M**	**70.3 (62.5)**	**34.9(14.2)**	**44.3(14.5)**	**5L/7R**

Table 4.1 Patient demographics for the Real (top) and Sham (bottom) group. Group means and standard deviations indicated where appropriate. F, female; M, male; L, Left; R, Right; Note: Fugl Meyer Upper Extremity assessment (FM) was scored by two independent assessors and the average score was taken. *indicates patient with cerebellum lesion where the affected hand is ipsilateral to the lesion.

Participants

Twenty-seven patients (22 males) aged between 36 to 86 years, met the initial screening criteria and were recruited into the trial. The trial was terminated in March 2020 due to the COVID-19 lockdown before the full sample (30 participants) could be collected. Sample size was based on previous NF studies using a similar study design (Neyedli et al. 2018; Sampaio-Baptista et al. 2020). All patients provided informed consent in accordance with

the Declaration of Helsinki and the ethics committee of the University of Oxford approved protocol (14/LO/0020). Two patients withdrew from the trial after the initial baseline day and were therefore not randomised (one due to M1 lesion, one did not tolerate the MRI environment). One patient's study participation was halted after the baseline day due to the COVID-19 breakout. Twenty-four patients (19 males) were randomised following the baseline day (12 in the real group and 12 in the sham group) and completed the neurofeedback training. Patients were randomised to reduce variance in time since stroke and baseline score on the Action Research Arm Test (ARAT) using a freely available randomization service (www.rando.la). However, the first three participants were forced into the Real group in order to ensure that sham videos were available. Baseline characteristics of all the randomised patients can be seen in table 4.1 and overlap of lesion volumes across all patients is shown in figure 4.2C.

4.2.2 Study design

Patients were invited to attend six testing days (see figure 4.1). During an initial baseline visit, clinical motor assessments of hand functioning were carried out, as well as baseline structural and functional MRI measurements and electroencephalogram (EEG). After the baseline testing day, patients were randomised and invited to attend three neurofeedback training days, during which they received either real or sham neurofeedback. Patients then attended two follow-up testing days – one at one-week post neurofeedback where all of the baseline measures were repeated, and another at one-month post neurofeedback where only the motor assessments were completed. The primary outcome measures of this trial were a) changes in lateralization of motor cortex activity and b) changes in motor performance on the Jebsen Taylor Test which was assessed on every testing day. Secondary outcome measures included measures of NF learning transfer, clinical measures of upper limb function and measures of brain structure. In this chapter I will focus on the

fMRI data acquired during the NF training days (Days 2-4), further details on the other testing days are described in Chapter 5.

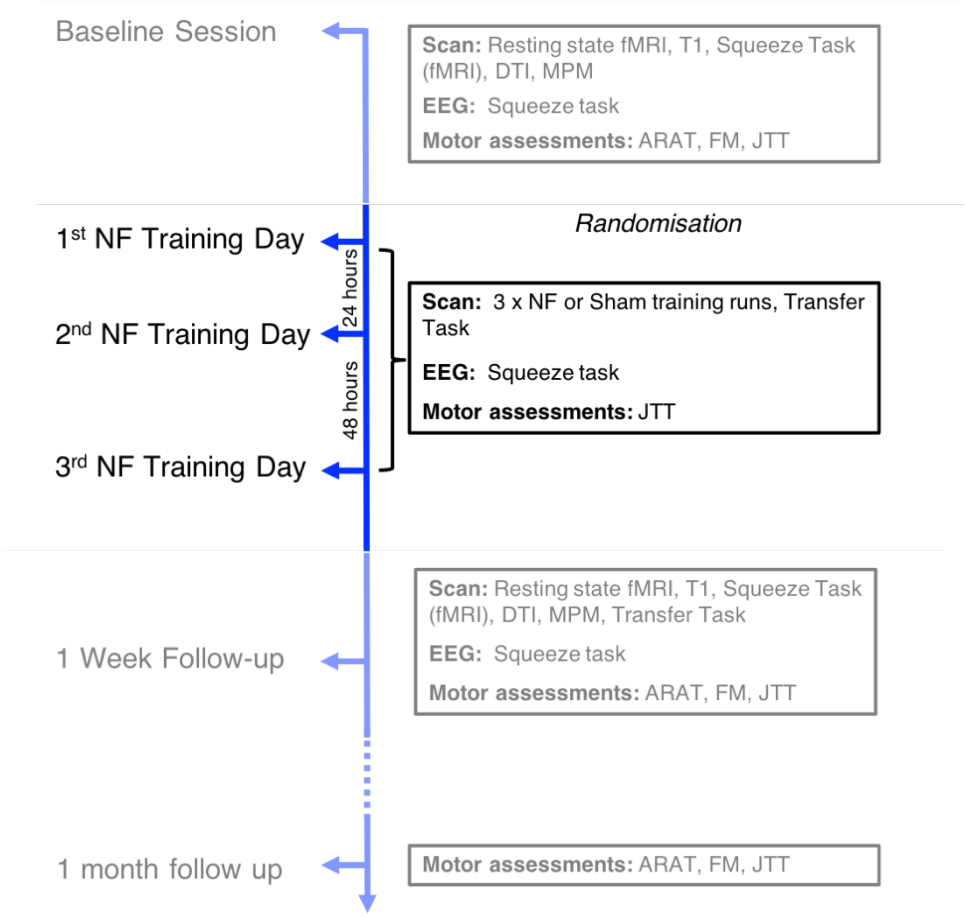


Figure 4.1 Full study timeline. Patients were invited to attend 6 testing days in total. In this chapter, only data from the three NF training days is presented (highlighted).

Neurofeedback training was carried out over three days, with 24 hours between the first two training days, and 48 hours between the second and third training days (see figure 4.1). During each neurofeedback training day, a functional localiser was initially carried out, during which patients were instructed to open and close their hands at a steady rate that was comfortable for them. There were three 15 seconds movement blocks for each hand (6 in total), interspersed with 15 seconds rest blocks. Patients saw the instructions 'Squeeze Right', 'Squeeze Left' or 'Rest' on the screen, displayed using TurboFeedback (v 1.0). A real-time GLM analysis was carried out and used to identify the peak activity in the hand

knob region of the sensorimotor cortex, and two ROIs (1.6x1.6x1cm) were centred on the peak activity in each hemisphere (figure 4.2A). A probability map of the placement of the ROIs in MNI space for all the patients is show in appendix B figure S4.2.

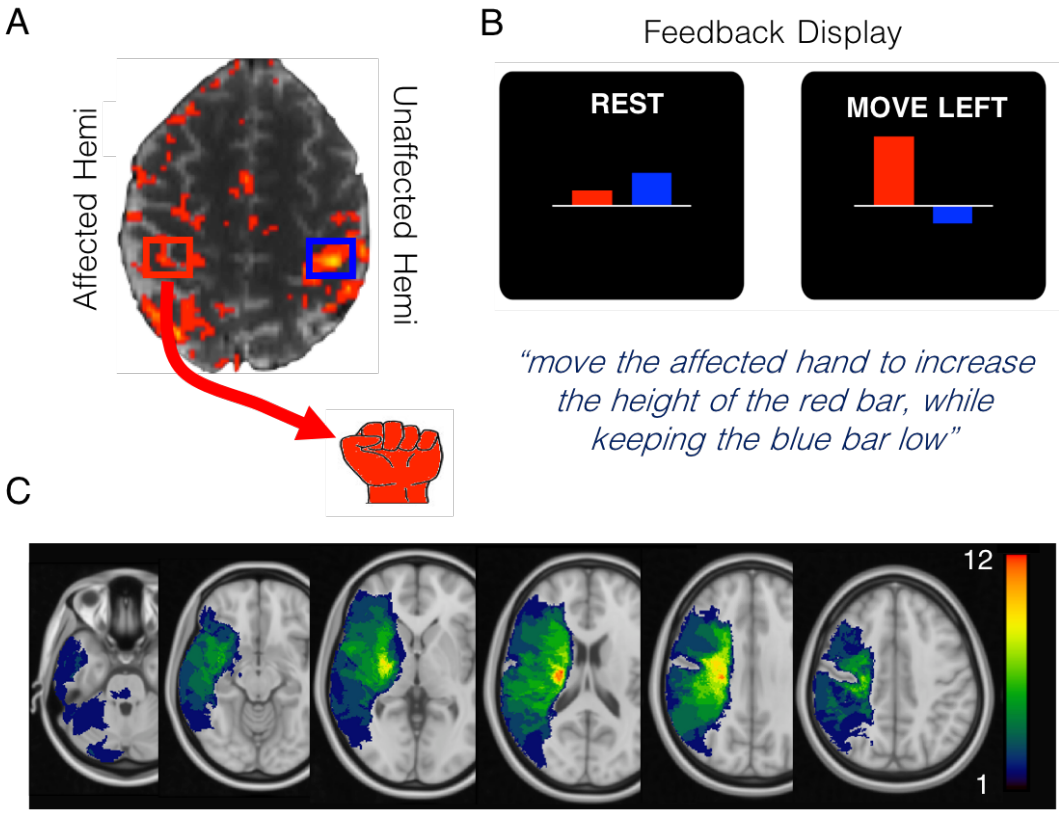


Figure 4.2 Neurofeedback display and Lesion overlap maps A) Example data from a localiser scan and ROI placement over peak activity in hand knob region. **B)** NF display and instructions. Patients saw a red and blue bar on the screen reflecting activity in their stroke affected and unaffected ROI respectively. **C)** Lesion overlap map in standard space with warmer colours indicating greater lesion overlap, left sided lesions flipped.

Patients experienced three NF training runs on each day. During NF training runs patients saw two bars on the screen (figure 4.2B), the height of which was determined by the measured percent signal change (PSC) in each of the ROIs. Patients were instructed to try different movements with their stroke affected hand to increase the size of the red bar, whilst keeping the blue bar as low as possible, with the height of the bars being updated with each TR (for full instructions, see Appendix A: supplementary information). This corresponded to increasing activity in the stroke-affected ROI, while keeping activity in

the stroke-unaffected ROI to a minimum. Patients were given some suggested strategies that they could try, including opening and closing their stroke-affected hand, tapping individual fingers or moving their wrist (see Appendix A). Each NF training run consisted of six 30-second NF blocks during which the instruction "Move Left" or "Move Right" (depending on which hand was stroke-affected) was displayed above the bars, and seven interspersed 30-second rest blocks, during which the instruction changed to "Rest". Patients were explicitly instructed not to move their stroke-unaffected hand at any point, and electromyography (EMG) was acquired to confirm this. After each NF training run the patients were allowed to rest until they felt ready to continue to the next run.

Before and after the NF training runs, patients completed Pre- and Post- Transfer runs. During these runs patients were instructed to use movement strategies that they had found to be successful during the NF training runs, when they saw the instructions "Move Right" or "Move Left", depending on which arm is stroke affected, on the screen. (Note: on the first neurofeedback training day, before having experienced any NF training runs, patients were instructed to practise movements with their stroke affected hand and received some suggested movements to try). However, unlike the NF training runs, they did not see the bars on the screen. During the transfer runs, patients received four 30-second movement blocks which were interspersed with five 30-second rest blocks during which the instructions changed to "Rest". These transfer runs were used to assess whether patients are able to maintain any changes in lateralisation achieved during the NF, when NF is no longer available. Patients in the Sham group received identical instructions and tasks as patients in the Real group, however instead of seeing their own brain activity displayed in the NF bars, they saw a video of a previous patient's feedback. This allowed us to match the motivation and perceived success across groups (Sorger et al. 2019).

After each NF training day, all patients completed a debriefing questionnaire (see Appendix A: supplementary information) in which they were asked about their perceived control over the two bars on a 5-point scale ranging from 1 (not in control) to 5 (fully in control). They were also asked which strategies they had used and how successful these strategies were from 1 (unsuccessful) to 5 (successful). Finally, at the end of the study, patients were asked whether they felt like they had received feedback of their own brain activity in order to assess if any unblinding had occurred. The experimenter who interacted with the patients, providing instructions, conducting debriefing questionnaires and carrying out behavioural assessments (described in chapter 5), was blind to which condition the patient was assigned to. A separate experimenter was in charge of running the NF software itself and therefore was not blinded to the condition, however their interactions with the patients was kept to a minimum.

4.2.3 MRI acquisition

MRI data was acquired on a 3 Tesla Siemens Magnetom Prisma MRI scanner (Siemens AG, Erlangen, Germany) using a 32-channel head coil. During the NF training days all functional MRI data was acquired using multiband gradient EPI (TR=933ms, TE=33.4, flip angle=60°, slice thickness=2mm, in-plane resolution=2 x 2, 72 slices, multiband factor=6). Additionally a high-saturation (single-band) whole brain EPI image was acquired for registration purposes at the start of the scan. A dual echo fieldmap was collected for fieldmap unwarping (TR = 482ms, TE1 = 4.92ms, TE2 = 7.38ms, flip angle=46°,slice thickness = 2mm, 49 slices). A whole brain T1-weighted (MPRAGE) image acquired during the baseline scan was used for registration purposes (resolution=1mm isotropic, TR = 1900ms, TE = 3.97ms, flip angle = 8°, TI = 904ms, slices = 192).

4.2.4 Online data analysis

Functional images acquired during the NF training days were processed in real-time using Turbo-BrainVoyager 3.2 (Brain Innovation, Maastricht, Netherlands). This was achieved by transferring dicom images via a direct TCP/IP network link from the MRI console computer to the real-time computer. Images were then pre-processed in real-time, including head motion correction, spatial smoothing with a gaussian kernel of 3mm full-width-half-maximum (FWHM) and linear temporal drifts were removed from the data. A recursive GLM was carried out to compute T-Statistics for the contrast of Movement > Rest. The height of the bars (feedback) displayed to the patients was updated with each TR (933ms) and was calculated as follows:

$$FB = (CurrValue - Baseline) / Baseline * 100$$

Where CurrValue is the current BOLD signal in the ROI and Baseline is the average BOLD signal in the ROI during the last 20 TRs of the preceding Rest block. Only the last 20 TRs were included in the baseline calculation in order to exclude any possible BOLD decay from the previous movement block at the beginning of the Rest block. This resulted in a percent signal value that determined the height of the bar with respect to the maximum bar height which was set to double the PSC measured in the ROI during the functional localiser. Positive values were represented above the centre line and negative values below it. The side on which the bars were displayed depended on which hand was stroke-affected, with the red bar always being displayed on the same side as the stroke-affected hand, and the blue bar on the same side as the stroke unaffected hand.

4.2.5 Offline fmri data analysis

Offline data analysis was conducted using FSL version 6.00 (FMRIB, Oxford). Images were first pre-processed using FEAT (FMRIB's Expert Analysis Tool), including carrying out motion correction using MCFLIRT (Jenkinson et al. 2002), brain extraction using BET (Smith 2002), fieldmap-based EPI distortion correction using FUGUE, spatial smoothing using a 5mm FWHM Gaussian kernel and high pass temporal filtering with a cut-off of 90 seconds. Co-registration was achieved by first using linear registration (FLIRT; Jenkinson et al., 2002; Jenkinson and Smith, 2001) to register the multiband functional data to a single volume, high-contrast (single-band) EPI image. Next, the high-contrast EPI image was linearly registered to the T1-weighted image using boundary-based registration (6 degrees of freedom; Greve and Fischl, 2009). For voxel-wise group analysis, nonlinear registration (FNIRT) into MNI space was carried out using lesion masks to exclude voxels in the lesioned area from the normalization process. Data from right-hand affected patients was mirror flipped. A first level GLM was carried out in FEAT for each fMRI run in order to compute task-based statistical parametric maps. A double gamma-HRF convolved boxcar regressor, as well as its temporal derivative, was used to model the movement and rest blocks with contrasts set to Movement > Rest. Intermediate fixed effects analysis steps were carried out in order to average activity within (average of runs per day) or across (average of each run over days) NF days and to create difference maps between days and runs (e.g. Day1 > Day2; Run 1 > Run 2). All group level analysis was carried out using FMRIB's Local Analysis of Mixed Effects (FLAME) to compare difference maps between the Real and the Sham groups. Group Z-statistic images were thresholded using clusters determined by $Z > 3.1$ and a family-wise corrected cluster significance threshold of $p<0.05$ was applied.

4.2.6 Laterality Index Calculation

In order to assess changes in laterality of motor cortex activity (one of the primary outcome measures), a laterality index (LI) was calculated based on the magnitude of the fMRI signal change in the ROIs used for NF training. Laterality indices are traditionally calculated as:

$$LI = \frac{ROI_{aff} - ROI_{unaff}}{ROI_{aff} + ROI_{unaff}}$$

Where ROI_{aff} and ROI_{unaff} are fMRI activity in the affected and unaffected ROIs. This calculation yields a number between -1 and 1, with positive values indicating lateralisation towards the affected hemisphere (higher values indicating stronger lateralisation), and negative values indicating lateralisation towards the unaffected hemisphere (lower values indication stronger lateralisation). As it was observed that the average magnitude of activity in ROIs (as assessed using the average T-Stat value) was occasionally negative, which resulted in incorrect or meaningless LI values (Seghier 2008), a threshold was applied to the activity in each ROI using the same approach as in work by Fernandez and colleagues (2001). In brief, for each ROI on each NF run, the mean maximum activity was calculated as the mean T-stat value of the top 5% of voxels showing the highest activity in the ROI. The threshold of activity voxels needed to achieve for inclusion in the LI calculation was then set to 50% of this mean maximum activity. This ensured that only activity from the most active voxels was included in the LI calculation and that the average activity for each ROI was above 0. This approach has previously been validated as more robust and reliable than LI based on unthresholded signal intensity or voxel counts (Jansen et al. 2006). This approach deviates from the described calculation of LI in the trial description (*'The activation in the region of interest is calculated for each hemisphere and the laterality index calculated as: (ipsilesional hemisphere – contralesional hemisphere) / (ipsilesional hemisphere + contralesional hemisphere)'*). Using a thresholded approach

allowed us to still use a LI to quantify lateralisation of motor cortex activity, without having to discard data due to negative values.

4.2.7 Statistical Analysis

Due to excessive motion, defined as average absolute motion >2 standard deviation above group mean motion, 7 individual NF runs (2 in the real group from the same patient, 5 in the sham group with three runs from the same patient and two from different patients) were removed. One run was removed from the real group due to a technical issue with the NF software. Additionally, two transfer runs were removed from the sham group due to excessive motion in separate patients, one transfer run was not collected due to the patient stopping the scan early and all of the transfer data for one patient in the real group was removed as 5/6 transfer runs had excessive motion.

Because of this missing data for some of the subjects on some of the runs, Linear Mixed Models (LMM) were conducted where appropriate using RStudio (version 1.2.5) and the lme4 package (Bates et al. 2015). All models used Restricted Maximum Likelihood (REML) estimation. LMMs allow for incomplete data, as well as greater flexibility in analysis and better handling of repeated-measures dependencies (Gueorguieva & Krystal 2004; Misangyi et al. 2006; Quené & Van Den Bergh 2004), giving this approach advantages over the traditional Analysis of Variance (ANOVA; Boisgontier and Cheval, 2016). Random intercepts per patient were included in the random effects structures and the addition of random slopes per patient was assessed using a likelihood ratio test (Matuschek et al. 2017). There were no cases where the addition of random slopes significantly improved the model fit. P-values for fixed effects were derived using the Satterthwaite approximation for degrees of freedom, as implemented in the package

lmerTest (Kuznetsova et al. 2017). This approach has previously been shown to be less prone to type I errors, and is less sensitive to sample size than other methods (Luke 2017). Post-hoc comparisons were carried out using the emmeans package, with tukey method for multiple comparisons. We report unstandardized effect sizes (parameter estimates; *b*) whenever possible in line with general recommendations (Pek & Flora 2018), as there is currently no agreed upon way to calculate standard effect sizes for individual terms in LMMs.

4.3 Results

4.3.1 Patients perceived equal control over the bars, irrespective of group

At the end of the study patients were asked whether they felt that they had received real feedback of their brain activity; three patients replied that they felt this had varied or that they were not sure. Two of these patients were in the sham group and one was in the real group. All the remaining patients reported that they felt they had received real feedback of their brain activity, indicating that blinding was largely maintained.

Additionally, patients in both groups were also asked to rate their perceived control over the two bars during the NF training runs on a scale of 1 – 5 (not in control – fully in control). Patients generally reported more control over the red bar (see Appendix B, figure S4.3), reflecting activity in the ipsilesional hemisphere (median = 3, STD = 1.02), than over the blue bar, reflecting contralesional hemisphere activity (median = 2, STD = 1.19). This was confirmed using a related-samples Wilcoxon signed ranks test which showed significantly lower perceived control for the blue bar than the red ($Z=-3.17, p=0.002$). On average, over

all of the training days, patients in the real and in the sham group reported similar levels of perceived control over the bars, with a Mann-Whitney U test revealing no significant differences between the two groups for the two bars (Red bar: U = 51, Z=-1.317, p=0.188, Blue bar: U = 49, Z=-1.389, p=0.165). This indicates that whether or not the patients saw real feedback of their brain activity, they felt they had similar control of the feedback.

4.3.2 Primary outcome: Patients increased laterality of brain activity within, but not between, NF training days

For the first primary outcome measure, in order to assess whether patients were able to use NF to change lateralisation of brain activity, laterality of brain activity in the two ROIs was calculated (see section *Laterality Index Calculation*) and is displayed in figure 4.3A.

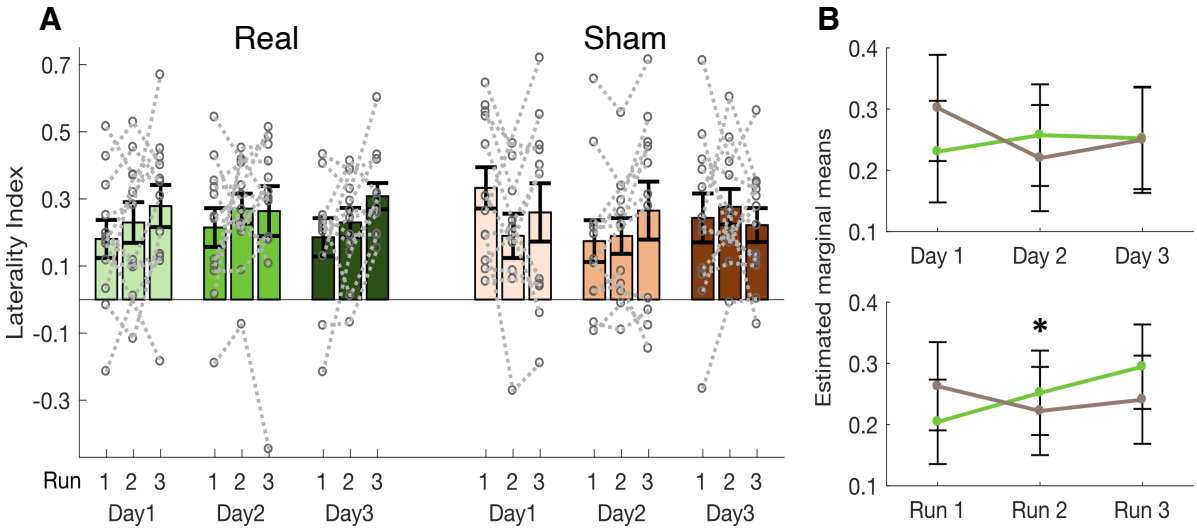


Figure 4.3: NF learning occurs within but not across training days. (A) Laterality of M1 activity (LI) is displayed for the Real (green/left, n=12) and Sham (orange/right, n=12) group over all the training runs and days. Bars represent group means, grey lines show individual participant data and error bars represent s.e.m. **(B) Top**: When assessing NF learning *across* training days by averaging LI for each day (pooled over runs), there were no significant interaction or main effects. **Bottom**: When considering NF learning *within* training days by averaging LI for each run (pooled over training days), a significant interaction could be observed between Run and Group (p=0.037), with the Real group increasing LI over runs, whilst the Sham group didn't change. Estimated marginal means and confidence intervals are shown for each run. *p<0.05

We first assessed whether there was evidence of NF learning over training days. In order to assess this, LI was averaged over runs within days for each patient, resulting in three average LI values (one per day; Figure 4.3B Top). A LMM was fit to the data using the lme4 package (Bates et al. 2015). LI was included as the outcome variable with Group (Real vs Sham), Day (3 training days) and their interaction added as fixed effects. Additionally, baseline LI was added as a covariate to adjust for baseline differences in LI. Baseline LI was assessed during the first transfer run before any NF was experienced. Random intercepts per patients were included in the random effects structure. The complete fitted model was:

$$LI \sim Group * Day + Baseline + (1|Patient)$$

Using p-values derived with the Satterthwaite approximation for degrees of freedom, we did not find a significant Group*Day interaction ($F(2,46)=1.061$, $p=0.355$), or main effect of Group ($F(1,23)=0.065$, $p=0.800$) or Day ($F(2,46)=0.262$, $p=0.771$), suggesting that LI scores did not change over days or between the two groups. Estimated model fixed effects are displayed in table 4.2.

Table 4.2 Estimated model fixed effects estimates, standard error and T-statistic. Reference group for the model was Real group Day 1.

Effect	**Estimate**	**Std. Error**	**t**
Intercept	0.134	0.045	2.97
Group	0.071	0.060	1.19
Day 2	0.027	0.053	0.509
Day 3	0.022	0.053	0.411
Baseline	0.466	0.092	5.08
Group*Day2	-0.109	0.076	-1.427
Group*Day3	-0.074	0.076	-0.968

Next, we assessed whether there was evidence of NF learning *within* training days. This was assessed by averaging the LI for each NF run between days for each patient, resulting in average LI values for the first, second and third NF runs, pooled over days (Figure 4.3B Bottom). Then a LMM was fit to the data in the same way as described above, but with the fixed effect of Run rather than Day:

$$LI \sim Group * Run + Baseline + (1|Patient)$$

P-values were derived using the Satterthwaite approximation for degrees of freedom and a significant Group * Run interaction was found ($F(2,46)=3.556$, $p=0.037$). (NOTE: we did not carry out multiple comparison correction across the LMMs in order to minimize false negatives due to a lack of power. This could lead to increased false positives therefore results should be interpreted with caution.) Post-hoc pairwise comparisons (using Tukey correction for multiple comparisons) showed that this interaction was driven by a significant increase in LI between Run 1 and Run 3 in the real group ($t(50.4)=2.815$, $p=0.019$), whereas no significant differences between runs were found in the sham group (all: $t(50.4)<1.5$, $p>0.1$). This suggests that LI increases over runs in the real group, whereas no significant differences in LI over runs are observed in the sham group. Estimated model fixed effects are shown in table 4.3.

Table 4.3 Estimated model fixed effects. Reference group was the Real group on Run 1.

Effect	Estimate	Std. Error	t
Intercept	0.105	0.040	2.69
Group	0.058	0.050	1.16
Run 2	0.047	0.031	1.55
Run 3	0.090	0.031	2.95
Baseline	0.479	0.096	4.97
Group*Run2	-0.088	0.044	-1.99
Group*Run3	-0.112	0.044	-2.53

As the LI is a composite measure, changes in LI could be driven by either activity changes in the affected or the unaffected hemisphere. Therefore, in order to shed light into what activity changes may be driving the change in LI over NF runs (up-regulation in the affected hemisphere, or down-regulation in the unaffected hemisphere), an exploratory LMM was conducted on the thresholded activity in each of these hemispheres separately over the runs (see Appendix B figure S4.4 for raw data). The LMM was conducted in the same way as the models described above, with Group and Run as fixed factors, baseline activity as a covariate and random intercepts per patient in the random effects structure. When looking at activity in the unaffected hemisphere (represented by the blue bar), although activity was lower in the Real group (after adjusting for baseline), there were no significant interactions or main effects (all F's < 0.4 , all p's > 0.1), with activity staying largely the same across the runs and across the groups (see estimated marginal means in table 4.4). When looking at activity in the stroke affected hemisphere, the Real group has lower activity but appears to increase activity over runs, whereas the Sham group stayed the same (table 4.4), however the interaction between group and run failed to reach significance ($F(2,48)=2.74$, $p=0.075$). There was also no significant main effects of Group or Run (both $F < 2$, both $p > 0.1$).

Table 4.4 Estimated marginal means for the Affected and Unaffected Hemisphere ROI (used for the NF training) over the average three NF runs displayed for the Real and the Sham group

Unaffected			Affected	
	Real	Sham	Real	Sham
Run 1	4.42	5.63	6.93	9.43
Run 2	4.68	5.61	7.60	8.56
Run 3	4.38	5.57	7.92	9.07

Increased activity in the putamen during initial neurofeedback learning

Exploratory voxel-wise whole brain analysis was carried out to assess any changes in brain activity that may have occurred outside of the ROIs used for the LI calculation. Similar to the ROI analysis, here we focused on within- and between-days effects. Therefore, data was first averaged either across days (mean of all runs in one day) or across runs (mean of each run across days). Following this, difference maps were created for each comparison (e.g. Day1 vs Day3; Run1 vs Run 2) which were then compared between the Real and the Sham group. No evidence was found for differences between the Real and the Sham group when comparing differences between average day activity. When comparing differences between runs, a significant cluster was found in the putamen of the unaffected hemisphere (figure 4.4A; peak coordinates based on MNI 152 brain template: -18 16 -4), when comparing differences in activity between Run 1 and Run 3 between the two groups (Real > Sham). This suggests that there is a greater difference in activity in this region in the Real group than the Sham group between these runs. In order to visualise this effect, the PSC was extracted from the significant voxels in the putamen and is displayed in figure 4.4B. It can be seen that activity was high in this cluster during the first NF runs in the Real group and that this activity has reduced at Run 3. In comparison for the sham group, activity slightly increases from Run 1 to Run 3, but on average remains lower than what is observed on the first runs of the Real group. This indicates increased activity in the putamen during the initial feedback learning stage of the Real group, that is not seen in the Sham group. No other significant differences were found.

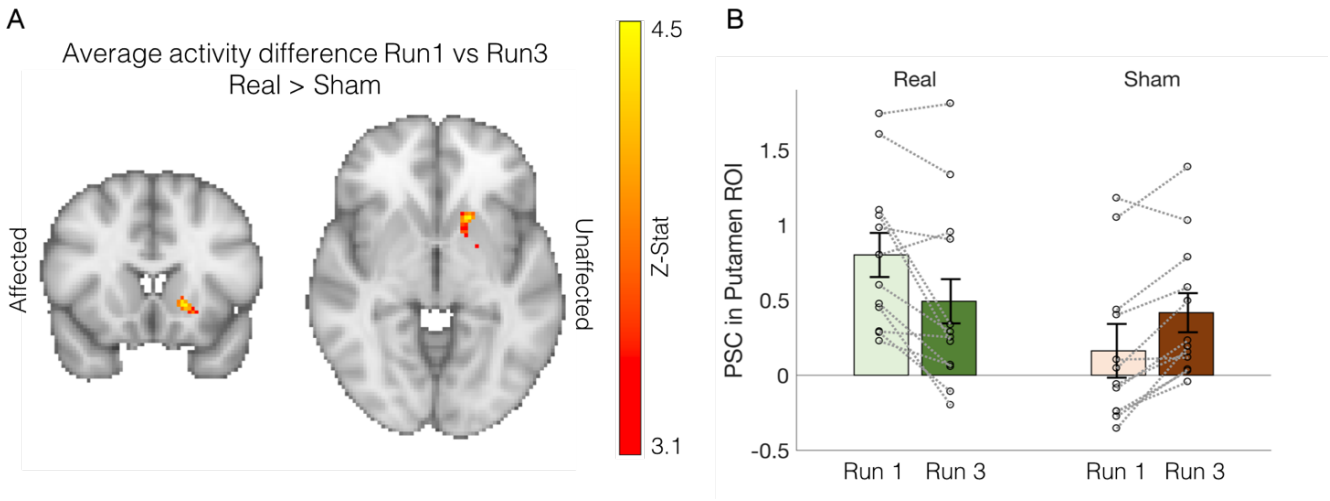


Figure 4.4 Increased activity in the putamen for the Real NF group during the first NF runs. A) A significant cluster was found in the putamen of the unaffected hemisphere where the change in average activity between Run1 and Run3 was greater in the Real group than the Sham group. **B)** For visualisation purposes the PSC values were extracted from the significant cluster for each participant.

4.3.3 Secondary outcome: No evidence of transfer of learning when neurofeedback is removed

Secondary outcome measures included *changes in lateralisation of brain activity* when NF is removed. In order to test for this transfer effect , a LI was calculated from the activity in the ROIs used during the NF training, for the pre- and post- transfer runs on each day (Figure 4.5A&C). To assess whether there was a within-day transfer effect, a change score was calculated between pre- and post-transfer LI on each day. A LMM was fit to this data with fixed effects of Group and Day, and random intercepts included in the random effects structure.

$$\Delta\text{TransferLI} \sim \text{Group} * \text{Day} + (1|\text{Patient})$$

P-values derived using Satterthwaite approximation for degrees of freedom indicated that there was no significant interaction ($F(2,44)=0.417$, $p=0.662$) or main effects (Group: $F(1,23)=1.642$, $p=0.213$; Day: $F(2,44)=2.276$, $p=0.115$). Therefore there was no evidence that the real group had a larger transfer effect (change in LI from pre- to post-transfer run)

than the sham group, or that there were any changes over the NF training days (Estimated model fixed effects are shown in table 4.5). However, a large amount of variability in transfer of NF learning was observed with some patients showing large increases in LI from pre to post transfer runs, and others showing no change or a decrease in LI. Furthermore, a substantial amount of variability is also seen in patients' ability to use the NF during the training runs. Therefore it was hypothesised in an exploratory analysis that patients who were more successful at using NF would also show the largest increase in LI on the transfer task.

Table 4.5 Estimated model fixed effects. Reference group was the Real group on Day 1

Effect	Estimate	Std. Error	t
Intercept	0.097	0.061	1.59
Group	-0.086	0.087	-0.98
Day 2	0.088	0.062	1.43
Day 3	-0.038	0.062	-0.62
Group*Day2	-0.043	0.088	-0.49
Group*Day3	0.035	0.088	0.39

To explore this possibility, transfer success (average post-pre difference in LI per patient, adjusted for baseline LI), was correlated against a measure of NF success (calculated as a regression slope over LI of NF runs for each day, averaged over training days; see chapter 6 for more details). Where values were missing, the average of the remaining transfer runs was taken. Two patients (one in each group) were excluded from the correlation analysis due to missing data (one in the real group due to motion artefacts in almost all transfer runs, and one sham patient for which it was not possible to calculate a NF success score due to motion corrupted runs). When these values were correlated using Spearman's rho the predicted positive correlation could be observed in the Real group (figure 4.5B), with higher NF success correlated with a larger transfer effect (rho = 0.59, p = 0.03, one-tailed) whereas a negative correlation (figure 4.5D) was observed in the sham group (rho = -0.66,

p = 0.03, two-tailed). A Fisher's Z-test confirmed that the two correlations were significantly different from each other (Z=2.94, p =0.002).

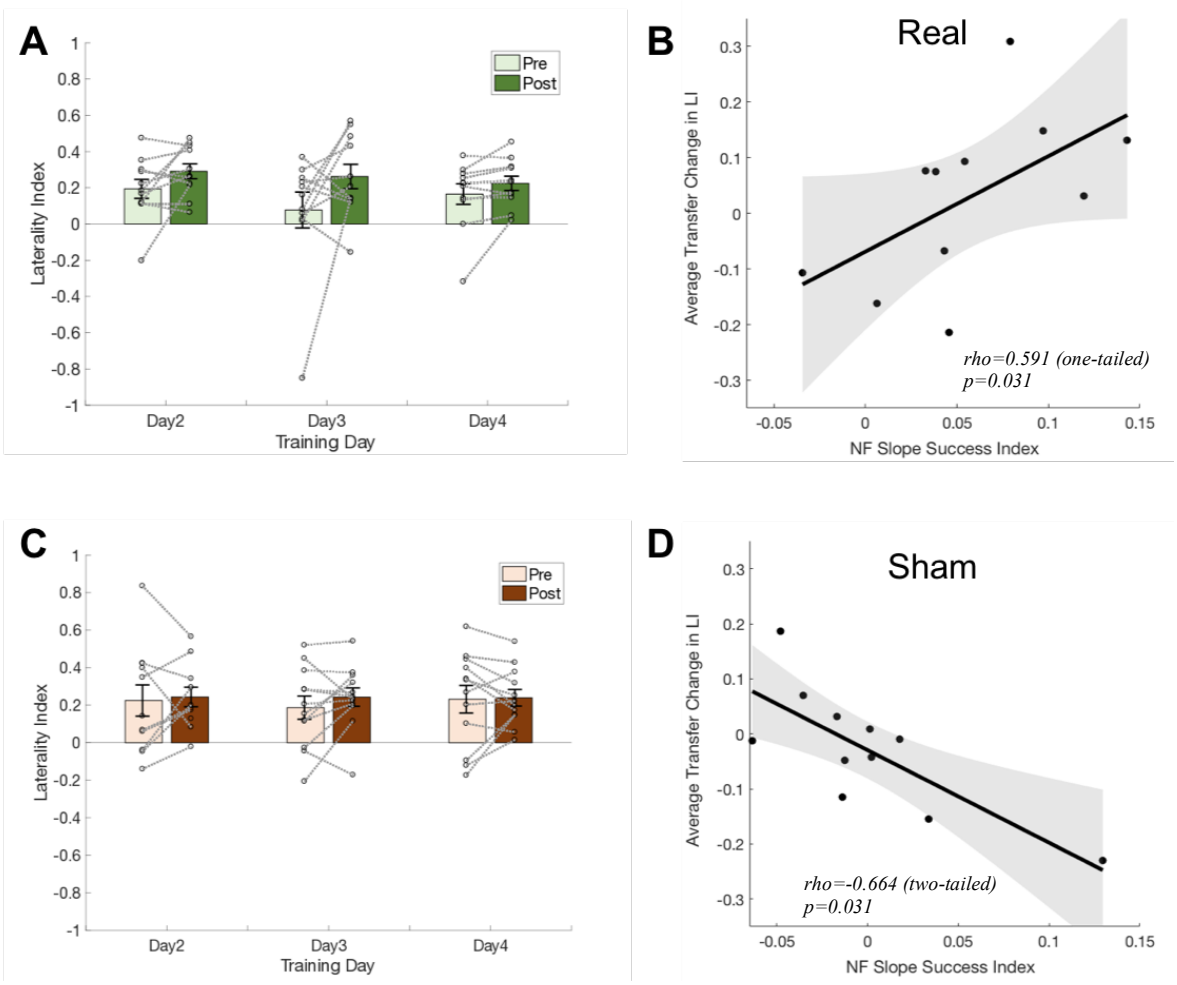


Figure 4.5 No evidence of a group level transfer effect. A) LI for the pre- and post-transfer run on each training day plotted for the Real group, including data points for individual patients. Error bars represent s.e.m. **B)** Correlation between the Real NF groups change in LI between the transfer runs (averaged over days) and patients NF success. Shaded areas represent 95% confidence intervals. **C)** and **D)** The same information is presented as in A and B but for the Sham group.

Finally, exploratory whole-brain voxel-wise analysis was carried out in order to assess any changes in brain activity that may have occurred elsewhere in the brain between transfer runs. Average difference maps between the Pre- and Post- transfer runs were compared between the two groups. No significant clusters were found in which the real or the sham group showed greater differences in activity, either when comparing the average Pre-Post difference across all the days, or when comparing the Pre-Post differences on each day.

4.4 Discussion

In this chapter I explored chronic stroke patients' ability to use real-time fMRI NF to increase laterality of brain activity in primary motor cortex. For this, I focused on the results of a double-blind, sham-controlled RCT, in which patients' ability to use NF to increase laterality of brain activity was assessed (primary outcome measure), as well as whether they can continue to modulate brain activity when NF was removed (secondary outcome measure). The main findings of the study are discussed below:

4.4.1 Laterality of M1 activity increases with Real NF within, but not across, training days

The first primary outcome measure of this trial was changes in lateralization of brain activity during movements of the affected hand throughout the NF sessions. The results of the study suggest that patients were able to use neurofeedback within NF training days to increase laterality of brain activity, however there was no evidence of carry-over effects onto the next training days. This result is in line with the work presented in chapter 3, in which a carry-over effect was only found in the condition that required co-activation of the two motor cortices. Although multiple training days do not appear to benefit the NF learning per se in this study, it is possible that having multiple training days may increase the likelihood of inducing a behavioural effect or brain changes. Previous NF studies have used a wide-range of training schedules from single days of NF training, to training over multiple days or weeks, with some studies reporting learning as early as the first NF run (e.g. Neyedli et al., 2017), and other showing gradual learning over multiple training days (e.g. Sitaram et al., 2012). The optimal dose of NF training has not yet been established, and may well vary depending on the population being studied, and the NF target.

Although fMRI Neurofeedback has previously been suggested as a potentially useful tool for stroke rehabilitation (Linden & Turner 2016; Wang et al. 2018), there have not been many previous studies investigating stroke patients' ability to use fMRI-NF for motor rehabilitation. Neyedli and colleagues (2017) demonstrated that older individuals, of a more similar age as typical stroke patients, were able to use NF to modulate laterality of motor cortex activity. In this older cohort, compared to a younger cohort, this effect was primarily driven by down-regulation of the ipsilateral motor cortex, which was hypothesised to be increased in older adults in line with previous literature (Ward & Frackowiak 2003). In the current study, we did not find significant effects when looking at activity in the two hemispheres separately, which may be an indication that the observed change in LI is driven by more subtle changes occurring in both hemispheres.

There have also been some small pilot studies examining fMRI NF in stroke patients. A recent study conducted in four stroke patients, combined fMRI and EEG NF to modulate activity in ipsilesional motor cortex and SMA using motor imagery (Lioi et al. 2020). This study found that two of the patients succeeded in increasing ipsilesional activity and hand function, with the authors suggesting that successful modulation may depend on the intactness of the CST. Other fMRI-NF pilot studies have targeted different areas of the motor system (Mehler et al. 2020). For example, one study that included two stroke patients and four controls looked at modulation of ventral premotor cortex and found that ability to modulate activity seemed to be associated with baseline intracortical facilitation (Sitaram et al. 2012). Another study investigated four stroke patients' ability to modulate connectivity between ipsilesional M1 and thalamus (Liew et al. 2016). Three patients were able to successfully modulate connectivity and the authors suggested that modulation

success may be dependent on baseline impairment level (a hypothesis explored in chapter 6). Therefore, previous literature looking at motor cortex modulation with fMRI-NF in stroke patients is sparse and the current work is, to the best of my knowledge, the first larger scale, sham-controlled study addressing this question. However, in the pilot studies conducted to date, the variability in NF response is a common theme and studies have hinted at factors that may be related to NF learning success. In the current study, although on average patients succeeded in using the NF, there was also evidence of variability in patients' ability to use NF, and this will be further explored in chapter 6.

4.4.2 Increased activity in the putamen during initial neurofeedback learning

When looking at whole-brain activity changes during NF training in an exploratory analysis, putamen activity appeared to be increased during the initial NF training runs in the real group compared to later training runs, and compared to the sham group. This may suggest that the putamen could play a role during early NF learning. The dorsal striatum (including the putamen) has previously been implicated in motor learning (Doyon et al. 2009), reward processing and decision making (Balleine et al. 2007), and reinforcement learning (O'Doherty et al. 2004). It has also been suggested as a neural substrate that may be important in neurofeedback learning (Emmert et al. 2016; Shibata et al. 2019; Sitaram et al. 2017; Zhao et al. 2021). Indeed, previous work in rats that learnt to self-regulate activity in M1, has implicated the dorsal striatum in playing an important role in neurofeedback learning (Koralek et al. 2012, 2013). In particular, it was demonstrated that both dorsal striatum activity, and the coherence between dorsal striatum and M1 neurons, increased with NF learning. Additionally, when long-term potentiation was blocked in the dorsal striatum, the rats were impaired in their ability to learn to self-regulate brain activity.

This suggests that the dorsal striatum may be a crucial neural substrate for NF-related learning, with some recent work demonstrating a relationship between right putamen volume and NF learning success (Zhao et al. 2021). Involvement of the striatum or cortico-striatal loops in NF learning is in line with mechanistic models of NF learning, such as operant learning or skill learning, which also rely on striatal involvement (Sitaram et al. 2017). Importantly, this may have implications for stroke patients with lesions in the striatum, who may be impaired in NF learning. This would be in line with findings in Parkinson's patients, demonstrating impaired learning on feedback-based tasks (Shohamy et al. 2004), although this stands in contrast to previous NF studies in Parkinson's patients demonstrating their ability to self-regulate activity in SMA with NF (Subramanian et al. 2016). Future work should aim to shed light on the neural mechanisms underlying NF learning in humans, in particular what neural substrates may be critical in order to achieve NF learning.

4.4.3 No evidence that patients can continue to modulate brain activity without neurofeedback

A secondary outcome measure of this trial was whether there were any changes in lateralisation of brain activity after NF training. Before and after the NF training transfer runs were carried out during which lateralisation of motor cortex activity was assessed in the absence of NF to test for transfer effects. We found no evidence of transfer of learning when assessed immediately after NF training. This suggests that in the absence of the NF display, patients were not able to maintain any learning achieved during NF training. A successful transfer may indicate that patients were able to learn specific strategies that they could employ to re-create brain changes achieved using NF. This could theoretically allow

patients to use these strategies outside of the scanner and potentially further boost any NF effect.

As mentioned in the introduction, a large proportion of fMRI NF studies have not addressed whether participants can successfully continue to modulate brain activity in the absence of feedback. Of the neurofeedback studies investigating motor cortex NF that also included transfer runs, three studies find evidence of successful transfer (Auer et al. 2015; DeCharms et al. 2004; Yoo et al. 2008), whereas three other studies did not find that people were able to transfer learning (Berman et al. 2012; Neyedli et al. 2018; Perronnet et al. 2017). The studies that demonstrated a successful transfer effect, often involved more extensive online or offline training schedules over several weeks (Auer et al. 2015; Yoo et al. 2008). Therefore, in healthy individuals, findings seem to be inconsistent, but there is some indication that more extensive training increases the likelihood of transfer success. However, more work is needed to address this question, and to date no previous studies have looked at transfer ability in stroke patients.

Although, as a whole group, the patients in the Real group did not show a successful transfer effect, it could be seen that there was significant variation in patients' change in LI from the pre- to post- transfer run. Additionally, patients' ability to use the NF was also variable, with some patients being more successful than others. When these two variables were correlated with each other in an exploratory analysis, it could be seen that those patients who performed well on the NF training, were also more likely to show a transfer effect. This finding hints that there may be a transfer effect in those patients who most successfully learnt to regulate motor cortex activity and that the null-finding on the group-level may be explained by high variability in NF learning success.

4.4.4 Limitations

This study had several limitations. Firstly, although the experimenter who carried out assessments and instructed the patients was blinded to condition, the second experimenter who operated the NF software was not blind to condition and their behaviour during the training days may have influenced the patient or the blinded experimenter. Software developments may allow future studies to be completely blinded. Secondly, in a deviation from the study protocol, a threshold was used in this study for the LI calculation, therefore making the LI dependent on an arbitrary statistical threshold (though one that had been validated in a prior study). This was a necessary step as occasionally average activity within a ROI was negative and this would lead to values outside of the -1 to 1 range, which may lead to misinterpretation (Seghier 2008). An alternative approach to calculating LI in a "threshold-free" manner, is to calculate LI at a range of statistical thresholds and plotting a LI curve, however interpretations of these curves may not always be straight-forward and this approach would be more computationally expensive in a real-time set up.

4.4.5 Future work

It has been demonstrated in the current and in previous studies that there is considerable variability in patients' ability to use NF. This is also the case in studies in healthy individuals with studies frequently reporting responders and non-responders to NF (Alkoby et al. 2018; Haugg et al. 2020). Therefore, future work may focus on identifying sub-populations of stroke patients who may benefit most from NF training. In particular, whether there are any factors which may hinder patients' abilities to use NF, such as severe damage to the CST or damage to brain areas critically involved in NF learning. Although

this will also be explored in this thesis, a study with a larger sample size, or future pooling of data across studies, would provide more power for identifying potential predictors of response.

4.5 Conclusion

In this chapter we investigated for the first time in a larger (not case report), randomized, sham-controlled clinical trial whether chronic stroke patients are able to use fMRI NF to modulate motor cortex activity. We found some evidence for the first primary outcome measure that patients were able to increase laterality of brain activity within NF training days. Additionally, exploratory analysis showed that during initial NF learning, there is increased activity in the putamen in the real group. We did not find strong evidence for the secondary outcome measure that patients were able to continue to self-regulate brain activity when NF is removed, however we did observe a large amount of variability in patients' ability to maintain self-regulation. This variability appears to be associated with initial NF learning success, with exploratory analysis showing that patients who were more successful at modulating brain activity with NF in the real group, were also most likely to be able to continue to modulate brain activity when feedback is removed.

5

Assessing Changes in Behaviour and Brain Structure and Function After Neurofeedback Training

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5.1 Introduction

An often important aim of NF training is to induce brain changes that will have a lasting positive influence on participants' behaviour or perception. This could potentially lend clinical utility to NF, if it can be used to induce brain activity changes that have a positive impact on patients' symptoms and improve well-being. Therefore, previous NF studies have typically targeted brain areas which are thought to be associated with disease-symptoms, such as the supplementary motor area in Parkinson's disease (Subramanian et al. 2016), the amygdala in depression (Young et al. 2017), or the anterior cingulate cortex in pain conditions (Guan et al. 2015).

Behavioural changes following NF training have been reported in several domains. For example, reductions in pain perception (Emmert et al. 2014; Guan et al. 2015), reduced nicotine cravings in smokers (Hartwell et al. 2016) and decreased fear responses (Taschereau-Dumouchel et al. 2018; Zilverstand et al. 2015). However, there has been little consistency in results (Thibault et al. 2018) and more work is needed to replicate

effects in robust well-controlled experiments. The primary motor cortex (M1) has been a common target of NF interventions due to its potential role in motor recovery after stroke (e.g. Chiew et al., 2012; Neyedli et al., 2017; Sampaio-Baptista et al., 2020). More bilateral activity in the primary motor cortices has previously been associated with worse outcomes in chronic stroke patients (Ward et al. 2003a), and reducing this bilateral activity is a feasible target for NF training, which may in turn increase motor functioning in these patients. However, to date there have been no large, sham-controlled fMRI NF studies in stroke patients targeting M1 activity.

One way through which NF may have an effect on behaviour is by triggering adaptive changes in brain function or structure, which may in turn have a positive influence on behaviour. Structural plasticity has previously been demonstrated to occur with new experiences and learning, as well as following injury (for review: Sampaio-Baptista et al., 2018). In stroke patients, while recovery depends largely on the severity of damage, there is evidence that intact areas of the brain can show adaptive plasticity that might contribute to recovery. For example, better motor functioning has previously been associated in cross-sectional studies with increased white matter integrity (compared to healthy controls) in several sensorimotor fibres, including the contralesional reticulospinal tract (Karbasforoushan et al. 2019) and the ipsi- and contralesional corticospinal tracts in well-recovered patients (CSTs; Schaechter et al., 2009). Additionally, grey matter volume in several cognitive and motor cortical regions is increased relative to controls (Diao et al. 2017; Wilkins et al. 2020), and has been related to motor functioning in stroke patients (Diao et al. 2017).

NF studies that include multimodal follow-up measures could potentially detect structural or functional brain changes resulting from NF training, and may shed light on mechanisms behind any observed behavioural effect. For example, previous studies on motor cortex NF in healthy individuals have shown that NF can drive rapid changes in WM microstructure in the corpus callosum (Marins et al. 2019; Sampaio-Baptista et al. 2020), as well as changes in functional connectivity (Marins et al. 2019). In the current study, in addition to assessments of motor functioning, several follow-up measures of brain structure and function were included in order to assess whether any brain changes can be detected 1-week post NF training in chronic stroke patients.

5.2 Aims

In this chapter I investigated whether three sessions of real-time fMRI NF in chronic stroke patients (described in the previous chapter) led to any improvements in their upper limb motor performance. The primary behavioural outcome measure was change in performance on the JTT. Secondary behavioural outcome measures included *changes in upper limb function and impairment* as assessed with the ARAT and the upper extremity Fugl-Meyer assessment (FM). Additionally, I explored changes on secondary outcome measures which included *changes in lateralisation of brain activity during visuomotor squeeze* task and changes in brain structure (in particular *changes in grey matter volume* and *changes in white matter microstructure*) following NF training.

5.3 Methods:

5.3.1 Participants

The patients in this chapter were the same as those described in chapter 4, however two patients did not complete the one-week follow-up scan (one due to feeling unwell, one due

to the scanner being out of use). Both of these patients still completed the follow-up motor assessments. Additionally, several patients did not complete the one-month follow-up. Occasionally, data from certain scans or tasks was missing, therefore the number of patients included in each analysis will be highlighted in the results section.

5.3.2 Study design

For this chapter I examined the data from the baseline and follow-up testing days of the randomized controlled trial described in chapter 4. During the baseline and the 1-week follow-up testing days patients underwent a MRI scan, EEG and motor assessment of their stroke-affected limb (see figure 4.1). At a 1-month follow-up, motor assessments only were repeated. Not all the modalities collected during these testing days will be included in this chapter, therefore I will focus only on those modalities that will be discussed:

5.3.3 Motor assessments

Multiple motor assessments were carried out to assess functioning of the stroke-affected limb. The primary behavioural outcome measure specified in the trial registration was the Jebsen Taylor Test (JTT). The JTT was carried out on each of the testing sessions (including the three NF training days), allowing for any potential changes to be tracked over time. The JTT measures performance of fine and gross hand function on a number of tasks that simulate activities of daily living. The JTT is a timed assessment in which patients complete a number of sub-tasks (such as stacking chequers), and are timed using a stop-watch on how long it takes them to complete the individual tasks.

Additionally, secondary outcome measures included clinical measures of upper limb activity and function assessed using the ARAT and the FM. These were carried out on the baseline and follow-up testing days. The ARAT is a well-established measure of stroke-related activity limitation during which patients have to complete a number of functional tasks (e.g. pinching a marble), and are scored based on the quality of the movement, the time taken and task completion. The FM is a measure of post-stroke impairment and assesses patients' ability to produce isolated and controlled movements of individual joints (e.g. shoulder abduction). Patients are scored based on completion of the tasks on a three-point scale.

5.3.4 Brain function during visuomotor squeeze task

Secondary outcome measures included changes in lateralisation of brain activity during a visuomotor squeeze task. Brain function during a controlled visuomotor squeeze task was assessed using fMRI during the baseline and the 1-week follow-up. During this task the patients held a MRI-compatible force transducer (BIOPAC, TSD121B-MRI) in their stroke affected hand whilst in the scanner. Before the start of the scan, the patient's maximum squeeze force was recorded and a target force was determined (~25% of max. force). During the scan participants saw an empty grey bar on the screen. Periodically, a yellow line, which represented their target force, would appear across the grey bar. Patients were instructed to squeeze on the force transducer until a blue bar (which represented the current force exerted on the force transducer) reached the yellow line, at which point they should release. The frequency with which the yellow line appeared (i.e. frequency of squeezing) was set to a comfortable rate for each patient but was typically between 0.25 and 0.35hz. Four 30-second squeezing blocks were interspersed with four 30-seconds rest blocks during which a fixation cross was presented on the screen.

5.3.5 Brain structure

Secondary outcome measures additionally included changes in white matter tract integrity and grey matter volume. White matter (WM) changes were assessed using diffusion weighted imaging (DWI) derived Fractional Anisotropy (FA). FA is a quantification of the directional dependence of water diffusion, and ranges from 0 (fully isotropic) to 1 (fully anisotropic). Therefore, in areas where water diffusion is unrestricted in all directions, such as in the cerebrospinal fluid, FA values would be expected to be close to 0, whereas in areas in which barriers to water diffusion in particular directions exist (such as myelin in WM), FA values would be greater than 0. Damage or degeneration of tissues can therefore decrease FA values by decreasing the barriers to diffusion, and reduced FA has been found in stroke patients in sensorimotor tracts (Borich et al., 2012; Mang et al., 2015; Rüber et al., 2012). Whole brain FA change across the groups was also compared using Tract-Based Spatial Statistics (TBSS), which overcomes registration issues with FA images (Smith et al. 2006).

Changes in the patients' Grey Matter Volume (GMV) was assessed using the T1-weighted images acquired during the baseline and follow-up scan, and voxel-based morphometry (VBM). VBM allows for whole brain voxelwise comparisons of tissue density, and has been commonly used to asses changes in GM volume and/or anatomy.

5.4 MRI acquisition

MRI data was acquired on a 3 Tesla Siemens Magnetom Prisma MRI scanner (Siemens AG, Erlangen, Germany) using a 32-channel head coil. Task fMRI was acquired using multiband gradient echo-planer imaging (TR=933ms, TE=33.4, flip angle=60°, slice

thickness = 2mm, in-plane resolution = 2 x 2, 72 slices, multiband factor = 6). Additionally, a high-saturation (single-band) whole brain EPI image was acquired for registration purposes and a dual echo fieldmap for fieldmap unwarping (TR = 482ms, TE1 = 4.92ms, TE2 = 7.38ms, flip angle=46°,slice thickness = 2mm, 49 slices).

Diffusion weighted imaging was acquired using a multi-shell, echo-planar imaging sequence (TR = 2483ms, TE = 78.2ms, voxel-size = 1.75 x 1.75 x 1.75 mm^3, 76 slices, multiband factor = 4) with 11 non diffusion-weighted images (*b*= ~0s/mm) and 60 diffusion-weighted images acquired with a b-value of 1500 s/mm and 60 with a b-value of 2500 s/mm. This data was acquired in both the A>P and the P>A phase encoding direction. A whole brain anatomical T1-weighted (MPRAGE) image was collected with 1mm isotropic resolution (TR = 1900ms, TE = 3.97ms, flip angle = 8°, TI = 904ms, slices = 192).

5.5 Analysis

5.5.1 Task fMRI Analysis

Task fMRI data acquired during the visuomotor squeeze task (see section *Brain function during visuomotor squeeze task*) was pre-processed using FEAT, including motion correction, brain extraction, fieldmap-based EPI distortion correction, spatial smoothing using a 5mm FWHM Gaussian kernel and high pass temporal filtering with a cut-off of 90 seconds. Linear registration was used to register the multiband functional data to a single volume high-contrast (single-band) EPI image. The high-contrast EPI image was then linearly registered to the T1-weighted image using boundary-based registration (6 degrees of freedom). Finally, nonlinear registration was carried out to register the T1-weighted structural image to MNI space.

FEAT was used to carry out a first level GLM for each fMRI run in order to compute task-based statistical parametric maps. A double gamma-HRF convolved boxcar regressor and its temporal derivative, was used to model the movement and rest blocks, and contrasts were set to Movement > Rest. Intermediate fixed effects analysis was carried out to create difference maps between the follow-up and baseline scans (Follow-up > Baseline). Group level analysis was carried out using FMRIB's Local Analysis of Mixed Effects (FLAME) to compare difference maps between the Real and the Sham groups (data from right-hand affected patients was mirror flipped). Contrasts were set to Real > Sham and Sham > Real. Group Z-statistic images were thresholded using clusters determined by $Z > 3.1$ and a family-wise corrected cluster significance threshold of $p<0.05$ was applied.

Additionally, average task activity was also extracted from an anatomical hand-knob ROI (see inset in Appendix B supplementary figure S5.1) and a laterality index was calculated (see chapter 4: *Laterality Index Calculation*). This allowed us to assess laterality of brain activity in the motor cortex during a tightly controlled visuomotor task before and after the NF training, where NF training was aimed at increasing laterality towards the stroke-affected hemisphere.

5.5.2 DWI Analysis

DWI data was analysed using FMRIB's Diffusion Toolbox (FDT). Pre-processing steps included distortion correction using *topup* and eddy-current correction using *eddy.* FA maps were then created by fitting a diffusion kurtosis tensor at each voxel using *dtifit,* and were brain extracted. Diffusion kurtosis imaging (DKI) allows quantification of non-gaussian diffusion and has previously been shown to produce more sensitive and accurate

tissue quantification (Cheung et al. 2009; Veraart et al. 2011). The halfway linear registration between the DKI-derived FA maps from the baseline and follow-up session was calculated, and the FA maps were linearly-registered to this halfway space and averaged to create a midspace image. The midspace image was then non-linearly registered to the FMRIB FA template in MNI space using ANTs (Advanced Normalization Tools; Avants et al., 2009) and the resulting warp was applied to the data from the individual timepoints. Lesion masks were used to exclude lesion areas from the registration and from group statistics. Once all the data was in the template space, it was averaged to create a mean FA image. This mean FA image was skeletonised and thresholded at FA > 0.2. FA data from individual patients and time points was then projected onto the skeleton by searching for the maximum FA value a short distance perpendicular to the skeleton tract. Group level voxel-wise statistics were carried out on difference maps created by subtracted the follow-up FA skeleton map from the baseline FA skeleton map. Permutation-based (5,000 permutations) nonparametric testing was used for statistical inference as implemented in FSL's *randomise*. Lesioned areas were excluded from the analysis by including Lesion maps as voxelwise regressors. Due to a large proportion of lesion overlap in certain voxels in the white matter – specifically the affected CST (see lesion overlap map figure 4.2), it was not possible to conduct voxelwise permutation testing, as implemented in TFCE (Threshold-Free Cluster Enhancement). Therefore, permutation testing was conducted on a cluster-wise level by setting a cluster forming threshold based on the t-value equivalent to $p<0.05$ given the degrees of freedom in the statistical test. Contrasts included in this analysis were: Real > Sham, Sham > Real, Real > 0 and Sham > 0.

Additionally to the whole brain TBSS analysis, a ROI based analysis was also carried out. The ROIs included the affected and unaffected CSTs, which are the major output fibres from the primary motor cortices, and the corpus callosum, which is the major trancallosal tract connecting the two primary motor cortices. These ROIs were derived from the JHU White-Matter Tractography atlas in standard space and were then registered into the native patient and timepoint specific space using ANTs. If the lesioned area encroached upon one of these ROIs, the overlapping voxels were removed. The mean FA of the voxels in these ROIs was then extracted and any outlier FA values (>3 IQR above median) were removed. Outlier values were usually restricted to the lesioned area, however in some patients these were also observed in perilesional tissue, near blood vessels or other abnormal tissues such as cavernomas. For the CSTs the FA asymmetry was calculated as the difference in FA between the two CSTs (Unaffected - Affected) / (Unaffected + Affected), where positive values indicate lower FA/more damage in the stroke affected CST. FA asymmetry has previously been shown to be correlated with motor functioning in stroke patients, and damage to the CST is considered to be a major contributor to post-stroke motor impairment (Lindenberg et al. 2010; Stinear et al. 2007; Zhu et al. 2010).

5.5.3 Grey-Matter Volume Analysis

T1-weighted images were analysed using FSL's voxel-based morphometry (VBM). A optimised FSL-VBM protocol was carried out for longitudinal analysis (Menke et al. 2014). T1-weighted images from the baseline and follow-up scans were bias-field corrected and the halfway linear registration between these two images for each patient was calculated. The native T1 images were then linearly registered to this halfway space and averaged to create a subject-specific midspace image. This midspace image was brain extracted and the resulting brain mask was registered back (using the inverse

transformation) into native space to brain extract the native baseline and follow-up T1 images. The non-linear transformation between the native T1-images to the midspace was additionally calculated using FNIRT. The bias field corrected and brain-extracted native T1 images were segmented using FAST (FMRIB's Automated Segmentation Tool; Zhang et al., 2001). The resulting grey matter partial volume estimates were used to create a study-specific grey matter template in MNI space after carrying out linear and non-linear registration to MNI space, and averaging across all patients and timepoint. Lesion masks were used to exclude voxels within lesioned areas and to improve the registrations.

Finally, the grey matter partial volume estimates in native space were non-linearly registered to the midspace, averaged and then the midspace average was non-linearly registered to the study-specific template. These transformations were combined and applied to the native space GM images, after which the registered images were modulated by the Jacobian and smoothed with a Gaussian kernel with a sigma of 3mm isotropic. Difference images were calculated between the follow-up and baseline testing sessions and were compared using permutation-based (5,000 permutations) nonparametric testing as implemented in *randomise*. Lesioned areas were excluded from the analysis by including the lesion maps as voxelwise regressors, additionally due to poor registration, the cerebellum was excluded from this analysis. The statistical threshold was set to $p < 0.05$ (family-wise error corrected) using Threshold-Free Cluster Enhancement (TFCE). Contrasts were set to: Real > Sham, Sham > Real, Real > 0 and Sham > 0.

Additionally, a ROI analysis was carried out looking at GMV changes in the primary motor cortices. For this analysis the mean GMV was extracted under the anatomically defined

hand-knob ROI (see Appendix B inset figure S5.1), in both the affected and unaffected hemisphere and was compared across the baseline and follow-up session.

5.6 Statistical analysis

For comparisons between baseline and follow-up behavioural and ROI results, Analysis of Covariance (ANCOVA) was used. Statistical tests were carried out comparing the Real and Sham group at follow-up, while accounting for baseline differences by including baseline as a covariate. The ANCOVA has previously been shown to be more sensitive in randomised studies compared to ANOVA of change from baseline (Van Breukelen 2006).

Where multiple follow-up timepoints were available (e.g. JTT) LMMs were carried out in the same way as described in Chapter 4.

5.7 Results:

5.7.1 Primary outcome: No improvement on overall JTT performance

The primary behavioural outcome measure was performance on the JTT, which was assessed during each testing day patients attended (6 in total). In order to assess for between group differences over the training days, the total time taken to complete the various subtasks on each day was calculated (see figure 5.1A). This timed data was log transformed to improve normality as the residuals were considerably skewed. A LMM was carried out on this data, with Group and Day as fixed factors, baseline time as a covariate and random intercepts per patient. This revealed a main effect of Time ($F(4,90.326)=3.207$, $p=0.017$), with patients in both groups improving over time (post-hoc linear contrast: $t(99.3)=-2.818$, $p=0.006$, $b=-0.205$, $SE=0.073$). However, there was no main effect of Group ($F(1,24.225)=2.660$, $p=0.116$) or Group×Time interaction ($F(4,90.326)=1.388$, $p=0.2445$).

In line with previous research (Hummel et al. 2010; Lee et al. 2018; Shin et al. 2016), performance on Gross (stacking checkers, moving heavy objects, moving light objects) and Fine motor sub-tasks of the JTT (card turning, picking up small objects, simulated feeding) was also analysed separately in an exploratory analysis. A LMM was carried out with Group, Day and Task (Gross, Fine) as fixed effects, baseline performance as a covariate and random intercepts. There was a significant main effect of Task ($F(1,168)=33.374$, $p<0.001$) and Group×Task interaction ($F(1,183.32)=42.05$, $p<0.001$). Post-hoc pairwise comparisons revealed that this effect was driven by faster performance in the Real group compared to the Sham group on the Gross motor tasks (Fig. 5.1B; $t(34.8)=-2.711$, $p=0.01$; Real–Sham: $b=-0.247$, $SE=0.091$). In contrast, there was not a significant difference between the two groups on performance of the Fine motor tasks (Fig. 5.1C; $t(34.8)=0.432$, $p=0.668$; Real-Sham: $b=0.039$, $SE=0.091$). Whilst the main effect of Day approached significance ($F(4,156.05)=2.412$, $p=0.051$), there were no other significant main effects of interactions (all $F<2$, $p>0.1$). These findings suggest that participants in the Real group had improved performance on gross motor tasks compared to the Sham group.

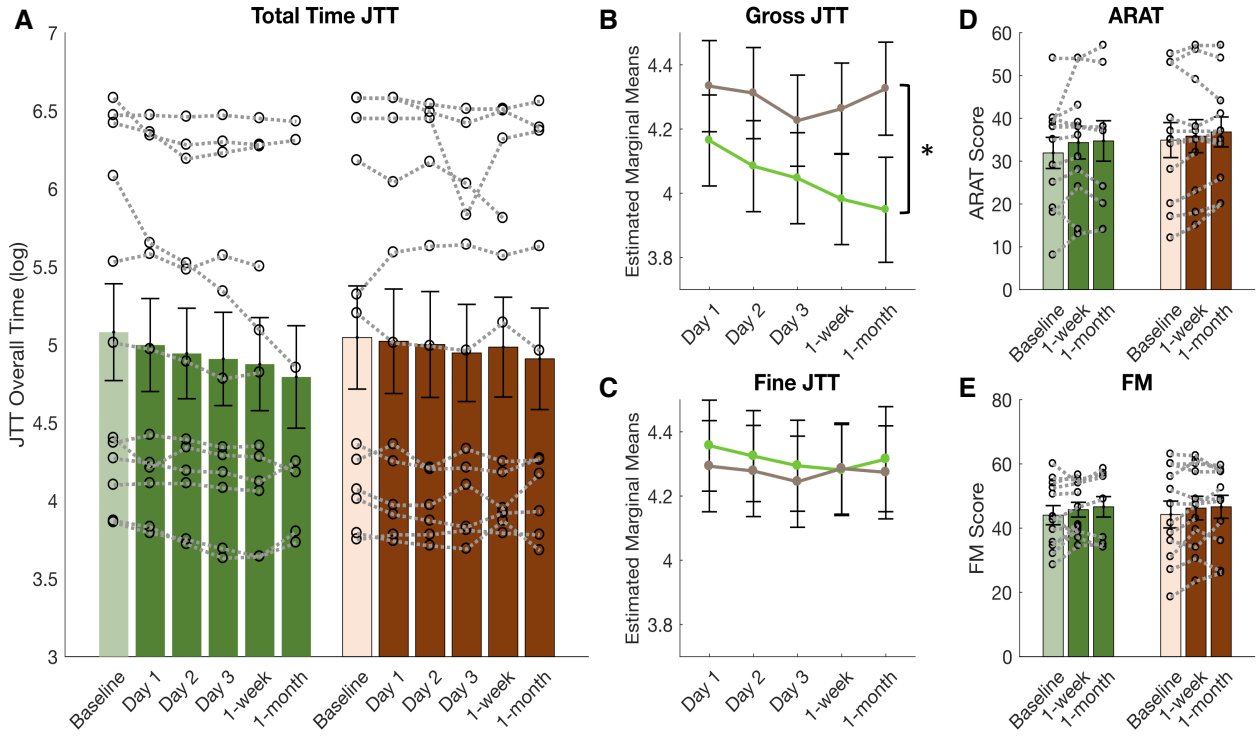


Figure 5.1 Participants in Real group improve on Gross motor performance (JTT) compared to Sham. (A) Participants in both the Real (green) and the Sham (brown) group got faster at performing the JTT over time when all subtasks were considered (p=0.017) and no group differences were found. Bars represent mean time to complete all subtasks (log), error bars represent s.e.m and data from individual participants is shown by the grey dotted lines. **(B)** When comparing performance on the Gross motor sub-tasks of the JTT, the Real group had faster performance compared to the Sham group (p=0.01). Estimated marginal means are shown with 95% confidence intervals at each follow-up timepoint. *p<0.05. **(C)** The same data is shown for Fine motor sub-tasks of the JTT where no significant group differences were found. There was no group effect on the ARAT **(D)** or the UE-FM (**E**) at 1-week or 1-month after NF training. Data displayed as in (A).

5.7.2 Secondary outcome: No evidence for NF effects on the ARAT or FM

Secondary behavioural outcome measures included changes in patients upper limb activity and functioning before and after NF training was assessed using the ARAT and the FM (see figure 5.1D&E). The two groups (both groups n=12) were not found to be significantly different from one another at the one-week follow-up when controlling for baseline differences (using ANCOVA) on the ARAT (F(1,21)=0.717, p=0.407, η_p^2=0.033), or the FM (F(1,21)=0.057,p=0.813, η_p^2=0.003). Similarly, when comparing the two groups at the

one-month follow-up (Real n=7 , Sham n=11), there was also no significant difference between groups on either measure (ARAT: $F(1,15)=0.308$, $p=0.587$, $\eta_p^2=0.020$. FM: $F(1,15)=0.055$, $p=0.818$, $\eta_p^2=0.004$).

However, when all time points (baseline, one-week and one-month follow-up) were considered in a LMM, both groups improved over time. Two LMMs were carried out with Group and Day, as well as their interaction were included as fixed factors, and random intercepts were included in the random effects structure. A significant main effect of Day was observed on the ARAT (F(2,42.136)=3.323, p=0.046, linear trend: t(46.4)=2.055, p=0.045; $b=1.86$, $SE=0.090$), whilst the main effect of time fell short of significance on the FM (F(2,42.225)=3.187, p=0.051). The main effect of Group and Group×Day interaction did not reach significance on either the ARAT or the FM (all $F< 1$, $p>0.5$). This suggests both groups improved over time on these measures.

5.7.3 Secondary outcome: NF training related changes in brain activity on controlled visuomotor task

A secondary outcome measure was change in lateralisation of brain activity during a visuomotor squeeze task. Visuomotor squeeze task data from three patients was not collected. Two patients did not complete a follow-up MRI scan, and for one patient the force transducer was not working during their baseline scan. Additionally, one patient's data was discarded due to excessive motion (mean absolute motion > 2SD above group mean) during the baseline scan. Therefore, analysis was conducted on the twenty remaining patients with eleven patients in the real group and nine in the sham group.

A ROI analysis was carried out where average activity was extracted from a hand-knob ROI. To compare with the NF runs analysis (Chapter 4, figure 4.3), a laterality index (LI) was then calculated and compared between the baseline and follow-up days and between the two groups (see Appendix B figure S5.1). A between-subjects ANCOVA on follow-up LI in the two groups, with baseline LI as a covariate, showed no significant differences between the two groups ($F(1,17)= 0.166$, $p=0.69$, $\eta_p^2=0.010$). LI at follow-up (adjusted for baseline LI) in the Real group was also not correlated with the NF success index (rho=-0.009, p=0.989, n=11, two-tailed). Additionally, when assessing changes in activity in each hemisphere separately (rather than the composite LI score), there were also no significant differences on day 5 between the two groups (Affected: $F(1,17)=0.460$, $p=0.507$, $\eta_p^2=0.026$; Unaffected: $F(1,17)=0.101$, $p=0.755$, $\eta_p^2=0.006$).

Additionally, exploratory whole-brain voxelwise analysis comparing the difference between the follow-up and the baseline brain activity (Follow-up vs Baseline) across the two groups (Real > Sham) revealed three significant clusters (Figure 5.2A). Significant clusters were all located in the unaffected hemisphere and included the putamen (p=0.015, MNI coordinate of max T-stat: -24 0 8), the lateral occipital cortex (p=0.041, max T-stat: -38 -82 -14) and the Parietal operculum cortex (p=0.047, -42 -38 26). In order to visualise these effects the PSC was extracted for each significant cluster at the baseline and follow-up session and is plotted in figure 5.2B.

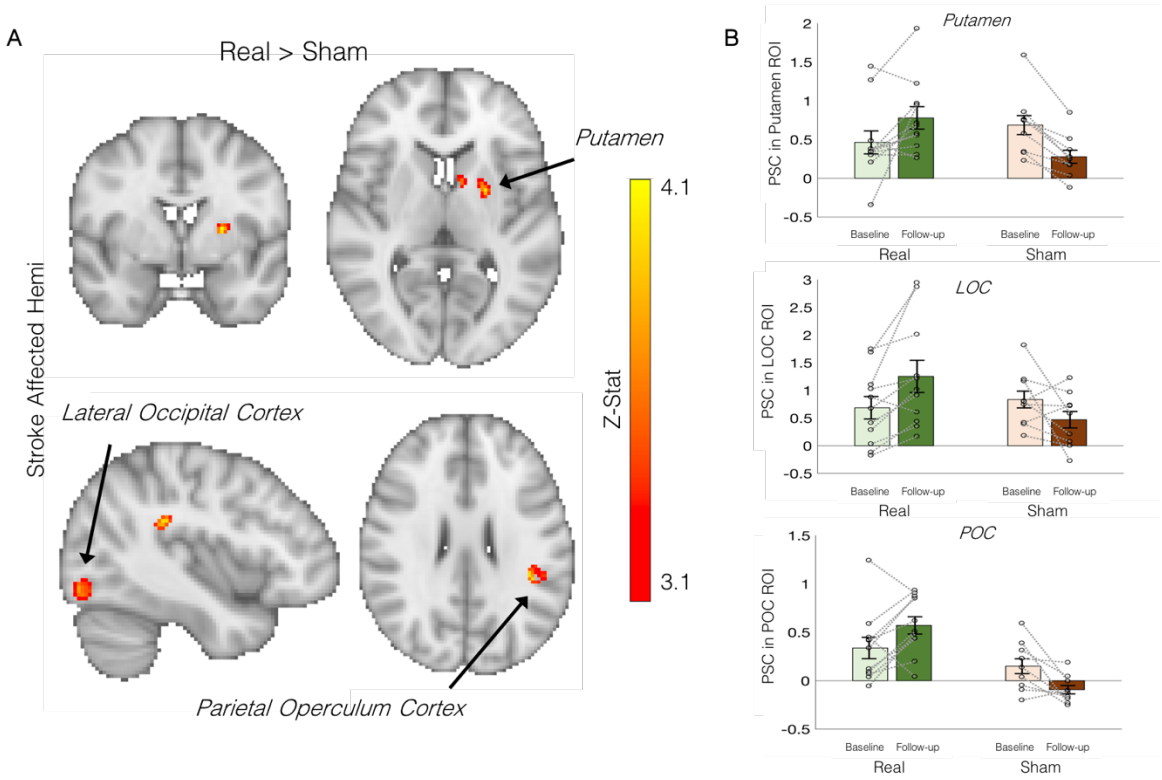


Figure 5.2 Changes in brain activity during controlled squeeze task before and after NF training. A) Three significant clusters were found where the Real group had a greater change than the Sham group, located in the putamen, lateral occipital cortex and the parietal operculum in the unaffected hemisphere. **B)** For visualisation purposes, the mean PSC of the significant clusters was plotted, as well as the data from individual patients (represented by open circles). Error bars represent s.e.m.

5.7.4 Secondary outcome: White matter changes in corticospinal tracts

Change in white matter tract integrity was a secondary outcome measure of this trial. In addition to the two patients who did not complete a follow-up scan, one patient requested to stop the scan during the DTI acquisition after feeling unwell. Therefore, analysis was conducted on the twenty-one remaining patients (ten in the real condition, eleven in the sham condition). Whole-brain voxel-wise analysis carried out using TBSS with a cluster forming threshold of T > 1.8 found no significant differences between the two groups when comparing the difference maps between Baseline and Follow-up FA (Real > Sham peak voxel p = 0.607, Sham > Real peak voxel p = 0.880).

FA asymmetry in the CST ROIs was also investigated. FA asymmetry decreased slightly in the Real NF group (median change = -0.005) and increased in the Sham group (median

change = 0.005; Figure 5.3A). Average FA asymmetry at baseline was 0.09, therefore a change (either increase or decrease) of 0.005 represents a relatively small change of 5.77%. FA asymmetry at follow-up was compared between the two groups in an ANCOVA with baseline FA asymmetry as a covariate. A significant group difference was found ($F(1,18)=8.99$, $p = 0.008$, $\eta_p^2= 0.333$), with the Real group having significantly lower FA asymmetry after NF training than the sham group (estimated marginal means: Real = 0.089, Sham = 0.098; difference between groups at follow-up = 0.009). As FA asymmetry is a composite measure, consisting of FA measured in both the affected and the unaffected CST, changes in FA in the separate CSTs was also investigated, in order to discern where changes may be occurring that are driving this effect. A 2x2 ANCOVA was conducted on follow-up FA, with baseline FA as a covariate in the two groups and the two CSTs. The Group x Hemisphere interaction was found to be strongly trending towards significance ($F(1,37)=3.99$, $p=0.053$, $\eta_p^2=0.097$). However, follow-up ANCOVAs comparing the two groups on each CST separately (Figure 5.3B) failed to reach significance (Group effect Affected Hemi: $F(1,18)=2.501$, $p=0.131$, $\eta_p^2=0.122$; Unaffected Hemi: $F(1,18)=1.442$, $p=0.245$, $\eta_p^2=0.074$). Exploratory paired T-Tests revealed that the interaction was most likely driven by increased FA in the stroke affected CST post intervention in the real group ($T(9)=-2.338$, $p = 0.044$, $d=-0.739$) compared to the Unaffected Hemisphere ($T(9)=0.841$, $p =0.422$, $d=0.266$) or changes in the Sham group (both T's(10)< 1, both p's > 0.1), however this result would not survive multiple comparisons correction.

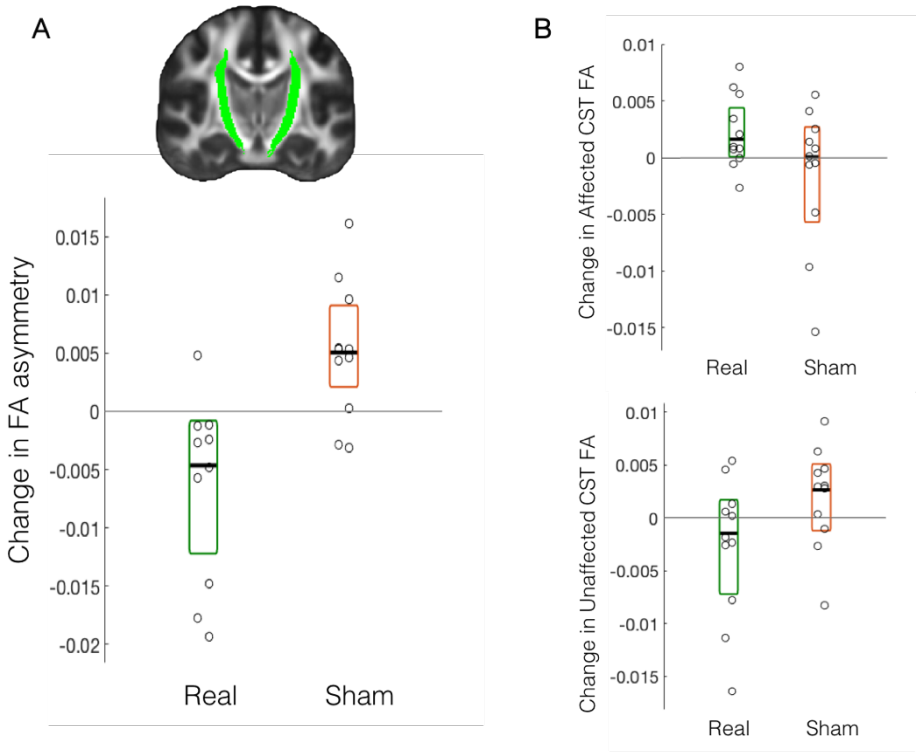


Figure 5.3 FA Asymmetry of CSTs is reduced after Real NF training. (A) The Real group (n=10) had lower FA asymmetry in the CSTs (shown in inset) after NF training compared to the Sham group (n=11). Change in FA asymmetry between baseline and 1-week follow-up is plotted for the Real (green) and the Sham (orange) group. The black line represents median change, coloured boxes represent 95% confidence intervals and individual participant data points are shown with open circles. **(B)** The same information is shown for FA change between baseline and 1-week follow-up in the affected (top) and unaffected (bottom) CSTs.

Additionally, the relationship between FA changes and how well participants performed on the NF training was explored. NF success was calculated as the average slope across NF runs (see chapter 6 for more details). When this success measure was correlated with changes in FA asymmetry (adjusted for baseline asymmetry) using Spearman's rho, there were no significant correlations for the Real group (rho = -0.297, p = 0.407), or the Sham group (rho = -0.164, p = 0.657). However, when NF success was correlated against changes in FA in the two CST's separately, there was a significant correlation between change in FA in the Affected CST (adjusted for baseline FA; figure 5.4) and NF success in the Real group (rho = 0.879, p = 0.002; Bonferroni corrected alpha for 6 comparisons =0.008) but not in the Sham group (rho = -0.030, p = 0.946). A Fisher's Z-test confirmed the two correlations were significantly different from each other (Z=2.622, p=0.004). In contrast,

correlations with change in FA in the Unaffected CST did not reach significance in the Real group (rho = 0.612, p = 0.067) or in the Sham group (rho = -0.297, p = 0.407).

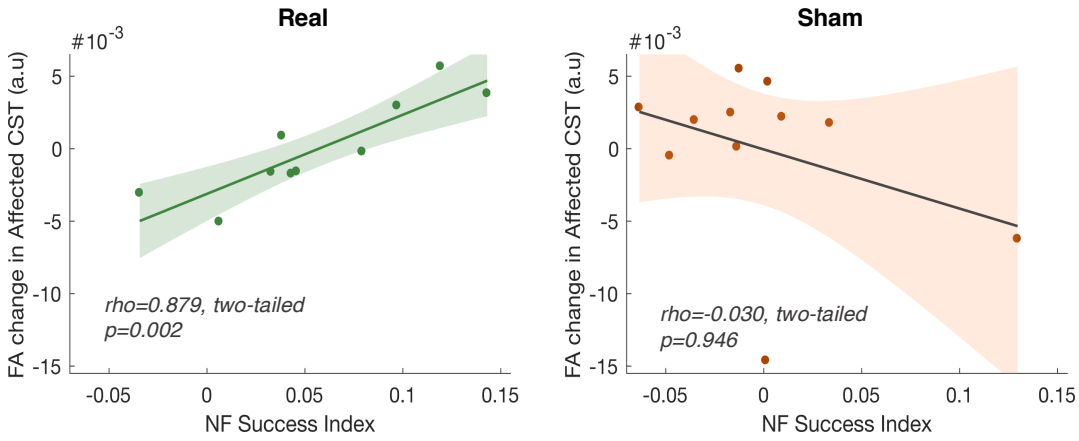


Figure 5.4 Correlation between change in FA after NF training in the stroke affected CST and NF learning success (as measured by the average slope across NF runs). A significant positive correlation was found in the Real group (left) with better NF performance associated with greater change in affected CST FA. In contrast no correlation was found between the same measures in the Sham group (right).

Finally, changes in FA in the body of the corpus callosum (delineated from the John Hopkins University ICBM-DTI-81 atlas) were examined to check for structural changes in the tracts that connect the motor cortices, where previously FA changes with NF have been demonstrated (Sampaio-Baptista et al., 2020). Analysis with ANCOVA on the Follow-up corpus callosum FA, with Baseline FA as a covariate found no significant group differences ($F(1,18)=3.589$, $p=0.074$, $\eta_p^2=0.166$) with both groups showing similar FA in the corpus callosum at the follow-up session (see Appendix B, supplementary figure 5.2; estimated marginal means Real=0.389, Sham=0.384). Similarly, in a control ROI in the superior longitudinal fasciculus (a region in which we would not predict changes with neurofeedback), there were no significant differences between FA asymmetry at follow-up between the two groups ($F(1,18)=0.597$, $p=0.450$, $\eta_p^2=0.032$; estimated marginal means: Real=0.100, Sham=0.103), with the median change in FA asymmetry being -0.002

in the Real group and 0.000 in the Sham group. Average FA asymmetry at baseline was 0.10, therefore the change of -0.002 in the Real group represents a change of -1.94%.

5.7.5 Secondary outcome: No changes in Grey Matter following training

Finally, secondary outcome measures also included changes in grey matter volume. Apart from the two patients who did not complete follow-up scans, all other patients were included in this analysis, with eleven patients in the Real group and eleven in the Sham group. Whole-brain voxelwise VBM analysis revealed no significant differences between the Real and the Sham group when comparing difference maps between follow-up and baseline (Real > Sham peak voxel p-value = 0.219, Sham > Real peak voxel p-value = 0.959).

A ROI analysis was additionally carried out by comparing the average GMV under the anatomical hand knob ROI between the Groups (Real vs Sham) and the Hemispheres (Affected vs Unaffected) in a 2x2 level ANCOVA with GMV at the follow-up assessment as the dependent variable and Baseline GMV as a covariate. There was no significant Group*Hemisphere interaction ($F(1,39)=1.017$, $p = 0.319$, $\eta_p^2 0.025$) or main effects of Group ($F(1,39)=0.149$, $p = 0.702$, $\eta_p^2=0.004$) or Hemisphere ($F(1,39)=.904$, $p = 0.348$, $p=0.023$).

5.8 Discussion

In this chapter, I explored whether three sessions of fMRI neurofeedback training in chronic stroke patients led to any changes in their motor performance, or in their brain function and structure. The main results of this chapter are discussed below:

5.8.1 Improvements in gross motor performance following NF training

A key challenge for the development of clinical NF interventions is to drive changes in brain activity that translate into meaningful and long-lasting improvements in patients symptoms. Here motor function was tested using a range of measures, and whilst no improvements were observed on the overall performance on the JTT (the primary outcome measure), exploratory analysis found improved performance on the gross motor sub-tasks of the JTT in the Real group compared to the Sham group. That improvements were found for gross rather than fine sub-tasks fits with extensive evidence that fine motor tasks are the hardest to recover following damage to the corticospinal system (Kuypers 1981; Lawrence & Kuypers 1968; Lemon 1993). There was not any evidence of NF training improving scores on secondary outcome measures which included clinical measures of impairment (FM) or activity (ARAT). This lack of an effect on clinical measures may be due to a number of factors. One possibility is that laterality of brain activity may not be a suitable intervention target for all stroke survivors. In particular, it has previously been suggested that more severely affected stroke survivors may instead benefit from boosting activity in both M1's (Buetefisch 2015). Additionally, neuromodulation approaches are often considered not as treatments per se, but rather as approaches that enhance the potential for use-dependent plasticity (Ward 2016). Therefore, the addition of further motor rehabilitation outside of the scanner, to drive the motor system while it is 'primed',

may be necessary to elicit observable effects of NF training on clinical measures. For example, a previous study in Parkinson's patients found clinically relevant improvements of symptoms after just two sessions of NF combined with extensive practice outside of the scanner using the successful strategies identified during training (Subramanian et al. 2011). However, the finding of an effect on the gross sub-tasks of the JTT is a promising first indication that NF can induce behavioural change in this population, and ways to bolster this effect should be explored further. Especially considering that the current study NF training was conducted on a relatively short timescale (three sessions of 20mins) and without further practice outside the scanner. Previous studies which demonstrated significant motor improvements in chronic stroke survivors are typically conducted over a much longer time period consisting of many hours of training over multiple days or weeks (for example ~9 hours using TDCS (Allman et al. 2016), ~7 hours of EEG NF (Mottaz et al. 2018), or between ~17 and ~90 hours of motor training) (Mawase et al. 2020; Ward et al. 2019).

Although behavioural results have been reported following fMRI NF training (for review see: Thibault et al., 2018), evidence for changes in motor functioning following fMRI NF training in controlled studies has been sparse. Work in Parkinson's patients has showed that patients improved in their symptoms with NF training of the supplementary motor cortex (Subramanian et al. 2011), although in a larger cohort they did not improve significantly more than an active control (Subramanian et al. 2016). In healthy subjects, Hui and colleagues (2014) found increased finger tapping speed after NF of the Premotor Cortex during motor imagery. However, in order for fMRI-NF to become a clinically useful tool in stroke patients its efficiency would need to be demonstrated, especially whether it is superior to more cost effective tools such as EEG-NF, brain stimulation or

behavioural and pharmacological interventions. Although there have been some promising results with brain stimulation techniques in stroke patients (Allman et al. 2016; Khedr et al. 2013) results have also been fairly mixed with some studies reporting no differences between real and sham stimulation (Di Lazzaro et al. 2016; Hesse et al. 2011; Pomeroy et al. 2007), and individual responses to brain stimulation also appear to be highly variability (Grefkes & Fink 2016). Therefore, variability in response and the need to find optimal intervention parameters to drive behavioural changes in stroke patients appear to be a more general issue for brain modulation approaches – whether externally or internally driven.

5.8.2 Increased activity in brain areas related to NF learning

A controlled visuomotor squeeze task was carried out as a secondary outcome measure during the Baseline and 1-week Follow-up scan. This allowed us to assess laterality of brain activity when patients were doing an identical motor task before and after NF training, as well as allowing us to assess any changes in brain activity occurring elsewhere in the brain. Although we did not find evidence for increases in the laterality of brain activity after NF training (secondary outcome measure), we did find evidence for more widespread changes in brain activity during an exploratory whole brain analysis. In particular, we found evidence of increased activity following NF training in the putamen (in the dorsal striatum), lateral occipital cortex and the parietal operculum cortex.

The dorsal striatum has previously been implicated in neurofeedback learning (Emmert et al. 2016; Shibata et al. 2019; Sitaram et al. 2017), and in chapter 4 we demonstrated increases in putamen activity early (i.e. during initial training runs on each day) during NF learning. The finding that activity in the putamen is also increased 1-week after NF training suggests that patients in the real group may have learnt to engage this area during initial

motor performance. Previous research has suggested that connectivity between the dorsal striatum and motor cortex increases with NF training, which may lead to increased activity in the dorsal striatum when the motor cortex is engaged. Although outside of the scope of the current thesis, future work could examine whether connectivity between these areas is increased in this sample, both in the resting-state data and during the task.

Additionally, there were several similarities between the squeeze task and the NF task, as in the squeeze task a bar that changed height based on motor output was displayed. Thus this bar also presented a form of real-time biofeedback to the patients. This similarity between the tasks may explain why altered activity in the putamen was present in both tasks. It is also possible that patients may have used strategies during the squeeze task that they found effective during NF training. Although this may have occurred subconsciously, patients were also explicitly asked after the scan if they had incorporated any strategies during the squeeze task. Although the majority of patients said that they did not incorporate strategies, three patients mentioned focusing more on the task or on relaxing the unaffected hand, and two patients mentioned incorporating different movements from the NF training. Finally two patients mentioned that they hadn't specifically tried to incorporate specific strategies but that squeezing had been a movement that they found effective during NF training.

The LOC has also previously been implicated in playing a role in NF control (when visual feedback displays are used), and has been demonstrated in a recent meta-analysis of twelve previous fMRI NF studies, to be a brain region consistently activated during NF learning irrespective of the NF target (Emmert et al. 2016). The LOC has been shown to be involved in directing attention to visual signals (Murray & Wojciulik 2004), and may reflect patients

in the Real group learning to pay more attention to the visual display after NF training. Additionally in this study we found increased activity in the parietal operculum cortex, where the secondary somatosensory cortex (SII) is located (Eickhoff et al. 2006). SII has previously been associated with tactile learning (Pleger et al. 2008; Ridley & Ettlinger 1978) and has also been proposed to play a role in tactile working memory (Condylis et al. 2020) and decision making (Romo et al. 2002).

5.8.3 White matter alterations following NF training

Secondary outcome measures also included changes in brain structure, specifically in white matter tract integrity and in grey matter volume. In this study we found evidence for changes in the WM microstructure of the CSTs after the NF intervention. Specifically, we found that FA asymmetry was significantly lower in the Real group after NF training than in the Sham group. However, it should be noted that the observed effect was relatively small and could also be driven by effects such as small differences in the co-registration between the baseline and follow-up time-points or noise. The use of a control ROI, which would presumably experience similar variations in these confounds helps us to somewhat decrease these concerns. When trying to ascertain what was driving this effect by looking at the two CSTs separately, there was stronger evidence that the effect was driven by FA changes in the stroke affected CST in the Real Group, than by changes in the unaffected CST, although this finding did not reach significance. However, changes in FA in the stroke affected CST strongly correlated with patients NF learning success, with patients who performed better on the NF training exhibiting greater changes in affected CST FA. There was no correlation between FA asymmetry change and NF success, which may be explained by the positive (although not significant) correlation between FA change in the unaffected CST and NF success.

In stroke patients, CST microstructure has previously been linked to motor recovery (Stinear et al. 2012), especially to dextrous hand function (Lemon 2008). Therefore finding ways to strengthen (or rewire) CST output could potentially lead to improved dextrous finger functioning (Wahl et al. 2014). Recent work in animals has shown that optogenetically activating the intact contralesional CST after stroke, in combination with intense rehabilitation, led to near-complete recovery of skilled forelimb function as well as corticospinal sprouting from the intact CST to the denervated tract (Wahl et al. 2017). In the current work, we found that FA asymmetry is reduced following NF, however increased FA in the contralesional CST may be compensatory in some patients and therefore the changes observed in this study may not be effective at inducing behavioural change. However, the change in FA asymmetry observed in this study appears to be primarily driven by changes in the ipsilesional CST. Recently, NF work using TMS NF has demonstrated the feasibility of directly modulating excitability of CSTs in healthy subjects (Ruddy et al. 2018), and it will be interesting in the future to see whether this is associated with behavioural changes in healthy people or in stroke patients.

In the current study we did not find any evidence of changes in GMV after NF, and there doesn't appear to be much evidence in the literature of NF-related GMV changes. A previous study using ipsilesional anodal tDCS in chronic stroke patients (Allman et al. 2016), which also measured brain structure before and after the intervention, found increases in GMV after tDCS in the ipsilesional motor and premotor cortices, and in the contralesional post-central gyrus. These changes in GMV were not correlated with changes in upper limb functioning. They did not find any changes in WM as measured by CST FA-asymmetry. Therefore, although both of these approaches targeted increasing activity in

stroke affected M1, they led to distinct changes in brain structure. This may be due to our NF training additionally targeting decreasing activity in contralesional M1, or may suggest that these approaches rely on different underlying mechanisms.

5.8.4 Limitations

A limitation in the current study is the small sample size, in particular for the follow-up MRI measures, due to the drop-out of patients and MRI artefacts. Especially when considering the one-month follow-up where six patients (25% of the sample size) did not complete the session. Recent work has highlighted that some NF-related changes develop over a longer time-scale, and that symptoms can continue to improve for several weeks following NF training (Rance et al. 2018). Therefore, it is possible that group differences may develop over time and may become apparent at later follow-up timepoints.

5.8.5 Future work

As mentioned above, if fMRI NF is to become a clinically feasible or attractive option, a convincing effect on behaviour would need to be demonstrated. Especially when compared to cheaper options such as brain stimulation techniques or EEG NF. Although the current study did not find any clinical effects, it could be that the current set-up was not ideal. In particular, combining NF training with motor training outside of the scanner, in which patients could practise successful strategies identified during NF, could boost NF effects on behaviour further.

One of the potential strengths of fMRI NF lies in the flexibility of this approach to spatially-selectively target different brain regions, as well as varying the modulation target

itself (e.g. whether to modulate activity, connectivity or multivariate patterns of activity). As chronic stroke patients are highly heterogeneous, both in terms of lesion characteristics (size, location, cause), but also in terms of patients age, medication, co-morbidities etc., they may benefit from a more individualised treatment approach. In the future, finding ways to identify 'ideal' training targets for individual patients, in combination with the flexibility of brain modulation that fMRI NF allows, could facilitate individualised treatment approaches, tailored to individual patients' needs.

5.9 Conclusions

In this chapter changes in behaviour and in brain structure and function were explored following three sessions of fMRI NF in chronic stroke patients. Whilst there was no improvement on the overall performance on the JTT (primary outcome measure), there was evidence of improved performance on the gross motor subtasks of the JTT with Real NF compared to Sham. However, there was no evidence of improvements on secondary outcome measures of upper limb activity and functioning. Furthermore, there was evidence of changes on secondary outcome measures in both brain functioning, as well as in WM microstructure. In particular, increased activity in brain areas associated with NF control and learning was found during a controlled squeeze task, and a decrease in CST FA-asymmetry was observed.

These findings suggest that fMRI NF in its current form still has a long way to go before becoming a potentially clinically useful tool in stroke patients. More work is needed to examine variability in response and to identify suitable targets for NF training in this highly heterogeneous group of patients. However, findings of motor performance improvements,

as well as functional and structural brain changes at 1-week post-NF training highlight the potential of NF in this population.

6
Exploring Variability in Stroke Patients Neurofeedback Learning

6.1 Introduction

Variability in response to a treatment or intervention is a common feature in many experimental and clinical studies. It has also been noted in the NF literature that typically not all participants appear to be able to achieve self-regulation of brain activity, with previous studies often dividing participants into 'responders' and 'non-responders'. It has been estimated in both fMRI and EEG NF that ~38% of participants do not seem to benefit from NF training to modulate their brain activity, although this figure varies from study to study (Alkoby et al. 2018; Haugg et al. 2020). The reasons why some participants do not seem to benefit from NF training remains unknown, but there has been increasing interest in identifying factors associated with successful NF learning in the hope of developing predictors that may be used to help target participant sub-groups who are more likely to benefit from NF training.

In order to achieve this, a robust and reliable measure of NF learning success (or a NF 'success index') has to be established, however there is currently no agreed-upon measure of NF success. Paret and colleagues (2019) recently summarised several different ways in which NF success can be assessed, but point out that the proposed methods all rely on assumptions about NF learning which have not yet been established and have several

drawbacks. Previous studies have, for example, counted the number of regulation blocks in which participants managed to increase activity above rest, or calculated the difference in performance between the first and last NF training run. Additionally, NF success can also be assessed by considering follow-up measures, such as changes in behaviour, brain connectivity or structure.

Despite the lack of consensus on an established measure of NF success, studies have started to explore relationships between measures of NF success and baseline measures. A recent meta-analysis across 24 fMRI-NF studies looked at associations between baseline activity in the ROIs used for NF training, and NF learning success (Haugg et al. 2020). NF success was measured in two ways, the slope over all NF runs and the difference between the first NF training run and the last. The authors were not able to find clear relationships between these factors, in either patient or healthy participant studies. The only significant finding was detected when only the active voxels were considered from functional localisers, in which case a relatively small positive relationship was found between activity during the localiser and NF success. Another systematic review that looked across fMRI and EEG NF, as well as BCI research (Kadosh & Staunton 2019), highlights the importance of attention, motivation and mood in determining NF success, while a smaller study combining across two fMRI-NF studies (Hellrung et al. 2020) found participants' reward sensitivity and prefrontal prediction error to be associated with successful Ventral Tegmental Area modulation.

Brain structure at baseline may also be a useful predictor of learning performance, with previous studies finding relationships between GM structure at baseline and training outcomes on different motor tasks (Gryga et al. 2012; Sampaio-Baptista et al. 2014).

Additionally, in a previous NF study using a similar design to the current study but in healthy adults, it was shown that NF success was associated with baseline motor network structural connectivity, specifically with FA in the superior longitudinal fasciculus and in contralateral CST (Sampaio-Baptista et al. 2020). Finally, small NF feasibility studies in stroke patients have pointed to various factors that may be associated with NF training success, including baseline impairment (Liew et al. 2016) and CST integrity (Lioi et al. 2020).

6.1.1 Aims

In this chapter, variability in patients' ability to use NF to modulate laterality of motor cortex activity, and whether this is related to baseline measures, was explored. In particular, based on previous literature, we chose to examine relationships between NF success and baseline measures of a) motor functioning, b) motor cortex activity, and c) brain structure.

6.2 Methods:

In this chapter the same patients were included as described in Chapter 4, but the main analyses relating baseline measures to NF success were solely focused on the patients who were allocated to the Real group (n=12). Patient characteristics can be found in table 4.1.

6.2.1 Success Index calculation

As mentioned in the introduction, there are several different ways to measure NF learning success, or to calculate a NF 'success index'. In the current study we opted for two separate approaches in the hope of better capturing the highly variable learning trajectories across patients (see figure 6.1). The first approach was a **NF slope success index**, where a regression slope was fitted across NF runs *within* training days and then averaged over the three training days. This was done as in Chapter 4 it was found that patients in the Real group primarily learnt to increase the laterality of brain activity within training days, and there was no evidence of a carry-over effect across training days.

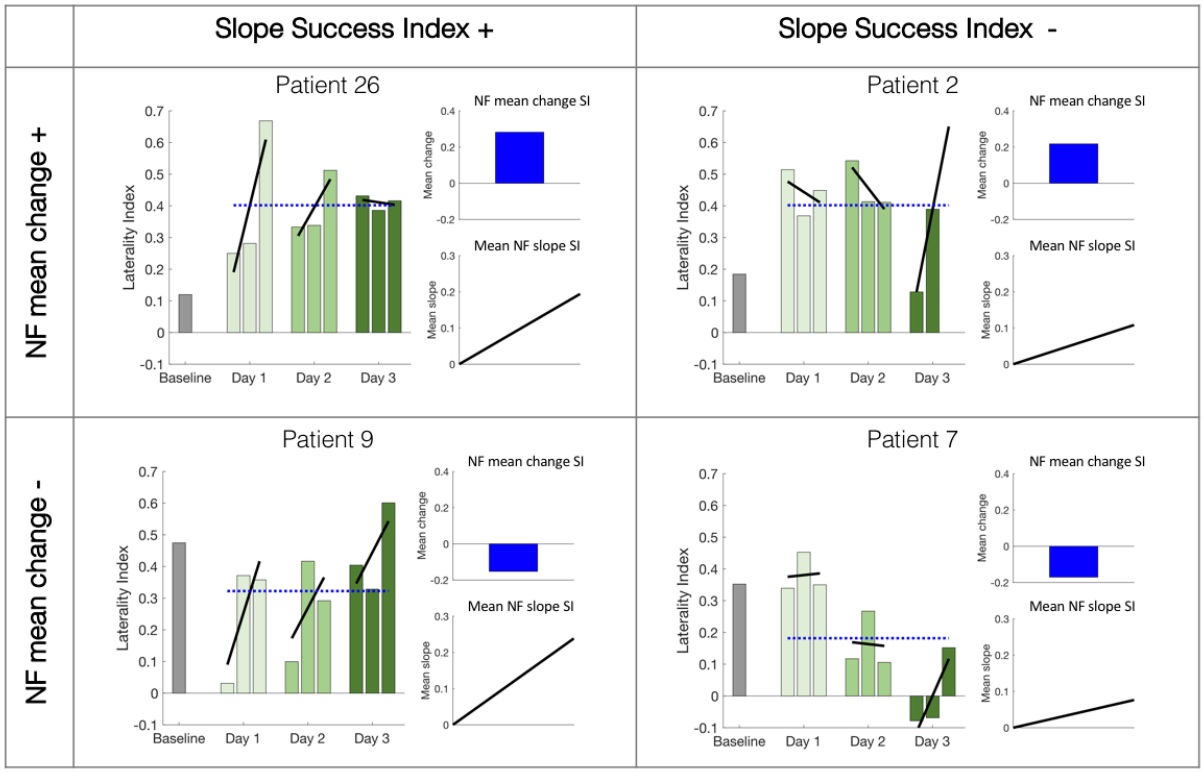


Figure 6.1*:* Success indices and example patients. NF performance (LI) over the three training runs per NF training day shown in shades of green for four example patients. Baseline LI measured during the first Pre-NF transfer run is shown in grey. The blue dotted line represents the average LI across all NF training runs and days, which was used to calculate the NF mean change success index. The black lines represent the slope across runs within training days which were averaged to calculate the mean NF slope success index. The two success indices are additionally shown for each patient on the right, with the **NF mean change success index** indicated by the blue bar and the **NF slope success index** indicated by the black line. Patients displayed can be classed as either responders (+) or non-responders (-) on either or both of the success index metrics used in this chapter. Note: Patient 2 is missing the third run on Day 3 due to technical issues.

Slope measures of NF success assume a linear increase in learning over runs or days. However, it may be the case that some subjects learn the most during the early/initial NF runs and then either remain high or even decrease in activity as they fatigue, which would result in a flat or negative slope. In order to capture this possibility, we also calculated a **NF mean change success index**, in which the mean LI across all of the NF runs was calculated, and baseline LI (as measured on the first Pre-NF transfer run) was subtracted from this. Thus, this measure captures the difference in LI between baseline and NF runs, without considering variation over time during NF runs. Although this approach makes fewer assumptions on how learning evolves over time, it is very dependent on accurately capturing baseline performance. If by chance activity or LI happens to be very high during the baseline run (patients by chance use a very effective strategy) then it will be much more difficult for patients to have an average LI during NF runs that is higher than this. This may also be problematic as the baseline run is always carried out first when the patients are less fatigued and possibly more attentive. Figure 6.1 displays individual NF data from four example patients who can be classed as either responders or non-responders on either or both of the success index metrics used in this chapter.

6.2.2 Modalities explored in relation to NF success

Several baseline measures were explored in relation to NF success. All of the baseline measures, acquisition thereof and pre-processing used in this chapter have been described in detail in Chapter 5. Firstly, relationships between **baseline motor functioning** and NF success was assessed by using Spearman's rho to correlate baseline FM and ARAT score of the patients with the NF success indices.

Secondly, in order to assess relationships between **baseline brain function during affected hand movement** and NF success, data from the *Pre-NF transfer run* and from the *visuomotor squeeze task* were correlated with the NF success indices. During the Pre-NF transfer run (see chapter 4), patients were allowed to make any movements they wanted to with their stroke-affected hand, and this task was carried out immediately before NF training. In contrast during the visuomotor squeeze tasks, which was carried out during the baseline session, all patients did the same movements where they squeezed on a force transducer at a given rate to ~25% of their maximum force output (see Chapter 5 Methods). First, a ROI analysis was carried out where a LI was calculated from the activity of either the ROIs used for the NF training for the Pre-NF transfer task, or an anatomical hand-knob ROI for the visuomotor squeeze task. This LI was then correlated with the NF success indices. Additionally, whole brain voxel-wise analyses were carried out. For both tasks separately the pre-processed first level task-based statistical parametric maps were fed into a group-level analysis using FEAT, where the NF success indices were demeaned and entered into the GLM. FLAME was used to test for both positive and negative associations with NF success indices using clusters determined by $Z > 3.1$ and a family-wise corrected cluster significance threshold of $p<0.05$.

Finally, relationships between NF success indices and brain structure at baseline were also examined. In order to assess relationships between **white matter microstructure at baseline** and NF success, FA in the CSTs at baseline was correlated with the NF success indices. Additionally, a whole brain voxel-wise analysis was carried out using TBSS on the baseline skeletonised FA data (see chapter 5). Permutation-based (5,000 permutations) nonparametric testing of the demeaned success indices was carried out using *randomise*. Lesioned areas were excluded from the analysis by including lesion maps as voxelwise

regressors. Permutation testing was conducted on a cluster-wise level by setting a cluster forming threshold based on the t-value equivalent to $p<0.05$ given the degrees of freedom in the statistical test.

For examining relationships between **grey matter volume at baseline** and NF success, a whole brain VBM approach was used. Permutation testing (5,000 permutations) of the demeaned success indices was carried out, with lesioned areas excluded from the analysis using a voxelwise regressor. The statistical threshold was set to $p < 0.05$ (family-wise error corrected) using Threshold-Free Cluster Enhancement (TFCE).

6.3 Results

6.3.1 Variability in NF learning success

Two separate measures of NF learning success were calculated based on the LI during NF training runs. When examining the **NF slope success index**, it could be seen that all of the patients in the real group, except two, had a positive slope over the NF runs on average within training days (Figure 6.2A), with one additional patient showing a fairly flat average slope (slope value close to 0). In comparison, when the same measure was calculated for the Sham group, half the patients (6) had a positive slope and half had a negative slope. When the slope success index was compared between the two groups it could be seen that it was significantly higher ($t(22) = 2.372$, $p = 0.027$, d=0.969) in the real group (average slope = 0.050, SD=0.054) than in the sham group (average slope = 0.000, SD=0.049).

When considering the **NF mean change success index,** in contrast to the slope success index, only six (out of eleven) patients in the real group had a positive NF mean change

success index, indicating that only half the patients managed to increase their average LI during the NF above their initial LI during baseline (Figure 6.2B). Similarly in the sham group also 6/11 patients had a positive mean NF success index. Note that one patient in the Real NF group and one in the Sham group had excessive head motion during their baseline pre-NF transfer run (absolute motion >2.5mm), therefore their data was excluded. The two groups were not significantly different from each other (t(20)= 0.265, p = 0.794, *d*=0.113), with the real group having an average NF mean change of 0.048 (SD = 0.154), and the sham group having an average of 0.0289 (SD = 0.175).

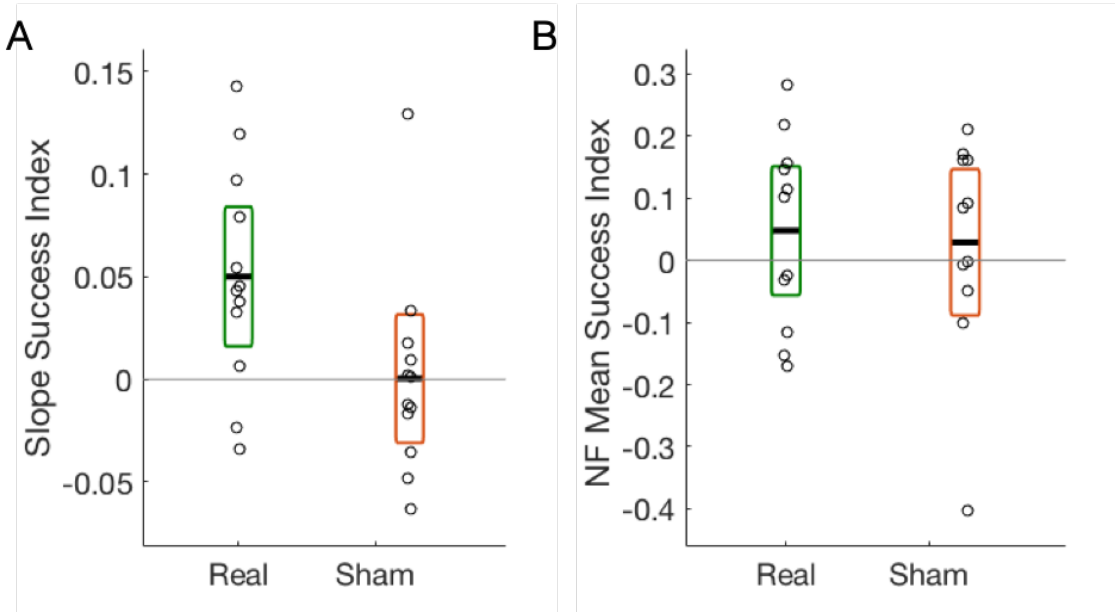


Figure 6.2 Success Index measures for the Real (green) and the Sham (orange) group with the black line indicating the median success index, boxes the 95% confidence intervals and individual patients data shown with open circles. **A)** NF slope success index **B)** NF mean change success index.

6.3.2 No association between NF learning success and baseline motor functioning

We first assessed whether baseline motor functioning, as assessed by the ARAT and the FM, was associated with patients' ability to use NF. In order to do this, four Spearman's rho correlations were carried out between the two success indices and the two baseline motor assessments. No significant correlations were found between the success measures

and baseline ARAT score (NF slope correlation: rho(10) = -0.404, p = 0.193, NF mean change correlation: rho(9) = -0.041, p=0.905). Additionally, there were also no significant correlations between the success measures and baseline score on the FM (NF slope correlation: rho(10)= -0.105, p=0.750, NF mean change correlation: rho(9) = 0.036, p=0.924)

6.3.3 Baseline brain activity during controlled squeeze task relates to NF success

In order to assess how brain function at baseline may relate to patients' ability to use NF, brain activity during the *Pre-NF transfer run* and during the *visuomotor squeeze task* was explored in relation to NF success. As the Pre-NF transfer run was used in the calculation of the NF mean change success index, activity during the Pre-NF transfer run was only correlated with the slope success index.

For the *Pre-NF transfer run*, no significant results were found when correlating the NF slope success index with ROI-based measures of baseline LI (rho(9)=0.082, p = 0.818) nor for the voxel-wise analysis.

When looking at the LI from the *visuomotor squeeze task* and NF success there were also no significant correlations when comparing the NF slope success index and LI at baseline (rho(10)=-0.396, p=0.203), or when comparing the NF mean change success index with LI at baseline (rho(9)=0.009, p=0.979). For the whole brain voxel-wise analysis no associations were found with brain activity and the NF mean change success index, however for the NF slope success index two significant clusters were found (Figure 6.3). One significant cluster (145 voxels, p = 0.001) was found in the Posterior Cingulate Cortex

(PCC; MNI coordinates of peak voxel: -4 -50 34) which exhibited a positive correlation between NF success and brain activity during the squeeze task. The second significant cluster (96 voxels, p = 0.015) was found in the primary motor cortex of the stroke-affected hemisphere (MNI coordinates of peak voxel: 28 -24 60), where a negative correlation with NF success was found.

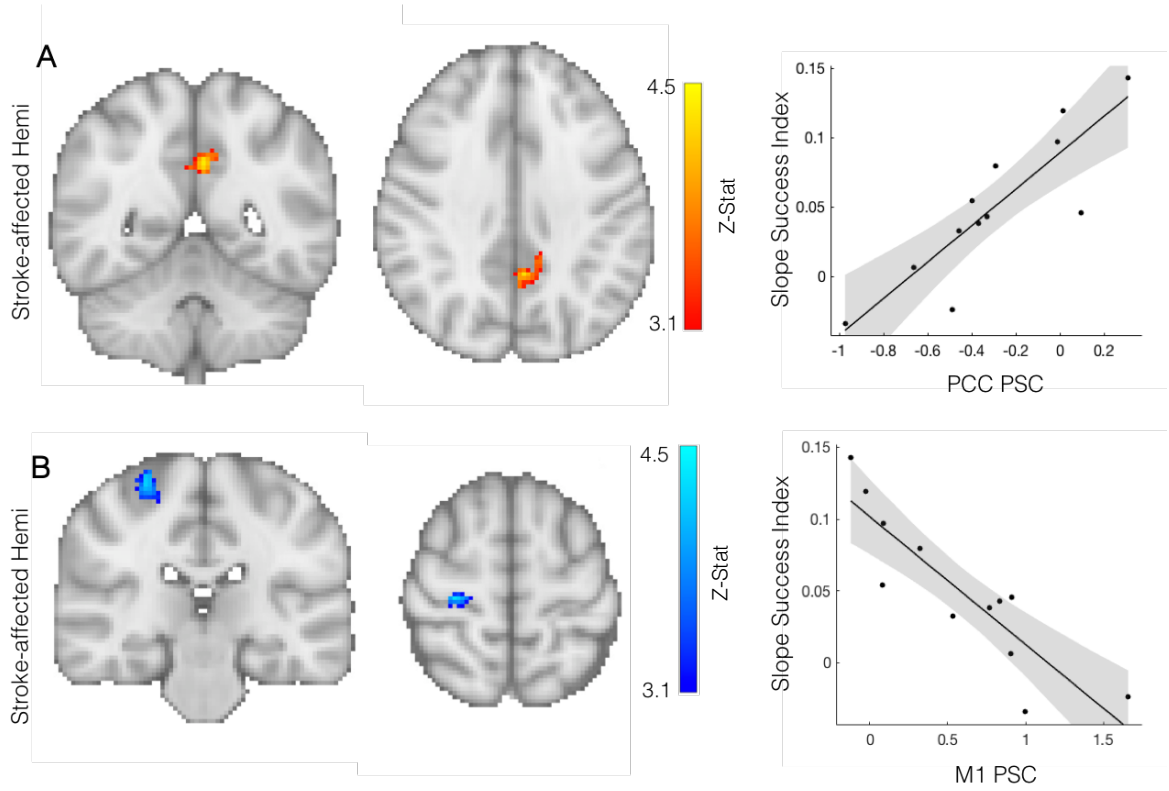


Figure 6.3 Correlation between brain activity during visuomotor squeeze task and NF success A) Voxel-wise analysis revealed a cluster in the PCC showing a significant positive correlation (displayed in red/yellow) between brain activity and NF success as measured by the NF slope success index. Scatter plot for visualisation purposes only (not inference) shows percent signal change (PSC) of voxels within the significant cluster in relation to the slope success index. **B)** Additionally a significant cluster was also found in the stroke-affected motor cortex where a significant negative correlation (displayed in blue) was found between brain activity at baseline and NF success as measured by the NF slope success index. Again the scatter plot shows the PSC of the significant voxels plotted against the success index for visualisation purposes only. Shaded area corresponds to 95% confidence interval.

6.3.4 NF success related to baseline brain structure

First, in order to examine associations between baseline white matter microstructure and NF success, FA from the CSTs was correlated with the NF success indices. There was missing DWI data from one patient who stopped the scan due to feeling unwell. There

were no significant association with FA in either CST for the NF mean change success index (affected CST (r(8)=-0.164, p=0.657); unaffected CST (r(8)=0.285, p=0.427)) or the slope success index (affected CST (r(9)= -0.282, p = 0.402); unaffected CST (r(9)= -0.555, p = 0.082)). When carrying out a whole-brain voxelwise analysis, a trend (687 voxels, p=0.07) towards a negative relationship was found for the NF slope success index and FA in the unaffected CST (figure 6.4A), with higher FA at baseline being associated with worse NF performance. No significant results were found when looking for whole brain associations with the NF mean change success index.

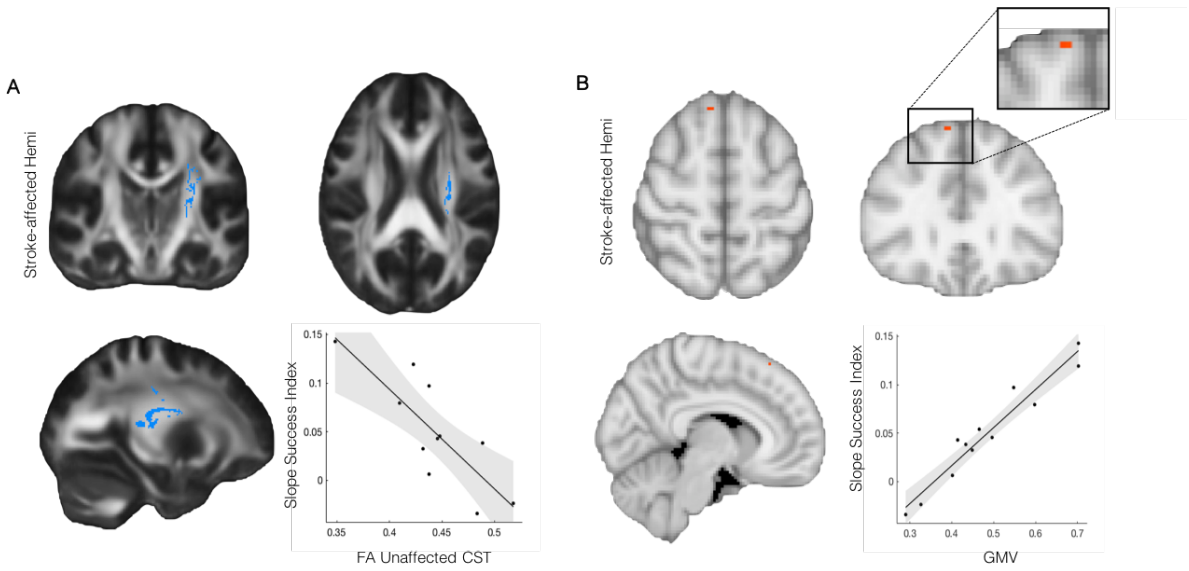


Figure 6.4 Correlation between baseline brain structure and NF success A) A trend was found (p=0.07, corrected) towards a negative correlation (shown in blue) between FA in the stroke-unaffected CST and NF success as measured by the NF slope success index. Scatter plot shows FA values from the cluster plotted against the success index for visualisation purposes. **B)** A significant cluster was found using VBM in the superior frontal gyrus/Pre-SMA, where grey matter volume at baseline showed a positive correlation (shown in red) with the NF slope success index. Scatter plot shows GMV in the significant cluster plotted against the NF slope success index for visualisation purposes. Shaded area shows 95% confidence interval.

Secondly, associations between GM density and NF success were also explored by carrying out a whole-brain VBM analysis with the demeaned success indices as regressors. No significant results were found for the NF mean change success index, however for the NF slope success index a small significant cluster (2 voxels, p<0.043) was found in the

superior frontal gyrus/Pre-SMA (MNI coordinates: 41, 78, 65), where greater grey matter density at baseline was associated with more successful NF performance (Figure 6.4B).

6.4 Discussion

Due to the high variability in participants' NF performance and a notable proportion of non-responders, there is increasing interest in trying to find factors associated with NF learning variability, and ways to identify subjects who are likely respond well to NF. In order to do this, studies have begun to investigate associations between participant characteristics at baseline and how these relate to subsequent performance during NF training. However, there currently is no consensus on how to best measure participants' performance during NF, and it is likely that there is considerable variability in participants' learning trajectories both across and within NF studies. In the current study we used two separate measures of NF success in the hope of capturing a broader range of potential learning trajectories. However, when comparing these success indices across participants in the Real and the Sham group, only the NF slope success index was significantly different across the two groups; this success measure also captured the main learning trends found during NF learning (see Chapter 4). The lack of a significant difference between the two groups on the NF mean change success index may indicate that this measure is not sensitive enough to pick up NF-related changes in the Real group. As the patients were not given specific movement instructions during the Pre-NF transfer task (which was used to calculate this success index), it may be that the baseline Pre-NF transfer run was too variable across patients to be useful in this calculation. In line with this, no significant associations were found between this success index and any of the baseline measurements. In contrast, several associations were found with the NF slope success index which will be discussed further.

6.4.1 No association with baseline upper limb function and NF success

In the current study we did not find any associations between patients' baseline upper limb function and NF success, suggesting that patients' baseline functioning is not related to how well they can learn to use NF. One previous NF study with stroke patients suggested that baseline impairment level may be related to NF success, however this study only included three stroke patients (Liew et al. 2016). It has been suggested in previous literature that stroke patients with greater impairments may be more reliant on compensatory activity in the stroke unaffected hemisphere (Buetefisch 2015; Rehme et al. 2011), therefore these patients may also be less able to increase lateralisation of brain activity towards the stroke-affected hemisphere. Previous work investigating variance in the response of stroke patients to brain stimulation found that FM score in combination with time since stroke predicted patients' response to cathodal TDCS (O'Shea et al. 2014), where patients who had higher scores on the FM many years after stroke were more likely to benefit from cathodal TDCS to the stroke unaffected motor cortex. The authors discuss that this result may be due to more impaired patients potentially benefiting from bilateral activity (see also Introduction). In the current study, we were not able to find associations between brain activity lateralisation during the visuomotor squeeze task and motor functioning as assessed by the FM and ARAT (see Appendix B figure S6.1), thus suggesting that patients' baseline scores on these tasks were not indicative of their motor cortex activity.

6.4.2 Baseline brain activity related to NF success

A previous study exploring relationships between baseline brain activity during functional localisers or Pre-NF transfer runs and NF success across a number of NF studies did not

find strong evidence for a relationship between these factors (Haugg et al. 2020). In the current chapter we explored brain activity during the Pre-NF transfer run, as well as during a visuomotor squeeze task, in relation to NF success. Whilst the Pre-NF transfer run is more similar to the NF runs that participants experienced, the participants were free to choose different movement strategies and therefore there was less control over what movements patients were making. Therefore, similarly to Haugg and colleagues (2020), we didn't find any relationships between Pre-NF transfer runs and NF success. The only significant, albeit small correlation that Haugg and colleagues (2020) previously found was between activity during functional localiser (considering just active voxels) and NF success. Typically during functional localizer runs participants are given explicit instructions on what to do in order to engage the ROI, whereas in the Pre-NF transfer run of the current study, patients were free to choose what movements to make.

In contrast, during the visuomotor squeeze task, all patients performed the same movements and when correlating brain activity during this task with NF success, two significant clusters were found. Firstly, there was a significant positive relationship between activity in the PCC and NF success. The PCC is a highly connected part of the default mode network (DMN) which is typically *deactivated* during tasks that require effort and attention, whilst becoming active during rest periods or during introspective tasks (for review: Leech and Sharp, 2014). Indeed in the current study the majority of the patients showed deactivations of PCC during the squeeze task (see figure 6.3A), with greater deactivation being associated with worse NF performance. It has previously been suggested that deactivation of the DMN during a task is related to task difficulty, with more difficult tasks leading to greater deactivation (McKiernan et al. 2003). Therefore, degree of task-related PCC deactivation may be a reflection of how difficult patients found

the visuomotor squeeze task, which may in turn be related to how well they did on the NF task. However, despite the PCC being implicated in many disorders (for example Alzheimer's disease, depression, schizophrenia etc), the exact role of the PCC remains unclear, and several functions have been ascribed to this region including arousal, switching between internal and external attention, and vigilance (Leech & Sharp 2014).

The second significant cluster found in relation to NF success was in the motor cortex of the stroke-affected hemisphere, where a negative correlation with NF success was found. Therefore, patients who had high activity in this area during the squeeze task at baseline were less likely to be successful in the NF training. This finding may be due to a ceiling effect where patients who start off with high activity in the stroke-affected motor cortex have less room to increase activity further through NF. In order for these patients to be more successful, they could have been instructed to focus more on decreasing activity in the stroke unaffected hemisphere. This finding contrasts with the previous findings of the meta-analysis from Haugg and colleagues (2020) where a small positive correlation was found between activity in the NF ROI at baseline (during the functional localiser) and participants' NF success. This previous study included results from NF studies with a variety of different target ROIs, with some aiming to up-regulate and others to down-regulate activity, as well as including studies targeting functional connectivity between two areas. Therefore, it may be the case that baseline activity in the ROI and its relation to later NF success varies depending on the ROI used and the aim of NF training.

6.4.3 Indications of associations between baseline brain structure and NF success

Relationships between baseline brain structure and NF success were additionally explored in the current chapter. When examining relationships between white matter microstructure at baseline and NF success, a trend was found in a large cluster in the stroke unaffected CST, where greater FA was associated with worse NF performance. This trend failed to reach significance and therefore should be interpreted with caution. However, the small sample size (11 patients) may have led to a decrease in power to detect such an effect. This finding may be due to some stroke patients having an increased reliance on the contralesional (ipsilateral) motor cortex and motor-related pathways. Using the same NF conditions as described in Chapter 3, it has previously been shown that higher FA in the superior longitudinal fasciculus was associated with worse performance when trying to decrease ipsilateral sensorimotor cortex activity (*dissociation condition*). In contrast when trying to increase activity in ipsilateral sensorimotor cortex (*association condition*), there was a trend towards higher FA in the contralateral CST at baseline being associated with worse performance (Sampaio-Baptista et al. 2020). Together this may suggest that the strength of the motor system structural connectivity (especially in motor-output pathways) may have a negative impact on performance and that these participants may benefit from having more training sessions.

When investigating relationships between GMV and NF success, a small significant cluster was found in the superior frontal gyrus/Pre-SMA, where higher GMV was associated with better NF performance. There currently is little consensus on the functional role of Pre-SMA (for review: Nachev et al., 2008), but it has been implicated in several cognitive/motor functions such as inhibition of responses, sequence learning, self-

generating actions and switching between actions. A rare lesion case study (Roberts & Husain 2015) found that a patient with a lesion in Pre-SMA showed marked impairment on switching between response plans. It may well be that some of these functions related to more cognitive aspects of motor control are also important for motor cortex NF, in which patients are required to initiate and select movements that they perceive as being more successful in controlling the height of the NF bars.

6.4.4 Limitations

A limitation of the current work is the small sample size used in this correlation based analysis, as smaller sample sizes are associated with increased variability and potentially inflated r-values (Button et al. 2013; Makin & Orban de Xivry 2019). Therefore, in order to find robust and reliable predictors of NF success, studies with larger sample sizes, or studies combining data across studies, are needed. Combining across multiple studies could elucidate predictors that translate across studies, targets and populations. However, while there are some factors that may be NF general, it may also be the case that different NF paradigms are associated with unique predictors. For example, signals relating to domain general functions such as attention or motivation might be relevant to any NF intervention. By contrast, motor network integrity may be an important factor specifically in NF training of motor regions, but not in studies targeting different areas. Additionally, combining across studies also limits the modalities and measures that can be investigated, as often the same modalities are not collected across studies. Smaller studies may help to highlight potential areas of interest and guide future studies.

In the current chapter an opportunistic analysis was used given the data that was available. Therefore, in the current study we did not collect data on patients' mood, personality or

motivation which may additionally be related to NF success. As these kinds of measures are cheaper and easier to collect, they would make attractive choices as potential predictors of NF success. Inclusion of these kinds of measures in future work across studies may help to identify general NF learning predictors.

6.5 Conclusion

In this chapter, variability in stroke patients' ability to use NF to self-regulate motor cortex activity was explored, and how this is related to baseline patient characteristics. In line with previous studies, there was considerable variability in how well patients were able to learn to use NF, however, there is no consensus yet on how to quantify NF success. Although upper limb motor function at baseline was not associated with later NF performance, associations were found between baseline brain activity and NF performance. Additionally, there appears to be some indication the motor network structural integrity, in particular in the stroke unaffected hemisphere, may be related to how malleable motor cortex activity is. Finally, an association was additionally found between grey matter volume in frontal cognitive-motor areas and NF success.

7
General Discussion

7.1 Thesis overview

The aim of this thesis was to explore the utility of real-time fMRI neurofeedback for motor rehabilitation after stroke. There has been increasing interest in developing rehabilitation interventions for stroke patients which target the brain and neurofeedback allows for flexible and spatially specific self-modulation of brain activity. Therefore, neurofeedback has previously been suggested as a potentially useful tool for stroke rehabilitation (Linden & Turner 2016; Wang et al. 2018).

In the first chapter of this thesis the time course of recovery and some common theories around how recovery after stroke may occur are discussed. Additionally, previous studies using brain stimulation techniques are considered, which typically focus on attempting to promote lateralisation of brain activity, either by increasing activity in the stroke-affected hemisphere, or decreasing activity in the stroke-unaffected hemisphere. However, this approach may not be suitable for all patients, in particular some patients may benefit from compensatory activity in contralesional motor cortex, or in secondary motor areas. One of the benefits of fMRI NF is that it allows a lot of flexibility in terms of what brain regions and functions can be targeted, as well as being endogenously driven.

In chapter 2 some methodological considerations are discussed for NF experiments. As this technique is still fairly new, there are several unanswered questions in terms of the optimal set-up of a NF experiment. These include issues such as how best to display the

feedback and what the optimal control condition is. These issues are discussed in reference to the studies that are presented in this thesis.

In Chapter 3 a study in healthy adults is presented in which the effect of having multiple training days on NF learning is explored. This was investigated in two separate conditions in which activity in ipsilateral motor cortex was driven in opposite directions by the participants. It was demonstrated that there were clear benefits of having multiple training days when trying to increase activity in ipsilateral motor cortex. However, when trying to decrease motor activity, there did not seem to be a benefit of having multiple training days on NF learning.

In Chapter 4, a double-blind, sham-controlled randomised control trial of fMRI NF in chronic stroke patients is introduced, which forms the basis of the remaining chapters. In this chapter stroke patients ability to use NF to increase lateralisation of brain activity over three training days is explored. We showed that patients appear to be able to use NF within each training day to increase laterality of brain activity, however there was no evidence of NF learning across training days. Additionally, we explored whether patients were able to continue to modulate brain activity when feedback was removed (transfer effect). This could have important clinical implications as it could allow patients to continue to modulate their brain activity outside of the scanner. Although no effect could be observed on the group level, there was a correlation between transfer success and how well patients performed on the NF, which was not present in the sham group. This intuitively suggests that transfer ability depends on how well patients learn during the NF training.

In chapter 5, changes in patients upper limb functioning and their brain structure and function after the NF training were explored. There were some promising indications of improvements on gross motor tasks of the JTT in the Real group compared to the Sham. However, there were no NF effects on clinical measures of upper limb activity and function. Additionally, there were indications that brain function, as well as brain structure were altered after NF training. In particular, activity appeared to be increased during a visuomotor squeeze task in brain regions which have previously been related to NF learning, including the putamen and lateral occipital cortex. Additionally, when examining white matter changes after NF, it could be seen that FA asymmetry in the CSTs (a measure that has previously been related to stroke impairment and recovery), was reduced after NF training in the real group. When looking at the two CSTs separately, there was some indication that this effect was more driven by increases in FA in the stroke-affected CST, rather than decreases in the stroke unaffected CST. Additionally, increases in FA in the stroke-affected CST was strongly correlated with patients performance on the NF training, suggesting that those patients who performed better during the NF training also exhibited greater changes in their stroke-affected CST.

Finally, in Chapter 6 variability in patients NF performance was explored and how this was related to baseline characteristics of the patients. There was no indication that NF performance was related to baseline upper limb functioning of the patients, however performance did seem to relate to brain activity at baseline. In particular, worse performance on NF was associated with higher activity in stroke-affected motor cortex during a visuomotor squeeze task (possibly due to a ceiling effect), as well as with greater deactivation in the posterior cingulate cortex (part of the default mode network). Additionally, there were some slight indications that NF performance is related to baseline

brain structure, in particular with baseline grey matter volume in a frontal cognitive-motor area and motor network structural integrity.

7.2 Overall comments

The work presented in this thesis included the first sham-controlled investigation of fMRI NF in stroke patients. Previous pilot and feasibility studies typically only included a handful of patients and were lacking a sham control. We were able to provide evidence that stroke patients are able to use NF within training days to increase lateralisation of brain activity, although it was also apparent that patients ability to use NF was highly variable. This suggests that NF could be a potential tool that can be used in this population in order to alter brain activity patterns that may be associated with worse functioning. However, in order for this to be achieved, appropriate targets need to be identified, and there is still discussion in the stroke literature about the role of contralesional motor cortex activity and its relation to motor recovery. One of the strengths of NF is that it allows greater flexibility in terms of areas that can be potentially targeted, as well as what function is being targeted, with recent studies starting to target functional connectivity between brain regions (Megumi et al. 2015; Zich et al. 2020) and multivariate activity patterns (Koizumi et al. 2016; Shibata et al. 2011; Taschereau-Dumouchel et al. 2018).

The observed effect on gross motor performance as assessed using the JTT is also a promising indication that NF training could drive improvements in motor functioning even after a relatively short amount of NF training. However, there was not any indications that NF improved performance on clinical measures of upper limb activity and functioning. In order for NF to be a useful tool in stroke rehabilitation it is important to demonstrate a clinically significant improvement in functioning. This lack of a clinical effect may be due

to the variability seen in patients ability to use NF and the small sample size. Additionally, as discussed above, increasing lateralisation of brain activity may not have been the optimal brain activity pattern for all of the patients in this study. Future studies could also explore NF in addition to a motor rehabilitation programme and explore whether NF boosts the effects of the rehabilitation programme. In particular, further practise outside of the scanner of strategies identified to be successful during NF runs may help to boost the effect of NF on behaviour further.

Furthermore, we found evidence of changes in brain function and structure after NF training. Our finding of increased activity after NF learning in areas such as the putamen and the lateral occipital cortex provides further support that these regions may play an important role in NF learning, and that these regions undergo some functional changes with NF. The putamen (part of the dorsal striatum) is thought to play an important role in NF learning. The dorsal striatum has been found to be activated across different NF studies targeting different cortical regions (Emmert et al., 2016, Shibata et al., 2019) and right putamen volume has been linked to NF success (Zhao et al. 2021). Additionally, when long term potentiation in the dorsal striatum is impaired, NF learning diminishes (Koralek et al., 2012). The dorsal striatum is also thought to play an important role in action selection and initiation (Balleine et al. 2007). In particular, the dorsal striatum has been implicated in learning action-reward associations (O'Doherty et al. 2004), which has been found to be impaired in patients with Parkinson's disease (Shohamy et al. 2004) a hallmark of which is striatal dysfunction. Therefore, changes in putamen activity in this study could also be due to its role in movement selection, as the patients in the current study would have to make decisions about what movements to make based on the neurofeedback display.

As mentioned in Chapter 5, the LOC's role in NF is thought to be through its role in directing attention to visual stimulus (Sitaram et al. 2017), and activity in this area has been demonstrated across a range of NF experiments during self-regulation (Emmert et al., 2016). However, the LOC, in particular the extrastriate body area (EBA), has also been demonstrated to respond to limb movements, even in the absence of visual feedback (Astafiev et al. 2004), and to contain overlapping visual- and motor-driven topographic maps of the body (Orlov et al. 2010). This has led to the suggestion that the EBA plays an important role in body representation by integrating visual and sensory motor signals (Astafiev et al. 2004). It has also been proposed that the EBA is involved in body ownership (Limanowski et al. 2014). Therefore, the increased activity observed in the LOC after NF training may be due to the limb movements made during the task, rather than visual attention to the NF signal.

There are several additional areas which may be important for motor NF but which we did not find in the current study. In particular, cerebellar activity has been consistently linked to motor control and motor learning, in particular to visuomotor adaptations and sequence learning (Bernard & Seidler 2013). The cerebellum is thought to be involved in predicting the consequences of motor commands and computing sensory prediction errors (Krakauer et al. 2019). This is supported by studies in patients with cerebellar deficits showing disrupted adaptation learning (Tseng et al. 2007), and imaging studies in healthy individuals showing increased cerebellum activity to motor errors (Grafton et al. 2008) and sensory prediction errors (Schlerf et al. 2012). Other brain regions that have previously been associated with NF learning include the Insula (Shibata et al. 2019; Emmert et al. 2016), which is thought to be involved in introspection and awareness of body movement (Craig 2008), and the anterior cingulate cortex which is thought to be involved in the

explicit processing of reward (Sitaram et al. 2017; Amiez et al. 2012). These areas may not have been picked up in the current study due to a lack in power from the small sample size.

Additionally, we were able to demonstrate NF-related changes in the patients' white matter microstructure of the CSTs. This result is in line with previous studies in healthy individuals which have shown that NF training of motor areas can lead to white matter changes in motor-related tracts (Marins et al. 2019; Sampaio-Baptista et al. 2020). White matter changes have been observed to occur with learning and experience in the adult brain (for review: Sampaio-Baptista & Johansen-Berg, 2017) and are thought to relate to the optimization of impulse transmission (Zatorre et al. 2012). However, neuroimaging derived measures of white matter, such as diffusion imaging, are sensitive to multiple different white matter tissue properties, making it difficult to determine what biological processes may underlie changes observed using diffusion imaging. One potentially attractive mechanism underlying diffusion changes is activity dependent myelination (Bonetto et al. 2021; Fields 2015). Studies have shown that electrical activity in axons can influence myelination of axons (Demerens et al. 1996; Gibson et al. 2014), and that environmental enrichment (Okuda et al. 2009) and physical activity in rodents (Simon et al. 2011) increases proliferation of oligodendrocyte progenitor cells, which mature into myelin forming oligodendrocytes. Furthermore, when oligodendrocyte production is impaired in adult mice, motor skill learning is additionally impaired (McKenzie et al. 2014; Xiao et al. 2016), highlighting the potentially important role myelination plays in motor skill learning. Whilst diffusion metrics have been shown in histological validation studies to be sensitive to myelin changes (Lazari & Lipp 2021), diffusion metrics are also modulated by several other biological features such as axon caliber and density, cell swelling or membrane permeability (Sampaio-Baptista & Johansen-Berg 2017) which

could also have led to the changes observed in the current study. Future studies using imaging measures with greater sensitivity to specific biological mechanisms (such as magnetization transfer or myelin water imaging (Heath et al. 2018) could help to shed light on the biological mechanisms underlying changes observed with diffusion imaging.

As discussed in the general introduction, alterations in white matter have previously been associated with better performance after stroke, in particular in descending motor pathways (Karbasforoushan et al. 2019; Schaechter et al. 2009). Additionally, in rodent stroke models, optogenetic stimulation has also been shown to lead to re-wiring within the motor system and restoration of function (Tennant et al. 2017; Wahl et al. 2017). The observed changes in white matter structure found in the current study indicates that NF may be a potential tool that could be harnessed to drive structural brain changes in humans.

7.3 Future directions

7.3.1 Personalised approach to rehabilitation

Stroke patients represent a highly heterogeneous group of individuals and it seems likely that there is not a one-size-fits-all solution when it comes to treatment approaches. Future work could attempt to personalise NF targets based on patients baseline characteristics. For example, the two approaches described in Chapter 3, where ipsilateral motor cortex activity was either increased or decreased, could be applied to more and less affected patients respectively. Additionally, NF can also be used to target other brain regions, including subcortical brain structures and connectivity between brain areas. Previous work has suggested that CST integrity may be a useful tool for stratifying patients into different groups. However, more work is needed in order to identify ways to stratify patients and

pinpoint what the optimal target for modulation is for individual patients. This may additionally require a deeper understanding of what mechanisms underlie recovery after stroke and how these can be targeted.

7.3.2 Explaining inter-individual differences in NF learning success

A common finding in the NF literature, which was also observed in the current study, is that individuals NF learning success is very variable, with some individuals seemingly unable to learn to self-regulate brain activity with neurofeedback, even after many training sessions. Future work exploring ways to identify these individuals would allow targeting of participants who are most likely to benefit from neurofeedback training.

However, one difficulty in studying and assessing NF success variability is that there is currently no agreed-upon measure of NF success. This could be measured in several different ways across NF training runs, or could be calculated based on changes on follow-up or behavioural measurements. Additionally, whilst individual studies (including the current study) can hint at some factors that may be associated with NF success, they are often under-powered to explore this variation in learning. Some initial work attempting to explore this issue have combined data across different NF studies. Whilst this increases the statistical power to detect effects by substantially increasing the sample size, this approach does limit which baseline characteristics can be assessed in relation to NF success, as the measurements need to be common across all the studies. Future cooperation and consensus across the NF community would allow progress in this research area by coming up with a common definition of NF success, and providing guidance on promising measures for predicting NF success that can be acquired in future studies.

7.3.3 Mechanisms underlying Neurofeedback learning

The mechanisms underlying NF learning remain relatively unknown. Previous work in rodents has provided strong evidence for the involvement of cortico-striatal plasticity in NF learning (Koralek et al. 2012). The authors observed increased correlations in activity between the target area (motor cortex) and the striatum during NF learning, and that deletion of striatal NMDA receptors significantly impaired learning. Work in humans has also demonstrated recruitment of the dorsal striatum during NF learning. Emmert and colleagues (2016) combined data across twelve NF studies and found that the striatum and anterior insula were consistently active across the different studies during NF learning. Similar results were found by Shibata and colleagues (2019) when combining across previous multivariate (decoded) NF studies, with activity also seen in the supplementary motor area, cerebellum and posterior parietal cortex.

Future work could build upon these previous findings and investigate how activity in these regions relates to NF learning success, or to the likelihood of successful transfer. Additionally, in line with the previous work in rodents, it would be interesting not only to examine where activity is increased during NF learning, but also whether any changes in brain connectivity occur and whether this relates to NF learning success.

8
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Appendix A

Chapter 4 supplementary information

Example participant instructions

1st Neurofeedback training day – LEFT HAND AFFECTED

Today we will perform a number of different scans. This will last about 45 minutes in total. Again, we will be able to speak to you through the intercom.

For the first few setup scans, you will simply lie still with your eyes open and relaxed. These will last for just a few minutes.

We will then ask you to perform a task where sometimes you need to move your left hand and sometimes you need to move your right hand.

Functional Localiser

In this scan, we will ask you to open and close your hands repeatedly, one at a time.



When you see the words **"SQUEEZE RIGHT**" we need you to open and close your **right hand** only at a steady pace for approximately 30 s. Try to keep your left hand relaxed and still.



When you see the words **"SQUEEZE LEFT"**, we need you to open and close your **left hand** only at a steady pace for approximately 30 s. Try to keep your right hand relaxed and still. At all times try to keep the rest of your body still.



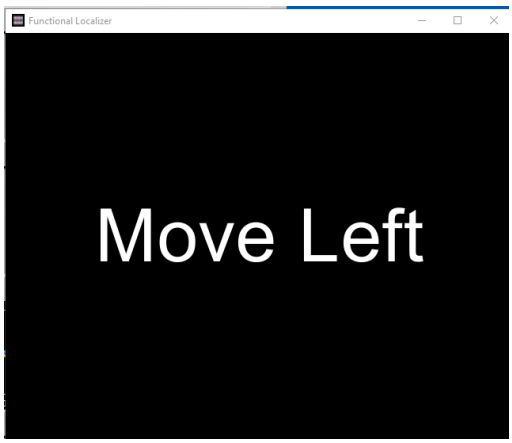
When you see the word "**REST**", you can relax. Keep your eyes open, but feel free to blink normally.

Note. The task might start with the right hand first or it might start with the left hand first. Just follow the instructions on the screen.
Prior to the scan starting, we will tell you that this is the scan where you need to open and close your right hand when you see the words "squeeze right", and to open and close your left hand when you see the words "squeeze left".

There will then be a short break while we set up a few things. You can just relax.

First Pre-Transfer Run

For this part of the scan, when you see the instruction "MOVE LEFT" you will need to move your **left hand** only. You can make any movements of the left hand that you want. However, do not move the rest of the arm as this will cause your head to move too much. Try to keep your right hand relaxed and still.





Please continue this for approximately 30 seconds and then you will see the word "Rest". When you see "Rest" you should take a break and lie still until the instruction "Move Left" reappears.

You can make any movements with your left hand that you want. Suggestions for movements include [experimenter should demonstrate each of these movements, as appropriate, and ask the participant to copy them if they can]:

- Opening and closing your affected (left) hand like this. *Could you copy what I am doing?*
- Moving individual fingers of your affected (left) hand like this.
- Moving your affected (left) hand at the wrist like this.
- Tapping the fingers of your affected (left) hand onto your thumb like this.

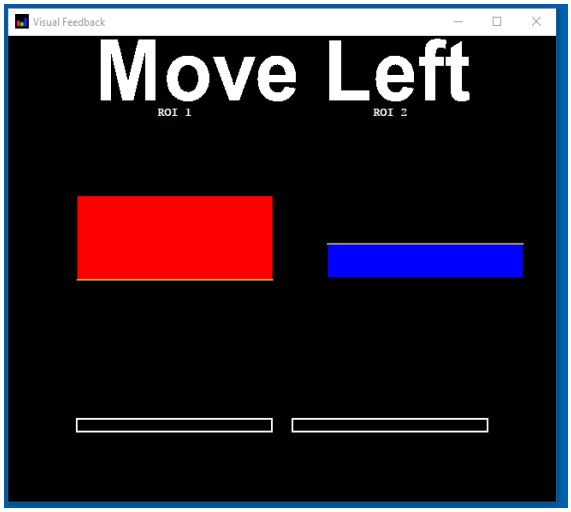
These are just suggestions for movements, you can do any movement of the left hand that you wish, so long as you do not move your right hand or the rest of your arm or body.

Prior to this scan starting, we will tell you through the intercom that this is the scan where you need to make different movements with your left hand only.

Neurofeedback Runs
(3 blocks of 6 minutes)

We will perform this scan 3 times, with breaks in between.

You will see two bars on the screen, which represent your brain activity. When you see the instruction "MOVE LEFT" you should move your **left hand only.** The bars will then start to move as your brain activity changes.



We want you to try to move your left hand so that the red bar on the left increases in height and the blue bar on the right stays as small as possible. This can be difficult to do – please try your best.

The red bar represents the activity in the affected side of your brain and the blue bar is the activity in the unaffected side.

We would like you to try to raise the red bar and decrease the blue bar because we're trying to see if you can use more of the affected part of your brain.

Please note: The bars may move slowly (not like the bar when you are doing the squeeze task with the plastic device in your hand).

Please continue making movements of your left hand for approximately 30 seconds and then you will see the word "Rest".

You may move the affected (left) hand in any way that you want. However, do not move the rest of the arm as this will cause your head to move too much.

Suggestions for movements include [experimenter should demonstrate each of these movements, as appropriate, and ask the participant to copy them if they can]:

- Opening and closing your affected (left) hand like this. *Could you copy what I am doing?*
- Moving individual fingers of your affected (left) hand like this.
- Moving your affected (left) hand at the wrist like this.
- Tapping the fingers of your affected (left) hand onto your thumb like this.

Other strategies you may want to try:

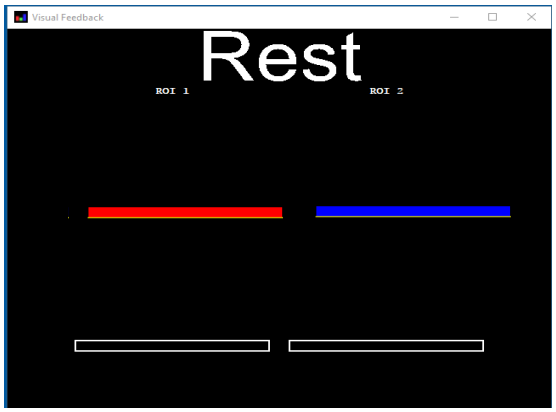
- Focusing less on your unaffected (right) hand
- Focusing more on your affected (left) hand
- Relaxing your right hand while moving your left hand
- Imagining movements of the left hand
- Combining different movements

These are just suggestions. Use any movement, imagined movement or other strategy that you find works to increase the height of the red bar on the left and decrease the height of the blue bar on the right.

Please note, the bars take approximately 5 seconds to respond to any changes in movement. So, if you change your movement pattern then ensure you continue for at least 5 seconds to see whether it alters the size of the bars as required.

When the scanner noises start you will see a blank screen for a while before the task starts. This is normal.

When you see "Rest" you should take a break. The bars should **slowly** start coming down. Stay relaxed until the instruction "Move Left" reappears.



This scan will last for approximately 6 minutes and will be performed 3 times in total.

There will be short break between blocks of this scan for you to relax again. We will ask you if you are ready to start again and if you need a longer break please say so.

For the second and third blocks of this task, you can repeat movements or strategies that you have found to be effective, and you can try new movements or strategies also. We know that this task can be difficult, so please just try your best. When the scanner noises start you will see the bars as they were at the end of the previous scan, after a few seconds they will start to move again. This is normal and please just relax until you see the "move left" instruction.

Prior to these scans, we will tell you through the intercom that this is the scan where you need to make movements with your left hand to make the red bar on the left as big as possible and the blue bar on the right as small as possible.

Post-transfer run

For this scan, we want to see your brain activity when the bars are no longer shown.

When you see the instruction "MOVE LEFT" you will need to move your **left hand only**. You should use the **movements or strategies that you found effective** in the previous scan for increasing the size of the red bar and keeping the blue bar as small as possible.

However, you will not see the bars this time.





Please continue this for approximately 30 seconds and then you will see the word "Rest". When you see "Rest" you should take a break and lie still until the instruction "Move Left" reappears.

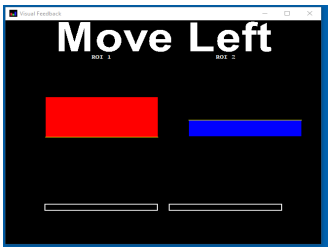
Prior to this scan starting, we will tell you through the intercom that this is the scan where you will move your left hand using the strategies or movements that you found most effective in the previous scans but there will be no bars this time.

FINALLY - At all times, please try to keep still in the scanner. I know it can be difficult but try to keep your head as still as possible, even when you are moving your affected hand. This is to ensure we get the best quality scans.

Debriefing Questionnaire

Participant ID:

Debriefing questionnaire



Please answer the questions below.

For question 1 and 2 please circle the number

1) How much control over the **red bar** did you feel you had?

Not in control		Somewhat in control		Fully in control
1	2	3	4	5

2) How much control over the **blue bar** did you feel you had?

Not in control		Somewhat in control		Fully in control
1	2	3	4	5

3) What sort of movements or strategies did you use to control the moving bars?
These could be particular ways of moving your hand or effort or relaxation strategies.

Please list **all strategies** that you tried and **rate on a scale of 1 to 5** how successful the strategy was in changing the bars in the correct way (1=unsuccessful, 5-successful).

Strategy	Effectiveness (1 to 5) 1=unsuccessful strategy 5=successful strategy

In this final section of the questionnaire we have listed some example strategies for controlling the bars. We realise that you may have listed some of these strategies on the previous page, but please fill in the below questions also.

- Please circle whether you used this strategy (yes or no).
- If you used this strategy then circle a number from 1 to 5 indicating how successful this strategy was (1=unsuccessful strategy, 5=successful strategy).

1) Focussing **more** on the **moving** hand YES NO

(Unsuccessful) 1 2 3 4 5 *(Successful)*

2) Focussing **less** on the **non-moving** hand YES NO

(Unsuccessful) 1 2 3 4 5 *(Successful)*

3) Focussing on **relaxing** the **non-moving** hand YES NO

(Unsuccessful) 1 2 3 4 5 *(Successful)*

4) Moving **more quickly** YES NO

(Unsuccessful) 1 2 3 4 5 *(Successful)*

5) **Opening and closing** the hand YES NO

(Unsuccessful) 1 2 3 4 5 *(Successful)*

6) Moving **individual** fingers YES NO

(Unsuccessful) 1 2 3 4 5 *(Successful)*

7) Moving the hand at the **wrist** YES NO

(Unsuccessful) 1 2 3 4 5 *(Successful)*

8) **Tapping** the fingers YES NO

(Unsuccessful) 1 2 3 4 5 *(Successful)*

Additional Questions

How tiring did you find it to concentrate on the moving bars?

(not at all) 1 2 3 4 5 (*very tiring)*

How was your sleep last night?

(very poor) 1 2 3 4 5 (*very good)*

Any other comments about how you felt during today's session?

Appendix B

Chapter 4 supplementary figures

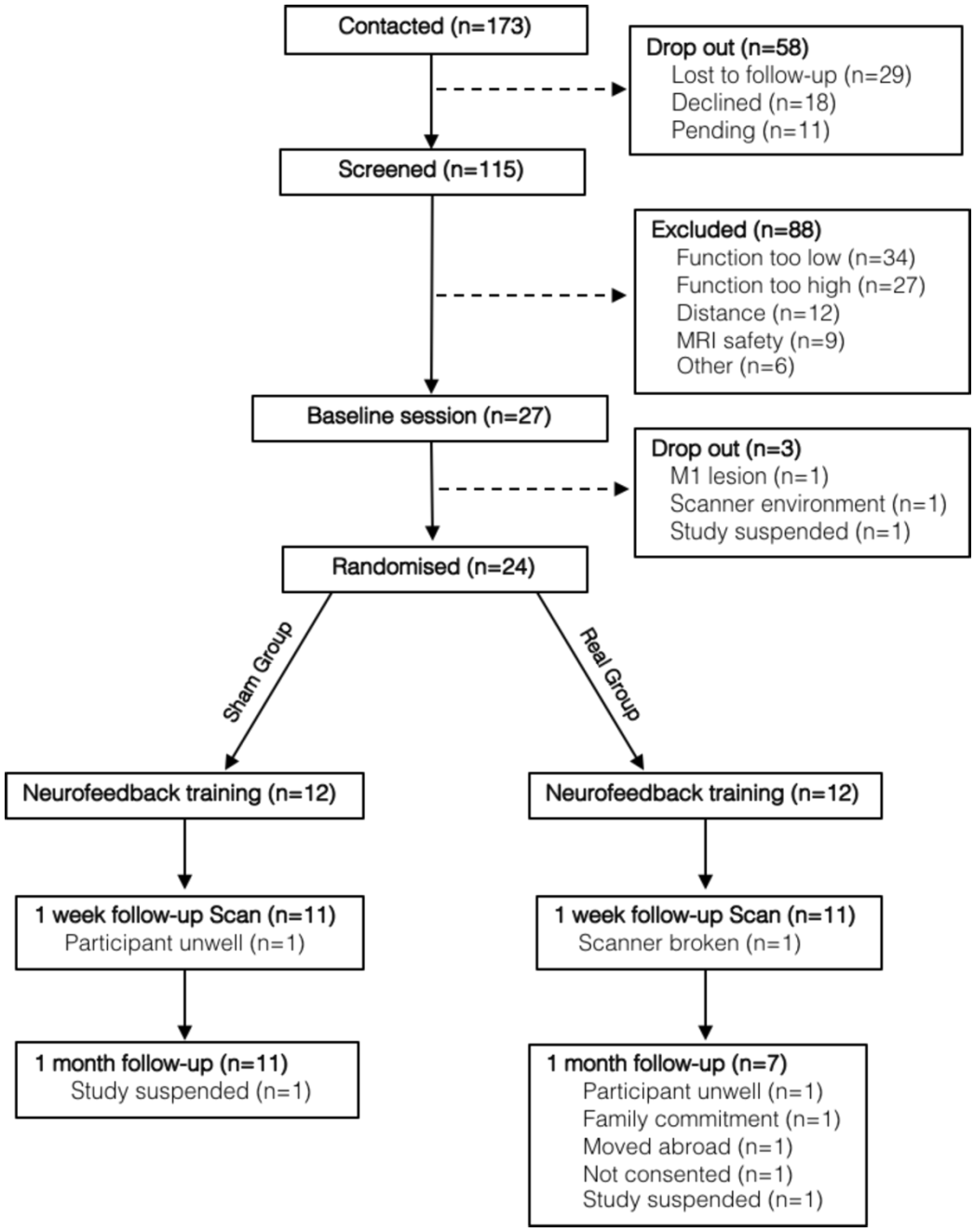


Figure S4.1 Recruitment flow diagram for RCT. Number of patients (n) indicated at each stage along with any reasons for drop out/exclusion. Note: all patients completed behavioural assessment at 1 week follow-up, but two didn't complete the scan due to the reasons given.

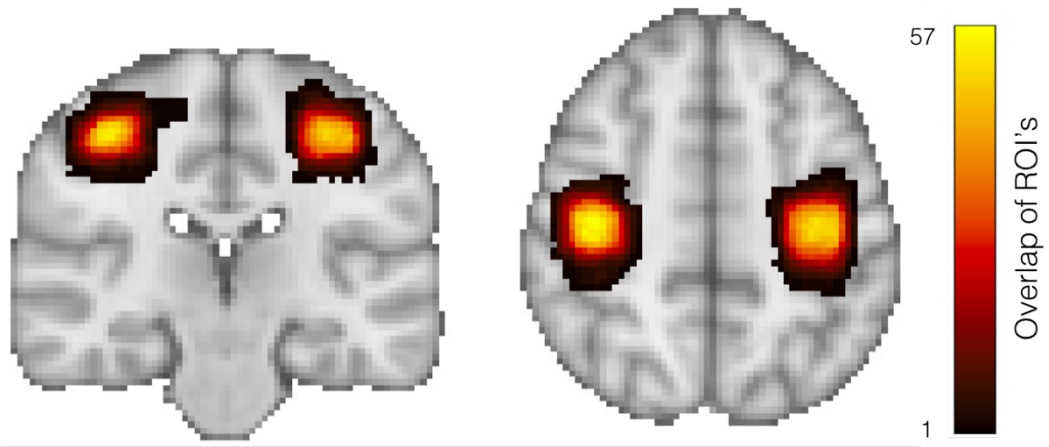


Figure S4.2 Probabilistic maps of subject specific ROI's used for NF training in MNI space. Note: maximum possible overlap would be 72 (24 patients x 3 NF sessions each).

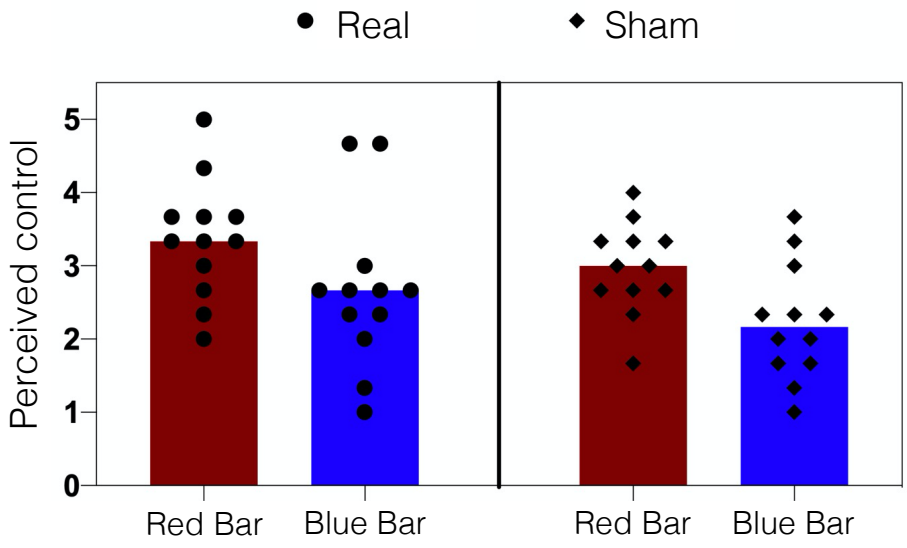


Figure S4.3 Patients rating (between 1 and 5) of their perceived control on average over the Red and Blue bars during the NF training runs. Data for the Real group shown on the left and the Sham group on the right.

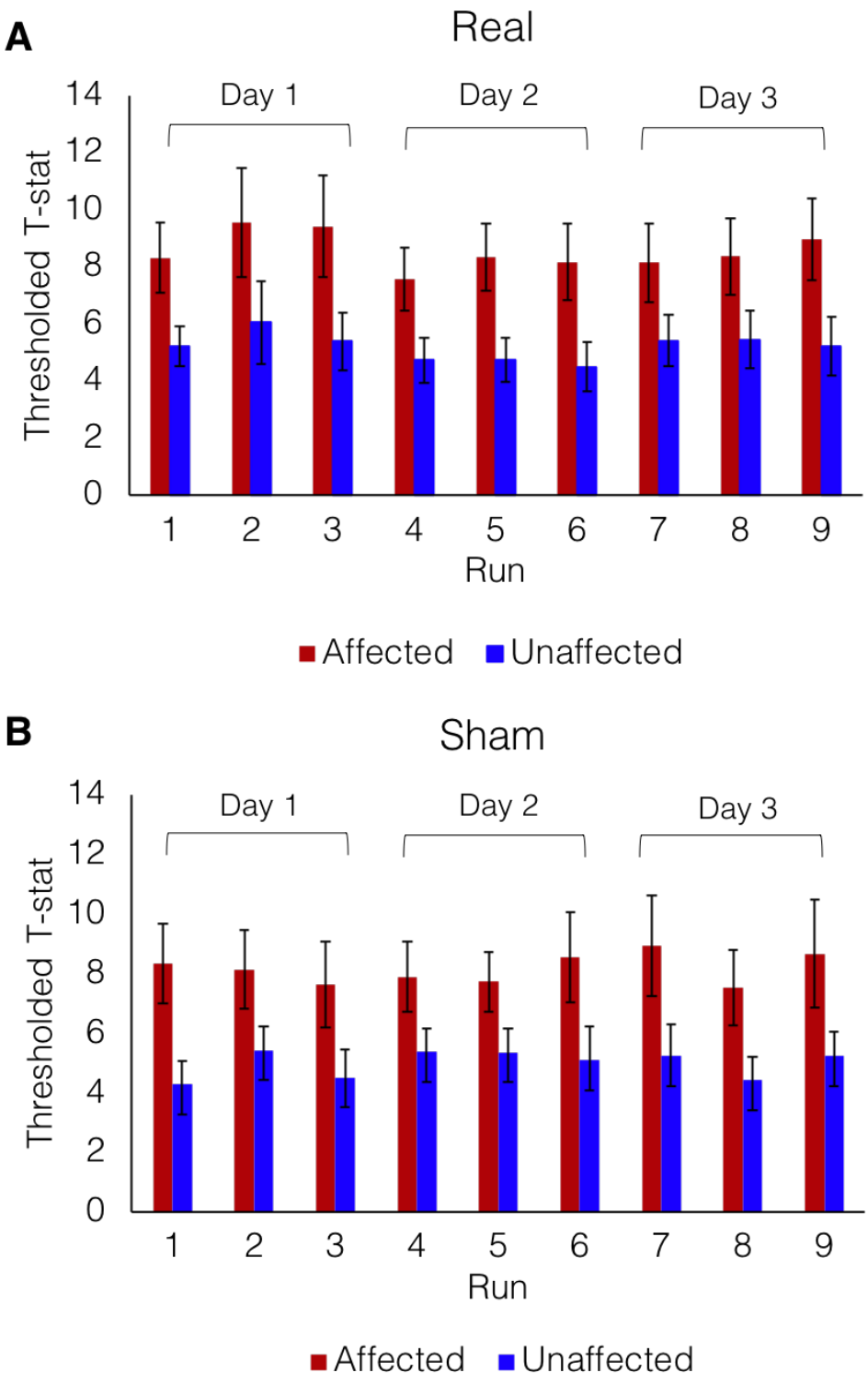


Figure S4.4 Average thresholded T-stat value plotted for both the ipsilesional (red) and the contralesional (blue) ROI over all the NF training runs and days. Values shown for the Real group in **A)** and the Sham group in **B)**. Error bars represent s.e.m.

Chapter 5 supplementary figures

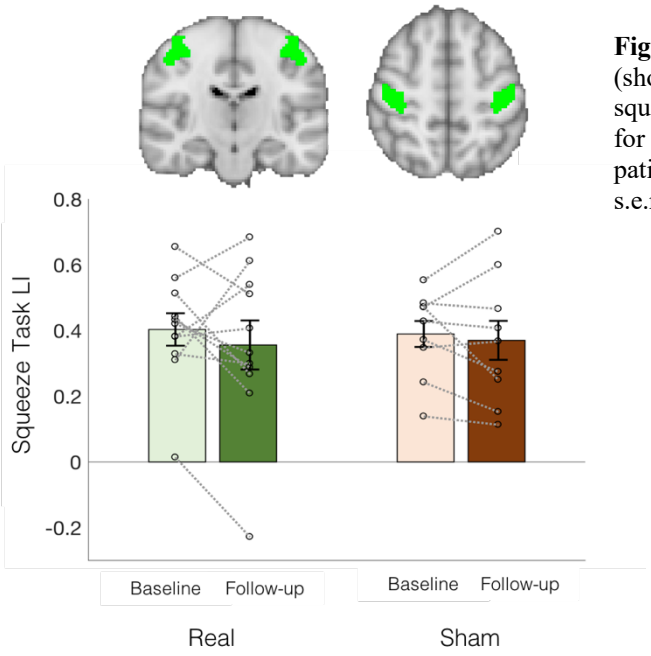


Figure S5.2 changes in LI in anatomical hand know area (shown in green in standard space) during visuomotor squeeze task. Mean LI shown during baseline and follow up for the Real (green) and the Sham (brown) group. Individual patient data shown with open circles. Error bars represent s.e.m.

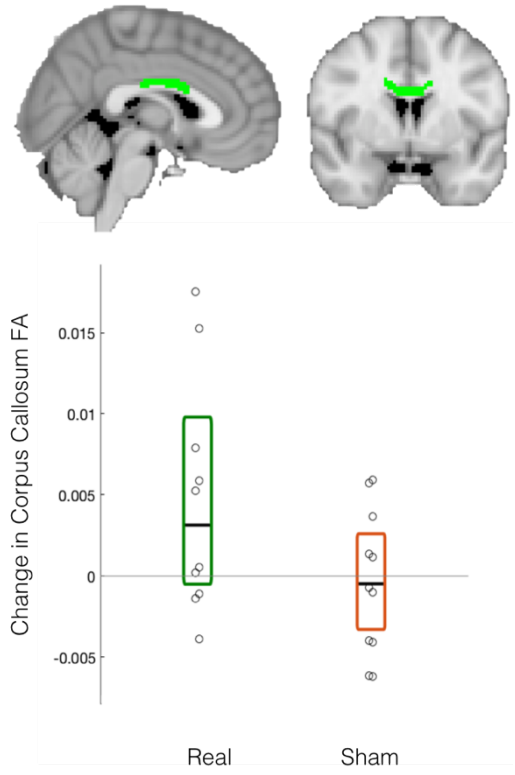


Figure S5.2 FA changes in corpus callosum ROI (shown in green in standard space). FA change is shown with the black line for the Real (green) and the Sham (orange) group. 95% confidence intervals are represented by the boxes. Individual patients data is shown with the open circles.

Chapter 6 supplementary figures

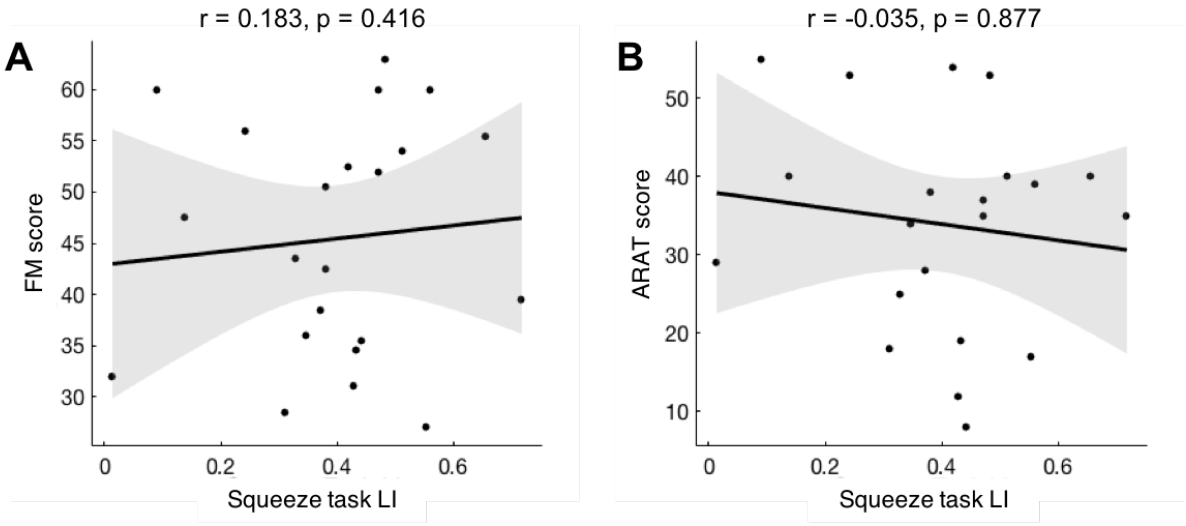


Figure S6.1 No correlation was found between baseline LI measured during the visuomotor squeeze task and baseline score on the FM **A)** or the ARAT **B)**. Correlations assessed using Spearman's rho (two-tailed). Shaded area shows 95% confidence intervals