Functions of the Orbital Prefrontal Cortex and Hippocampus in Goal-Directed Behavior and Memory

A thesis presented by

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to the

Department of Experimental Psychology

in partial fulfillment of the requirements

for the degree of

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in the subject of

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Christ Church Trinity Term 2006

Brief Abstract

Ailments such as Alzheimer's disease (AD) and frontotemporal dementia (FTD) are devastating conditions linked to deficits in the human prefrontal cortex, with some apparent localization to the orbital prefrontal cortex (OPFC). Work in rodent and primate animal models suggests structural and functional homology in the prefrontal cortex across species but many discrepancies remain. The primary aim of this project was thus to characterize the behavioral similarities and differences in both rat and monkey OPFC.

Thirty-five male Lister-Hooded rats received complete lesions to either the OPFC or hippocampus (HPC), permitting investigation of dissociations of function between the two structures. These experiments demonstrated that the rodent HPC was required for a variety of anxiety behaviors, whereas OPFC damage only had effects on a hyponeophagia test, perhaps suggesting a more complex modulatory role for that structure. The OPFC was relatively uninvolved in spatial learning and memory or in reversal learning, contrary to large deficits observed in all aspects of spatial behavior in rats with HPC lesions. Rats with OPFC lesions showed decreased levels of spontaneous locomotor activity; however, the age of the rats at the time of this test may have been a confounding factor. A test of goal-directed behavior—reinforcer devaluation—was unsuccessful due to deficiencies in the testing paradigm. Finally, a technical proof-of-concept demonstrated successful use of intra-orbital 5,7-dihydroxytryptamine (5,7-DHT) to produce selective serotonin (5-HT) depletions in the rodent OPFC for future studies.

Concurrently, eight experimentally-naïve male rhesus monkeys were trained in a variety of analogous tasks to assess goal-directed behavior, strategy implementation, and associative learning. They will receive selective neurotransmitter depletions—possibly of 5-HT—in the OPFC, allowing cross-species synthesis of results. Such integration may eventually aid in the development of better therapies for AD and FTD.

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Extended Abstract

Ailments such as Alzheimer's disease (AD) and frontotemporal dementia (FTD) are devastating conditions—with generally poor prognoses and relatively few therapeutic options—that have been linked to deficits in the human prefrontal cortex with some apparent localization to the orbital prefrontal cortex (OPFC). Prior work in rodent and primate animal models suggests a structural homology in the prefrontal cortex based largely on connectivity. There also is evidence for functional homology in executive function, but inter-species behavioral differences make direct comparison extremely difficult, a fact further compounded by conflicting reports in the literature. The primary aim of this project was thus to characterize these functional similarities and differences in studies of both rat and monkey OPFC.

The majority of this work was undertaken in a cohort of 35 male Lister Hooded rats that had received complete cytotoxic lesions to either the OPFC or hippocampus (HPC). Having two lesion groups allowed investigation of dissociations of function between the two structures. The results of these experiments showed that the rodent HPC was necessary for an appropriate anxiety response in both a hyponeophagia test and a successive alleys test, the latter being a novel procedure designed to provide a continuum of anxiety-inducing conditions. Thus, whereas the HPC seemed to be fundamental to generating anxiety behaviors, OPFC damage was effective in the hyponeophagia test only—in which novel foodstuffs were presented in an anxiogenic environmental

context—perhaps suggesting that the structure modulates an anxiety response generated in the HPC. The OPFC was found to be relatively uninvolved in spatial working memory, spatial learning, or reversal learning as assessed by tests of rewarded alternation on the T-maze and spatial behavior in the Morris watermaze, contrasting with consistent and profound deficits observed in all aspects of spatial behavior in rats with lesions of the HPC. Faster running and swimming speeds observed in these tasks also hinted at possible hyperactivity in the HPC group, but an explicit test of spontaneous locomotor activity showed no effect of HPC lesions. Unexpectedly, hypoactivity was demonstrated in rats with OPFC lesions, though the age of the rats at the time of the test may have been a confounding factor. Unfortunately, a key test of goal-directed behavior likely to be sensitive to OPFC damage—reinforcer devaluation—gave inconclusive results due to deficiencies in the testing paradigm. The test was unsuccessful despite using both selective satiety and Pavlovian aversive conditioning as means of devaluing one of the food stimuli. Rectifying these difficulties is an immediate direction for future experimental work.

A technical proof-of-concept was achieved with the successful use of intra-orbital injections of 5,7-dihydroxytriptamine (5,7-DHT) to produce selective serotonin (5-HT) depletions in the rodent OPFC. This will be a useful tool for future studies, although an improvement would be possible by using the anti-serotonin reuptake transporter (SERT)-saporin immunotoxin to produce the local depletions, as this toxin would selectively target serotonergic neurons without the need for noradrenergic protective drugs as is the case with 5,7-DHT.

Concurrent to the rat studies, eight experimentally-naïve male rhesus macaque monkeys were trained in a variety of tasks chosen to be broadly analogous to those employed in the rodent. Specifically, these tasks were designed to assess goal-directed

behavior, strategy implementation, and associative learning using a computerized touch-screen apparatus with which there is extensive prior experience at the Oxford University Department of Experimental Psychology. This preoperative training and testing phase continues at the time of writing. Once it is complete, these animals will receive lesions of the OPFC, likely including selective neurotransmitter depletions of the 5-HT system. The data generated by these studies will allow cross-species synthesis of results that will refine our understanding of the level of functional homology between the rodent and primate. Such integration of results and validation of animal models will ultimately allow extrapolation of findings to humans, aiding development of improved therapies for AD and FTD.

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Chapter I. General Introduction

Alzheimer's disease (AD) is a debilitating ailment, characterized by profound mental deterioration that affects decision-making, memory, language and personality, as well as by production of motor and sensory disturbances.[1] Although the clinical and pathological signs and symptoms have been known for close to a century, the ultimate prognosis remains substantially unchanged.[2] However, emerging drug therapies show promise in slowing the clinical progression of AD.[3] Development of such therapies, including cholinesterase inhibitors like donepezil and the low-affinity, uncompetitive, open-channel N-methyl-d-aspartate (NMDA) receptor antagonist memantine,[4] relies on an understanding of the underlying structural and neurochemical disturbances in AD. These disturbances are wide-ranging, involving imbalance of neurotransmitters including acetylcholine (ACh), serotonin (5-HT), dopamine, and norepinephrine,[5] structural deficits such as hippocampal cell loss,[6] and unique pathologic findings such as neurofibrillary tangles and beta-amyloid deposits.[7] Clarifying and synthesizing these findings to understand the pathogenesis of AD and design appropriate therapeutics will be a primary application for animal models.

By contrast, the etiology of Parkinson's disease (PD) is far better understood, allowing development of several targeted treatment options such as drug therapies and deep brain stimulation of the subthalamic nucleus.[8] The structural abnormalities of PD are more localized[9] than those of AD, and neurobiological and neuroanatomical studies have exploited this to guide the design of therapeutics. The damage observed in AD is by contrast diffuse and pervasive, compounded by a lesser understanding of the role of the prefrontal cortex vis-à-vis the basal ganglia. Developing a better understanding of the structures and processes involved in AD is a prerequisite for improving the clinical prognosis.

Past work has linked symptoms associated with AD and related conditions such as frontotemporal dementia (FTD) to functional losses in the prefrontal cortex. Past studies of adrenergic receptors in the brains of patients with AD have shown a prefrontal-cortex-specific pathology.[10,11] Additionally, a prefrontal-cortex-specific deficit in Glu-2 RNA editing has also been described.[12] Similarly, patients with mild frontal variant FTD (fvFTD) have shown deficits in tasks sensitive to functions linked to the orbital prefrontal cortex (OPFC or OFC), such as reversing previous visual discriminations and demonstrating appropriate risk-taking behavior in a decision-making task.[13,14,15] Structural evidence for this effect has been provided in the human by imaging work, such as a 99Tc-HMPAO-SPECT study[16] that demonstrated reduced cerebral bloodflow in the OPFC of patients with FTD. Additional human evidence indicates that damage to the OPFC is associated with impulsive behavior but not impairments of spatial working memory.[17] These clinical findings in the human suggest that the prefrontal cortex—and the OPFC in particular—are regions of primary interest for further study with animal models.

Two popular animal models in behavioral neuroscience are the rat and monkey, both of which have been used in the study behavior in the prefrontal cortex and its components. Primate work allows controlled studies to be performed in an animal with a cortical organization resembling that of the human, whereas rat experiments permit extrapolation of results across species. Rat studies should normally precede work in monkeys, as it would be ethically and financially unviable to perform experiments on primates without prior evidence to support the methodology of a proposed course of study. In addition, the relative ease with which rats can be trained and tested often makes them the more convenient model for focused behavioral studies. However, an immediate concern arises regarding the appropriateness of comparing results between rodent and

primate, as there must necessarily be preexisting evidence of structural or functional homology to justify such an integrative approach. As reviews of the literature have shown, addressing this issue is difficult and controversial.[18,19] Originally, the prefrontal cortex was defined solely by cytoarchitectonic criteria and the structure was seen as being unique to the primate brain.[19] However, a classic study by Rose and Woolsey redefined the structure based on its receipt of projections from the mediodorsal thalamic nucleus.[20] Modern technical refinements have expanded that definition to include other projections as well as reciprocal connections.[21] Based on these definitions, there is general agreement that both rats and monkeys have homologous regions that can be considered prefrontal cortex.[18,19,21] Further, despite their lesser cortical development relative to higher mammals, the rat prefrontal cortex can be subdivided into smaller distinct regions, one of them being OPFC.[22]

The question of functional homology is a thornier issue, however, as there are clear inter-species differences in behavior, compounded by a lack of consensus in the primate as to what functions are in fact controlled by the dorsolateral prefrontal cortex.[18,19] On a basic level, one can study the properties of individual neurons in brain regions considered to be homologous by the anatomical criteria discussed above. Such an analysis of fast-spiking interneurons versus pyramidal neurons in rat and monkey prefrontal cortex demonstrated an apparent functional homology.[23] Of greater interest to the behavioral neuroscientist, however, are higher-order cognitive functions that can be assessed through behavioral testing in the lab.

Fortunately, there is a broad consensus among studies in rats, monkeys, and humans that the OPFC is involved in executive function.[22] For example, bilateral OPFC lesions in rhesus monkeys attenuated the effects of reinforcer devaluation, a task in which the ability to alter the choices of objects is assessed by modifying the value of an

associated food.[24] An equivalent test with rats—in which there was a Pavlovian pairing of a light conditioned stimulus with a food pellet unconditioned stimulus—showed similar impairment in the reinforcer devaluation task.[25] In marmosets, selective lesions of the OPFC impaired performance of a task that involves reversing stimulus-reward contingencies while preserving attentional set shifting, a finding that was duplicated in rats.[26] Also in rats, electrolytic lesions to the *medial* prefrontal cortex impaired reversal of a non-match-to-sample rule in the delayed non-matching-to-sample (DNMS) task.[27] The rule reversal component of the task simulated rule-shifting in the Wisconsin Card Sorting Test (WCST), thus indicating a deficit that resembled pathology observed in humans. However, bilateral excitotoxic lesions of the rodent medial frontal cortex impaired extradimensional set shifting in a digging task without affecting discrimination reversals.[28] Taken together, these studies are consistent with a view that the OPFC is involved in reinforcer devaluation and discrimination reversals but not attentional set shifting whereas the medial frontal cortex is involved in rule reversals and attentional set shifting. Regarding the roles of specific neurotransmitter systems, evidence exists for similar contributions of dopamine in the prefrontal cortex to attentional control and working memory in both the rodent and primate.[29,30] Taking this evidence of functional homology—which is also addressed in Chapter II.1—together with the aforementioned structural homology, the neuroscientist can be at least partially assured of the scientific legitimacy of using rodent and primate animal models for prefrontal cortex.

However, the neuroscientist would be foolhardy to become complacent following the preceding discussion, as discrepancies and gaps remain. For example, in human patients with fvFTD, a sophisticated test of decision-making was performed using a computer simulation of a betting task.[13] The subject was required to choose which of a variable number of blue and red boxes displayed on a computer screen hid a randomly-

placed “token” while also betting a number of fictitious points on that choice. The objective of the task was to maximize the point total by the end of the session. It was found that subjects in the fvFTD group deliberately made more “risky” bets, pursuing a maladaptive strategy that was distinctly different from an impulsive approach. This apparent lack of impulsivity can be putatively associated with the bilateral frontal and/or temporal degeneration present in fvFTD. However, explicit tests of impulsivity in the rodent model have generated conflicting results in the literature. Two studies that used variants of an operant box paradigm for measuring impulsive choice by assessing intolerance to delay of reinforcement reported opposite effects following quinolinic-acid-induced lesions of the OPFC.[31,32] It should be noted that these studies used different genders of different rat strains coupled with slightly different behavioral testing methods. The discrepancy may thus have been a manifestation of the different paradigms employed or due to a bona fide behavioral phenomenon that is sex- or strain-dependent. Further investigation with rats will be needed to clarify the source of these differences. Additionally, explicit studies of impulsivity in the monkey and human would aid in making inter-species comparisons. In particular, use of a non-human primate animal model would allow testing of complex tasks well beyond the cognitive abilities of the rat. The facility to create localized lesions and selective neurotransmitter depletions within the monkey brain would also permit much finer experimental consistency than would be possible in a human cohort.

Characterizing the roles of various neurotransmitters in the mammalian brain—and in the OPFC specifically—is another area of active research, though past work again paints a disconcertingly inconsistent and incomplete picture. For example, the aforementioned studies of human patients with fvFTD additionally suggested that a lack of serotonin (5-HT) might be partially responsible for the observed deficits in executive

function and memory.[13,15] In contrast, examination of serotonin levels in the OPFC cortex of rats with in-vivo microdialysis has shown relatively little role for the chemical during a delay-discounting task for quantifying impulsive choice.[33] Similarly, studies of prefrontal serotonergic contributions to attentional control and working memory apparently dissociate between species, affecting reversal learning in humans and monkeys but impulsivity in rats,[29,30] though there is evidence that $5HT_6$ receptor antagonists improve reversal learning in rats on a set-shifting task.[34] Again, whether these differences are the result of true interspecies differences or of an artifact of the procedure or assessment is unknown and motivates additional study. Furthermore, although there is experimental evidence that hippocampal ACh is implicated—but not necessary—in memory in the intact brain,[35] the importance of ACh in the OPFC has received lesser attention, thus requiring further study. Expanding our knowledge of these brain regions and the neurotransmitter systems supplying them in a cross-species integrative manner will be instrumental in creating targeted therapies for disorders such as AD and FTD.

Therefore, the primary goal of this project was to use animal models to evaluate the role of the OPFC in tasks designed to assess executive function, learning, and memory with an eye to performing future studies on animals with selective neurotransmitter depletions and to making comparisons between rodent and primate data. Concurrently, dissociations of function between the OPFC and hippocampus (HPC) were studied. A two-pronged approach was taken in addressing these research questions. The bulk of the data presented in this project (Chapters III-VII) was generated with a cohort of rats that had received excitotoxic lesions of either the OPFC or HPC (along with a control group of SHAM surgeries). These animals were tested on a variety of tasks chosen to be potentially sensitive to functional deficits in the OPFC, including the hyponeophagia and successive alleys tests of anxiety (Chapter III), spatial working memory via rewarded

alternation (Chapter IV), reinforcer devaluation with both selective satiety and Pavlovian aversion paradigms (Chapter V), spatial learning and reversals in the Morris watermaze (Chapter VI), and spontaneous locomotor activity (Chapter VII). Successful pilot surgeries to deplete serotonin in the OPFC were also performed (Chapter VIII), laying the technical groundwork for future studies of selective neurotransmitter depletions. Concurrent with these rat studies, young rhesus macaque monkeys were trained in broadly analogous tests of goal-directed behavior and strategy as well as an associative learning task (Chapter IX). Use of the monkey affords a primate analog for the rodent studies in a species possessing a cortical organization similar to that of the human. Furthermore, the increased sophistication of the monkey brain allows use of more complex testing paradigms, resulting in greater insight into the relevant cognitive processes than would be possible with non-primate mammals.

Detailed explanations of each task or procedure appear within the relevant chapters (II-IX) along with more focused background, results, and discussion. Chapter X offers a general discussion that integrates all work encompassed by this project on a broader level. Chapter XI summarizes the main experimental conclusions of the work and proposes numerous avenues for future study.

Chapter II. General Methods and Results: Cytotoxic Ablations of Rat Orbital Prefrontal Cortex and Hippocampus

1. Background

The prefrontal cortex and hippocampus form parts of complex interlinked neural circuits involved in spatial learning and memory.[36,37,38,39,40] These circuits appear to contribute differentially to spatially-dependent behavior. For example, the dorsal hippocampus is necessary for rats to solve spatial learning tasks while the ventral hippocampus is not.[41,42,43,44] A mounting body of evidence additionally implicates the hippocampus in more varied functions, including memory of temporal sequences of events,[45] a time-dependent contribution of its subregions to contextual fear-conditioning,[46] and a role for the ventral hippocampus in fear and anxiety behaviors.[47,48] Identifying and characterizing specific functions of the hippocampus—and then dissociating these from functions of the prefrontal cortex—was a major goal of this first experiment.

Lesions of the OPFC in rats[25] and rhesus monkeys[24] as well as crossed lesions of the OPFC and amygdala in monkeys[35] have been shown to produce deficits in goal-directed behavior, as assessed by performance on a reinforcer devaluation task. Additionally, central muscarinic blockade impaired the ability to reverse previously learned discriminations,[49] but the specific contributions of OPFC activity—and especially the role of particular neurotransmitters within the structure—to reversal learning is not well characterized. There also is evidence that the OPFC may play a role in spatial orientation because rats with lesions to that structure are severely impaired in the radial arm maze and Morris watermaze tasks, though the relevant study utilized less specific aspiration lesions and suffered from relatively small experimental groups.[50] As a result, another key aim of the experiment was to perform a battery of tasks on the rats designed

to assess spatial reference and working memory, goal-directed behavior, and reversal learning. The tasks identified as most sensitive to orbital prefrontal ablation will then be used in subsequent experiments on the effects of serotonin depletions in the rodent OPFC.

2. Animals and Surgery

The current experiment utilized an existing cohort of 35 male Lister hooded rats. These rats were not experimentally naïve, as they had received testing (and surgery) prior to the start of this project. Their testing history consisted of delay decision-making on the T-maze, assessed both pre- and post-operatively with a nonspatial version of a previously described paradigm.[51] For the duration of the current project, animals were kept in cage groups of twos or threes, though one pair had to be separated into individual cages due to excessive fighting. Animals were maintained on a 12-hour light/dark cycle (7:00 AM to 7:00 PM), with all training and testing occurring during the light phase. Animals had free access to water at all times, but were reduced to between 80% and 85% of their free feeding weights for Chapters IV and V. All work was conducted in compliance with the UK Animals (Scientific Procedures) Act of 1986.

The cohort was divided into three groups that had received either OPFC cytotoxic ablation (n = 12), hippocampal (HPC) cytotoxic ablation (n = 12), or sham surgery (n = 12), operated prior to the start of this project. One sham animal died prior to the start of the current study, resulting in a final n = 11 for that group. Allotment to each of these three groups was counterbalanced based on pre-operative performance on the delay decision-making task. The experimenter remained blind as to which rats belonged to which lesion group. At time of surgery, the rats weighed between 315-387 grams. Excitotoxic lesions were produced in the OPFC with quinolinic acid (0.09 M) and in the HPC with N-methyl-D-aspartate (Sigma Chemical, Poole, UK) dissolved in phosphate-buffered saline (pH 7.4) at a concentration of 10 mg/mL (0.068 M). Surgeries occurred

under isoflurane anaesthesia (Animal Care, York, UK) with the head placed in a stereotaxic frame and secured with ear bars. The lambda-bregma axis was kept horizontal.

Table 1. Stereotaxic coordinates for excitotoxic lesions of the OFC and HPC, including volumes of toxin injected. AP and ML coordinates are in millimeters relative to bregma (with positive AP values anterior to bregma and positive ML values in the left hemisphere). DV coordinates are in millimeters relative to brain surface (with negative coordinates being deeper in the brain).

Lesion	Coordinates			Volume (µL)
	AP	ML	DV	
Orbital Prefrontal Cortex (bilateral, 3 sites/side)	+4.0	±0.8	-3.4	0.150
	+3.7	±2.0	-3.6	0.200
	+3.2	±2.6	-4.4	0.150
Hippocampus (bilateral, 18 sites/side)	-2.4	±1.0	-3.3	0.075
	-2.8	±1.8	-3.3	0.075
	-3.2	±1.4	-3.3	0.050
	-3.2	±1.4	-2.6	0.050
	-3.2	±3.0	-3.1	0.100
	-3.6	±3.5	-3.1	0.075
	-4.4	±2.8	-3.3	0.050
	-4.4	±2.8	-2.3	0.050
	-4.4	±4.0	-4.2	0.025
	-4.4	±4.0	-3.3	0.050
	-4.4	±4.0	-2.3	0.050
	-4.9	±4.8	-5.2	0.075
	-4.9	±4.8	-4.2	0.050
	-5.2	±4.0	-7.3	0.100
	-5.2	±4.0	-4.2	0.075
	-5.2	±4.0	-3.5	0.050
	-5.5	±5.0	-5.6	0.100
	-5.5	±5.0	-4.9	0.075

Following a midline incision, the scalp was reflected to expose the skull and the bone above the injection sites was removed. Injections were made with a 5 µL syringe (Scientific Glass Engineering, Milton Keynes, UK) and a custom 34 gauge needle mounted on the stereotaxic frame (Kopf Instruments, Clark Electromedical, Reading, UK and Stoelting Company, Illinois, USA). Injection coordinates and volumes appear in Table 1. The anterior-posterior (AP) and mediolateral (ML) coordinates were measured relative to the bregma skull landmark while the dorsoventral (DV) coordinates were relative to the brain surface. Infusions occurred at a rate of 0.1 µL/min. The needle was left in place for 1-2 minutes at the end of each injection to allow time for the toxin to diffuse away from the injection site. Sham surgeries (including both OPFC and HPC

controls) consisted of anaesthesia, incision, and craniotomy without needle insertion or injection of vehicle. Following the procedure, rats were sutured and given post-operative analgesia with Carprofen (4 mg/kg s.c.).

3. Post-Mortem Histology: Methods

At the conclusion of the project, all rats were perfused to allow histological analysis of the brains. The animals were deeply anaesthetized with an i.p. injection of pentobaribital sodium (Euthatal, 200 mg in 1 mL; Vericore Ltd., Dundee, UK), approximately 0.5 mL per animal. Following thoracotomy, normal saline (0.9%) was then introduced via an intracardial cannula inserted directly into the left ventricle while the descending aorta was clamped with a hemostat. Flow was maintained for approximately five minutes to ensure complete exsanguination. The cannula was then switched to a supply of 10% formol saline for at least another five minutes until the upper body became rigid. The entire brain was then removed by dissection and stored in 10% formalin. Coronal frozen sections were taken from all OPFC animals, horizontal frozen sections from the HPC animals, and a mixture of coronal and horizontal sections from the SHAM animals. Coronal sections were taken at 25 µm thickness and horizontal sections at 50 µm thickness. All sections were mounted on glass slides, stained with cresyl violet, and coverslipped.

4. Post-Mortem Histology: Results

The slides underwent detailed microscopic examination and photomicrographs were taken of several sections from a representative brain in each lesion group. These images appear in Figures 1 and 2 alongside corresponding photomicrographs from SHAM brains. Additionally, rats in the OPFC and HPC groups were rank ordered by extent of lesion. Reconstructions were then made to show minimum, representative, and

maximum lesions (Figures 3 and 4). Note that the representative lesion reconstructions are from the same brains used for the representative photomicrographs.

OPFC lesions were found to be somewhat variable, as can easily be seen in Figure 3. Damage consisted of areas of complete destruction (shaded gray) generally surrounded by a diffuse penumbra of reduced cell density (hatched). It should be noted that the total volume of the minimal lesion depicted in the left column of Figure 3 was noticeably less than the mean for the OPFC group as a whole. Damage commonly included portions of the overlying cortex, in a few instances with a clear needle track evident. In the majority of cases, lesions included the ventral and lateral areas of the OPFC with varying degrees of sparing in the medial and dorsolateral areas. Cell loss extended from 5.70 mm anterior to bregma to 3.20-2.70 mm anterior to bregma. Apart from the overlying cortical damage, the lesions exhibited reasonably good selectivity, only occasionally involving the ventral surface of the olfactory bulb. Lesions also did not extend to the nucleus accumbens core, though there was rare and minimal penumbral involvement of the shell. One OPFC rat was excluded from the analysis as its lesion was much smaller than the minimal reconstruction in Figure 3, resulting in a final $n = 11$.

HPC lesions were found to be highly selective and reproducible, with near-complete destruction of the hippocampus that was consistent with prior experience in the lab.[44] The representative photomicrographs in Figure 2 and reconstructions in Figure 4 clearly demonstrate the nature of the lesions. Specifically, the lesions almost always included the entirety of the fimbria at dorsal levels (i.e. no deeper than -4.60 mm from skull surface) and only minimally involved the subiculum. There was no visible damage to structures beyond the subiculum, including the amygdala. Minimal sparing was observed in the more dorsal portions of CA1 and the dentate gyrus (i.e. between -3.86 mm and -4.28 mm from skull surface). The lesions became complete as they continued

ventrally, though at the ventral tip there was found to be occasional sparing of the dentate gyrus and consistent sparing of CA3. All rats in the HPC group (n = 12) were retained in the analysis.

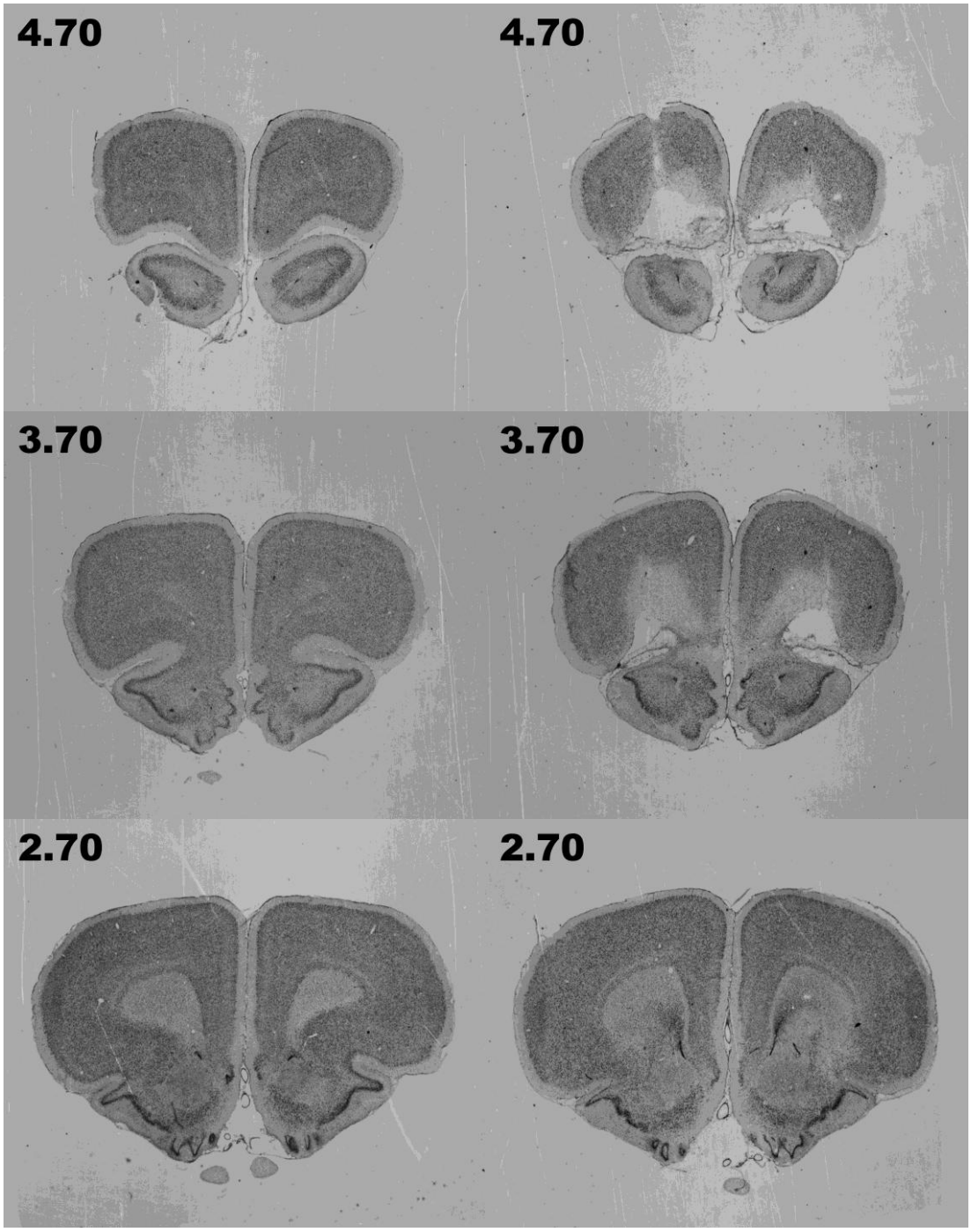


Figure 1. Grayscale photomicrographs of coronal sections from SHAM control (left column) and representative OPFC lesion (right column) brains with locations given in millimeters anterior to bregma.

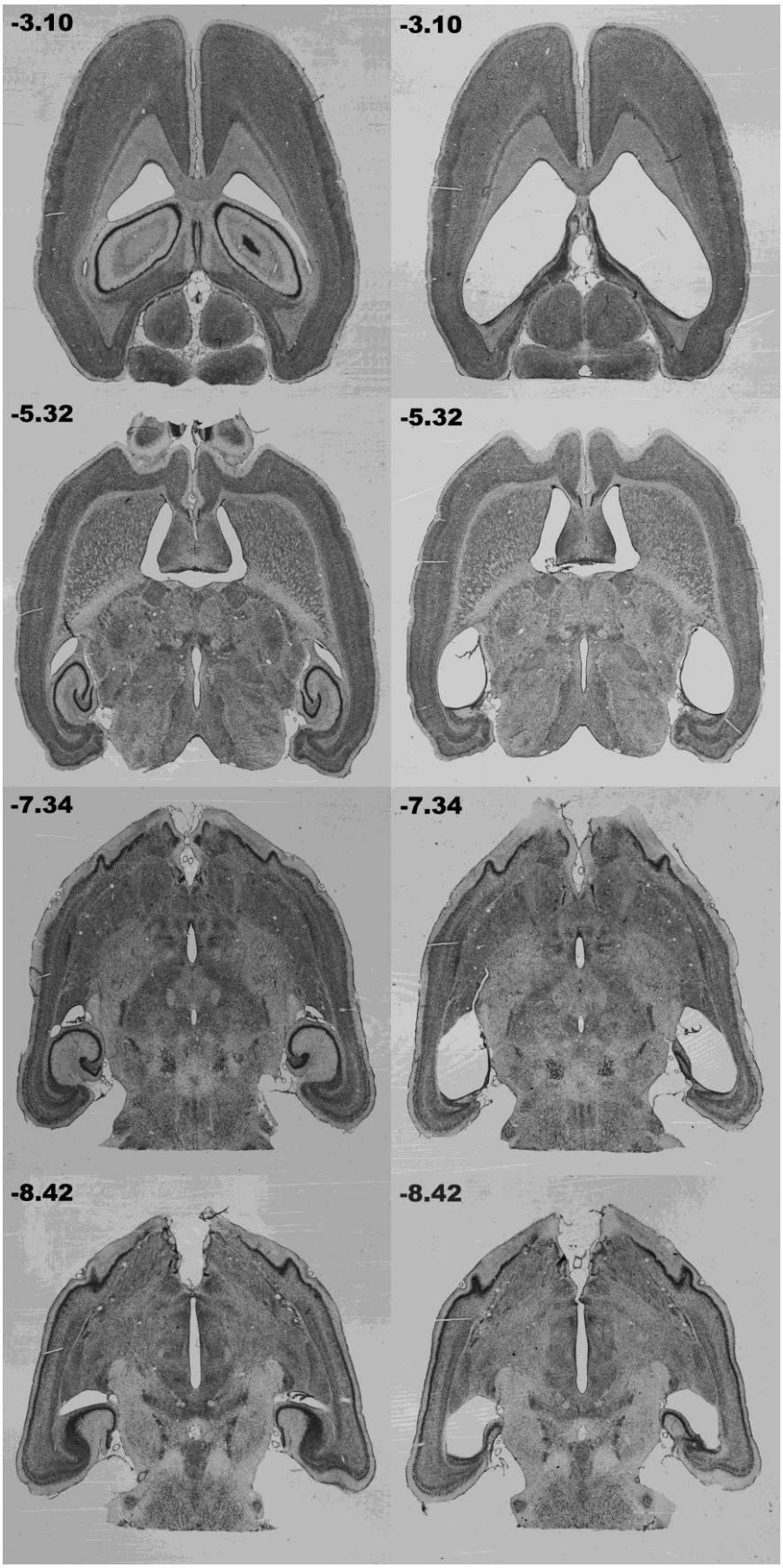


Figure 2. Grayscale photomicrographs of horizontal sections from SHAM control (left column) and representative HPC lesion (right column) brains with locations given in millimeters inferior to the skull surface.

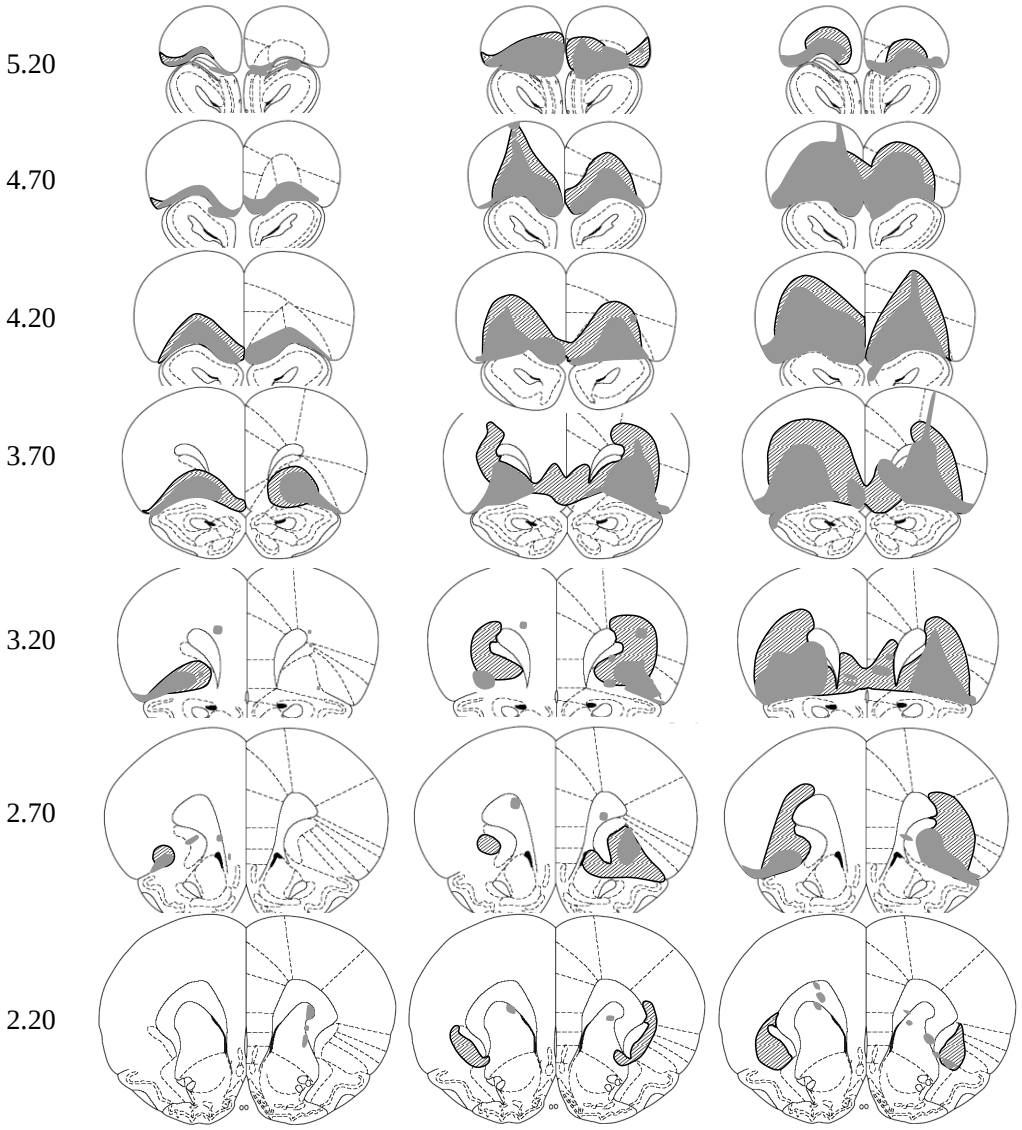


Figure 3. Reconstructions of the complete OPFC lesions, showing minimal (left column), representative (middle column), and maximal (right column) extent of damage in coronal sections. Gray color indicates total destruction whereas hatching indicates penumbrae of reduced cell density. The numbers at left indicate distance in millimeters anterior to bregma. Reproduced from *The Rat Brain in Stereotaxic Coordinates*, 4th ed., G. Paxinos and C. Watson, Figures 4-10, copyright 1998.

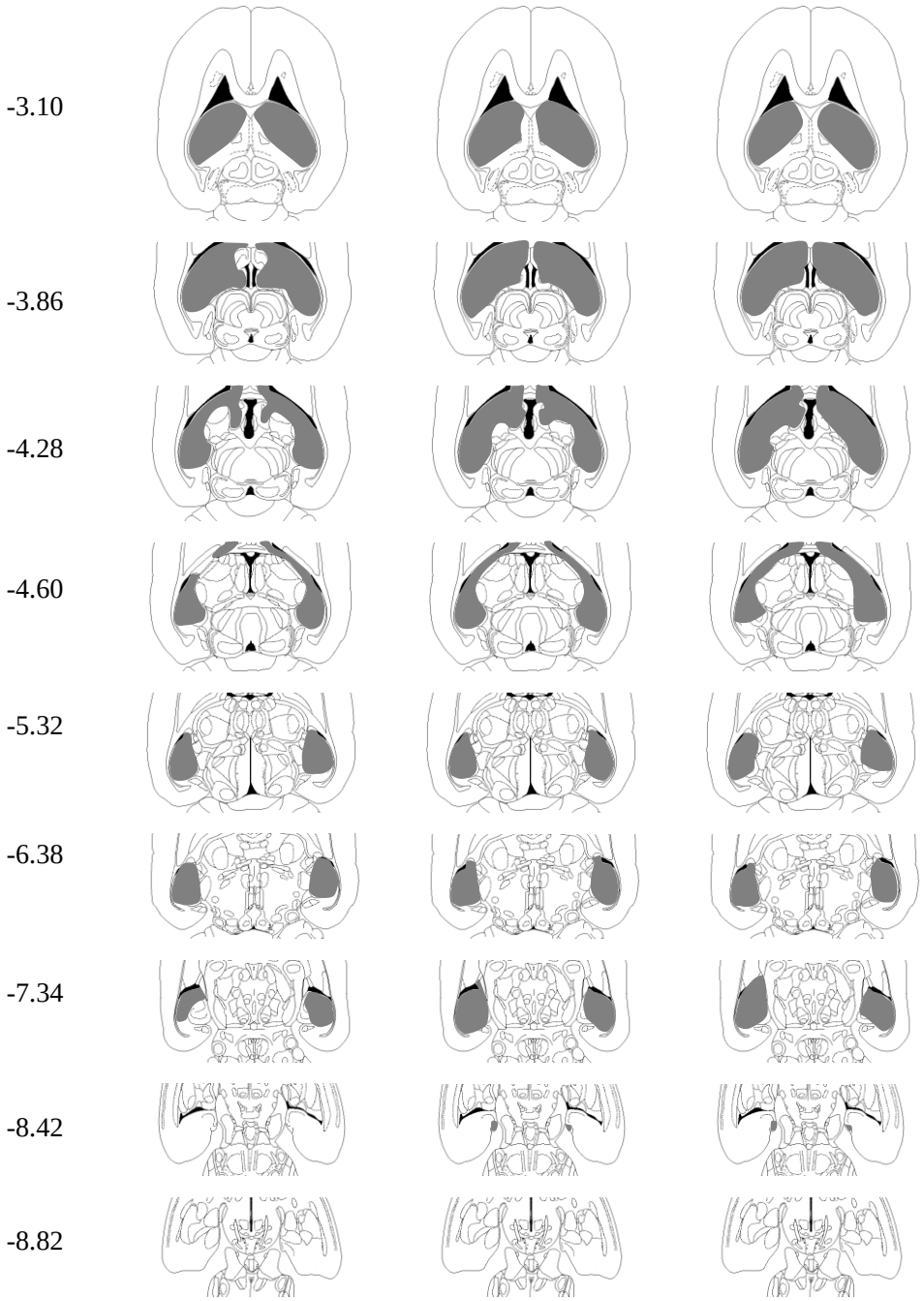


Figure 4. Reconstructions of the complete HPC lesions, showing minimal (left column), representative (middle column), and maximal (right column) extent of damage in horizontal sections. The numbers at left indicate distance in millimeters relative to skull surface. Reproduced from *The Rat Brain in Stereotaxic Coordinates*, 4th ed., G. Paxinos and C. Watson, Figures 93, 95, 99, 103, 107, 110, 111, 113, and 116, copyright 1998.

5. Statistical Analysis

The number of subjects retained in the analysis were OPFC $n = 11$, HPC $n = 12$, and SHAM $n = 11$ with two important exceptions. One SHAM rat was excluded from the

hyponeophagia test due to illness (Chapter III.1) but participated in the subsequent tasks. One OPFC rat was euthanized during the reversal learning phase of the watermaze study and was thus excluded from the analysis for that portion of the task, resulting in OPFC *n* = 10 in that instance (Chapter VI.5).

All data were initially organized and sorted with Microsoft Office Excel 2003 (version 11.8012.6568) SP2. Statistical analyses were performed with SPSS 13.0 for Windows and consisted of analysis of variance (ANOVA) with post-hoc testing via Tukey's HSD.[52] Figures 6, 9-15, and 17-19 were generated with MATLAB 6.5.0.180913a Release 13. Figures 5, 7, 8, 16, 21, and 22 were produced in Excel.

Chapter III. Anxiety: Hyponeophagia and Successive Alleys

1. Background

The emerging evidence for a role of the HPC in fear and anxiety behaviors was introduced in Chapter II.1. More specifically, impairments in both the hyponeophagia and successive alley tests have been clearly demonstrated for rats with ventral HPC lesions.[47] Thus, it is expected that replication of these tests with complete HPC lesions should produce similar anxiolytic effects. There is comparatively little published evidence for what role cortical structures might play in these behaviors, though an immediate-early gene study does suggest a possible involvement of the OPFC.[53] Recent work in the lab has specifically implicated the OPFC in the hyponeophagia test of anxiety, as rats with OPFC lesions ate a novel foodstuff much more quickly than controls without demonstrating hyperactivity.[54] The current study thus sought to validate and extend these results, effectively using the complete HPC lesion group as a positive control for characterizing the role of the OPFC in behaviors of fear and anxiety. Dissociations of function between the two brain regions were also explored.

2. Hyponeophagia

The hyponeophagia task has been proven to show a reliable anxiolytic effect in rats secondary to ventral HPC lesions[44,55] similar to that observed with benzodiazepines.[56,57] Hyponeophagia was assessed by recording the latency to eat one of two novel foods in one of two novel and potentially anxiogenic environments. These assessments were performed in two distinct tests. The first of these involved a circular table 38 cm in diameter, made from red plastic that flexed under a rat's weight. The table was elevated 65 cm from the ground and at its center was a food well 2.5 cm in diameter and 2 cm tall, in which were placed pieces of sweet corn (Green Giant Original Niblets).

At the start of a trial, the rat was placed at the periphery of the table facing away from the central food well. The second test used a single 50-cm long, 9-cm wide arm of a Y-maze raised 76 cm from the floor that also flexed under the weight of a single rat. The reward in this case was three Noyes sucrose pellets.

The rats were food deprived overnight prior to testing and were briefly habituated to the testing room before any trials commenced. Non-experimental rats were used to give the apparati a uniform rat odor prior to the start of testing. Each rat received up to 3 trials for each of the two tests, with each trial lasting up to a maximum of 300 seconds.

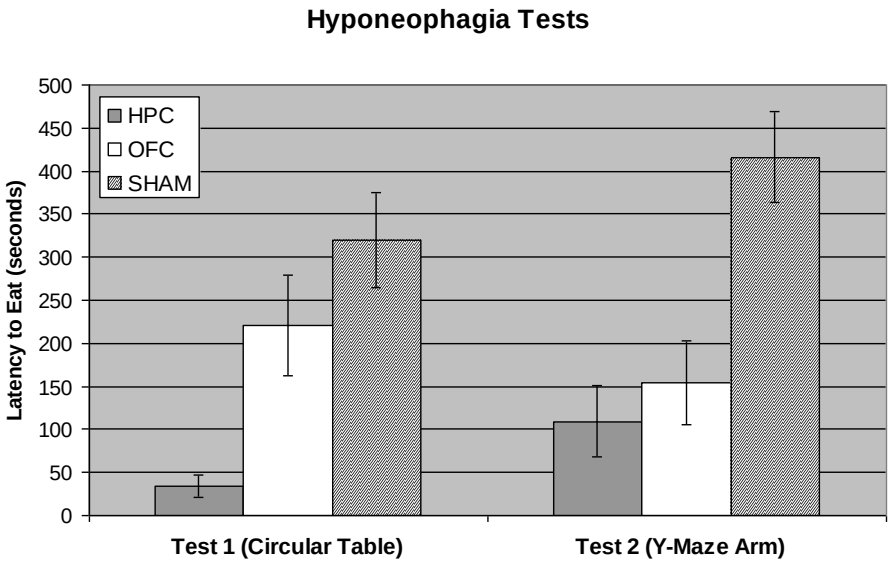


Figure 5. Latency to eat for each of the two hyponeophagia tests, presented as group averages. Error bars indicate ±1 standard error of the mean.

The results of both hyponeophagia tests appear in Figure 5. One SHAM rat was excluded from the study because of a middle ear infection that later resolved. This was in addition to the excluded OPFC animal, resulting in HPC n = 12, OPFC n = 11, SHAM n = 10. Visual inspection of Figure 5 readily shows that the HPC group was much quicker to eat than the SHAM group in both tests. The OPFC group was also quicker to eat than the SHAM group, particularly in the single Y-arm test.

A two-way ANOVA was performed on these data, with group as the between-subjects factor and test as the within-subjects factor. There was a significant effect of

group ($F_{(2,30)} = 15.216$, $p < 0.0005$). There were no other significant main effects or interaction terms; with the next-highest F-value corresponding to the test by group interaction ($F_{(2,30)} = 2.795$, $p = 0.077$). Tukey's HSD post-hoc tests showed that the HPC and OFC groups both significantly differed from the SHAMs ($p < 0.0005$ and $p = 0.007$, respectively) but not from each other ($p = 0.087$).

The key result is the presence of a clear impairment of both the OPFC and HPC groups in the hyponeophagia task, indicating the necessity of either structure to this particular anxiety behavior. The lack of a significant difference between the OPFC and HPC groups further suggests that the level of impairment produced by lesions to either structure is comparable.

3. Successive Alleys

The successive alleys task is a relatively novel procedure for testing anxiety and was designed as an alternative to the plus-maze, as it avoids interpretation difficulties resulting from the central square[58,59] and provides a continuum of anxiogenic conditions. The task assesses a rat's anxiety response and has also been validated by experimental work with benzodiazepine-treated animals.[47] The wooden apparatus consisted of four 25-cm long linearly connected alleys—or sections—designed to be of increasingly anxiogenic character. The first of these was 8.5 cm wide with walls 25 cm high. Following a downward step of 0.5 cm, the second alley was again 8.5 cm wide but had walls only 1.3 cm high. The third alley followed after a downward step of 1.0 cm and was 3.5 cm wide with walls 0.8 cm high. After a final downward step of 0.4 cm, the fourth alley was 1.2 cm wide with walls 0.2 cm high. The first alley was painted black, the second was painted grey, and the third and fourth were painted white. The first alley was raised 80 cm above the floor, and padding was placed underneath the third and fourth alleys in case a rat fell from the apparatus. An overhead camera connected to a VCR and

video monitor was used to record all trials remotely, avoiding any distractions from having the experimenter visible to the animals.

Prior to the first experimental trial, a non-experimental rat was allowed to run up and down the alleys for several minutes to give them a rat odor. Once removed, the alleys were wiped with a damp cloth to leave a uniform trace background odor. The apparatus was similarly wiped—and then dried—between subjects.

Each rat was allowed one trial of five minutes (300 seconds) to explore the alleys. Each rat was released at the closed end of the first alley facing the wall. The total amount of time spent in each alley was recorded, aided when necessary by subsequent playback of the video tape for off-line analysis. A rat was considered to have entered an alley when all four of its feet were clearly on the floor of that alley. For any rat that fell from the apparatus, the five-minute timer was halted and the rat was placed at the point from which it fell with its nose facing the first alley. The latency to enter each alley initially, the number of crossings into each alley, the number of fecal boli, and the presence or absence of urination were also recorded, but as portions of the video record were lost, these data were not analyzed. The time spent in each alley was recorded in real-time during the experimental trials, allowing analysis.

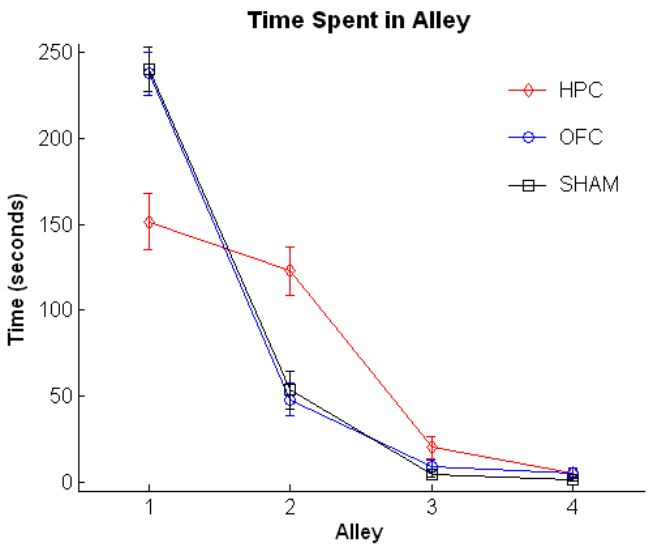


Figure 6. Time spent in each of the four successive alleys, represented as means for each experimental group. Alleys 1-4 are of increasing anxiogenic character. Error bars represent ±1 standard error of the mean.

Figure 6 plots the mean time (in seconds) spent in each alley for the three experimental groups. Note that for each rat, the sum of the time spent in each alley necessarily summed to 300 seconds. Visual inspection shows virtually no difference in performance of the SHAM and OPFC groups, regardless of alley. The HPC group was clearly different in its traversal of the first three alleys, spending less time in the first alley—but more in the second and third—relative to the other groups, suggesting an impaired anxiety response.

The above was confirmed by a two-way ANOVA with experimental group as a between-subjects factor and alley as a within-subjects factor. There was a significant main effect of alley ($F_{(2,93)} = 220.056$, $p < 0.0001$) and a significant interaction between alley and experimental group ($F_{(4,93)} = 12.089$, $p < 0.0001$). Note that the numerator degrees of freedom and p-value were adjusted to account for the fact that the time spent in each of the alleys always summed to 300 seconds due to the design of the task and thus were not truly independent.

Testing for simple main effects (without pooled error term) showed significant group differences for the first ($F_{(2,31)} = 13.064$, $p < 0.0005$), second ($F_{(2,31)} = 12.557$, $p < 0.0005$), and third ($F_{(2,31)} = 3.296$, $p = 0.050$) alleys. Separate one-way between-subjects ANOVAs were then performed on the data for each alley, duplicating the F-statistics and p-values produced by the simple main effects test. More helpfully, Tukey's post-hoc tests showed that in Alleys 1 and 2, there were significant differences between the HPC and OPFC groups and between the HPC and SHAM groups ($p \leq 0.001$ in all cases) but not between the OPFC and SHAM groups ($p > 0.9$ in all cases). The post-hoc analysis for Alley 3 revealed a significant difference between the HPC and SHAM groups only ($p = 0.047$; all others $p > 0.2$).

There is a clear difference in behavior between animals with hippocampal cytotoxic lesions and those with orbital prefrontal and sham lesions. This difference is most robust in the initial two alleys and diminishes as the alleys become more distal, eventually disappearing on the fourth alley. If one assumes the successive alleys task to be an adequate metric of anxiety behaviors, these findings indicate that ablation of the hippocampus bilaterally has an anxiolytic effect while that of the orbital prefrontal cortex does not. These data suggest that damage to the entirety of the hippocampus significantly disrupts anxiety behaviors, a notion that is in rapport with current literature regarding selective lesions to regions of the hippocampus.[47,48] Additionally, this study suggests that the orbital prefrontal cortex is relatively uninvolved in such behaviors.

4. Conclusion

The results of both the hyponeophagia and successive alleys tests show a consistent and profound impairment of rats with HPC lesions in both types of anxiety behaviors. Rats with OPFC lesions are impaired in the hyponeophagia test—performing no differently from animals with HPC lesions—but completely unaffected in the successive alleys test. This dissociation of function suggests that the HPC may have a primary role in generating anxiety responses, with removal of the structure severely impairing the associated behaviors globally.

The OPFC also plays a role in the anxiety response, but its contribution is more subtle, as an impairment become evident only when the rat was confronted with a novel foodstuff in the a potentially anxiogenic environment. Thus, the hyponeophagia test differs fundamentally from the successive alleys test, as the latter provides an anxiogenic environment without presentation of a novel food. Given the OPFC's involvement in executive function and decision-making as well as its interconnections with the HPC (Chapter I), one could postulate a role for the structure in modulating anxiety responses

initially generated elsewhere—such as in the hippocampus—with consequent effects on externally observable behaviors. This modulation may be based on the integration of information from multiple stimuli, such as the novel foodstuff and anxiogenic environment used in this study. Damage to the OPFC would cause a failure of this integrative activity, attenuating the response modulation and resulting in the behavioral manifestation observed in this experiment. One potential counterargument is that OPFC damage simply creates a fear of novel foods irrespective of the environmental context. This concern could be easily addressed by performing a new hyponeophagia test with presentation of novel foods in a familiar environment and noting if the OPFC effect remains.

Chapter IV. Spatial Working Memory: Rewarded Alternation

1. Background

The hippocampus has been widely implicated in numerous tasks that assess spatial memory—such as the Morris watermaze—as previously addressed in Chapter II.1.[36,37,38,41,42,43] More specifically, rewarded alternation (also called non-matching-to-position) on a T-maze is a test of spatial working memory that has shown impairment secondary to both complete and dorsal hippocampal lesions.[44,60] More poorly understood is the role of the OPFC in such tasks, though there is evidence in the rhesus monkey for impairment in delayed non-matching-to-sample secondary to orbital frontal aspiration lesions.[61] Therefore, the primary goal of this study was to characterize any effect of OPFC lesions in the rat on the rewarded alternation task, dissociating if possible from the expected deficiencies secondary to HPC lesions. The effect of introducing a delay into the task was also examined.

2. Rewarded Alternation without Delay

The rewarded alternation task utilized an elevated T-maze built from unpainted wood and had a start arm 80 cm long and 10 cm wide and identical goal arms 60 cm long and 10 cm wide, all surrounded by a 1-cm high raised lip. Metal food wells of 3-cm diameter were centered 3 cm from the end of each goal arm and the entire maze was elevated 1 m above the floor in a room well-lit by fluorescent lamps. All 35 rats were maintained between 80% and 85% of their free feeding weight. They were habituated to the maze over 8 days until they were running freely and consuming food rewards (45-mg Noyes pellets) from both food wells.

Testing consisted of a sample run followed immediately by a choice run, though in practice there was a minimal delay of approximately 5-10 seconds between runs. For a

sample run, a wooden block forced the rat down either the left or right arm for a single reward pellet, following a pseudorandom sequence. The block was removed and the animal immediately placed at the far end of the start arm. If the rat chose the previously unvisited arm, a reward of two pellets was earned. No reward was located in the previously visited arm. The rat was considered to have "chosen" an arm when all four of its feet advanced over a mark 45 cm from the far end of the reward arm. Each of the 35 rats received a total of 30 trials. Rats were tested in squads of 5-6 animals, each receiving 3-5 trials per day. The intertrial interval varied from 5 to 10 minutes depending on the running speed of individual animals in the squad.

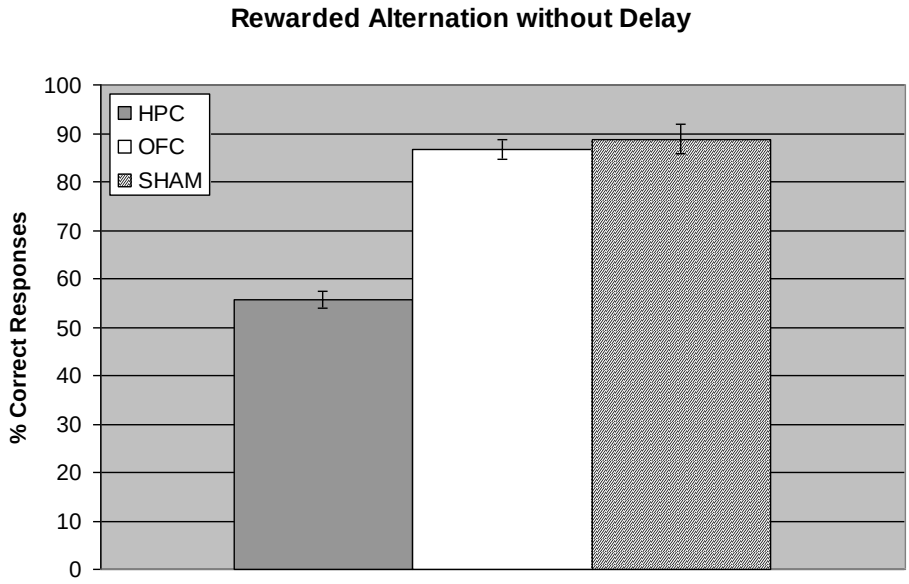


Figure 7. Rewarded alternation without delay between sample and choice runs, presented as group average "correct" scores (as described in text). Error bars indicate ±1 standard error of the mean. There is a statistically significant difference between the HPC group and each of the sham and OPFC groups, but no statistically significant difference between the sham and OPFC groups.

Rats in the HPC group were profoundly impaired in the without-delay condition relative to the sham and OPFC groups (Figure 7), though their performance was above chance levels as confirmed by a one-way t-test with 50% as the test value ($p < 0.01$). While the HPC group ($n = 12$) had on average 55.6% correct responses, the SHAM ($n = 11$) and OPFC ($n = 11$) groups had 88.8% and 86.7% average correct responses,

respectively. One-way between groups ANOVA showed there to be a significant difference between the three groups ($F_{(2,31)} = 68.902$, $p < 0.0005$). Tukey's HSD post-hoc tests showed significant differences between SHAM and HPC groups and between OPFC and HPC groups ($p < 0.0005$ in both cases) but no difference between SHAM and OPFC groups ($p > 0.7$).

The rats with hippocampal lesions exhibited a profound impairment in spatial working memory. OPFC lesions, by contrast, produced no discernible effect, suggesting that the structure is not required for spatial working memory. However, in all cases the choice run immediately followed the forced run, possibly masking any delay-dependency of the effect. Therefore, it was decided to modify the task to determine if an effect of OPFC lesions would be revealed if the delay between sample and choice were extended.

3. Rewarded Alternation with Delay

The task was thus repeated with two clearly different delay conditions—either 30 or 600 seconds—introduced between the sample and choice runs, as work in the monkey has implicated the ventromedial prefrontal cortex—which encompasses the OPFC—in the ability to perform spatial delayed-response tasks.[62] During the delay period, rats were separated in holding cages (24 cm long by 13 cm high by 13 cm wide) with unpainted wooden walls, wire mesh bottoms, and clear plastic hinged covers. All rats were habituated to these cages before testing began. The remainder of the procedure was identical to the no-delay condition described in the preceding section (Chapter IV.2). Delays were interleaved and each rat received a total of 20 trials (10 at each delay). Rats were again tested in squads of 5-6 animals receiving 2 trials each per day. The intertrial interval varied from 10-15 minutes.

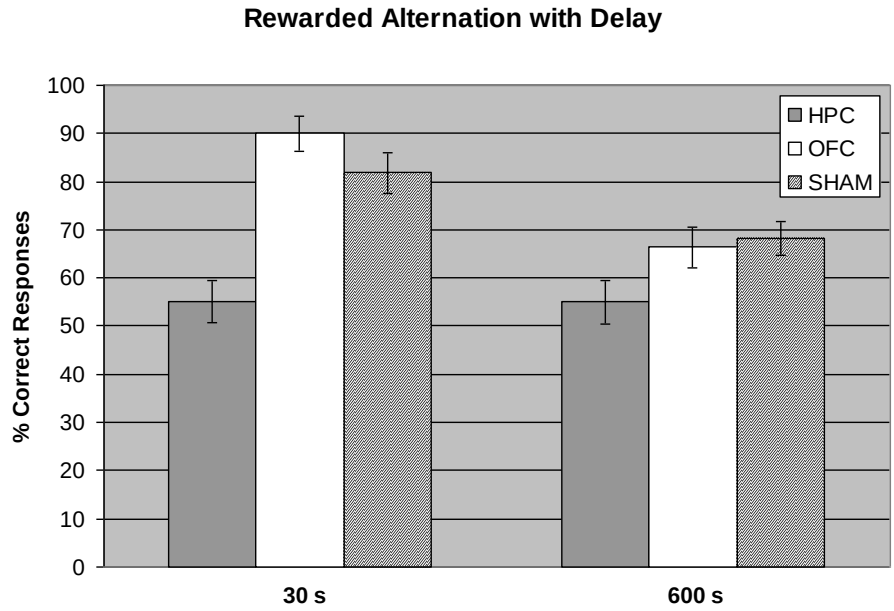


Figure 8. Rewarded alternation with variable delay (either 30 or 600 s), scored and presented as in Figure 7. Error bars again indicate ±1 standard error of the mean. There is a statistically significant difference in the score for the HPC group versus the sham and OPFC groups for both delay conditions. There is a statistically significant decrement in performance of the sham and OPFC groups as delay changes from 30 to 600 s. There is no statistically significant difference in the scores for the sham and OPFC groups within the same delay condition.

The percentage of correct responses for each combination of delay condition and lesion group appears in Figure 8. The HPC group was consistently impaired for the 30 and 600 second delays (55.0% and 55.0% correct, respectively), their performance this time being at chance levels according to one-sample t-tests with 50% as the test value ($p > 0.25$ for both delay conditions). The SHAM and OPFC groups again performed comparably for each delay condition, but performance for both decreased for the 600 second delay (68.2% correct sham and 66.4% correct OPFC) as compared to the 30 second delay (81.8% correct SHAM and 90.0% correct OPFC). A two-way mixed design ANOVA again verified a significant main effect of group ($F_{(2,31)} = 21.180$, $p < 0.0005$) and of delay condition ($F_{(1,31)} = 19.329$, $p < 0.0005$). The interaction of delay and group was not significant ($F_{(2,31)} = 2.859$, $p = 0.073$). Tukey's HSD post-hoc tests again showed significant differences between HPC and SHAM and between HPC and OPFC groups (p

< 0.0005 in both cases) but no significant difference between SHAM and OPFC groups ($p > 0.75$).

These results echo those of the no-delay version of the task (Chapter IV.2), showing a profound deficiency of spatial working memory in rats with complete HPC lesions. The OPFC was again shown not to be required for spatial working memory—regardless of delay.

4. Conclusion

Comparing the results of both the no-delay and delay versions of the task, it appears that hippocampal lesions produce consistent and profound impairment in the rewarded alternation task without any delay-dependency, indicating that the structure plays a fundamental role in spatial working memory. Conversely, the orbital prefrontal cortex seems to play no such role, also without any delay-dependency. This finding is notable as the delay conditions were chosen to be widely different, including a lengthy delay of 600 seconds introduced under conditions of potentially high interference from the constant alternation between two locations.

Chapter V. Reinforcer Devaluation: Selective Satiety and Pavlovian Aversion

1. Background

Work in both the rat and monkey has implicated the OPFC in goal-directed behavior such as reinforcer devaluation, as mentioned in Chapter II.1.[25,24] Often, the incentive value of one of the foodstuffs is altered by injecting the rat with lithium chloride (LiCl) producing a profound and lasting Pavlovian association between the aversive stimulus and the devalued foodstuff[25,63,64] that is dose-dependent.[65]

For this task, however, selective satiety—in which the animal is allowed to eat its fill of a reward food to decrease that food's incentive value—was initially chosen, as it has also been successfully used with the rat.[66] Selective satiety also is a more humane testing paradigm, as it does not devalue a food by pairing it with physical illness. Its theoretical reversibility additionally offers a more naturalistic control of goal values. Furthermore, the reinforcer devaluation task as performed in monkeys also employs selective satiety (see Chapter IX).

2. Reinforcer Devaluation: Selective Satiety

A wooden plus-maze was utilized, adapting a paradigm successfully applied to the mouse.[67] The plus maze had four arms of 66 cm long and 10.5 cm wide (painted grey) that comprised two intersecting alleys. Unpainted wooden start platforms of length 23.5 cm were added to each alley and food wells 2 cm in diameter were centered 3 cm from the opposite ends. The arms and platforms were surrounded by a 2-cm high lip and the entire maze was elevated 65 cm above the floor. The maze was instrumented with infrared breakbeam sensors connected to an in-house data acquisition system and IBM compatible laptop. Existing custom software developed with Microsoft Visual C#.NET (Microsoft Development Environment 2003 Version 7.1.3088) was adapted for use in this

task in consultation with the author and then further modified by the author directly. Three sensors were placed on each alley, located at (1) the end of the start platform, (2) 20 cm from the opposite end of the alley, and (3) immediately above the food wells. Latency to first beambreak at each sensor was recorded. The rats were habituated to each of two reward foods (chocolate buttons and sweet corn) in their home cages (presented in the sequence corn, chocolate, corn, chocolate) and on the plus maze (one food at a time) until they readily ran down the alley to the food well. Each rat was trained to run down a particular alley for a specific foodstuff. The combination of food and alley applied to each rat followed a counterbalanced, pseudorandom pattern.

Training consisted of using two wooden blocks to confine a rat to one alley. The rat was then placed on the start platform and the computer set to record when it broke each of the sensors on its way to the food well. Only the first instance of a beambreak was recorded. The combination of food and alley allotted to each rat followed the same counterbalanced and pseudorandom pattern used during habituation. A single food was presented until a block of 10 trials was completed for each rat; the order of food presentation was counterbalanced and pseudorandom, repeating after 10 blocks were completed. At the start of the task, the rats were initially run for only 5 trials per testing day (specifically on days 1, 2, 5 and 6), but were increased to 10 trials per day for all other days. The maze was wiped clean with wet paper towels at the end of each testing day. Each of the 35 rats received 160 trials in total (80 for each reward food). All rats were maintained at between 80% and 85% of their free feeding weight for the duration of this task.

Once the recorded latencies had reached a plateau for two or three consecutive days, rats were considered to have successfully completed training and the reinforcer devaluation test was performed. The rats were allowed unlimited access to one of the two

rewards for 120 minutes in bare cages, and the amounts eaten were recorded. This pre-feeding was designed to induce selective satiety, thus decreasing the given reward's incentive value. Immediately following the devaluation treatment, the rats received a series of test trials to both foods on the plus-maze in extinction, modeled after the procedure of Johnson et al.[67] Each rat was allowed to run up to 18 trials on each alley; testing was immediately discontinued if an animal failed to run in 2 minutes, in which case it was considered to have extinguished responding. However, the first trial on each alley was untimed to account for the fact that some rats ran more slowly on their first trial of the day irrespective of devaluation treatment. After receiving a set of trials on one alley, each rat was subsequently tested that same day on the alternative alley. The allocation of food to be devalued and pairing of food and alley were counterbalanced within each experimental group. Half of the rats were tested initially on the devalued alley and the other half on the non-devalued alley. The testing was run in two waves, such that for the first wave, each rat was devalued to one of the two rewards (the assignment being pseudorandom and counterbalanced), and for the second wave, each rat was devalued to the remaining reward. Two days of retraining (one block of 10 trials for each reward food) were performed between waves to verify that alley-running latencies were comparable to those at the end of the initial training.

Figure 9 plots the average latency (in seconds) to traverse the entire alley for each combination of experimental group and reward food during the training phase of the task. Trial blocks were paired, such that one "session" represented each rat receiving 10 trials of training to chocolate and 10 trials to sweet corn. The latencies to the first and second breakbeam sensors are not plotted. It was found that the latency to the first breakbeam sensor—located at the end of the start platform—was not useful, as the rats often actuated the sensor with their snouts or tails while exploring the start platform. While traversing

the alley, the majority of rats actuated the third breakbeam sensor—found directly over the food well—immediately after actuating the second—located 17 cm before the food well. Thus, the latency to the third sensor was most instructive for assessing alley traversal and thus motivation to run.

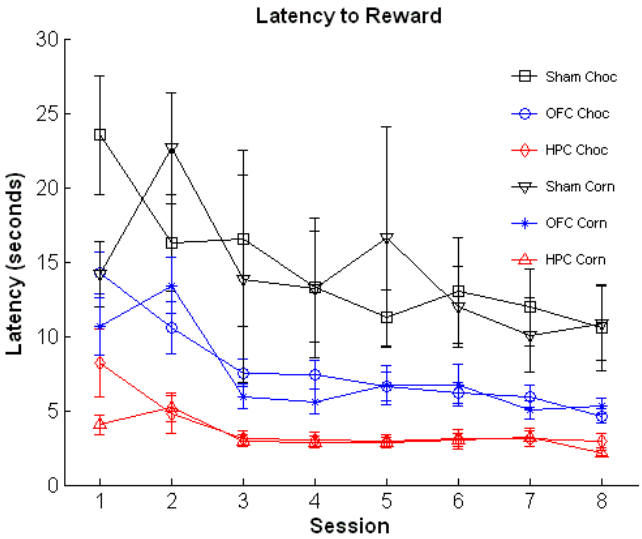


Figure 9. Average latency to reward (in seconds) during the training phase, presented for each combination of experimental group and reward food. Error bars indicate ±1 standard error of the mean for the average latencies. Each session consists of 20 trials (10 for each food).

Visual inspection of Figure 9 shows that the latencies decreased as training continued, reaching asymptotes by session 6 or 7. The HPC group is notable for significantly shorter latencies, with an asymptote reached by session 3. After this point the latencies were consistently near 4 seconds, a value that appeared to be the minimum possible time required for a rat to traverse an alley.

A three way mixed-design ANOVA—with experimental group as the between-subjects factor and session and reward food as within-subjects factors—showed significant main effects of session ($F_{(7,217)} = 12.190$, $p < 0.0005$) and group ($F_{(2,31)} = 7.646$, $p = 0.002$). The interaction of session and food was also significant ($F_{(7,217)} = 6.644$, $p < 0.0005$). No other main effects or interaction terms were significant, with the largest F-value being for the interaction of session, food, and group ($F_{(14,217)} = 1.533$, $p >$

0.1). Tukey's HSD post-hoc tests noted a significant difference between the HPC and SHAM groups ($p = 0.001$) but no significant difference between OPFC and SHAM groups or between OPFC and HPC groups ($p > 0.06$ in both cases).

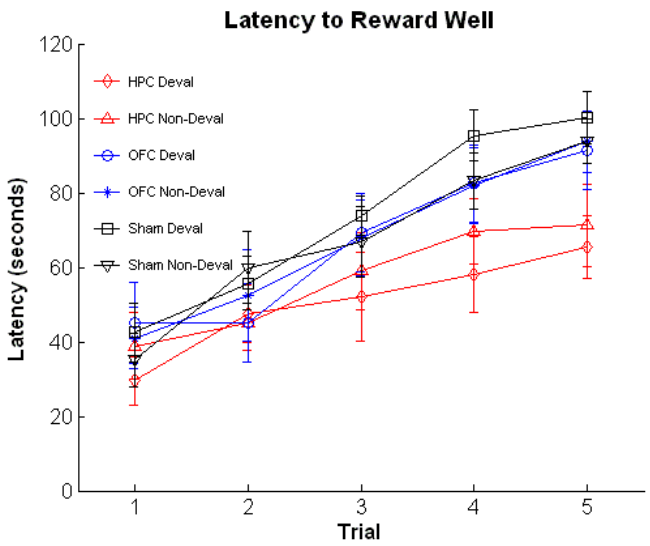


Figure 10. Latency to reward well during the extinction test, collapsed across foods. Error bars indicate ±1 standard error of the mean for the average latencies.

Figure 10 plots the average latency (by experimental group and devaluation condition) to traverse the entire alley as a function of trial number during the extinction test. As increasing numbers of rats reached the 2-minute latency criterion as the test continued, only the first five trials were displayed and analyzed. Rats reaching the latency criterion within these five trials had all subsequent trials scored as having a "latency" of 120 seconds. The data have been collapsed across foods in the figure but not in the analysis immediately below.

Visual inspection of Figure 10 showed a striking near-linear increase in latency with each subsequent trial, with the only apparent difference being slightly reduced latencies for the HPC group. Curiously, the latencies start at 25 seconds, reaching maxima of 80-100 seconds by trial 5. This is clearly far different from the training sessions, in which all latencies were *below* 25 seconds, reaching minima in session 8 below 15 seconds (Figure 9).

A four-way mixed-design ANOVA—with experimental group as the between-subjects factor and trial number, satiety food, and devaluation condition (i.e. devalued versus non-devalued) as within-subjects factors—showed a significant main effect of trial ($F_{(4,124)} = 49.448$, $p < 0.0005$) and group ($F_{(2,31)} = 3.424$, $p = 0.045$). The interaction of satiety food, devaluation condition, and trial number was also significant ($F_{(4,124)} = 2.580$, $p = 0.041$). No other main effects—including devaluation condition—or interaction terms were significant, with the largest F-value being for the food by trial number interaction ($F_{(4,124)} = 1.709$, $p = 0.152$). Tukey's HSD post-hoc tests showed a barely-significant difference between the HPC and SHAM groups ($p = 0.049$) only (all others $p > 0.1$).

It would appear that the rats quickly learned that no reward was being associated with either alley, causing the extinction effect to generalize to both alleys, overshadowing any more subtle devaluation effect. The main effect of trial number was due to the rats' latencies steadily increasing as the sessions progressed and the observed group effect was simply the result of the higher HPC group running speeds apparent during training and not of any OPFC-specific effect. It is also possible that allowing 120 minutes of unrestricted access to the reward foods during the devaluation treatment led to excessive satiety that similarly generalized to both foods and alleys, again masking other effects.

3. Reinforcer Devaluation: LiCl Aversion

Noting these ultimately inconclusive results, it was decided to modify the experimental paradigm to use lithium chloride (LiCl) instead of selective satiety for the devaluation treatment. Intraperitoneal (i.p.) injections of LiCl immediately after food access have successfully produced taste aversion and the technique of Hatfield et al. was adapted for this study.[68] After another two days of retraining on the plus-maze (one block of 10 trials for each reward), rats were assigned—in a pseudorandom and

counterbalanced fashion—to receive aversion sessions to devalue either the chocolate or sweet corn reward foods. Three aversion sessions were performed, staggered such that all rats assigned to be devalued to chocolate received injections on days 1, 3, and 5 while those assigned to be devalued to corn were injected on days 2, 4, and 6. For each aversion session, the designated rats were moved from their home cages and placed in bare cages with water bottle access for 5-6 hours. After this habituation period, a known amount of the appropriate food was placed in the cage for 10 minutes and the water bottles were removed. Food weights before and after the feeding session were measured and the amount eaten was calculated. Immediately after the food access, each rat received an i.p. injection of 0.2 M LiCl solution (5 mL/kg as in the Hatfield study[68] for the first aversion sessions and 7.5 mL/kg for subsequent sessions) and was returned to its home cage. This 50% dose increase after the first session was done because little to no change in behavior was noted in the 30-60 minutes following injection, with coprophagia occurring soon after return to home cages.

After one day of rest following the third aversion session, the rats received a second devaluation test on the plus-maze. Several important modifications were made to the paradigm, however. Given the rapidity with which the rats reached the extinction criterion in the satiety devaluation test, only 10 trials total were run, interleaved by devaluation condition. Thus, each rat received 5 trials on the devalued alley and 5 on the non-devalued alley, with the order of trials (i.e. on the devalued versus the non-devalued alley) occurring in a counterbalanced, pseudorandom fashion. It was ensured that each rat was not run on a particular alley more than twice consecutively. It was also ensured that the first two trials for a given rat would consist of one trial on the devalued alley and one on the non-devalued alley. The two-minute limit for traversing an alley was still used, but the criterion was changed such that a rat had to reach this limit three times

consecutively on a given alley before testing was discontinued. For trials in which this limit was reached or that did not occur because testing was discontinued early, the latency was scored as 120 seconds. This more rigorous extinction criterion was designed to reduce confounding effects of inter-trial running speed variability not due to devaluation.

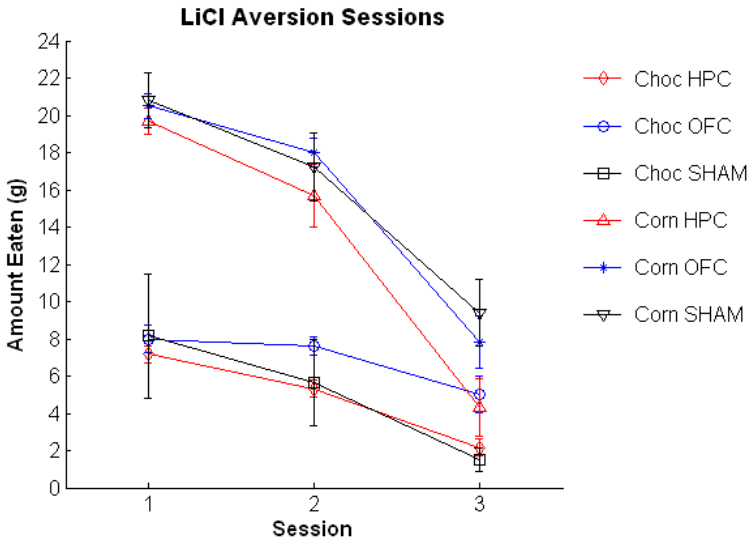


Figure 11. Results of the LiCl aversion sessions, showing amount of food eaten in grams. Means are presented for the devalued food only but for all combinations of devalued food and experimental group. Note the large decrease between the second and third sessions and the overall difference in the amounts of chocolate and sweet corn eaten. Error bars indicate ±1 standard error of the mean.

Figure 11 shows the results of the three aversion sessions, plotting the amount of food eaten (in grams) prior to each round of LiCl injections. Each rat was devalued to only one food and the displayed results are thus for that food only. Results are presented for all six combinations of devalued food and experimental group. The rats were observed to eat far less chocolate than sweet corn on average, eating more than twice as much corn as chocolate. This finding is consistent with the satiety devaluation, in which the rats consumed the two foods in similar proportion during the satiety sessions. Consumption of either food decreased significantly with subsequent aversion sessions, suggesting a successful Pavlovian association between the aversive stimulus and the devalued food.

A three-way mixed-design ANOVA—with experimental group and devalued food as between-subject factors and session as the within-subject factor—yielded a significant

main effects of session ($F_{(2,56)} = 319.039$, $p < 0.0005$) and food ($F_{(1,28)} = 158.458$, $p < 0.0005$). There were significant interactions of session and food ($F_{(2,56)} = 69.198$, $p < 0.0005$) as well as session, group, and food ($F_{(4,56)} = 3.199$, $p = 0.020$). No other main effects or interaction terms were significant, with the next-highest F-value corresponding to the session by group interaction ($F_{(4,56)} = 2.115$, $p = 0.091$). The main effect of session gives clear evidence of successful taste aversion induced by LiCl. Although the two foodstuffs were not equally preferred (due to the main effect of food), the ratio of sweet corn consumed to chocolate consumed was consistent with the satiety sessions of the preceding section. The lack of a main effect of group suggests that devaluation was successfully demonstrated irrespective of lesion status, though the session by group by food interaction does create some doubt. A future replication of this experiment would benefit from the use of alternative foods that are consumed in equal quantity.

Figure 12 plots latency for alley traversal in seconds, averaged by group and devaluation condition. The data have been collapsed across foods for the plot but not for the analysis. Once again, latencies had a tendency to increase with subsequent trials and were in general far above those encountered during training. No group difference is immediately obvious from inspection of the plot.

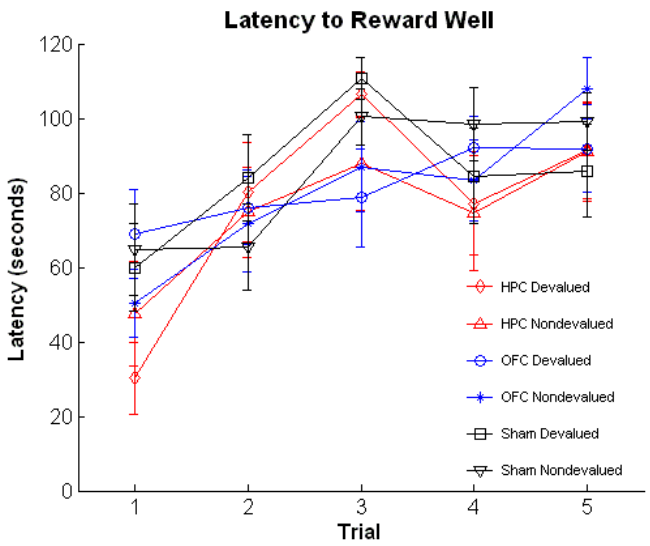


Figure 12. Latency to reward well (in seconds) for the LiCl devaluation test, collapsed across foods. Error bars indicate ±1 standard error of the mean.

A four-way mixed-design ANOVA—with experimental group and food as between-subject factors and trial number and devaluation condition (i.e. devalued versus non-devalued) as within-subject factors—demonstrated a significant main effect of trial ($F_{(4,112)} = 16.847$, $p < 0.0005$). Significant interaction terms included trial by food ($F_{(4,112)} = 2.609$, $p = 0.039$) as well as trial by group by food ($F_{(8,112)} = 2.773$, $p = 0.008$). No other main effects or interaction terms were significant, with the next-highest F-value corresponding to the interaction of group and food ($F_{(2,28)} = 2.919$, $p = 0.071$). The lack of any effect of devaluation condition or any interaction of devaluation condition with group suggests that the task was again unsuccessful.

However, because each rat was devalued to a single food during the course of this test and because the rats in all cases ate roughly half as much chocolate as sweet corn, it was decided to perform separate analyses for the chocolate and corn sub-cohorts to determine if devaluation may have been successfully elicited with one food but not the other. The relevant counterbalancing and pseudorandomization were preserved within each sub-cohort. Experimental group was the between-subjects factor; devaluation condition and trial were the within-subjects factors. Performing this three-way mixed ANOVA on the chocolate sub-cohort ($n = 17$), there was a significant main effect of trial ($F_{(4,56)} = 12.635$, $p < 0.0005$) and a significant interaction of trial and group ($F_{(8,56)} = 2.106$, $p = 0.050$ exactly). No other main effects or interaction terms were significant, with the next-highest F-value corresponding to the group main effect ($F_{(2,14)} = 2.404$, $p = 0.127$). The analogous three-way mixed ANOVA for the sweet corn sub-cohort ($n = 17$) yielded a similar result, again with trial being the only clearly significant main effect ($F_{(4,56)} = 6.529$, $p < 0.0005$). The next-highest F-value was for the interaction of trial and group ($F_{(8,56)} = 1.949$, $p = 0.070$). The group main effect was clearly not significant ($F_{(2,14)} = 1.272$, $p = 0.311$).

Throughout the analyses for the LiCl devaluation test, trial was the only consistently significant effect. Despite the modifications and increased rigor of the paradigm employed for the test, it appears that extinction again became consistently generalized to both alleys after a few trials. This theory is supported by the larger latencies (relative to training) that increased with subsequent trials just as occurred with satiety devaluation, suggesting a similar failure mode for both paradigms. The strikingly slow running speeds additionally suggest that there may have been an overall decrease in motivation for running on the maze, possibly due to the absence of training during the aversion sessions or alternatively to a failure in associating the aversive stimulus with an alley. Furthermore, the age of the rats and their prior training and testing experience might have rendered them less likely to display a devaluation effect even with successful taste aversion. Specifically, the majority of the rats' testing lives had occurred on mazes of various types, possibly leading to a generalized habituation to maze-running tasks. The combination of these confounding effects could easily have masked any more subtle devaluation effect, leading to the inconclusive results. In light of this overall lack of significant group differences for the reinforcer devaluation task (as the group effect seen in the satiety devaluation was the result of the high running speeds of HPC rats evident during training), an initially planned discrimination test between the chocolate and sweet corn rewards was deemed futile and omitted.

4. Conclusion

As described in the preceding sections, both variants of the reinforcer devaluation task were unsuccessful. The selective satiety paradigm apparently suffered from generalized extinction possibly combined with a lack of motivation induced by excessive satiety. The LiCl aversion paradigm, despite numerous improvements in rigor, generalized extinction again seemed the chief difficulty.

Potential improvements for future work would most logically focus on LiCl aversion as the means of decreasing the incentive value of a foodstuff, as this more robust devaluation effect would be less susceptible to other confounding factors. Modifications to this task could include providing rats with their non-devalued food on their off days during the aversion sessions to balance their exposure. To combat the initially slowed running speed, the rats could also be habituated and trained to a third neutral food, such as Noyes pellets. This food would not be devalued, instead being used for brief retraining between any aversion or satiety sessions and the true experimental trials. The scheme would necessitate a six-arm radial maze, with pairs of opposing arms forming three separate alleys. Alternatively, one could use operant boxes instead of mazes, further adapting a proven technique such as that of Hatfield et al. Additionally, it would be prudent to select two foodstuffs that are consumed in roughly equal quantity, as the lack of this was another potential difficulty in the current study. With many of the maze-specific confounding effects thus eliminated, definite results showing more robust effects would be likely. Once successful results are thus obtained, the selective satiety method of devaluation could then be explored in greater detail.

Chapter VI. Spatial Memory Acquisition and Reversal Learning: The Watermaze

1. Background

The Morris watermaze is a classic paradigm specifically developed for the purpose of studying spatial memory.[69,70] It has successfully demonstrated the ability of the rat to utilize distal room cues and adopt a search strategy for locating a hidden platform in a fixed location.[71] Furthermore, the task is quite sensitive to hippocampal lesions.[37,38] However, as before, the role of the OPFC is less well understood. Furthermore, the rewarded alternation task failed to show an OPFC deficit in spatial working memory. The watermaze allows testing of both spatial memory acquisition and reversal learning, accessing a different aspect of spatially-dependent behavior. An OPFC deficit observed in the watermaze would thus allow a functional dissociation to be made. A strong deficit in the HPC group was expected, providing a basis for comparison with any effect observed in the OPFC group.

2. Methods

In the current study, a pool 2 m in diameter with walls 60 cm high was used. The bottom of the pool was raised 42 cm above the floor. The entire pool was painted white. Four 240V, 500W floodlights in the corners of the testing room and various posters and shelves on the walls served as distal visual cues. A cylindrical white plastic platform, 10 cm in diameter and 30 cm high, was hidden 2 cm below the water surface. Wire mesh (also painted white) affixed to the platform provided a gripping surface for the rats. Two liters of whole milk were used to opacify the water in the pool and fresh water was used for each testing day. Water temperature was maintained at 25.5 ± 2ºC for all trials. Rat movement was tracked with an overhead black and white camera (HR-J220, JVC) connected to an HVS Image VP116 Classic Tracker (Hampton, UK). The tracker's

output was fed to a BBC Acorn RISC PC 600 computer with a data acquisition card (Paul Fray Ltd., Cambridge, UK) and running version 2.12 of the Watermaze program (also Paul Fray Ltd.). Samples were taken at a frequency of 10 Hz. The software reported a number of measures, including latency to platform, time spent in each of four imaginary quadrants in the pool, path length, and swim speed. A folding screen separated the equipment and experimenter from the pool, serving to minimize any transient visual distractions to the rats.

The training phase of the task was broken into two 5-day blocks, each comprising 4 days of acquisition followed by a transfer test (or probe trial) conducted 24 hours after the preceding training trial. Such division allowed two snapshots of performance to be taken via the transfer tests occurring at two distinct stages of acquisition. The blocks were separated by a 2-day rest period.

Rats were assigned in a counterbalanced fashion to receive their training with the platform in one of the four quadrants of the pool, arbitrarily designated northeast, southeast, southwest, and northwest. For a particular rat, the location of the platform was kept constant throughout acquisition. For all rats, the radial distance between the center of the pool and the platform was fixed at 50 cm (which is also 50 cm radially from the inner wall of the pool). At the start of each acquisition trial, the rat was released by hand while facing the pool wall. The location of release was pseudorandom and corresponded to one of the eight cardinal and ordinal directions (north, northeast, east, southeast, south, southwest, west, northwest) of the pool, with north arbitrarily assigned. Each rat received four acquisition trials on a given day, with all of its trials performed sequentially before the next rat was tested. Each acquisition trial had a maximum length of 90 seconds and was ended when the rat reached the platform. If a rat failed to reach the platform within the 90 second limit, it was guided to the platform by the experimenter. Once the platform

was reached—either alone or with experimenter guidance—the rat was allowed 30 seconds to stand on it undisturbed before removal by hand. The inter-trial interval was approximately 15 seconds. At the end of the fourth day of training, 16 acquisition trials had been completed by each rat.

The fifth and final day of a block was devoted to a transfer test, in which the rat swam in the pool with the platform absent for the duration of the trial. Time spent in each quadrant was used as a measure of the extent to which the rat had learned the platform location during acquisition. Successful learning would be suggested by a majority of time spent in the training quadrant (i.e. the quadrant in which the platform was located during acquisition). The procedure for releasing the rat was identical to that of the acquisition trials. Each rat received a single transfer test trial 60 seconds in duration with removal from the pool immediately following. If the rat was not in the training quadrant after 60 seconds had elapsed, it was guided to the proper quadrant and then removed by hand from the pool. For both acquisition and transfer test trials, the rat was thoroughly dried with a towel and placed in a warming chamber before being returned to its home cage. The rats were allowed to feed ad libitum in their home cages for the duration of the task.

Following the second transfer test on the tenth day of the experiment, the rats in the HPC group (n = 12) were identified and their testing was discontinued, as the expected profound deficit in water maze acquisition[37,38] would necessarily impair performance on the reversal learning phase. The remaining rats (n = 22) received four days of retraining, each day composed of four trials given in the established manner. This brief retraining was designed to combat any extinction effects caused by the transfer tests, as this is a known issue with repeated no-platform probe trials.[72]

On the day following the fourth retraining day, serial reversals were begun. The reversals were performed in four-day blocks, with each day consisting of four trials. The

trials were run in the manner established for acquisition. No transfer tests were performed. For the first reversal, the platform was moved to the "Opposite" quadrant for each rat (i.e. it was moved 180° away from the training position). The radial distance from the center of the pool was unchanged at 50 cm. For the second reversal block, the platform was moved to the "Adjacent-Right" quadrant with respect to the training quadrant, meaning that the platform was located 90° clockwise from its initial position. Additionally, the radial distance between the pool center and the platform was changed to 75 cm (i.e. 25 cm radially from the inner wall of the pool). During the third reversal block, the platform was located in the "Adjacent-Left" quadrant with respect to the training quadrant (so moved 90° counter-clockwise from its initial position). The radial distance from pool center to platform was also reduced to 25 cm. The experiment was concluded with a single day (four trials) of a fourth reversal. For each rat, the platform was moved 135° clockwise with respect to its training position, so that it was on the imaginary border separating the Adjacent-Right and Opposite quadrants. The radial distance from pool center to platform was changed to 62.5 cm as well.

One rat belonging to the OPFC group was euthanized during the course of the serial reversal phase of the task following discovery of a rapidly-growing tumor. The animal was therefore excluded from the reversal data (OPFC: $n = 10$) but kept in the acquisition data (OPFC: $n = 11$).

3. Acquisition

For the acquisition phase—consisting of two training blocks with all three experimental groups and one retraining block with the OPFC and SHAM groups—three measures of performance were examined: the distance swum during a trial (referred to as "path length"), latency for each trial, and mean swim speed (calculated as path length divided by latency). These data are plotted in Figures 13, 14 and 15, respectively. Note

that a one-day transfer test was inserted between days 4 and 5 of acquisition and another between days 8 and 9. These are represented by gaps in the plots.

By inspection, one sees a generally steady decrease in path length and latency—with a commensurate increase in average swim speed—during the two training blocks. The HPC group diverges from the OPFCs and SHAMs with longer path lengths, larger latencies, and higher mean swim speeds—particularly during the second training block. For the first two days of retraining, the SHAM group appeared less efficient at rediscovering the platform, as evidenced by longer path lengths and larger latencies. Mean swim speed appeared slower on days 9, 10, and 12.

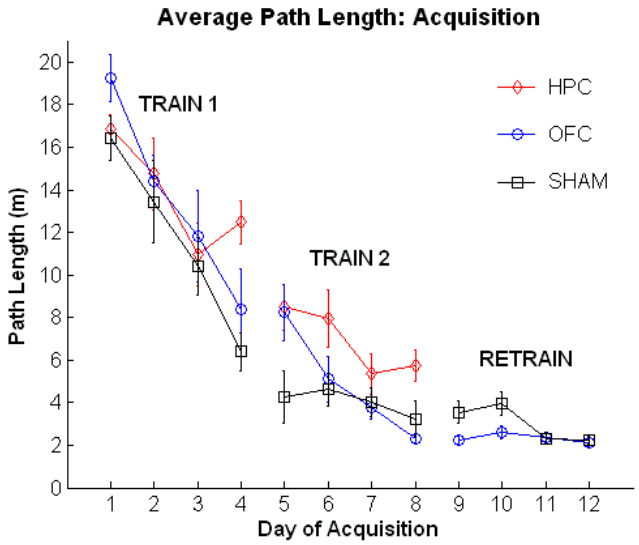


Figure 13. Path lengths per trial, plotted by group and averaged over each day (there were four trials in a day). Note that all three groups received training in the first two blocks; the HPC group was omitted from the third retraining block. Two transfer tests—were inserted between acquisition days 4 and 5 and between days 8 and 9. Error bars represent ±1 standard error of the mean.

A two-way ANOVA—with experimental group as the between-subjects factor and day of acquisition as the within-subjects factor—was performed on the path length data for the first two training blocks (i.e. the first 8 days of acquisition). There were significant main effects of day ($F_{(7,217)} = 58.627$, $p < 0.0005$) and group ($F_{(2,31)} = 3.702$, $p = 0.036$). The interaction of day and group was not significant ($F_{(14,217)} = 1.593$, $p = 0.083$). Post-hoc analysis with Tukey's HSD confirmed a significant difference between the HPC and SHAM groups ($p = 0.028$) but no other significant differences (all $p > 0.3$).

The retraining path lengths for the OPFC and SHAM groups were also examined by two-way ANOVA, showing significant main effects of day ($F_{(3,60)} = 5.930$, $p = 0.001$) and group ($F_{(1,20)} = 5.381$, $p = 0.031$). The day by group interaction was also significant ($F_{(3,60)} = 2.912$, $p = 0.042$). A test of simple main effects reported a significant group differences on day 9 only ($F_{(1,20)} = 5.681$, $p = 0.027$), with the next-highest F-value for day 10 ($F_{(1,20)} = 4.263$, $p = 0.052$). The group effect was thus driven primarily by day 9 (and to a weaker extent by day 10), during which the OPFC group re-acquired the platform position more accurately than the SHAM group.

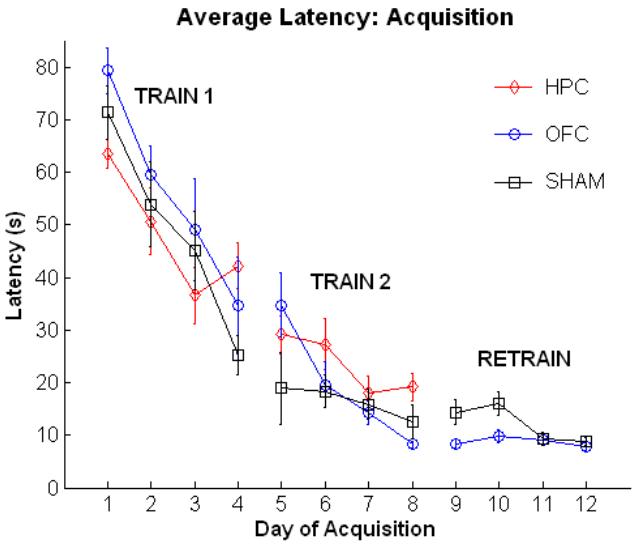


Figure 14. Latencies are plotted by group and averaged over each day, like in Figure 13. Two transfer tests were inserted between days 4 and 5 and between days 8 and 9. Error bars indicate ±1 standard error of the mean.

The average latencies for both training blocks and the retraining block appear in Figure 14. A two-way ANOVA—with group as the between-subjects factor and day as the within-subjects factor—performed on the 8 days of training revealed a significant main effect for day ($F_{(7,217)} = 52.632$, $p < 0.0005$). No other main effects or interactions were clearly significant, with the next-highest F-value corresponding to a day by group interaction trend ($F_{(14,217)} = 1.698$, $p = 0.058$). A two-way ANOVA performed on the retraining latencies for the OPFC and SHAM groups, however, showed significant effects of both day ($F_{(3,60)} = 4.963$, $p = 0.004$) and group ($F_{(1,20)} = 8.137$, $p = 0.010$), as well as a significant day by group interaction ($F_{(3,60)} = 2.755$, $p = 0.050$). A test for simple main

effects revealed significant group differences on days 9 ($F_{(1,20)} = 5.855$, $p = 0.025$) and 10 ($F_{(1,20)} = 5.438$, $p = 0.030$), with all other days being clearly not significant ($F < 0.9$, $p > 0.35$). The observed group effect during retraining was thus mainly due to the OPFC rats outperforming the SHAMs on days 9 and 10 in a manner similar to that seen with the path lengths.

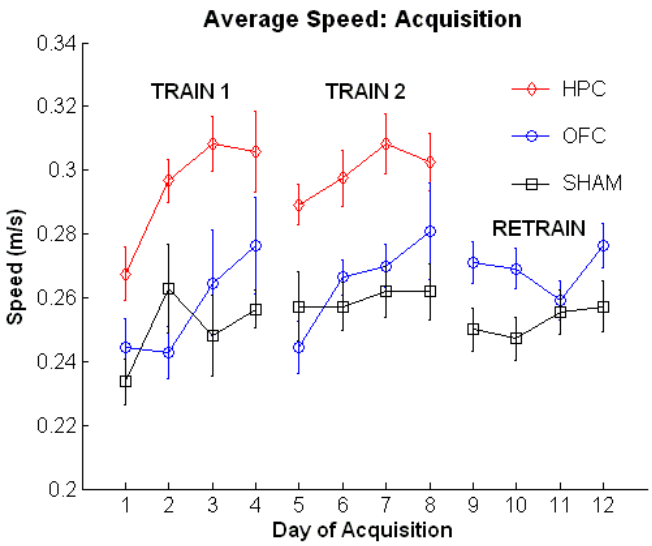


Figure 15. Swim speeds are plotted by group and averaged over each acquisition day. Speed was calculated from the path length and latency data. Two transfer tests were inserted between days 4 and 5 and between days 8 and 9. Error bars indicate ±1 standard error of the mean.

The calculated swim speeds appear in Figure 15. Once again, it is clear from visual inspection that the HPC group swam at higher speeds for all eight days of training. A two-way ANOVA performed on the 8 days of training showed significant effects of day ($F_{(7,217)} = 4.562$, $p < 0.0005$) and group ($F_{(2,31)} = 17.652$, $p < 0.0005$), but the day by group interaction was not significant ($F_{(14,217)} = 0.903$, $p > 0.5$). Post-hoc analysis with Tukey's HSD confirmed clear group differences between HPCs and OPFCs ($p < 0.0005$) and between HPCs and SHAMs ($p < 0.0005$) but not between OPFCs and SHAMs ($p = 0.700$). This group effect is clearly the result of the HPC rats swimming much faster overall than either OPFC or SHAM rats, as is clearly visible in Figure 15. During retraining of the OPFCs and SHAMs, ANOVA revealed a significant main effect of group only ($F_{(1,20)} = 5.118$, $p = 0.035$), due to the OPFC animals swimming faster than the

SHAM controls. No other main effects or interaction terms were significant, with the next-highest F-value corresponding to the main effect of day ($F_{(3,60)} = 1.357$, $p > 0.25$).

Looking at the training data as a whole, latency was an uninformative measure of performance, showing in all cases only an expected significant main effect of day. Path length was a more helpful measure, revealing an overall group difference (in addition to the expected day difference) for the two training blocks analyzed together. Furthermore, longer path lengths can be considered to reflect inaccuracy in finding the platform. The calculated swim speeds showed robust day and group differences for both training blocks together due to the faster swimming of the HPCs. Regarding retraining, OPFC animals outperformed the SHAMs, with the measures of latency and path length both showing significant group and day main effects as well as significant day by group interactions. The observed effects were driven by the first two days of retraining and may have reflected a mild resistance to extinction in the animals with OPFC lesions, though it is conceivable that this effect began to manifest itself late during the initial training. Retraining speeds did not differ significantly from day to day, but did differ between experimental groups, with OPFCs swimming faster than the SHAMs.

4. Reference Memory: Transfer Tests

The two transfer tests performed during the acquisition phase occurred 24 hours after the last training trial and were an assessment of reference memory retention. For the two transfer tests—occurring after 16 and 32 trials, respectively—analysis was performed on a measure of time spent in each quadrant that was normalized and reported as a percentage by the Watermaze software (Figure 16). There was a clear tendency for rats to spend more time in the training quadrant, an effect most apparent during the second transfer test.

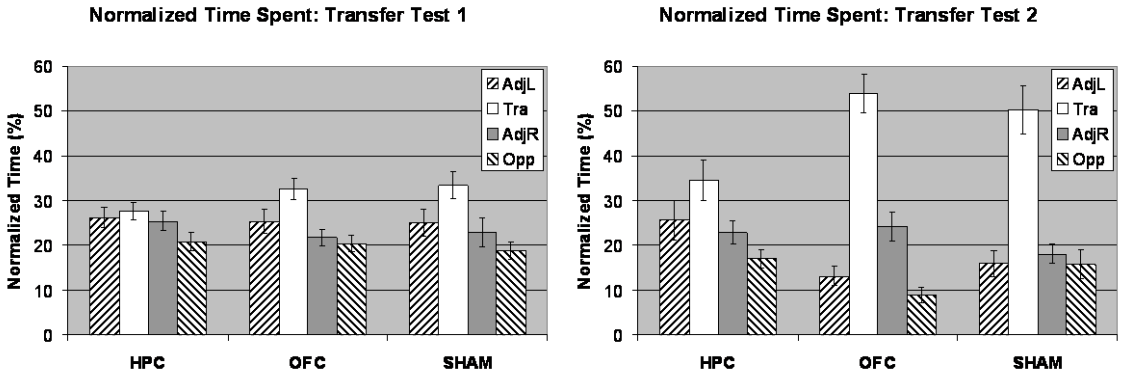


Figure 16. Amount of time spent in each quadrant during both transfer tests, normalized and presented as a percentage. The results for the first transfer test—occurring after 16 trials—appear in the left panel and those for the second transfer test—occurring after 32 trials—in the right panel. Error bars indicate ±1 standard error of the mean.

Two-way ANOVAs were performed with group as the between-subjects factor and quadrant as the within-subjects factor. Note that because the percentages reported for the four quadrants always summed to 100% and thus were not truly independent, the numerator degrees of freedom and p-value for ANOVAs had to be adjusted. For the first transfer test, there was a significant effect of quadrant only ($F_{(2,93)} = 8.709$, $p < 0.0005$) due a tendency for the OPFC and SHAM rats in particular to linger in the training quadrant. The quadrant by group interaction term was not significant ($F < 1$, $p > 0.35$). For the second transfer test, there again was a significant effect of quadrant ($F_{(2,93)} = 39.539$, $p < 0.0001$), but it was this time accompanied by a significant quadrant by group interaction ($F_{(4,93)} = 3.796$, $p = 0.007$). A test for simple main effects for the second transfer test showed significant group effects for the Training quadrant ($F_{(2,31)} = 4.760$, $p = 0.016$) and for the Adjacent-Left quadrant ($F_{(2,31)} = 3.748$, $p = 0.035$). The next-highest F-value corresponded to the Opposite quadrant ($F_{(2,31)} = 3.207$, $p = 0.054$). Noting the significant effect of training quadrant, a binned time analysis in that quadrant only was next performed.

The time spent by each rat in the training quadrant was calculated in 15-second time bins as represented in Figure 17. Each 60-second trial was thus composed of four such bins. The performance of all three experimental groups appeared to be roughly

consistent during the first transfer test. However, by the second transfer test, the HPC rats had clearly diverged from the OPFC and SHAM rats, spending less time in the training quadrant regardless of bin.

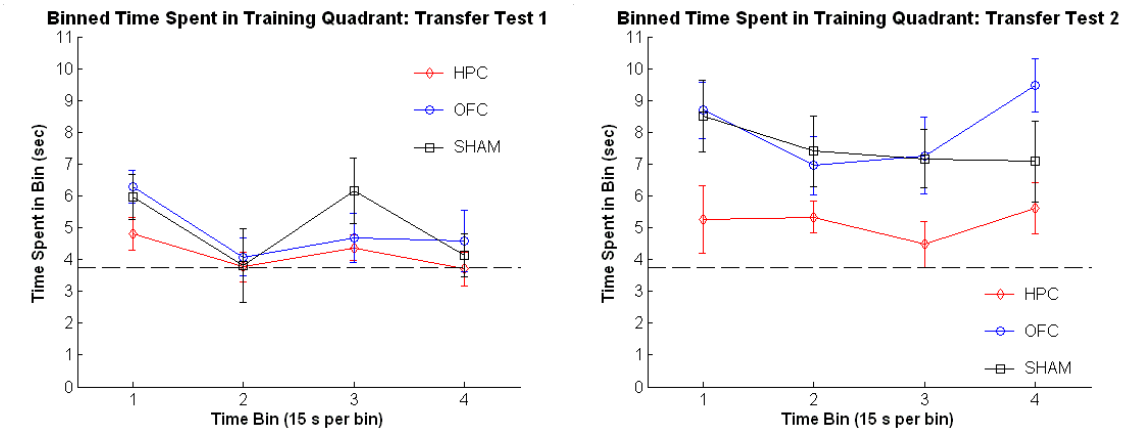


Figure 17. Time spent in training quadrant, presented in four bins of 15 seconds each. The results for the first transfer test—after 16 trials—appear in the left panel; those for the second—after 32 trials—appear in the right panel. Dashed horizontal lines indicate chance performance. Error bars indicate ±1 standard error of the mean.

Analysis by two-way ANOVAs—with group as the between-subjects factor and bin as the within-subjects factor—showed a split result. For the first transfer test, there was a main effect of bin only ($F_{(3,93)} = 3.927$, $p = 0.011$). There were no other significant main effects or interaction terms, with the next-highest F-value corresponding to the main effect of group ($F_{(2,31)} = 1.805$, $p > 0.15$). The second transfer test showed a main effect of group only ($F_{(2,31)} = 5.107$, $p = 0.012$). No other main effects or interactions were significant, with the next-highest F-value corresponding to the main effect of bin ($F_{(3,93)} = 1.805$, $p = 0.152$). Tukey's HSD post-hoc tests confirmed a group difference between HPCs and OPFCs ($p = 0.015$) but a barely not significant difference between HPCs and SHAMs ($p = 0.054$) and a clearly not significant difference between OPFCs and SHAMs ($p > 0.8$).

Thus, looking at the full analysis of the two transfer tests presented in Figures 16 and 17, a reliable effect of quadrant was seen for both when normalized time was used as a measure, indicating that the animals unsurprisingly had a preference for their old training quadrant. This preference was confirmed by a test for simple main effects

carried out on the data for the second transfer test, motivating the subsequent binned time analysis. That analysis yielded a clear group difference for the second transfer test, occurring after 8 days of acquisition (32 trials).

Examining the results of the transfer tests and the initial 8 days of acquisition training together shows that group differences were seen in measures of path length and swim speed only. The HPC animals swam faster than the OPFCs and SHAMs during training (Figure 15) but less accurately than SHAMs, as shown by the significantly increased path lengths, especially during the second training block (Figure 13). These results indicate that rats with complete cytotoxic hippocampal lesions are impaired in this type of spatial learning task, a finding that is consistent with prior work.[37] Conversely, animals with cytotoxic OPFC lesions are unimpaired in spatial learning. As for the no-platform transfer tests, during both tests the animals tended to spend more time searching for the platform in the old quadrant. When the time spent in the training quadrant was analyzed in 15-second bins, a dissociation was observed. During the first transfer test, rats spent roughly the same amount of time in the training quadrant during each 15-second bin, regardless of group. However, after four additional days of training, the HPC group diverged, spending much less time in the training quadrant for all bins as compared to either the OPFCs or the SHAMs. Because the HPC rats were clearly unable to remember the platform location—and presumably other visual cues as well—their optimal strategy for escaping from the water was to employ an alternative search strategy immediately upon release in the water, thus resulting in less time spent in the training quadrant. As for the OPFC and SHAM groups, they showed statistically identical performance, suggesting that the OPFC is not involved in spatial reference memory acquisition as tested in the Morris watermaze.

5. Serial Reversals

The four reversals—three of which were full four-day blocks and the final of which was a single day—concluded the test. Recall that one OPFC rat was euthanized during the course of reversal testing; its data were thus excluded from the analysis of the reversal learning phase, leaving OPFC $n = 10$ and SHAM $n = 11$. Figure 18 plots the path lengths for the serial reversals, showing a dramatically increased average path length for the OPFC group versus the SHAM group on the first day of the second reversal (day 5 overall). There was also a possible modest reduction in OPFC path lengths relative to SHAMs on days 9 and 10.

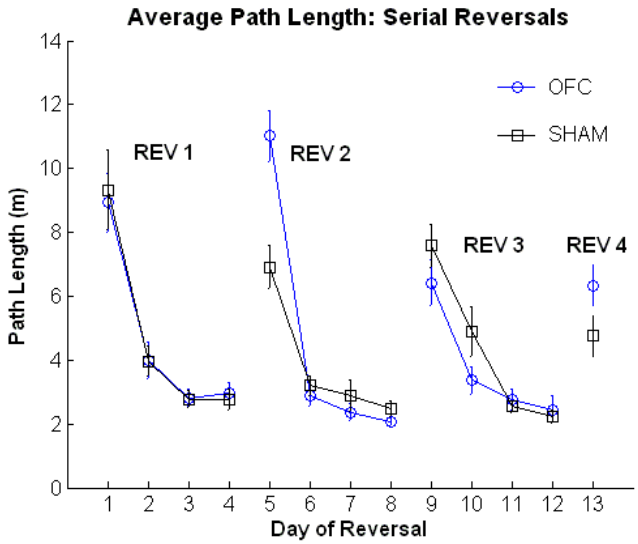


Figure 18. Path lengths during serial reversals, plotted by group and averaged over each reversal day. Reversal 4 consisted of only one day. Error bars indicate ±1 standard error of the mean.

Initially, a two-way ANOVA was performed on the full 13 days of serial reversals, with group as the between-subjects factor and day as the within-subjects factor. This overall analysis showed a significant main effect of day ($F_{(12,228)} = 40.073$, $p < 0.0005$) and a significant interaction of day and group ($F_{(12,228)} = 3.207$, $p < 0.0005$), but no main effect of group ($F_{(1,19)} = 0.461$, $p > 0.5$). Based on the significant interaction term and the transient differences in group performance apparent from visual inspection of Figure 18 (particularly on day 5, the first day of the second reversal), it was decided to test for simple main effects on the complete data set. The analysis reported a significant

group difference on day 5 ($F_{(1,19)} = 15.816$, $p = 0.001$). The next-highest F-value was for day 10 ($F_{(1,19)} = 3.136$, $p = 0.093$).

As a result, further analysis was focused on reversal 2 (days 5-8). ANOVA performed on the full four-day block—with group as the between-subjects factor and day as the within-subjects factor—revealed significant main effects of both day ($F_{(3,57)} = 95.101$, $p < 0.0005$) and group ($F_{(1,19)} = 4.898$, $p = 0.039$) as well as a significant interaction ($F_{(3,57)} = 12.073$, $p < 0.0005$). Examining Figure 18, it is clear that the effect is driven by the performance on Day 5, as revealed by the preceding test for simple main effects. Thus, the group difference seen in the reversal 2 and the interaction effect apparent across all 13 days of the serial reversals was driven by an effect manifested very early in the second reversal.

Given that the effect seen in reversal 2 was primarily the result of a major group difference on the first day of that block, it was decided to analyze the first days of each reversal block (i.e. days 1, 5, 9, and 13) to search for any possible early effects that would be apparent only at the beginning of a given reversal block. The two-way ANOVA had group as the between-subjects factor and reversal (with one level for the first day of each reversal) as the within-subject factor. There was a significant main effect of reversal ($F_{(3,57)} = 8.609$, $p < 0.0005$) and a significant reversal by group interaction ($F_{(3,57)} = 4.045$, $p = 0.011$). There was no significant main effect of group ($F_{(1,19)} = 3.514$, $p = 0.076$). A test for simple main effects revealed significant effects during reversal 2 ($F_{(1,19)} = 15.816$, $p = 0.001$) with the next-highest F-value for reversal 4 ($F_{(1,19)} = 3.136$, $p = 0.093$). Although the first day of reversal 2 exhibited a group difference, it was insufficient to drive an overall group difference for the entire first days analysis.

Dissecting out meaning from these results is complicated by the fact that while reversal 2 exhibited a major group difference caused by the considerable day 5 path

length difference, this effect did not produce an overall group difference for the full serial reversals set or the first days analysis. It should be noted that all visual cues and techniques were kept identical throughout the test and that platform location and release point were randomly varied, making it unlikely that some spatial bias or temporary disturbance would have distracted the rats on any single day. Rather, these results suggest that there may be a subtle and short-lived intrinsic difference in the performance of OPFC animals when approaching the watermaze reversal task. During the retraining that occurred after the second transfer test, the significant group differences observed in path lengths, latency, and swim speed were the result of the OPFC rats swimming more quickly and accurately to the platform, primarily during the first two days of the retraining block. This behavior might suggest that the OPFC rats were mildly resistant to the extinction effect of the second no-platform transfer test. Such an effect could impair performance on reversals, as the animals would tend to continue swimming to the old platform position as opposed to abandoning it and employing an alternative cognitive strategy for ascertaining the new platform position. The reversal data are partly consistent with this notion, but the simple main effects result and the subsequent group effect seen in reversal 2 is almost wholly the result of one day's data.

6. Conclusion

Sections 3 and 4 provide evidence that rats with OPFC lesions are unimpaired in tests of spatial learning and reference memory, in contrast to the profound impairments seen in rats with HPC lesions. Section 5 hints at a possible weak OPFC effect in reversal learning, but the results obtained are due to longer path lengths observed in the OPFC group on day 5 only.

For a possible explanation of this isolated effect, it should be noted that the first reversal occurred after 12 days (48 trials) of training to a platform in a constant location

for each rat. After that much experience, it is possible that the rats habituated to the training aspect of the task—with a concurrent gradual shift in their cognitive representation of the training phase. The first day of reversals would thus have introduced a novel situation requiring the rats not only to develop a strategy for tackling reversals but also to break any habituation that may have developed. If this latter process had a large time constant—such as a day—it could have masked a subtle and transient OPFC-sensitive effect. Reversal 2 may have occurred after this habituation had diminished, unmasking the OPFC effect because the rats' behavior was dominated by a cognitive representation of the reversal phase alone. However, by reversal 3, the rats had experienced 8 days of reversals, possibly causing a new habituation to develop to the reversal phase as before. This habituation again may have been accompanied by a concurrent shift in the cognitive representation—perhaps causing the rats to treat the task as a test of spatial alternation like that of Chapter IV—thus re-masking the OPFC-sensitive effect.

Alternatively, it is possible that OPFC lesions may simply have rendered the rats impulsive and hyperactive, the latter claim supported by the higher mean swim speeds of OPFC rats during retraining. However, if this were the case, impulsivity would be expected to interfere with performance on the reversal task in a consistent and profound way—unlike what was observed.

These two alternative explanations for the transient OPFC effect would best be addressed by replication of the watermaze experiment. The methodology could be adjusted to employ an Atlantis platform during the transfer tests, as this would permit repeated transfer tests without danger of extinction. Alternatively, the transfer tests could be omitted altogether and the training phase shortened to a more focused single block of 8 days.

Chapter VII. Spontaneous Locomotor Activity

1. Background

During many of the studies undertaken, it was observed that there was a tendency for rats in the HPC group to behave in what appeared to be a hyperactive manner. These observations included significantly higher running speeds during reinforcer devaluation training (Chapter V.2) and noticeably faster average swim speeds during watermaze acquisition (Chapter VI.3). Additionally, the anxiolytic effects seen in the successive alleys test (Chapter III.3) could also be partially explained by a general increase in activity levels. Therefore, it was decided that a useful final test for this cohort of rats would be a quantitative assessment of hyperactivity via a test of spontaneous locomotor activity that utilized a paradigm successfully employed in the lab.[44]

2. Methods

The spontaneously-generated locomotor activity task is designed to quantify such activity by recording how many times a rat traverses a small wire cage. The rack-mounted cages were 25 cm high by 23 cm wide by 39 cm long (Modular Systems Developments Co. Ltd., Hereford, UK) and located in a darkened room. Infrared breakbeam sensors were placed at the sides of the cages 1.5 cm above the floor and were centered 13 cm from the front and rear ends of each cage. The emitter/detector pairs were connected to a Photobeam/Rotometer Activity Sensor (Paul Fray Ltd.). The output was then fed to an Acorn Archimedes RISC PC 600 computer running the Arachnid Activity Monitor software program (Paul Fray Ltd., Cambridge, UK). The software recorded total number of beambreaks as well as the number of crossovers, with a crossover event defined as a rat actuating a pair of breakbeam sensors sequentially. The test was

conducted in a series of 24 5-minute bins, for a testing session totaling two hours. Rats were allowed ad libitum access to food in their home cages prior to testing.

3. Results

The collected data appear in Figure 19. Of immediate impact is the reduced number of both beambreaks and crossovers for the OPFC group, particularly apparent in the first 10 intervals. There were no obvious visual differences between the plotted performance for the HPC and SHAM groups.

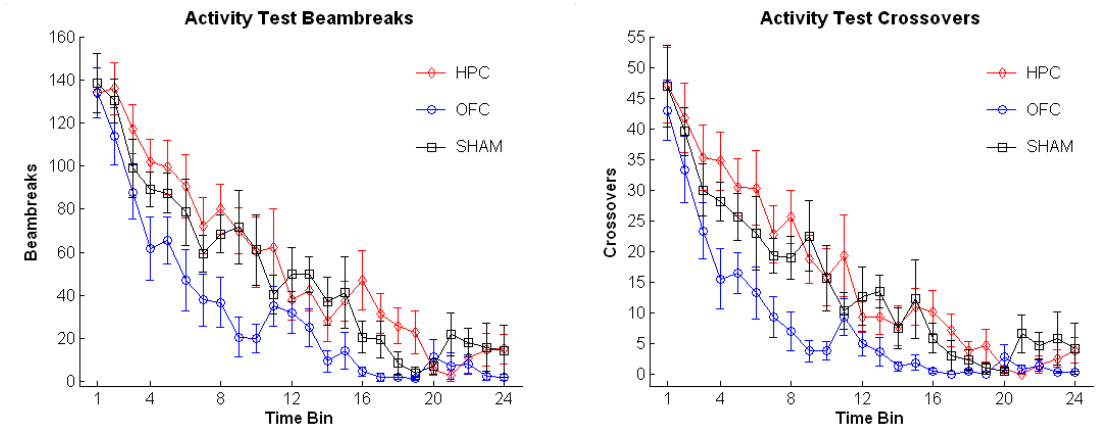


Figure 19. Activity test data, plotted across all 24 5-minute bins. Beambreak data appear in the left panel with crossover data in the right panel. As usual, error bars represent ±1 standard error of the mean.

A two-way ANOVA—with group as the between-subjects factor and time bin as the within-subjects factor—performed on beambreaks for the entire data set showed significant main effects of time bin ($F_{(23,690)} = 49.534$, $p < 0.0005$) and group ($F_{(2,30)} = 5.315$, $p = 0.011$). The group by time bin interaction was not significant ($F_{(46,690)} = 1.091$, $p = 0.318$). Post-hoc analysis with Tukey's HSD showed a significant difference between the HPC and OPFC groups only ($p = 0.011$) with the difference between the OPFC and SHAM groups almost significant ($p = 0.052$) and that between the HPCs and SHAMs clearly not significant ($p > 0.75$). Another ANOVA performed on crossover events for the full 24 time bins again showed significant main effects of bin ($F_{(23,690)} = 42.957$, $p < 0.0005$) and group ($F_{(2,30)} = 5.450$, $p = 0.010$). There was not a significant bin by group

interaction ($F_{(46,690)} = 1.190$, $p > 0.15$). Tukey's HSD post-hoc tests showed significant differences between the OPFCs and HPCs ($p = 0.010$) and between the OPFCs and SHAMs ($p = 0.042$) but not between the HPCs and SHAMs ($p > 0.8$).

The significant group effect was thus not the result of increased activity of the HPC group but the result of *reduced* activity of the OPFC group. This was most pronounced in the crossover test, in which the OPFC group's performance differed significantly from both the HPCs and SHAMs. The beambreaks test, although showing a significant group effect, was only able to differentiate OPFC performance from that of HPCs, though the OPFC-SHAM difference was barely not significant.

4. Conclusion

The lack of any increased activity for HPC rats was surprising, as at least some amount of hyperactivity might have been expected. As mentioned in Section 1, the HPC group displayed fewer anxiety-related behaviors, ran more quickly during devaluation training on the plus maze, and swam more quickly during watermaze acquisition trials. Prior studies confirm these predictions.[44,73] However, it must be remembered that the rats were over 12 months of age by the time locomotor activity was assessed. Studies have demonstrated age-related declines in locomotor activity in a variety of rat strains.[74,75] An overall decrease in activity due to advancing age may have blunted any HPC effect.

The significantly reduced OPFC activity levels were unexpected. Some prior work has paradoxically suggested that OPFC lesions might increase activity, although the relevant study employed a distinctly different assessment paradigm.[76] In contrast, other work has indicated no effect for OPFC lesions (Rudebeck PH et al., in preparation). It cannot be known what effect the age of the rats might have had on activity levels. It is conceivable that the observed results are due to an age-related decline in activity that is facilitated or amplified by OPFC lesions as opposed to their being an intrinsic result of

OPFC damage. A rigorous confirmation would best be obtained with a new cohort of rats destined for similar memory and goal-directed behavioral tasks that would receive activity tests at both the beginning and end of testing.

Chapter VIII. Producing Serotonergic Depletions in the Rat Orbital Prefrontal Cortex

1. Background

The next logical step following the work described in Chapter II with cytotoxic ablations in the OPFC and HPC will be to deplete neurotransmitters selectively in those regions to isolate any effects that are linked to local deficits in specific neurotransmitters, such as acetylcholine or serotonin. Prior work has shown that global pharmacological blockade of the muscarinic ACh receptor impairs executive function and attentional set shifting[49] whereas OPFC-specific cholinergic depletion impairs odor-guided associative learning.[77] Furthermore, serotonin in the OPFC has been implicated in frontal lobe function.[14,33,78] These studies suggest that neurotransmitters in the OPFC play an important role in the behaviors assessed via the experimental work described in Chapters III-VII. Although there is evidence for the specific contributions of both ACh and 5-HT to functions associated with the OPFC, less is known about the role of 5-HT in that region. It was thus decided that developing a technique for generating rats with selective 5-HT depletions in the OPFC would be a sensible choice for producing novel results, particularly if combined with a revised testing paradigm for the devaluation task of Chapter V.

2. Animals and Surgery

To that end, a cohort of six rats received unilateral pilot lesions to target 5-HT neurons in the OPFC using a variety of drugs and dosages. Surgery for all rats occurred during one session with the animals weighing 349-371 grams. Each rat was anaesthetized with 2.5% isoflurane, mounted in a stereotaxic frame, and received midline incision and craniotomy as described in Chapter II.2. Two rats received intra-orbital injections of 0.6 µg/µL anti-serotonin reuptake transporter (SERT)-saporin toxin (Advanced Targeting

Systems, San Diego, CA) and two received intra-orbital injections of 0.3 µg/µL anti-SERT-saporin toxin. The anti-SERT-saporin toxin conjugates a monoclonal antibody to the fourth extracellular domain of the serotonin re-uptake transporter with the ribosome-inactivating cytotoxin saporin and is a relatively novel molecule.[79] The final pair received intra-orbital injections of 2 µg/µL 5,7-dihydroxytryptamine (5,7-DHT) toxin. These final two rats also received i.p. injections of 10 mg/kg nomifensine and 10 mg/kg desipramine 10-15 minutes prior to toxin injection in order to protect noradrenergic neurons.[80,81] In all cases, the toxin was delivered in three separate injections, with coordinates and volumes as in Table 2. Injection occurred at a rate of 0.1 µL/minute with the needle left in place for 3 minutes after injection to allow diffusion of the neurotoxin away from the injection site. The syringe and needle were as described in Chapter II.2. After the procedure, the rats were sutured, injected with metacam (0.1 mL in 1 mL saline, s.c.), and returned to home cages. They received wet mash (made from a mixture of food pellets and glucose solution) as well as bottles of glucose solution for four days, after which they were returned to a standard ad libitum feeding regimen of hard food pellets and freely accessible water.

Table 2. Stereotaxic coordinates for unilateral selective 5-HT depletions of the OPFC, along with volumes of toxin injected. AP and ML coordinates are in millimeters relative to bregma (with positive AP values anterior to bregma and positive ML values in the left hemisphere). DV coordinates are in millimeters relative to the brain surface (with negative coordinates being deeper in the brain).

Lesion	**Coordinates**			**Volume (µL)**
	AP	**ML**	**DV**	
Orbital Prefrontal Cortex (left side only, 3 sites)	+4.0	+0.8	-3.4	0.150
	+3.7	+2.0	-3.6	0.200
	+3.2	+2.6	-4.4	0.150

3. Post-Mortem Histology

After an additional recovery period of three weeks, all rats were perfused following the procedure described in Chapter II.3. Histological analysis was performed on the brains of all rats using a 5-HT immunostain to assess the level of serotonergic

depletion. Additionally, cresyl violet stain was used to check for any nonspecific damage from the drugs or surgical techniques. Slides were created from coronal frozen sections 50 µm thick.

Representative photomicrographs from slides showing successful lesions with 5,7-DHT appear in Figure 20. It was found that a low-magnification view of the entire slice was most effective for qualitatively assessing the level of depletion. The 5,7-DHT injections (with nomifensine and desipramine premedications) resulted in a clear reduction in the number of surviving serotonergic projections, evidenced by a unilateral zone of pallor localized to the OPFC in the hemisphere that received toxin injection (the right hemisphere in all cases). This zone was apparent only in the immunostained sections; none of the cresyl violet stained sections showed any damage, verifying the specificity of the 5,7-DHT toxin. Neither dose of the anti-SERT-saporin toxin produced any discernible effects, nonspecific or otherwise (not pictured).

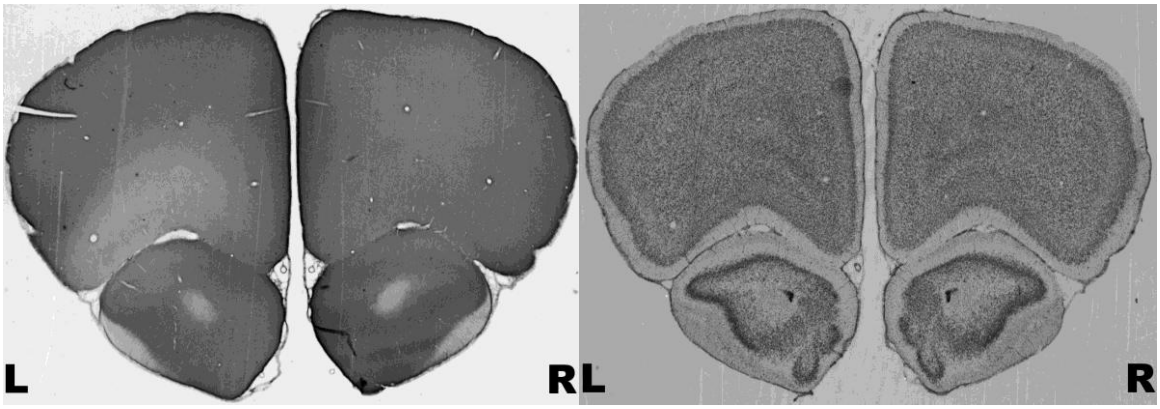


Figure 20. Representative photomicrographs from a rat that received unilateral 5,7-DHT injections (2 µg/µL) in the left hemisphere. A 5-HT immunostained section appears in the left panel, showing a distinct zone of pallor in the left OPFC (left-hand side of panel). An adjacent section stained with cresyl violet appears in the right panel, showing a lack of any nonspecific damage from the procedure.

The use of either toxin intra-orbitally is a novel procedure and based on the above results, it seems most sensible to use the regimen of nomifensine, desipramine and 5,7-DHT (at the dosages mentioned) to generate future rats with OPFC-specific 5-HT depletions for behavioral testing. However, the possibility of resprouting of 5-HT terminals is a concern that will have to be addressed in experimental design. Thus, it

would still be prudent to perform additional pilot surgeries with serially increased dosages of the SERT-saporin toxin, as the nomifensine and desipramine premedications are rather poorly tolerated in the rat. These drugs are also poorly tolerated in the monkey, which is an important concern in the likely event that serotonin depletions are desired in a primate model.

4. Future Work

Also worth consideration would be creation of animals with hippocampal 5-HT depletions. These might prove useful for assessing potential impairments in watermaze reversals, as rats with such lesions might further elucidate the complex results of Chapter VI. A review of the recent literature concerning serotonin depletions and spatial learning yielded conflicting reports. Two studies showed that loss of serotonin in the HPC following intracisternal 5,7-DHT injection[82] and direct bilateral 5,7-DHT injection into the CA1 region of the HPC[83] yielded no impairments in Morris watermaze (MWM) performance. However, the former study did not assess any global effects of the procedure and the latter only depleted a specific region within the hippocampus. Interestingly, a study in which the $5HT_3$ receptor antagonist granisetron was injected bilaterally into the CA1 region of the hippocampus[84] showed definite MWM impairments. Administering the 5HT1A agonist 8-OH-DPAT also impaired MWM performance.[85] Three additional studies[86,87,88] used 3,4-methylenedioxymethamphetamine (MDMA) to produce global 5-HT depletions, two of which showed some deficits in MWM transfer tests[86,88] while the remaining study[87] showed some impairment in reversals dependent on the MDMA treatment. In all studies the drug was administered subcutaneously and the depletions, when statistically significant, did not exceed 50% (and often were far less). While such depletions were of course sizable, they nonetheless left a nontrivial amount of 5-HT available in the brain.

Furthermore, MDMA has significant extra-serotonergic effects[89,90] that may have further confounded the experimental results. Further testing utilizing rats with specific 5-HT depletions throughout the entirety of the hippocampus would likely be helpful in clearing some of these discrepancies.

Thus, ideas for the composition of a new experimental cohort begin to take shape. Given the success of the 5,7-DHT OPFC depletions, several rats should be generated with the surgical procedure described above. Combined with a group with cytotoxic OPFC lesions and a group of controls, testing could then begin on a devaluation task using operant boxes and a selective satiety paradigm. A repeat of the watermaze task could also prove useful. At the same time, it would be useful to generate pilot lesions using 5,7-DHT in the HPC. If successful, future testing could then occur with the successive alleys and rewarded alternation tasks.

Chapter IX. The role of the Primate Orbital Prefrontal Cortex in Goal-Directed Behavior, Strategy Implementation, and Associative Learning

1. Background

A logical extension of this project would be to study the effects of excitotoxic lesions and selective neurotransmitter depletions in the OPFC of the primate brain. The region has already been implicated in goal-directed behavior[24] as well as spatial memory, discrimination reversals, and attentional set shifting,[61,91,92] while the whole frontal cortex has been implicated in performance of nonspatial tasks such as strategy implementation.[93] The use of primates is critical given the potential clinical applications of the work to neurodegenerative diseases such as AD. Monkeys' cortical complexity and organization more closely resemble those of humans than do those of the rodent brain. To that end, concurrent with the rat studies described in Chapters II-VIII, eight young, experimentally-naïve male rhesus macaque monkeys (*Macaca mulatta*) were preoperatively trained on a variety of tasks chosen in part to be broadly analogous to those employed with the rats. Additional tasks were selected as a bioassay for OPFC function. It is important to note, however, that for cases in which analogous tasks were used in both species, the paradigms were necessarily different when testing a primate versus a rodent. For example, when testing goal-directed behavior, the monkeys were trained on a selective satiety reinforcer devaluation task assessed using images on a computer touch screen as opposed to targets in a plus-maze.

Additional preoperative training occurred in tests of strategy implementation and object-in-place associative learning. Held in reserve were memory tasks for which training could occur postoperatively. These tasks included delayed non-matching-to-sample (DNMS) and discrimination reversals, versions of which were tested in the rat (Chapters IV and VI) and have been demonstrated to be susceptible to OPFC damage in

the monkey.[61,94] The increased complexity of all of these tasks as compared to rodent testing paradigms further underscores the necessity of a primate model.

2. Methods

All training utilized equipment and techniques already in existence at the Department of Experimental Psychology at Oxford University.[93,95] The apparatus consisted of six isolated testing chambers each containing a 38 cm by 28 cm touch-sensitive cathode ray tube display for stimulus presentation. In each chamber, the display was driven by a computer that also served as the data acquisition system. During a testing session, a monkey was placed in a wheeled metal transport cage that was fixed 15 cm from the display, sufficient for allowing the monkey to touch the screen by reaching between the horizontal bars of the cage. Activity was monitored by the experimenter from a separate control room via closed-circuit television. Reward pellets (190 mg) formulated for monkeys were delivered under computer control to a metal hopper centered below the display. At the conclusion of a session, a metal "lunchbox"—located below the display and to the left of the hopper—opened automatically, revealing a large reward consisting of proprietary monkey food, various fruits, and nuts. The monkeys received no other food on testing days apart from a small amount of forage mix (consisting of small seeds and rice grains) given in their home cages. Water was freely available in the home cages. The monkeys were group housed at all times except for the testing sessions.

As with the rats, the work was conducted in accordance with the UK Animals (Scientific Procedures) Act of 1986. The key elements of training and testing for the various tasks are explained below with appropriate references to detailed descriptions in the scientific literature. Prior to this training, all monkeys were habituated to the testing apparatus following methods previously developed.[96]

Goal-directed behavior was assessed with a reinforcer devaluation paradigm. Similar to rats,[25] devaluation is impaired in rhesus monkeys after bilateral lesions of the OPFC even though object discrimination and satiety appear to be preserved.[24] For this task, a paradigm originally developed for studying excitotoxic lesions of the amygdala was employed.[97] The monkeys were initially trained to discriminate between small images presented side-by-side on the touch-display. For each trial, one object was consistently associated with a reward, evenly distributed between two equally-preferred food items identified beforehand. Training was run in blocks of 60 trials per day until criterion was reached, after which point sessions of critical trials were inserted between test sessions. In these critical trials, the images were re-paired such that for each trial the monkey had to choose between two images, each being associated with one or the other of the foodstuffs.

In a paradigm analogous to that used with the rats (Chapter V), one of the two foodstuffs could then be devalued via selective satiation. The computer would then record how many times the monkey chose the devalued and non-devalued foods, providing a quantitative measure of any altered food preference.

Monkeys with lesions including—but not limited to—the OPFC have shown impairment in a strategy implementation task.[93] That task was employed in the current study, which was designed to assess the specific contributions of the OPFC itself—as well as neurotransmitter levels within the structure—to the strategy task. The task involved the monkey choosing between two images presented side-by-side on the display. One object had to be chosen persistently (P) to receive a food reward and the other sporadically (S) for the same reward. The number of times that the P object had to be touched for delivery of a single reward was a user-settable parameter in the control program. After receiving a P reward, the monkey received an additional reward for

touching the S object. The ratio of total trials to rewarded trials was the quantitative measure of performance on the task, with criterion set at 2.94 based on prior simulation.[93] Monkeys were usually trained in sessions ending after a predetermined number of rewards (usually no greater than 50) had been collected.

Training began with a single pair of images repeatedly displayed until the monkey reached criterion, with the number of pairs and the number of P touches necessary to receive a reward advanced in a systematic manner.[93] For the critical trials, all monkeys learned four pairs of P and S objects, with four P touches required per single reward. The order in which the P-S pairs were presented was randomized during test sessions to remove any sequence-learning bias. Thus, the monkeys' most efficient strategy for the task was to use the sequence *pppPSpppPS*, where capitalized letters indicate an earned reward. The ratio of total trials to rewarded trials corresponding to this optimal strategy is 5:2 or 2.5.

Bilateral lesions including—but not limited to—the OPFC of the rhesus monkey have been shown to impair performance in a test of associative learning of objects in scenes.[95] This test was also used in the current study, again with the aim of assessing the role of the OPFC itself along with neurotransmitter levels within the structure. Two computer-generated typographic characters of variable size and color appeared in a constant foreground location on the display. They were presented against a background of ellipses and ellipse segments of random location, size, and color. Training consisted of presenting a list of new scenes with a predetermined number of repetitions per scene, the eventual goal being 20 new scenes repeatedly presented in eight blocks for a given daily testing session. The order of scenes was the same for all blocks. One typographic character in each scene was consistently rewarded with a food pellet. Presses of the other, "incorrect" character in the first block resulted in 20 seconds of a blank screen

followed by a repeat presentation of the scene, but with only the correct character in the foreground. In the remaining blocks, touching an incorrect character resulted in the 20 second delay followed by the next scene in the list. If a monkey touched a part of the display not corresponding to one of the foreground characters, an "inaccurate" touch was registered, followed by 20 seconds of blank screen and a subsequent repeat presentation of the scene. The computer provided separate measures of the number of incorrect trials and inaccurate touches from the second block onwards in a given session.

3. Preliminary Data

At the time of writing, all monkeys remained in the preoperative phase of the experiment. For descriptive purposes, Figure 21 shows preoperative training data for the scene learning and strategy tasks from 16 monkeys, with training to scene learning preceding strategy training. Monkey numbers were assigned based on preoperative training performance. For reference, monkeys not part of the cohort are included in the plot. Specifically, monkeys 1 and 3-12 are not members of the cohort. Only monkeys that had completed the scene learning training phase are included in Figure 21. Monkeys 1-12 have completed both phases of preoperative training—scenes and strategy—whereas monkeys 13-16 are still in the strategy training phase. The number of sessions required to reach criterion for training to scenes ranged from approximately 20 to 120. The number of sessions needed to reach criterion for the more challenging strategy training ranged more widely from approximately 80 to 250.

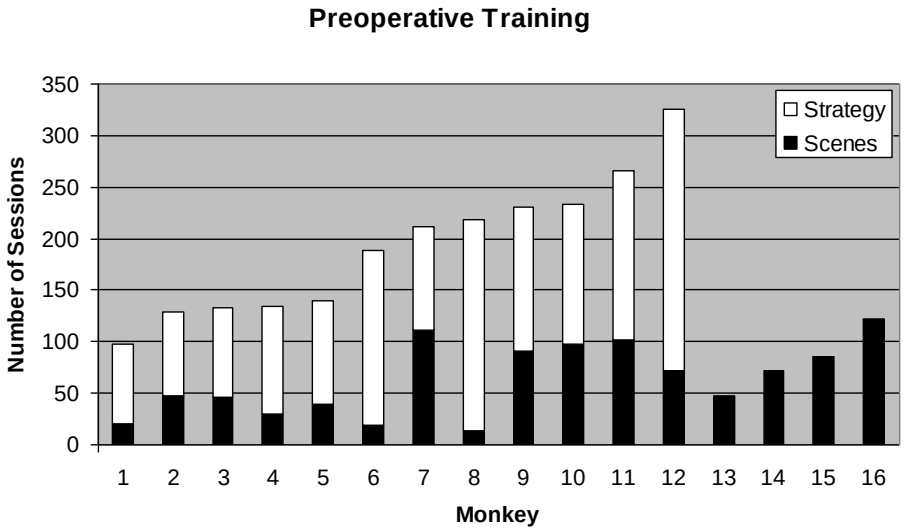


Figure 21. Preoperative training data for the scene learning and strategy tasks from 16 monkeys including some members of the current cohort (monkeys 2 and 13-16). The data are arranged by performance with monkey numbers assigned on that basis. Monkeys 1-12 have completed preoperative training; monkeys 13-16 are still training to the strategy task at time of writing.

Also for descriptive purposes, Figure 22 shows results of actual preoperative experimental trials for the scene learning task from 11 monkeys, arranged in order of increasing percentage error. Monkey numbers are as assigned for Figure 21. Note that these data are from monkeys 1 and 3-12; no members of the current cohort have completed this testing phase at the time of writing. The error rate in this task ranged from just under 2 percent to over 11 percent.

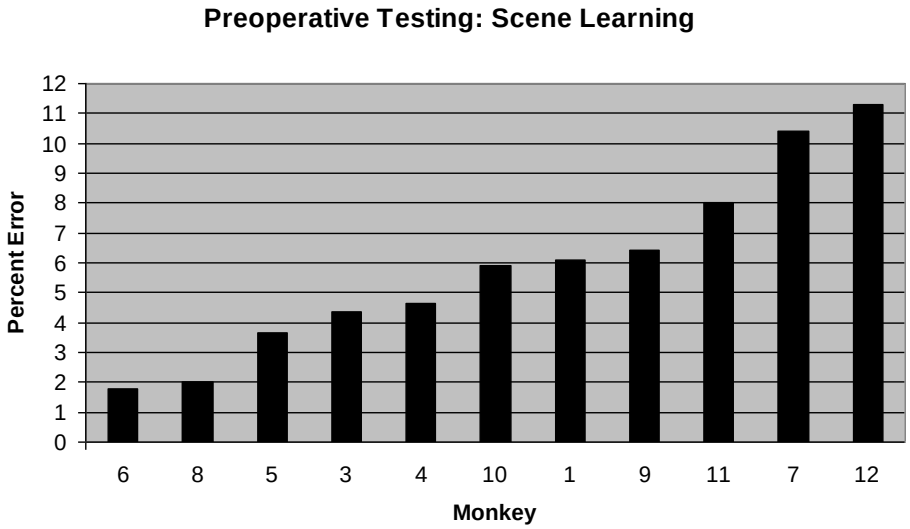


Figure 22. Performance in preoperative testing trials of scene learning from 11 monkeys, arranged in order of increasing percent error. Monkey numbers are as assigned in Figure 20 and do not include data from members of the cohort (i.e. all data are from monkeys 1 and 3-12).

4. Future Work

Preoperative training and testing of the monkeys will continue in the manner previously described. Unpublished data collected in the lab have suggested that bilateral OPFC lesions create deficits in the reinforcer devaluation task consistent with prior work[24] and moderately impair scene learning, but do not disrupt performance on the strategy task (M.G. Baxter, personal communication). The deficit in the reinforcer devaluation effect agrees with prior work,[24] and the negative effects on scene learning suggest future directions for continued assessment of the role of the OPFC in associative learning. The lack of an effect on strategy implementation is particularly notable as it contradicts the current understanding of the OPFC as being involved in behavioral inhibition[91,94,98] but does find support in other recent work.[99] These findings provide numerous avenues for extending study of the primate OPFC.

Once the cohort's preoperative phase is complete, some monkeys will receive selective neurotransmitter depletions in the OPFC. Serotonergic depletion would be an ideal choice given its correlate in the pilot lesions already generated in rats (Chapter VIII). Additionally, serotonergic depletions in the entire prefrontal cortex have been implicated in reversal learning in the marmoset,[92] leaving open the question of the specific role of 5-HT in the OPFC of the rhesus monkey. However, the premedications required when using 5,7-DHT are often poorly tolerated in monkeys. Use of this toxin therefore risks creating unacceptable sequelae to the surgical intervention. Furthermore, there is the possibility of resprouting of 5-HT terminals, just as with the rats. The anti-SERT-saporin immunotoxin is a superior option from an animal welfare standpoint, but additional work is required to determine appropriate dosages for intra-orbital injection. Alternatively, a different neurotransmitter system could be explored by creating OPFC-specific cholinergic depletions with another immunotoxin such anti-p75-saporin. Both

options are viable as our knowledge of the relative contributions of 5-HT and ACh to OPFC function in the primate brain remains incomplete. A reasonable testing cohort might then consist of some animals with selective neurotransmitter depletions, some with nonspecific OPFC lesions—generated by surgical removal, aspiration, or excitotoxin—and some control animals.

Given the preoperative training history of these monkeys, all subjects in this theoretical cohort would then be tested postoperatively on reinforcer devaluation, strategy implementation, and object-in-place learning. Statistical comparison would be made either to preoperative performance in the same animals or to postoperative performance in the control group. With the preoperative training on the strategy task, the experimental cohort could also be trained and tested postoperatively on the aforementioned memory tasks—DNMS and reversals—following the reinforcer devaluation. An important caveat would be the potential effect of the lesions on acquisition of the task, as impaired acquisition would cast doubts on the relevance of any experimental results. At the conclusion of the monkeys' testing careers, all generated lesions would be verified by histology. Regardless of the specific experimental choices, the battery of tests available for postoperative use allows the attractive opportunity to compare and synthesize data across three different mammalian species—rat, monkey, and human.

Chapter X: General Discussion

In this project, a variety of investigations were undertaken in both the Lister hooded rat and rhesus macaque monkey. The general aim was to better characterize the relative functions of the OPFC and HPC in these species and attempt to address some of the discrepancies in the literature (Chapter I). Doing so would extend our incomplete knowledge of how damage to these regions can impair many aspects of behavior with applications to understanding the etiology of neurodegenerative diseases such as AD. The bulk of the experimental results were derived from studies with rats (Chapter II-VIII), though additional studies were begun with monkeys (Chapter IX). In the rat, the experimental program was intended to dissociate the functions of the OPFC and HPC as well as to identify tasks expressly sensitive to OPFC damage, as OPFC-specific tasks would be useful in future investigations of selective neurotransmitter depletions. The monkey studies are ongoing due to the significantly greater timescale for training and testing of primates. For both species, there are clear directions for future work, as addressed in Chapter XI.

The first results obtained demonstrated the relative contributions of the OPFC and HPC to anxiety behavior in the rat. Tests of hyponeophagia and successive alleys (Chapter III) clearly showed that bilateral excitotoxic HPC ablation was consistently anxiolytic. HPC rats were quicker to eat a novel food in a potentially anxiogenic environment during the hyponeophagia test and spent greater time in the more anxiogenic distal alleys of the successive alleys apparatus. The OPFC group showed a dissociation, performing no differently than HPC animals in the hyponeophagia test but no differently than controls in the successive alleys task. Thus while the HPC appears fundamental to generating appropriate anxiety behavior, the OPFC appears to play a different role. In particular, these findings suggest that the OPFC may modulate the anxiety response

produced by the HPC based on environmental context and other stimuli, serving in an integrative capacity rather than a generative one. Such a theory would take into account the known importance of the OPFC in executive function and decision-making as well as its connectivity with the HPC and other structures.

Tests of spatial memory and behavioral flexibility next occurred in the rats using rewarded alternation (Chapter IV) and the Morris watermaze (Chapter VI). In the former task, the no-delay version was essentially a non-matching-to-position task, broadly analogous to the non-matching-to-sample task on which the monkeys were trained, whereas the with-delay version was more akin to a delayed response task. HPC rats were found to be consistently and profoundly impaired in both no-delay and with-delay variants of the rewarded alternation task. Regarding the watermaze study, the initial eight days of training also showed a clear deficit in acquisition for the HPC group. Taken together, these findings underscore the key role of that structure in spatial memory. The parallel lack of impairment in the OPFC group—especially despite the rewarded alternation task's lengthy delay and potentially high interference—additionally shows the relative unimportance of that structure in spatial memory. Similarly, in the monkey surgical ablation of the OPFC did not impair spatial working memory in the delayed response task, [62] but lesions to the ventromedial and dorsolateral prefrontal cortex did impair spatial delayed response—though with slightly different effects.[61] Work with the current cohort of monkeys will thus help to verify these results and further dissociate between the local contributions of different neurotransmitter systems.

The combination of much higher swim speeds during watermaze acquisition and higher running speeds during devaluation training (explored further below) additionally suggested a role for the hippocampus in suppressing hyperactive behavior, as absence of the structure appeared to promote hyperactivity. The acid test explicitly designed for

assessing hyperactivity is the spontaneous locomotor activity task performed in Chapter VII. Although that task demonstrated a significant group main effect, the difference was due to *hypoactivity* of the OPFC group relative to controls with no difference observed between the HPC and SHAM groups. It is possible that the increasing age of the rats may have confounded the study,[74,75] as the lack of hyperactivity in the HPC group is consistent with neither prior observations in the earlier phases of the experimental program nor prior experimental work.[44,47]

Reinforcer devaluation (Chapter V) is a classic test of goal-directed behavior—and thus OPFC function—and was of particular relevance to this project. Unfortunately, the use of a plus-maze testing paradigm with the rats proved to be problematic. Devaluing a food by either selective satiety or LiCl-induced aversion failed, even though taste aversion was successfully produced in the latter case. There are a number of reasons why the plus-maze paradigm may not have been successful, chief among them being a generalized habituation to maze-running based on the rats' prior testing history or a generalized extinction overshadowing a subtler devaluation effect. These confounding factors may have resulted in a failure to associate the reduced incentive value of a foodstuff with the appropriate alley on the maze. Age-related effects are also possible, as are specific procedural errors including excessive satiety or lack of running experience during devaluation sessions. Improvements in the technique could be achieved by using a three-alley (six-arm) radial maze, as that apparatus would allow continual maze-running experience during devaluation by training the animals to a third neutral foodstuff. More drastically, the devaluation test could be adapted to operant boxes.[68] The latter case is most likely to produce conclusive results, especially when coupled with Pavlovian aversion conditioning produced by LiCl injections. Given the importance of reinforcer devaluation testing to the aims of this project and the fact that the monkeys will also be

tested on devaluation by selective satiety, there is a strong impetus for rectifying the difficulties encountered. Testing of monkeys is necessary because translating results from the laboratory to the clinic will first require studies in an animal model exhibiting cortical sophistication approaching that of the human. Analogous studies in rats would directly aid such primate studies, as the relative speed with which rats can be trained and tested would help refine the monkey paradigm with a minimal ethical and financial toll. With the task operating in the rodent and primate, there would be potential for synthesizing results across mammalian species. Analogous results in the rat and monkey would provide a strong case for devaluation behavior being a fundamental product of mammalian evolution.

Reference memory was assessed in the rats with the two watermaze transfer tests after 4 and 8 days of training in acquisition (Chapter VI.4). In the second transfer test, the HPC animals more readily abandoned the training quadrant (as shown by the binned time analysis). Because HPC lesions rendered the rats unable to recall the platform position, this abandonment of the training quadrant might be a reflection of these rats immediately switching to a search mode, increasing their chances of escape but reducing the time spent in the training quadrant. Nonetheless, bilateral damage to the HPC disrupts reference memory in addition to the other forms of spatial memory already discussed. As before, OPFC damage exhibited no discernible effect, thus suggesting a lack of involvement of that structure in reference memory.

Reversal learning in the watermaze (Chapter VI.5) also showed a weak potential deficit in the OPFC group, though that effect was only apparent when examining the second reversal block and was driven by a single day's data. Overall, the OPFC animals were relatively unimpaired in serial reversals, a surprising result in light of prior work in the marmoset implicating the prefrontal cortex[92,98,100]—and more recently the OPFC

specifically[101]—in suppressing impulsivity and permitting behavioral flexibility. Although these conflicting findings could be the result of inter-species differences, further study is clearly required. It should be noted that the OPFC rats exhibited faster swim speeds, shorter path lengths, and shorter latencies when reacquiring the platform position during the four days of retraining, suggesting that OPFC damage may have produced a slight resistance to extinction. Such an effect would impair performance on reversals, as it would render the OPFC animals less likely to abandon the old quadrant in search of the new platform position. However, the lack of a reliable group effect throughout the entirety of the reversal phase of the task suggests that this theorized extinction resistance is at best highly transient. This transience might be the result of broad shifts in the animals' cognitive approach to the serial reversals problem due to increasing experience with the task, but it is impossible to make a credible statement without replication studies.

In a more technical vein, this project also verified the technique of intra-orbital injection of 5,7-DHT in the rat, successfully creating selective depletions of 5-HT in the OPFC as verified by immunohistology (Chapter VIII). These selective depletions are a potentially useful experimental tool for both the rat and the monkey. The need for noradrenergic protective drugs is suboptimal, however, motivating continued investigative work with the anti-SERT-saporin toxin.

Testing monkeys will allow comparisons with rats along several dimensions of behavior. Reinforcer devaluation will follow a selective satiety paradigm similar to that used in the rats, serving in both cases as a measure of goal-directed behavior. DNMS in the monkeys could be compared to the no-delay version of the rewarded alternation task in rats, while the with-delay version would be akin to a test of delayed response. Alternatively, the rats could be tested on a delayed non-matching to place task using the plus-maze to allow explicit comparison to DNMS in the monkey. Finally, discrimination

reversals in the monkey could be broadly related to the watermaze spatial reversals assessed in the rat, both serving as measures of behavioral flexibility. There are of course major differences in how the mechanics of how the tasks are conducted in each species. For example, all of the tests on the rat rely on the animals' movement through space. Such movement is impossible with the monkeys as they are confined within small transport cages fixed in front of a touch-activated computer screen. Although there is still a spatial component to these tasks, it is putatively less pronounced when the animal is stationary within a static environment as opposed to moving through it. Nonetheless, the tasks have broad similarities across both species and useful integrative comments are still possible. Of primary experimental importance is the fact that the increased sophistication of the monkey brain allows testing of more complex behaviors, such as strategy implementation and associative learning.

The monkey studies, when complete, would thus allow cross-species comparisons of functional homology of the OPFC between rats, monkeys, and humans, complementing the structural homology—as primarily defined by connectivity—described in Chapter I. The studies begun in this project will continue to delineate these behavioral similarities and differences, allowing improved extrapolation from one animal model to another. With an improved understanding of these animal models in place, experimental results could be related in a more sophisticated way to similar deficits in humans resulting from ailments such as AD.[14,17] Ultimately, targeted therapeutic methods might be developed, helping to enhance a clinical armamentarium that currently offers little hope to patients.

Chapter XI. Conclusions and Future Work

This project strove to characterize several functions of the orbital prefrontal cortex and hippocampus in anxiety behavior, spatial learning and memory, and goal-directed behavior. Dissociations of function were demonstrated between the two regions, verifying that the HPC plays a fundamental role in anxiety behavior (hyponeophagia, successive alleys) and spatial learning and memory (rewarded alternation, watermaze acquisition). The OPFC was necessary in anxiety behaviors involving presentation a novel foodstuff in a potentially anxiogenic environmental context (hyponeophagia) but was otherwise unimpaired in a strict anxiety test (successive alleys), possibly suggesting a higher-level modulatory role for the structure. Spatial learning and memory were unimpaired by OPFC lesions. Retraining in the Morris watermaze following two transfer tests revealed an OPFC-dependent effect that may hint at a role for the structure in generating appropriate extinction behaviors. OPFC lesions also apparently resulted in locomotor hypoactivity, though this finding may have been confounded by the age of the rats. Additionally, the project successfully demonstrated proof-of-concept for using intra-orbital 5,7-DHT frontally in the rat to produce selective 5-HT depletions. Finally, training and testing of rhesus monkeys was begun on a variety of tasks chosen to be putatively sensitive to OPFC damage as well as analogous to tests used in the rats.

There are numerous opportunities for continued work in both the immediate and distant future. Replication studies of the watermaze task—perhaps with focused training and no transfer tests—would be prudent in clarifying the observed OPFC effect during retraining and determining if the OPFC effect in reversal 2 is legitimate and reproducible.

A better assay for OPFC function is reinforcer devaluation. Thus, the unsuccessful plus-maze devaluation paradigm would ideally be adapted to operant boxes—successfully used in the past—to generate useful data. A new cohort of animals

could be prepared with experimental groups consisting of selective serotonergic OPFC depletions, total OPFC excitotoxic lesions, and sham controls. Testing these animals on devaluation and reversals would allow a focused study of OPFC function with the ability of localizing effects to orbital prefrontal serotonin levels.

Pilot surgeries could also be performed with 5,7-DHT in the HPC and anti-SERT-saporin in both OPFC and HPC. If these are successful, new cohorts could be generated, as having animals with selective and total lesions of the OPFC and the HPC would allow detailed separations of function between brain regions as well as characterization of the specific role of the serotonergic neurotransmitter system.

The ongoing work with the monkeys allows further opportunities for future research. Once the preoperative phase is complete, the animals will receive surgery and postoperative testing. Numerous surgical options exist, including complete or selective lesions of the OPFC, with the selective lesions being to either the serotonergic or cholinergic systems. Selective 5-HT depletions would ideally use a toxin that does not require the premedications necessary with 5,7-DHT. After surgery, testing would include tasks of reinforcer devaluation, strategy, associative learning, DNMS, and discrimination reversals. Comparisons between different experimental groups or between preoperative and postoperative performance levels would allow further elucidation of the functions of the OPFC and the neurotransmitter systems serving it.

These future studies will produce a wealth of data in both rat and monkey models, allowing integrative statements to be made about functional homology of brain regions—chiefly the OPFC—between species. Synthesizing with data from human patients would allow the possibility of further extension of this homology and may ultimately offer clinical insight into the development of better treatments for AD and other neurodegenerative diseases.

References

[1] Cummings, JL. Alzheimer's disease. *N Engl J Med.* 1 Jul 2004. 351(1):56-67.

[2] Norman, HJ. *Mental Disorders: A Handbook for Students and Practitioners*. 1928. Edinburgh: E. & S. Livingstone, 231-232.

[3] Tariot PN. Contemporary issues in the treatment of Alzheimer's disease: tangible benefits of current therapies. *J Clin Psychiatry.* 2006. 67 Suppl 3:15-22; quiz 23.

[4] Lipton SA. The molecular basis of memantine action in Alzheimer's disease and other neurologic disorders: low-affinity, uncompetitive antagonism. *Curr Alzheimer Res.* Apr 2005. 2(2):155-65.

[5] Lanari A, Amenta F, Silvestrelli G, Tomassoni D, Parnetti L. Neurotransmitter deficits in behavioural and psychological symptoms of Alzheimer's disease. *Mech Ageing Dev.* Feb 2006. 127(2):158-65. Epub 16 Nov 2005.

[6] Attems J, Jellinger KA. Hippocampal sclerosis in Alzheimer disease and other dementias. *Neurology*. 14 Mar 2006. 66(5):775.

[7] Wenk GL. Neuropathologic changes in Alzheimer's disease: potential targets for treatment. *J Clin Psychiatry*. 2006. 67 Suppl 3:3-7; quiz 23.

[8] Pahwa R, Factor SA, Lyons KE, Ondo WG, Gronseth G, Bronte-Stewart H, Hallett M, Miyasaki J, Stevens J, Weiner WJ. Practice Parameter: treatment of Parkinson disease with motor fluctuations and dyskinesia (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology*. 11 Apr 2006. 66(7):983-95.

[9] Fearnley JM, Lees AJ. Ageing and Parkinson's disease: substantia nigra regional selectivity. *Brain*. Oct 1991. 114 (Pt 5):2283-301.

[10] Kalaria RN, Andorn AC, Tabaton M, Whitehouse PJ, Harik SI, Unnerstall JR. Adrenergic receptors in aging and Alzheimer's disease: increased beta 2-receptors in prefrontal cortex and hippocampus. *J Neurochem.* Dec 1989. 53(6):1772-81.

[11] Kalaria RN, Andorn AC. Adrenergic receptors in aging and Alzheimer's disease: decreased alpha 2-receptors demonstrated by [3H]p-aminoclonidine binding in prefrontal cortex. *Neurobiol Aging*. Mar-Apr 1991. 12(2):131-6.

[12] Akbarian S, Smith MA, Jones EG. Editing for an AMPA receptor subunit RNA in prefrontal cortex and striatum in Alzheimer's disease, Huntington's disease and schizophrenia. *Brain Res.* 20 Nov 1995. 699(2):297-304.

[13] Rahman S, Sahakian BJ, Hodges JR, Rogers RD, Robbins TW. Specific cognitive deficits in mild frontal variant frontotemporal dementia. *Brain*. Aug 1999. 122 (Pt 8):1469-93.

[14] Rahman S, Robbins TW, Sahakian BJ. Comparative cognitive neuropsychological studies of frontal lobe function: implications for therapeutic strategies in frontal variant frontotemporal dementia. *Dement Geriatr Cogn Disord.* 1999. 10 Suppl 1:15-28.

[15] Rahman S, J Sahakian B, N Cardinal R, Rogers R, Robbins T. Decision making and neuropsychiatry. *Trends Cogn Sci*. 1 Jun 2001. 5(6):271-277.

[16] Starkstein SE, Migliorelli R, Teson A, Sabe L, Vazquez S, Turjanski M, Robinson RG, Leiguarda R. Specificity of changes in cerebral blood flow in patients with frontal lobe dementia. *J Neurol Neurosurg Psychiatry*. Jul 1994. 57(7):790-6.

[17] Berlin HA, Rolls ET, Kischka U. Impulsivity, time perception, emotion and reinforcement sensitivity in patients with orbitofrontal cortex lesions. *Brain*. May 2004. 127(Pt 5):1108-26. Epub 25 Feb 2004.

[18] Brown VJ, Bowman EM. Rodent models of prefrontal cortical function. *Trends Neurosci*. Jul 2002. 25(7):340-3.

[19] Uylings HB, Groenewegen HJ, Kolb B. Do rats have a prefrontal cortex? *Behav Brain Res*. 30 Nov 2003. 146(1-2):3-17.

[20] Rose JE, Woolsey CN. The orbitofrontal cortex and its connections with the mediodorsal nucleus in rabbit, sheep and cat. *Res. Publ. Assn. Nerv. Dis*. 1948. 27:210-232.

[21] Uylings HB, van Eden CG. Qualitative and quantitative comparison of the prefrontal cortex in rat and in primates, including humans. *Prog Brain Res*. 1990. 85:31-62.

[22] Dalley JW, Cardinal RN, Robbins TW. Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. *Neurosci Biobehav Rev*. Nov 2004. 28(7):771-84.

[23] Povysheva NV, Gonzalez-Burgos G, Zaitsev AV, Kroner S, Barrionuevo G, Lewis DA, Krimer LS. Properties of excitatory synaptic responses in fast-spiking interneurons and pyramidal cells from monkey and rat prefrontal cortex. *Cereb Cortex*. Apr 2006. 16(4):541-52. Epub 20 Jul 2005.

[24] Izquierdo A, Suda RK, Murray EA. Bilateral orbital prefrontal cortex lesions in rhesus monkeys disrupt choices guided by both reward value and reward contingency. *J Neurosci*. 25 Aug 2004. 24(34):7540-8.

[25] Gallagher M, McMahan RW, Schoenbaum G. Orbitofrontal cortex and representation of incentive value in associative learning. *J Neurosci.* 1 Aug 1999. 19(15):6610-4.

[26] McAlonan K, Brown VJ. Orbital prefrontal cortex mediates reversal learning and not attentional set shifting in the rat. *Behav Brain Res*. 30 Nov 2003. 146(1-2):97-103.

[27] Joel D, Weiner I, Feldon J. Electrolytic lesions of the medial prefrontal cortex in rats disrupt performance on an analog of the Wisconsin Card Sorting Test, but do not disrupt latent inhibition: implications for animal models of schizophrenia. *Behav Brain Res*. May 1997. 85(2):187-201.

[28] Birrell JM, Brown VJ. Medial frontal cortex mediates perceptual attentional set shifting in the rat. *J Neurosci*. 1 Jun 2000. 20(11):4320-4.

[29] Robbins TW. Chemistry of the mind: neurochemical modulation of prefrontal cortical function. *J Comp Neurol*. 5 Dec 2005. 493(1):140-6.

[30] Chudasama Y, Robbins TW. Functions of frontostriatal systems in cognition: Comparative neuropsychopharmacological studies in rats, monkeys and humans. *Biol Psychol*. Jul 2006. 73(1):19-38. Epub 20 Mar 2006.

[31] Winstanley CA, Theobald DE, Cardinal RN, Robbins TW. Contrasting roles of basolateral amygdala and orbitofrontal cortex in impulsive choice. *J Neurosci.* 19 May 2004. 24(20):4718-22.

[32] Mobini S, Body S, Ho MY, Bradshaw CM, Szabadi E, Deakin JF, Anderson IM. Effects of lesions of the orbitofrontal cortex on sensitivity to delayed and probabilistic reinforcement. *Psychopharmacology* (Berl). Mar 2002. 160(3):290-8. Epub 25 Jan 2002.

[33] Winstanley CA, Theobald DE, Dalley JW, Cardinal RN, Robbins TW. Double dissociation between serotonergic and dopaminergic modulation of medial prefrontal and orbitofrontal cortex during a test of impulsive choice. *Cereb Cortex*. Jan 2006. 16(1):106-14. Epub 13 Apr 2005.

[34] Hatcher PD, Brown VJ, Tait DS, Bate S, Overend P, Hagan JJ, Jones DN. 5-HT6 receptor antagonists improve performance in an attentional set shifting task in rats. *Psychopharmacology (Berl)*. Sep 2005. 181(2):253-9. Epub 14 Oct 2005.

[35] Parent MB, Baxter MG. Septohippocampal acetylcholine: involved in but not necessary for learning and memory? *Learning and Memory*. 2004. 11:9-20.

[36] Floresco SB, Seamans JK, Phillips AG. Selective roles for hippocampal, prefrontal cortical, and ventral striatal circuits in radial-arm maze tasks with or without a delay. *J Neurosci.* 1 Mar 1997. 7(5):1880-90.

[37] Morris RG, Garrud P, Rawlins JN, O'Keefe J. Place navigation impaired in rats with hippocampal lesions. *Nature.* 24 Jun 1982. 297:681-683.

[38] Morris RG, Hagan JJ, Rawlins JN. Allocentric spatial learning by hippocampectomised rats: a further test of the "spatial mapping" and "working memory" theories of hippocampal function. *Q J Exp Psychol B.* Nov 1986. 38(4):365-95.

[39] Squire LR. Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. *Psychol Rev*. Apr 1992. 99(2):195-231. Erratum in: *Psychol Rev*. Jul 1992. 99(3):582.

[40] Sutherland RJ, Whishaw IQ, Kolb B. Contributions of cingulate cortex to two forms of spatial learning and memory. *J. Neurosci*. Jun 1988. 8(6):1863-1872.

[41] Moser MB, Moser EI, Forrest E, Andersen P, Morris RG. Spatial learning with a minislab in the dorsal hippocampus. *Proc Natl Acad Sci U S A*. 10 Oct 1995. 92(21):9697-701.

[42] Moser MB, Moser EI. Functional differentiation in the hippocampus. *Hippocampus*. 1998. 8(6):608-19.

[43] Moser MB, Moser EI. Distributed encoding and retrieval of spatial memory in the hippocampus. *J. Neurosci.* 15 Sep 1998. 18(18):7535-42.

[44] Bannerman DM, Deacon RM, Offen S, Friswell J, Grubb M, Rawlins JN. Double dissociation of function within the hippocampus: spatial memory and hyponeophagia. *Behavioral Neuroscience*. 2002. 116(5):884-901.

[45] Fortin NJ, Agster KL, Eichenbaum HB. Critical role of the hippocampus in memory for sequences of events. *Nat Neurosci*. May 2002. 5(5):458-62.

[46] Lee I, Kesner RP. Differential contributions of dorsal hippocampal subregions to memory acquisition and retrieval in contextual fear-conditioning. *Hippocampus*. 2004. 14(3):301-10.

[47] McHugh SB, Deacon RM, Rawlins JN, Bannerman DM. Amygdala and ventral hippocampus contribute differentially to mechanisms of fear and anxiety. *Behav Neurosci*. Feb 2004. 118(1):63-78.

[48] Bannerman DM, Rawlins JN, McHugh SB, Deacon RM, Yee BK, Bast T, Zhang WN, Pothuizen HH, Feldon J. Regional dissociations within the hippocampus—memory and anxiety. *Neurosci Biobehav Rev*. May 2004. 28(3):273-83.

[49] Chen KC, Baxter MG, Rodefer JS. Central blockade of muscarinic cholinergic receptors disrupts affective and attentional set shifting. *European J. Neuroscience*. 2004. 20:1081-1088.

[50] Kolb B, Sutherland RJ, Whishaw IQ. A comparison of the contributions of the frontal and parietal association cortex to spatial localization in rats. *Behavioral Neuroscience.* Feb 1983. 97(1):13-27.

[51] Rudebeck PH, Walton ME, Bannerman DM, Rushworth MF. Contrasting decision making in rat orbital frontal and anterior cingulate cortices. *Society for Neuroscience*. 2004. 30.

[52] Howell DC. *Statistical Methods for Psychology (Fifth Ed)*. Duxbury. 2002. 320-466.

[53] Duncan GE, Knapp DJ, Breese GR. Neuroanatomical characterization of Fos induction in rat behavioral models of anxiety. *Brain Res*. 25 Mar 1996. 713(1-2):79-91.

[54] Walton ME, Rudebeck PH, Rushworth MF, Bannerman DM. The anterior cingulate and not the orbital frontal cortex is important for social behavior in rats. Program No. 323.10. *2005 Abstract Viewer/Itinerary Planner*. Washinton, DC: Society for Neuroscience. 2005. Online.

[55] Bannerman DM, Grubb M, Deacon RM, Yee BK, Feldon J, Rawlins JN. Ventral hippocampal lesions affect anxiety but not spatial learning. *Behav Brain Res*. 17 Feb 2003. 139(1-2):197-213.

[56] Britton DR, Britton KT. A sensitive open field measure of anxiolytic drug activity. *Pharmacol Biochem Behav*. Oct 1981. 15(4):577-82.

[57] Shephard RA, Broadhurst PL. Effects of diazepam and picrotoxin on hyponeophagia in rats. *Neuropharmacology*. Aug 1982. 21(8):771-3.

[58] Deacon RM, Croucher A, Rawlins JN. Hippocampal cytotoxic lesion effects on species-typical behaviours in mice. *Behav Brain Res*. 14 May 2002. 132(2):203-13.

[59] Shepherd JK, Grewal SS, Fletcher A, Bill DJ, Dourish CT. Behavioural and pharmacological characterisation of the elevated "zero-maze" as an animal model of anxiety. *Psychopharmacology (Berl)*. Sep 1994. 116(1):56-64.

[60] Bannerman DM, Yee BK, Good MA, Heupel MJ, Iversen SD, Rawlins JN. Double dissociation of function within the hippocampus: a comparison of dorsal, ventral, and complete hippocampal cytotoxic lesions. *Behav Neurosci*. Dec 1999. 113(6):1170-88.

[61] Meunier M, Bachevalier J, Mishkin M. Effects of orbital frontal and anterior cingulate lesions on object and spatial memory in rhesus monkeys. *Neuropsychologia*. 1997. Vol. 35, No. 7:999-1015.

[62] Bachevalier J, Mishkin M. Visual recognition impairment follows ventromedial but not dorsolateral prefrontal lesions in monkeys. *Behav Brain Res*. Jun 1986. 20(3):249-61.

[63] Paredes-Olay C, Lopez M. Comparison of the instrumental reinforcer devaluation effect in two strains of rats (Wistar and Lister). *Behav Processes*. 17 Aug 2000. 50(2-3):165-169.

[64] Rescorla RA. Depression of an instrumental response by a single devaluation of its outcome. Q J Exp Psychol B. Feb 1992. 44(2):123-36.

[65] Paredes-Olay C, Lopez M. Lithium-induced outcome devaluation in instrumental conditioning: dose-effect analysis. *Physiol Behav*. 15 Apr 2002. 75(5):603-9.

[66] Corbit LH, Balleine BW. The role of the hippocampus in instrumental conditioning. *J Neurosci*. 1 Jun 2000. 20(11):4233-9.

[67] Johnson AW, Bannerman DM, Rawlins NP, Sprengel R, Good MA. "Impaired outcome-specific devaluation of instrumental responding in mice with a targeted deletion of the AMPA receptor glutamate receptor 1 subunit." *J Neurosci.* 2 Mar 2005. 25(9):2359-65.

[68] Hatfield T, Han JS, Conley M, Gallagher M, Holland P. Neurotoxic lesions of basolateral, but not central, amygdala interfere with Pavlovian second-order conditioning and reinforcer devaluation effects. *J. Neurosci.* 15 Aug 1996. 16(16):5256-65.

[69] Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods*. May 1984. 11(1):47-60.

[70] Stewart CA, Morris RGM. The watermaze. *Behavioral Neuroscience: A Practical Approach (Vol I)* A Sahgal (ed). Oxford University Press. 1993. 107-122.

[71] Morris RG. Spatial localization does not require the presence of local cues. *Learning and Motivation*. 1981. 12:239-260.

[72] Markowska AL, Long JM, Johnson CT, Olton DS. Variable-interval probe test as a tool for repeated measurements of spatial memory in the water maze. *Behav Neurosci.* Aug 1993. 107(4):627-32.

[73] Mundy WR, Tilson HA. Behavioral impairment in the rat after colchicine lesions of the hippocampus and nucleus basalis. *Neurotoxicology*. Fall 1988. 9(3):511-9.

[74] Jucker M, Oettinger R, Battig K. Age-related changes in working and reference memory performance and locomotor activity in the Wistar rat. *Behav Neural Biol.* Jul 1988. 50(1):24-36.

[75] Casadesus G, Shukitt-Hale B, Joseph JA. Automated measurement of age-related changes in the locomotor response to environmental novelty and home-cage activity. *Mech Ageing Dev.* Oct 2001. 122(15):1887-97.

[76] de Bruin JP, van Oyen HG, Van de Poll N. Behavioural changes following lesions of the orbital prefrontal cortex in male rats. *Behav Brain Res*. Dec 1983. 10(2-3):209-32.

[77] Ross RS, McGaughy J, Eichenbaum H. Acetylcholine in the orbitalfrontal cortex is necessary for the acquisition of a socially transmitted food preference. *Learning and Memory*. May-Jun 2005. 12(3):302-6.

[78] Dringenberg HC, Vanderwolf CH. Neocortical activation: modulation by multiple pathways acting on central cholinergic and serotonergic systems. *Exp Brain Res*. Aug 1997. 116(1):160-74.

[79] Nattie EE, Li A, Richerson G, Lappi DA. Medullary serotonergic neurones and adjacent neurones that express neurokinin-1 receptors are both involved in chemoreception in vivo. J Physiol. 1 Apr 2004. 556(Pt 1):235-53. Epub 14 Jan 2004.

[80] Winstanley CA, Dalley JW, Theobald DE, Robbins TW. Fractionating impulsivity: contrasting effects of central 5-HT depletion on different measures of impulsive behavior. *Neuropsychopharmacology.* Jul 2004. 29(7):1331-43.

[81] Winstanley CA, Dalley JW, Theobald DE, Robbins TW. Global 5-HT depletion attenuates the ability of amphetamine to decrease impulsive choice on a delay-discounting task in rats. *Psychopharmacology*. Nov 2003. 170(3):320-31. Epub 2 Sep 2003.

[82] Ueda S, Sakakibara S, Yoshimoto K. Effect of long-lasting serotonin depletion on environmental enrichment-induced neurogenesis in adult rat hippocampus and spatial learning. *Neuroscience*. 2005. 135(2):395-402.

[83] Majlessi N, Kadkhodaee M, Parviz M, Naghdi N. Serotonin depletion in rat hippocampus attenuates L-NAME-induced spatial learning deficits. *Brain Res.* 14 Feb 2003. 963(1-2):244-51.

[84] Naghdi N, Harooni HE. The effect of intrahippocampal injections of ritanserin (5HT2A/2C antagonist) and granisetron (5HT3 antagonist) on learning as assessed in the spatial version of the water maze. *Behav Brain Res.* 28 Feb 2005. 157(2):205-10.

[85] Luttgen M, Elvander E, Madjid N, Ogren SO. Analysis of the role of 5-HT1A receptors in spatial and aversive learning in the rat. *Neuropharmacology*. May 2005. 48(6):830-52.

[86] Able JA, Gudelsky GA, Vorhees CV, Williams MT. 3,4-Methylenedioxymethamphetamine in Adult Rats Produces Deficits in Path Integration and Spatial Reference Memory. *Biol Psychiatry*. 29 Nov 2005. Epub ahead of print.

[87] Cohen MA, Skelton MR, Schaefer TL, Gudelsky GA, Vorhees CV, Williams MT. Learning and memory after neonatal exposure to 3,4-methylenedioxymethamphetamine (ecstasy) in rats: interaction with exposure in adulthood. *Synapse.* 1 Sep 2005. 57(3):148-59.

[88] Sprague JE, Preston AS, Leifheit M, Woodside B. Hippocampal serotonergic damage induced by MDMA (ecstasy): effects on spatial learning. *Physiol Behav.* Jul 2003. 79(2):281-7.

[89] Thiriet N, Ladenheim B, McCoy MT, Cadet JL. Analysis of ecstasy (MDMA)-induced transcriptional responses in the rat cortex. *FASEB J.* Dec 2002. 16(14):1887-94.

[90] Quate L, McBean DE, Ritchie IM, Olverman HJ, Kelly PA. Acute methylenedioxymethamphetamine administration: effects on local cerebral blood flow and glucose utilisation in the Dark Agouti rat. *Psychopharmacology (Berl)*. May 2004. 173(3-4):287-95. Epub 20 Feb 2004.

[91] Dias R, Robbins TW, Roberts AC. Primate analogue of the Wisconsin Card Sorting Test: effects of excitotoxic lesions of the prefrontal cortex in the marmoset. *Behav Neurosci*. 1996. Vol. 110, No. 5: 872-886.

[92] Clarke HF, Walker SC, Crofts HS, Dalley JW, Robbins TW, Roberts AC. Prefrontal serotonin depletion affects reversal learning but not attentional set shifting. *J Neurosci*. 12 Jan 2005. 25(2):532-8.

[93] Gaffan D, Easton A, Parker A. Interaction of inferior temporal cortex with frontal cortex and basal forebrain: double dissociation in strategy implementation and associative learning. *J Neurosci*. 15 Aug 2002. 22(16):7288-96.

[94] Jones B, Mishkin M. Limbic lesions and the problem of stimulus-reinforcement associations. *Exp Neurol*. Aug 1972. 36(2):362-77.

[95] Browning PG, Easton A, Buckley MJ, Gaffan D. The role of prefrontal cortex in object-in-place learning in monkeys. *Eur J Neurosci*. Dec 2005. 22(12):3281-91.

[96] Gaffan D, Saunders RC, Gaffan EA, Harrison S, Shields C, Owen MJ. Effects of fornix transection upon associative memory in monkeys: role of the hippocampus in learned action. *Q J Exp Psychol B*. Aug 1984. 36(3):173-221.

[97] Malkova L, Gaffan D, Murray EA. Excitotoxic lesions of the amygdala fail to produce impairment in visual learning for auditory secondary reinforcement but interfere with reinforcer devaluation effects in rhesus monkeys. *J Neurosci*. 1 Aug 1997. 17(15):6011-20.

[98] Dias R, Robbins TW, Roberts AC. Dissociation in prefrontal cortex of affective and attentional shifts. *Nature*. 7 Mar 1996. 380(6569):69-72.

[99] Chudasama Y, Kralik JD, Murray EA. Rhesus Monkeys with Orbital Prefrontal Cortex Lesions Can Learn to Inhibit Prepotent Responses in the Reversed Reward Contingency Task. *Cereb Cortex*. 14 Jun 2006. Epub ahead of print.

[100] Clarke HF, Dalley JW, Crofts HS, Robbins TW, Roberts AC. Cognitive inflexibility after prefrontal serotonin depletion. *Science*. 7 May 2004. 304(5672):878-80.

[101] Clarke HF, Walker SC, Dalley JW, Robbins TW, Roberts AC. Cognitive inflexibility after prefrontal serotonin depletion is behaviorally and neurochemically specific. *Cereb Cortex*. 20 Feb 2006. Epub ahead of print.

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[*] The hyponeophagia data discussed in Chapter III.2 were collected by Thomas Campbell; analysis of those data was performed by the author. All other data described in this thesis were collected and analyzed by the author.

Appendix I. Accepted Abstract for the Fifth Forum of European Neuroscience, July 8-12, 2006, Vienna, Austria[†]

Dissociations of function between the rodent orbital frontal cortex and hippocampus.

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The orbital frontal cortex and hippocampus form parts of interlinked neural circuits involved in spatial learning and memory. The various portions of these circuits contribute differentially to spatially-dependent behavior. The goal of this study was to characterize the dissociations of function between these brain regions as well as to assess their contributions to goal-directed behavior, the latter portion being in progress at time of writing.

Thirty-five male Lister Hooded rats were randomly assigned to one of three experimental groups, receiving either cytotoxic orbital frontal (OPFC) ablations, cytotoxic hippocampal (HPC) ablations, or sham surgeries. Rewarded alternation on a T-maze was then used to test spatial working memory. The task was performed without a delay between sample and choice trials, as well as with a variable delay of either 30 or 600 seconds. The trials were pseudorandom, counterbalanced, and interleaved as appropriate.

Rats with HPC lesions were profoundly impaired in the no delay condition as compared to sham and OPFC groups; there was no statistically significant difference in sham and OPFC group performance. When variable delays were introduced, the HPC group was consistently impaired for both delay conditions. The sham and OPFC groups again performed comparably for each delay condition, exhibiting no statistically significant difference within a delay condition. However, performance for both sham and OPFC groups decremented significantly with the 600 second delay as compared to the 30 second delay.

Hippocampal lesions produce consistent impairment in the rewarded alternation task, suggesting that the hippocampus plays a key role in spatial working memory—irrespective of delay—while the orbital prefrontal cortex does not.

[†] Mariano TY, McHugh SB, Rudebeck P, Baxter MG, Bannerman DM. Dissociations of function between the rodent orbital frontal cortex and hippocampus. *FENS Abstr*, *vol. 3*, *A089.18*. 2006.