The role of frontal-striatal circuits in instrumental behaviour

Yiran (Lisa) Duan

Christ's College

Department of Psychology

University of Cambridge

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This dissertation is submitted for the degree of Doctor of Philosophy

For my mother and father,

致我亲爱的母亲和父亲

Declaration

I hereby declare that my thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text.

It is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text.

It does not exceed the prescribed word limit for the School of Biological Sciences Degree Committee.

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Abstract

Behaviour can be goal-directed, when performing an action to obtain a specific goal, and it can be habitual, whereby a stimulus in the environment can trigger a response regardless of the outcome. Behavioural output can be a mixture of goal-directed and habitual aspects and there may be a competitive balance between these dual influences. Degrading contingencies between actions (A) and their outcomes (O) challenges beliefs about cognitive control and can be used to distinguish between behaviour that is goal-directed or habitual. In this thesis, the possible causal role of specific brain regions in controlling this balance between goal-directed and habitual behaviour, as measured in the contingency degradation paradigm, were determined. As argued in the thesis Introduction, studies of the marmoset help to bridge rodent and human studies, including possible clinical translation, one of the reasons being the homologies that exist for the prefrontal cortex (PFC). A novel touchscreen-based contingency degradation task was developed for the common marmoset monkey, a New World non-human primate. The possible roles of the primate medial PFC (area 32), anterior cingulate cortex (area 24), ventromedial PFC (area 14-25), anterior orbitofrontal cortex (OFC, area 11), medial OFC (area 14) and the caudate nucleus (CN) were then compared following training, using reversible pharmacological inactivation and activation of these structures via implanted cannulae. None of the studies in the literature had examined the causal role of these brain regions in the expression of A-O associations as measured by contingency degradation.

Inactivation of either area 24 or the CN significantly impaired the animals' sensitivity to contingency degradation, as did activation of area 24. These findings suggest that area 24 and the CN may form part of a neural circuit that mediates the expression of A-O contingencies, a hypothesis supported by an anatomical tracing study. By contrast, inactivation of area 11 apparently enhanced sensitivity to instrumental contingency degradation, possibly by blocking competing pavlovian associations. Manipulations of area 14, 25 or area 32 did not affect sensitivity, indicating the neuroanatomical specificity of the contingency degradation deficits. Control experiments ruled out the possible

contribution of effects on primary motivation or any non-specific effects of inactivation or activation.

The findings are interpreted in the light of literature suggesting that the PFC sub-regions have largely distinctive but overlapping roles in controlling goal-directed behaviour. Additionally, a specific PFC sub-region, area 24, may work together with anterior CN to maintain and utilise learned causal relationships between actions and outcomes. The current thesis's study on the expression of goal-directed knowledge may be useful for explaining the chronic psychopathology of several psychiatric disorders, including obsessive-compulsive disorder, as well as its possible neural substrates.

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Chapter 1

General Introduction

1. 1. Goal-directed and habit systems

1.1.1. Goal-directed vs. habitual behaviours

There are two forms of learned behaviours: pavlovian and instrumental. In pavlovian behaviours, a response (R) is learned through a stimulus (S) that signals the occurrence of an outcome (O) (Pavlov, 1927). Over time, the stimulus alone is enough to automatically trigger the response regardless of the presence of the outcome. Even though the animal could understand the causal relationships between a stimulus and a response, a pavlovian-elicited response is based on automatic reflexes. Contrarily,

instrumental behaviour is not performed automatically but with an autonomous intent to reach a certain outcome.

Instrumental behaviour can be divided into goal-directed and habitual (Figure 1.1A). Goal-directed behaviours are learned and are performed as actions (A) to achieve certain consequences or outcome (O) (Figure 1.1A). Voluntary actions are chosen and can be adjusted based on the expected value of the outcome and whether the action would yield the outcome (probability of performing an action that leads to a specific outcome; Figure 1.A). Another important quality of instrumental actions is when behaviour becomes habitual. Animals initially associate a stimulus with a response due to the meaningful outcome generated by that response, but then the behaviour gradually becomes habitual through repetition (Figure 1.1B). That is, when a stimulus, environmental cue or context is repeatedly occurring together with a response, animals gradually associate their responses with the stimuli and not with the outcome generated by the response (reinforcement of stimulus-response associations). Eventually, the stimuli or contexts themselves suffice to trigger the responses and the animals can become insensitive to the goal and fail to adapt to changes in the value of the outcome generated by their actions, or the contingent relationship between the action and the outcome. The behaviours are now habitual. Habitual behaviour is different from learned skills, such as when we ride a bike without conscious awareness of the performance. Skilled behaviour improves the more one practises and is eventually performed automatically. Unlike habits, skills can be performed in a goal-directed manner, such as when one uses the skill to ride a bike to reach a meaningful destination. Habits behave more autonomously, where it is an independently controlled system by itself that generates its own rules and has its own neurological underpinnings.

Overall, in goal-directed behaviour, an action is performed to cause the desired consequence; however, in habit-driven behaviour, the consequence is not important as the response elicited is essentially a reflex to the triggering context. Like Yin and Yang, the two systems are not always opposed to one another; they rather work sometimes together and only sometimes in competition with one another. An absence of goal-directed actions does not necessarily entail a complete 'take-over' by the habitual system of behavioural output, and habit-dominated behaviour does not mean the complete disappearance of goal-directed actions.

The goal-directed system is needed to adapt flexibly and the habit system is needed to off-set cognitive load. In our everyday lives, we must make decisions constantly based on our goals or go on “autopilot” to get through the day. Having a dominant goal-directed system can have its advantage, in which we can adapt and remain flexible to changing environments and goals. For instance, when the goal is to enter a building that was recently modified to have an automatic door, we will adapt our behaviour appropriately to not reach for a door handle (action no longer leads to the outcome), but instead, simply approach the door for it to open. However, if our goal-directed system is still dominant, we will evaluate and plan our actions whenever we approach the automatic door, which would be tiresome and inefficient. Indeed, almost half of our daily actions are repeated in reliable contexts (Wood et al., 2002). Through repetitions of performing the same action to achieve the same goal in the same context, a habit is formed. Thus, we will approach the automatic door not looking for door handles and without the need to think unduly. The behaviour becomes more efficient and the cognitive burden is reduced.

However, an excessively dominant habit system could be maladaptive. Using the example in the previous paragraph, once an excessive habit has been formed from approaching the automatic door and the goal-directed system is weak, one could not adapt readily if the door was switched back to a manual one. That is, one might continue to wait or wave the door open instead of trying to push or pull it. Impairments in the coordination and competition between the goal-directed and habitual systems are manifested in neuropsychiatric disorders such as obsessive-compulsive disorder (OCD). Classic examples include excessive handwashing and checking if the doors are locked. OCD patients were impaired in tasks that measure goal-directed actions (Gillan et al., 2011; Gillan et al., 2014a; Gillan and Robbins, 2014; Robbins et al., 2019; Vaghi et al., 2019). Overall, to choose the optimal behaviour to reach goals, the goal-directed and habit systems need to work both in collaboration and competition. The following section describes the behavioural tasks used to measure the 'balance' between these two systems.

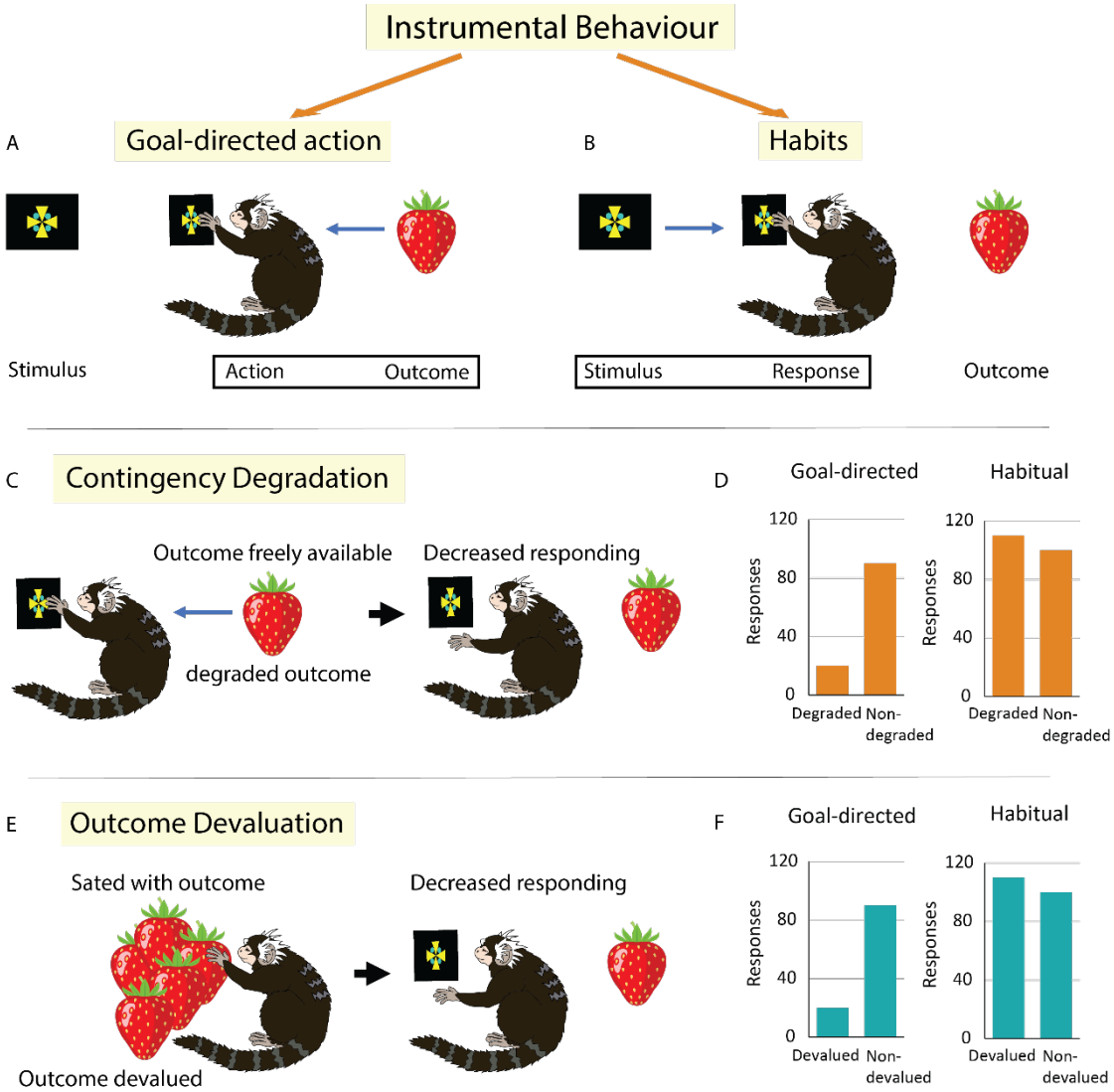

Figure 1.1 Instrumental behaviour can be divided into goal-directed and habitual behaviours. Two tasks are usually used to measure goal-directed and habitual behaviour: contingency degradation and outcome devaluation. A). Goal-directed behaviour. The action of touching the stimulus is performed because the marmoset wants to achieve the outcome of receiving strawberry juice reward. B). Habitual behaviour. The marmoset's response to touch the stimulus is driven by the presence of the stimulus. C). Contingency degradation. The association between the action and the outcome is degraded. The act of touching the stimulus becomes less predictive of the outcome because the juice reward could be delivered freely, that is, regardless of the animal's action. D). If the marmoset is goal-directed, he/she will decrease the action of touching the degraded stimulus. E). Outcome devaluation. The value of the outcome has decreased. The marmoset is sated with the strawberry juice reward; thus, the value of strawberry reward is decreased, which subsequently decreases the need to perform the action to obtain the devalued outcome. F). If the marmoset is goal-directed, he/she will decrease the action of touching the devalued stimulus.

1.1.2. Tasks for measuring the balance between goal-directed and habitual behaviour

There are two commonly used methods to measure goal-directed and habitual behaviour across rodents, non-human primates and humans: outcome devaluation and contingency degradation (Hammond, 1980; Adams and Dickinson, 1981; Balleine and Dickinson, 1998; Balleine and O'Doherty, 2010) (Figure 1.1C, E). These two tasks encapsulate what has been described as the desire and belief psychology of goal-directed behaviour, that is, the intention to act is the interaction between the desire or 'wanting' to obtain the desired value of the outcome and the 'belief' that the action will cause the outcome to happen (Heyes and Dickinson, 1990; de Wit and Dickinson, 2009).

1.1.2a. Outcome devaluation

The test of outcome devaluation was first developed in rats (Adams and Dickinson, 1981). In a basic task design, the rats were trained to press either of the two levers on the left or right side of the testing apparatus. Each lever was associated with a different food outcome. Animals were trained to press a specific lever to receive a specific type of food outcome on separate days. One of the outcomes was then devalued in the home cage, either through selective satiation by free access to the reward or through nausea by pairing that reward with unpleasant experiences (e.g. injection of lithium chloride). After the devaluation, the animals were tested under extinction in the presence of both levers simultaneously. Animals dominated by goal-directed systems respond more to the non-devalued outcome; animals dominated by the habit system are unable to distinguish between valued and devalued outcomes, making them insensitive to change of the outcome value (Figure 1.1F). This task was adopted in rhesus macaques (Chudasama et al., 2013; Rhodes and Murray, 2013), healthy humans (Valentin et al., 2007; de Wit et al., 2009; Tricomi et al., 2009) and humans with pathology (de Wit et al., 2011; Gillan et al., 2011; Sjoerds et al., 2013). The rhesus macaque studies used selective satiation to devalue the rewarded food outcome that was obtained by either selecting an object or performing a specific action. In humans, the devaluation was achieved either by selective satiation or instruction. In the latter situation, the participants were either told explicitly by the

experimenter or by the task itself that one outcome (usually pictures of food) was devalued. Outcome devaluation taps into the subject's "wanting" of or "desire" for the outcome based on changing the value of the outcome.

1.1.2b. Contingency degradation

On the other hand, contingency degradation taps into animals' "beliefs" about whether their action remains predictive in receiving a specific outcome, while the value of the outcome is not changed (Figure 1.1C). A more detailed description of the task used in this thesis can be found in Chapter 2.5. Animals associate one action with one outcome and another action with a different outcome. One of these action-outcome associations is degraded while the other association is not. In the degraded condition, both performing or not performing the response yields the same outcome; in the non-degraded situation, the outcomes yielded by performing or not performing the response are not the same. Thus, subjects dominated by goal-directed systems respond more in the non-degraded sessions compared to the degraded sessions; if the habit system is dominant, such difference is not observed, making them insensitive to contingency degradation (Figure 1.1D). This is based on the premise that in response-outcome learning processes, if the probability of an outcome is more likely given the performance of a specific action, then the strength of association between the response and the outcome increases. If the probability of an outcome is equally or more likely to occur in the absence of a response, the strength of association between the response and the outcome decreases, thus reducing the likelihood of performing that response (Hammond, 1980).

While both tests of outcome devaluation and contingency degradation measure goal-directed behaviour, the underlying neurobiological and psychological mechanisms are likely to differ. First, different brain regions are probably involved in processing contingency information compared to value information. Second, they also differ fundamentally with respect to the nature of the updating process itself. For instance, Bradfield et al. (2015) proposed that outcome devaluation is sensitive to changes in reward value that occur outside of the testing session, thus making updated value information unobservable during the task. On the other hand, contingency degradation is sensitive to the changes in action-outcome contingencies that occur during the session,

thus making updated action-outcome relationships observable during the task. Indeed, medial orbitofrontal (mOFC) lesion impaired outcome devaluation acquisition (the outcome is unobservable) but not contingency degradation (the outcome is observable).

Across species, studies have consistently implicated the dorsal striatum and various prefrontal cortex (PFC) sub-regions located closer to the medial surface (rather than more lateral), such as the medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC), ventromedial PFC (vmPFC) and anterior cingulate cortex (ACC), as the neural substrates underlying goal-directed behaviour involving A-O contingencies and value updating (Balleine and O'Doherty, 2010; Dolan and Dayan, 2013). To properly comprehend and compare different studies on goal-directed behaviour across species, I will examine the importance of studying the PFC and the homologies, especially the PFC sub-regions mentioned above and the dorsal striatum, between non-human primates, humans and rodents in the following section.

1.2 Homologies between non-human primates, human and rodent

1.2.1. The Prefrontal Cortex

The prefrontal cortex (PFC) in humans and non-human primates is part of the frontal lobe, the largest of the four brain lobes, with the most differentiated cytoarchitectonic structure. The PFC is responsible for a wide repertoire of functions and any damage to it could lead to a diverse range of deficits. It integrates information coming from the sensory, motor, parietal and temporal cortices to aid the animals to plan and execute behaviours using internally and externally generated information, appropriate to the environmental and physiological context. The diverse range of deficits that can be observed following frontal lobe damage is seen in the following examples. One of the early pieces of evidence on the consequences of frontal lobe damage was the case of Phineas Gage, who damaged his frontal cortex in the mid-19th century in a rail-work accident (Harlow, 1993). Despite the recovery, he was reported by those close to him as a changed man: he became rude, hot-tempered and could not follow or plan a coherent course of actions in work and social settings.

A classical neuropsychological test to assess cognitive flexibility and planning is the Wisconsin Card Sorting Task, where the participants are asked to place cards to match the sorting rule of already displaced cards according to colour, shapes or number of shapes on the cards. The rule of sorting could be changed by the experimenter and the participants needed to place cards pertaining to the new rule. Frontal lobe damaged subjects consistently performed poorly in this task (Milner, 1963; Milner and Petrides, 1984). Patients with frontal lobe lesions were also impaired in conditional associative-learning tasks, where they needed to associate a set of stimuli with a set of responses, either spatially or non-spatially (Petrides, 1985). Frontal lobe patients were also impaired in the Stroop task that involved identifying conflicts and attending the correct information, for instance, saying the word “blue” printed in green ink (Perret, 1974).

One fundamental role of the PFC is in the conscious planning of actions, that is, the retention of learned information and the execution of behaviour that leads to the

desired goal or outcome, which is essential for the performance of goal-directed actions. In the Tower of London task, subjects need to use higher-order planning to perform a series of actions to reach an end goal (Shallice et al., 1982). Such goal-directed planning was impaired in frontal lobe patients (Shallice et al., 1982; Owen et al., 1990). Another important element to support goal-directed actions is working memory, where the information is retained in short-term storage to aid current and future behaviour (Baddeley, 1986; Fuster, 1995). Both lesion and human imaging studies have indicated the importance of the frontal lobe in working memory (Owen et al., 1990; Fletcher and Henson, 2001). Understanding how the frontal cortices affect the way we use goal-directed actions to optimise our daily decision-making is not only pertinent to our everyday life but also in the alleviation of maladaptive behaviour expressed in frontal lobe-related neuropsychiatric disorders. The medial and orbital PFC rather than the lateral PFC, and the cortico-striatal network, were apparently implicated in instrumental goal-directed actions relating to value updating and action-outcome contingencies (Balleine and O'Doherty, 2010). The following section will discuss the homologies of medial PFC, orbital PFC and the cortico-striatal connectivity between non-human primates, humans and rodents.

1.2.2. Homologies

The terms used to describe PFC sub-regions in rodents, monkeys and humans are variable and often confusing. Often in published studies, generalised and imprecise terms such as anterior cingulate cortex (ACC), orbitofrontal cortex (OFC), ventromedial PFC (vmPFC), or medial PFC (mPFC) are used, which makes it more difficult for other researchers to specify the precise location of a targeted brain region within a large area such as the primate mPFC. Researchers investigating goal and habit systems are trying to better clarify this cross-species brain comparison at various levels: anatomical, functional and molecular (Balleine and O'Doherty, 2010; Wallis, 2011; Vogt et al., 2013; Heilbronner et al., 2016; Laubach et al., 2018).

We need to find similarities but also consider the differences between human, non-human primates and rodent PFC. Rodent PFC only has agranular areas, whereas in non-human primates these only make up the caudal third of the orbito-medial PFC and

even less in humans (Figure 1.2). Agranular means that there is a lack of granule cells in the external granular layer II and internal granular layer IV. Rodent PFC does not have dysgranular and granular cortices, whereas they are prominently featured in primate PFC. Dysgranular cortex contains sparse granule cells in layer IV; the more recently evolved granular cortex contains prominent granule cells in layer IV.

In human mPFC, the agranular area 32 lies between the cingulate sulcus and paracingulate sulcus and is bounded ventrally by the agranular area 24 (anterior cingulate cortex), which lies on the cingulate gyrus. The definition of ventromedial PFC in humans often shifts between studies, but generally it includes areas 10, 11, 14, 25, ventral 32 and ventral pregenual 24. In marmosets, area 32 is lightly granular and comprises the largest area in mPFC. It is bounded caudally by the perigenual part of the agranular area 24, rostrally by granular area 10, dorsally by granular area 8b and 9 and ventrally by lightly granular area 14 (Figure 1.2A). Area 24 that belongs to mPFC situates anterior to, and surrounds, the genu of the corpus callosum. Ventral to the genu of the corpus callosum is area 25, also called the subgenual anterior cingulate cortex. The medial part of area 10 lies anterior to area 32. The Brodmann terms, such as area 32, are used to address the sub-regions in the medial surface of the PFC in humans and non-human primates (NHP); in rodents, the newest edition (7^{th}) of the commonly used atlas by Paxinos and Watson (2017) has begun to use terms such as area 32, area 24 and area 25 to roughly replace the former terms prelimbic cortex (PL), anterior cingulate cortex (Cg) and infralimbic cortex (IL), respectively (Figure 1.3). Consistent with this change in the atlas, through comparing rodent, human and non-human primate mPFC-striatal connectivity, Heilbronner et al. (2016) postulated that the rodent anterior PL is more similar to area 32, posterior PL is more similar to anterior area 24 and rodent IL is more similar to area 25. Although the most recent rat brain atlas changed the terms of the PFC to match that of the primates, many recent publications still use the older labels (e.g. PL, IL) (Laubach et al., 2018). Two most commonly used primate species are the rhesus macaques and common marmosets. Great similarities in areas 25, 32 and 24 were also found between the primate species, especially when comparing macaques with marmosets (Burman and Rosa, 2009; Paxinos et al., 2011; Vogt et al., 2013; Roberts and Clarke, 2019) (Figure 1.2).

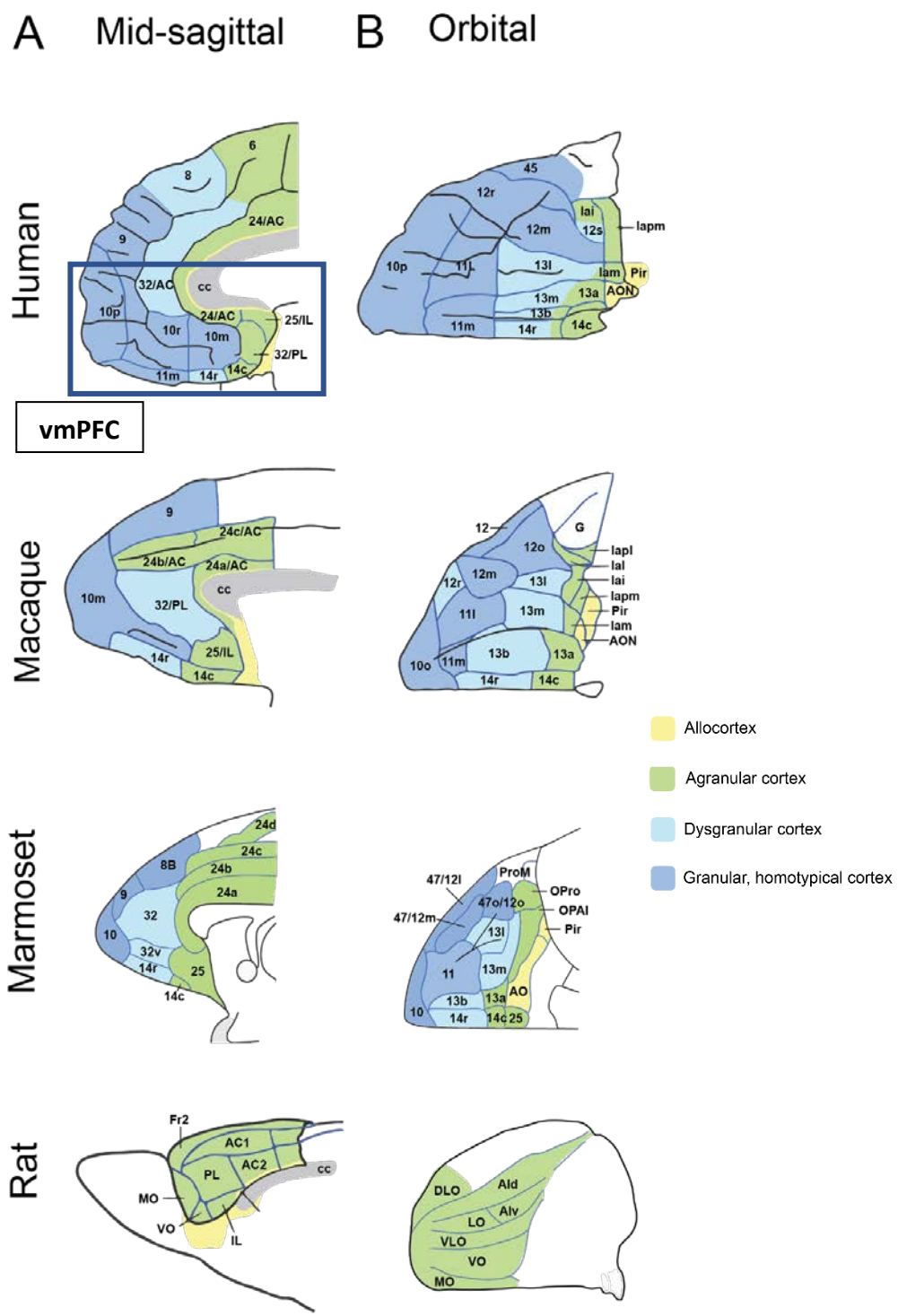

Figure 1.2. Cross-species comparison of prefrontal cortex (PFC): human, macaque, marmoset and rat. Compared to primates, rat does not have dysgranular or granular cortex. A). Mid-sagittal section of PFC. B). Orbital section of PFC.

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Modified from Dr. Nicole Horst's artwork. Referenced from Roberts and Clarke (2019).

p: posterior; r: rostral; c: caudal; AC: anterior cingulate; cc: corpus callosum; IL: infralimbic cortex; PL: prelimbic cortex; m: medial; v: ventral; MO: medial orbital; VO: ventral orbital; Fr: fasciculus retroflexus; DLO: dorsolateral orbital; VLO: ventrolateral orbital; LO: lateral orbital.

OFC lies on the ventral surface of the PFC, cuts into the medial edge and lateral surface of PFC (Figure 1.2B). Medial PFC, such as area 32, 24 and 25, is not very interconnected with OFC except for areas such as 11, 13, and 14. Area 11 and 14 are primarily connected with mPFC (Price, 2007). In marmosets, Roberts et al. (2007) used retrograde and anterograde tracing to investigate the medial dysgranular part of the OFC, similar to the area 11 and 13 of the macaque monkey. They found that this part of the OFC is not connected with dorsolateral PFC, but reciprocally connected with caudal OFC and ventromedial PFC. Like macaques, area 11 and 13 of the marmoset is interconnected with ventromedial PFC areas, such as area 14 and ventral area 32. Krettek and Price (1977) subdivided this region in the rodent into medial, ventrolateral, and lateral orbital areas (MO, VLO, LO) (Figure 1.2B); these may be comparable to areas 14, 13a and 13m/l, respectively, in macaque monkeys (Price, 2007). Similar positions of brain regions and connectivity in the dysgranular cortex (area 25 and posterior area 13, 14) between species might indicate their relative comparability (Price, 2007). However, the comparison between rodent and primate species is unclear for the dysgranular or granular cortices, given that the rodent PFC is only agranular.

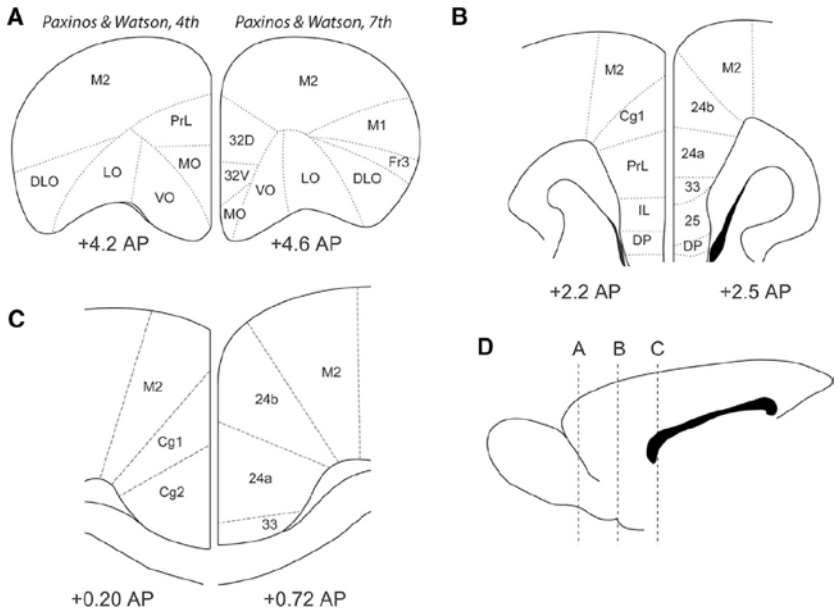

Figure 1.3. Changes of mPFC organisation in rodent atlas. A). In Paxinos and Watson 7th edition, rostral PL is changed to 32dorsal and 32ventral. B). IL is changed to 25, caudal PL is changed to 24a and 33, Cingulate1 (Cg1) to 24b. C). Further changes from cg1 and cg2 to 24a, 24b and 33. D). sagittal view of the brain, indicating where the cross section of A, B and C are taken.

Cortico-striatal connectivity across primate species is very similar, and similar projection patterns were also found between primates and rodents (Haber et al., 1995; Ferry et al., 2000; Heilbronner et al., 2016) (Figure 1.4). However, differences between primate and rodent still exist. Schilman et al. (2008) addressed a "ventral shift" in the projection from cortical regions to striatum in primates, in that an area in primates sends its projection more ventrally to the striatum compared to a homologous area in rats. Moreover, the percentage of medium spiny neurons (MSNs) that predominates in the striatum is different between rodent and primates. In non-human primates, ~77% of neurons in the striatum are MSNs, while over 90% are MSNs in rodents (Graveland and DiFiglia, 1985). Therefore, in terms of anatomical similarities, comparing non-human primates with humans (also primates) could yield more translational power instead of comparing rodents with humans. Despite anatomical and functional similarities between the rodent and primate PFC and dorsal striatum, it is still quite difficult to map out precisely which brain region is homologous to which, across rodent and primates. The fact that rodents and primates are relatively far apart in the evolutionary tree compared to non-human primates and humans may mean that there will not be suitable homologies across rodents and primates that will satisfy everyone. Therefore, a bridging species such as the non-human primate would have more translational power to understand the neurological underpinnings of human behaviour. In the next section, I will explore functional homology and review the neural substrates of goal-directed behaviour across rodents, non-human primates and humans.

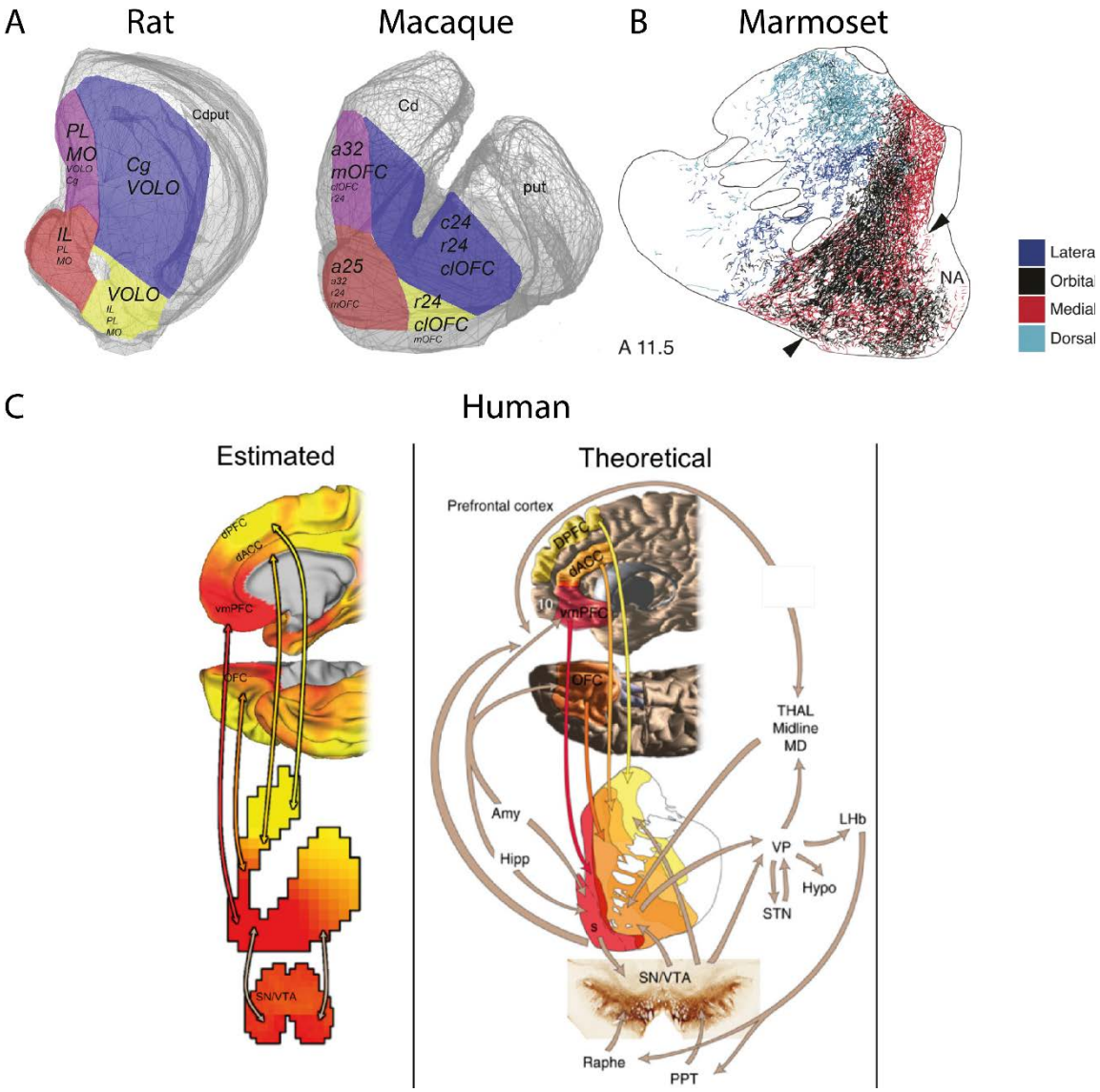

Figure 1.4. Corticostriatal connectivity across species. Similar topographical organisation of PFC-striatal connectivity, where the vmPFC projects to ventromedial regions of the striatum, mPFC projects to medial striatum, and more lateral PFC projects to lateral striatum. There are also many overlaps between PFC projections within the striatum. A). rat and macaque connectivity data from tracing studies. B). Marmoset connectivity data from tracing studies. C). human connectivity estimates from resting state fMRI.

A). Reprinted from Biological Psychiatry, vol. 80, issue 7, Sarah R. Heilbronner, Jose Rodriguez-Romaguera, Gregory J. Quirk, Henk J. Groenewegen, Suzanne N. Haber, Circuit-Based Corticostriatal Homologies Between Rat and Primate, 509-521, Copyright (2016), with permission from Elsevier.

B). Reprinted from Journal of Comparative Neurology, vol. 502, issue 1, Barry J. Everitt, Trevor W. Robbins, David J. Cutter, et al, Forebrain connectivity of the prefrontal cortex in the marmoset monkey (Callithrix jacchus): An anterograde and retrograde tract-tracing study, 27, Copyright (2007), with permission from John Wiley and Sons.

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1.3. Neural substrates of goal-directed actions

1.3.1. The role of PFC and dorsal-striatum in goal-directed behaviour: human studies

Medial PFC (mPFC), ventromedial PFC (vmPFC) and OFC are thought to influence behavioural flexibility, reward value prediction and action-outcome (A-O) associations (Balleine and O'Doherty, 2010; Dolan and Dayan, 2013). The more associative region of the dorsal striatum (caudate nucleus in primates) is linked with goal-directed behaviour (Graybiel, 1998; Liljeholm and O'Doherty, 2012). Human fMRI studies have demonstrated the functional specialisation of these striatal sub-regions. In the following section, I will describe the neural correlates affecting goal-directed behaviour through alterations in A-O contingencies and outcome valuation.

1.3.1a. Action-outcome contingencies

Several human functional imaging studies have investigated the role of PFC sub-regions and the caudate nucleus (CN) in learning and expressing the causality between an action and an outcome. Tanaka et al. (2008) used a contingency degradation task in healthy subjects to investigate brain regions involved in instrumental contingencies. Subjects were asked to respond freely on a button that, depending on the schedule used, gave them a monetary reward (25 cents) across the session. The authors used a variable ratio (VR) and a variable interval (VI) schedule to differentiate high vs. low contingencies. The VR schedule, where the numbers of responses are proportional to the outcome delivery, is more likely to develop goal-directed behaviour, while the VI schedule, where the outcome is delivered after a certain time regardless of responding, is more likely to develop habitual behaviour (Dickinson and Nicholas, 1983; Yin and Knowlton, 2006; Rossi and Yin, 2012). In this study, a VR schedule indicated high contingency because the outcome can only be obtained by button presses; by contrast, the VI indicated low contingency because the reward delivery depended on the time passed and not on button presses. At the end of each session, subjects were asked to rate the causality of their action in providing a reward, from 0 to 100 (100 most causal, 0 least causal). They found

no between-subject differences in the degree of contingency or causality judgement across schedules, so a within-subject analysis was used. Subjects were able to distinguish between high vs. low contingency situations in that the objective contingency in high contingency conditions was significantly associated with high subjective judgement compared to low contingency conditions. They showed that portions of the ventromedial PFC, the medial OFC and the anterior medial caudate nucleus were more active (blood-oxygen-level-dependent; BOLD signal) when the subjects' actions were based on a high-contingency schedule than the low-contingency schedule (Figure 1.5A, B) (Table 1.1). They then looked at how the brain activity of these regions changed over time in tracking the action-outcome contingencies. Only the average BOLD signal in mPFC, but not mOFC or caudate, was significantly correlated with objective contingency. In terms of subjective contingency, mPFC, dorsomedial PFC and lateral OFC activity correlated with the increased causality judgement of subjects across sessions. The authors only assessed changes in the probability (P) of the outcome (o) when the action (a) was present [$P(o|a)$] and did not evaluate the situation in which the probability of the outcome occurred in the absence of the action [$P(o|{\sim}a)$]. Thus, they did not compute the change in contingency, that is, $\Delta P = P(o|a) - P(o|{\sim}a)$.

Liljeholm et al. (2011) took this into account and computed the change in contingency (ΔP) (Table 1.1). The subjects were instructed to press the button freely to receive the 25 cents reward, but each press cost them 1 cent and, depending on the session, the reward could also be delivered without any response. The task followed the equation $\Delta P = P(o|a) - P(o|{\sim}a)$, where the contingency (ΔP) is the difference in the probability (P) of receiving the outcome in the presence of an action (a) and in the absence of that action (~a). There were 6 sets of contingency situations with varied probabilities. The frequency of non-contingent reward delivery was adjusted individually to accommodate differences in response rate across subjects. Subjective contingency judgement was also collected at the end of each block (6 blocks in one session). Both response rate and contingency judgement decreased with decreasing objective contingency. They found that activity in the left middle frontal gyrus, left superior and inferior parietal lobules increased with a decrease in contingency (ΔP). The non-contingent condition ($P(o|{\sim}a)$) was related to the activity in the lateral frontal cortex bilaterally, the medial frontal cortex, the right posterior superior temporal gyrus, the right

posterior intraparietal sulcus, and the left posterior caudate. After masking for an exclusive negative contrast for contingency, only the right inferior frontal cortex and left posterior caudate activity survived. The contingent condition ($P(o|a)$) was related to activities in the right mPFC, and right anterior caudate nucleus, with only the caudate activity surviving after an exclusive positive contrast for contingency (Figure 1.5C). Further region-of-interest analysis indicated significant effects in the left caudate nucleus for the non-contingent condition (($P(o|\sim a)$), and significant effects bilaterally in the caudate and the vmPFC in the contingent condition (($P(o|a)$). In a subsequent study using a task assessing response-outcome and stimulus-response conditions, Liljeholm et al. (2015) showed that during response-outcome conditions vmPFC and anterior caudate nucleus had greater activity.

Table 1.1. Neural correlates from A-O contingency tasks.

	$P(o|a)$	**$P(o|\sim a)$**	**$\Delta P = P(o|a) - P(o|\sim a)$**	**Subjective contingency**
Tanaka et al. (2008) imaging	• mPFC (also tracks A-O contingencies overtime) • mOFC • anteromedial CN	N/A	N/A	• mPFC • dorsomedial PFC • lOFC
Liljeholm et al. (2011) imaging	• vmPFC • right anterior CN	• right inferior frontal cortex • left posterior CN	• left middle frontal gyrus • left superior parietal lobules • left inferior parietal lobules	N/A
Tricomi et al. (2004) imaging	• CN	N/A	N/A	• CN
O'Callaghan et al. (2019) lesion				• vmPFC: $P(o|a) > P(o|\sim a)$

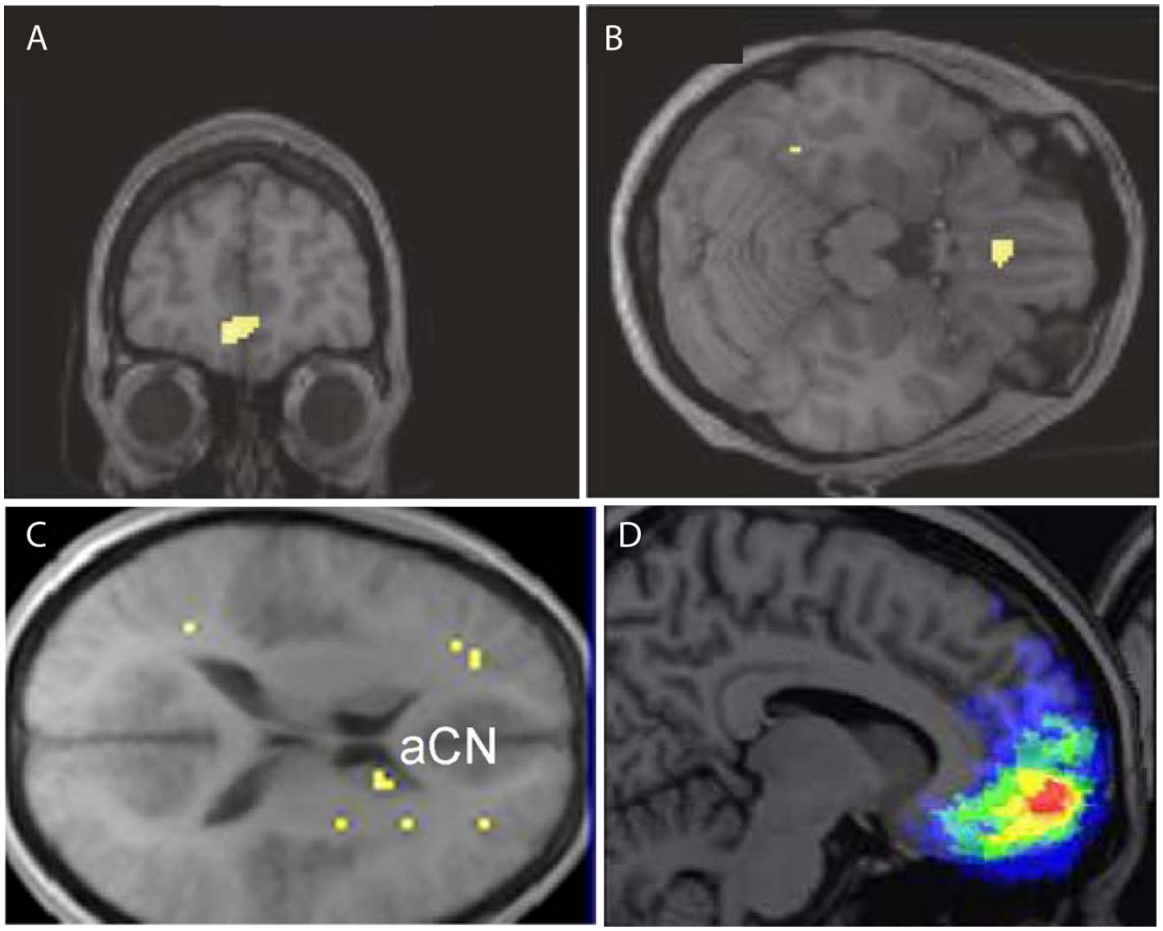

Figure 1.5. Human neuroimaging and lesion studies that indicated the involvement of mPFC, OFC and anterior CN in performing A-O contingencies and vmPFC in the subjective judgement of A-O contingencies. A). mPFC activity correlated with $P(o|a)$ (Tanaka et al., 2008). B). mPFC activity correalted with $P(o|a)$ (Tanaka et al., 2008). C). anterior CN activity correlated with $P(o|a)$ (Liljeholm et al., 2011). D). vmPFC lesioned patients were not impaired in contingency degradation performance but were impaired in subjective judgement (O'Callaghan et al., 2019).

A, B). Images from Tanaka et al. (2008). ("Copyright [2008] Society for Neuroscience"). For full citation of article please see the bibliography.

C). Image from Liljeholm et al. (2011). Article is under a CC-BY-NC-SA liscence. For full citation of article please see the bibliography.

C). Image from O'Callaghan et al. (2019). Article is under a CC-BY liscence. For full citation of article please see the bibliography.

Tricomi et al. (2004) used oddball paradigms to understand whether the caudate nucleus would respond to pseudorandomly presented stimuli that signalled reward, the anticipation of reward and the perceived A-O contingencies in human participants. The caudate nucleus was only active when the subjects perceived a contingent relationship between the action and outcome, and not in any other conditions, including non-contingent conditions. Thus, they concluded that the caudate nucleus is implicated in perceiving the likelihood of an action leading to an outcome and not for the reward itself. Other fMRI studies had demonstrated caudate activity when the subjects perceived that their actions, such as button pressing, led to rewards or punishments (Delgado et al., 2000; Elliott et al., 2000; Knutson et al., 2001; Delgado et al., 2003; O'Doherty, 2004).

Overall, the neuroimaging studies revealed two brain circuits at work: 1). mPFC/vmPFC/mOFC and anteromedial CN circuit mediated the probability of response-contingent reward delivery (i.e. instrumental action-outcome associations). 2). Inferior frontal cortex and posterior CN circuit mediated the probability of receiving a reward in the absence of action (i.e. pavlovian context-reward associations). The middle frontal gyrus and parietal lobules are the integration centre to calculate the overall change in contingency from the above two circuits. It seems that in addition to regions such as the parietal lobules, PFC sub-regions in the medial surface and anteromedial CN are good candidates that affected instrumental A-O contingencies. However, vmPFC lesion in patients did not affect their ability to learn contingency relationships (for the lesion site see Figure 1.5D). O'Callaghan et al. (2019) found that vmPFC lesioned subjects can learn A-O contingencies as well as age-matched, elderly healthy controls, but have reduced awareness of such relationships when the changes in contingency are positive [i.e. the performance of action increases the probability of outcome; $P(o|a) > P(o|\sim a)$] when compared to lateral PFC lesioned and elderly healthy subjects. Although vmPFC activity in neuroimaging studies was implicated in A-O contingency, vmPFC lesion did not impair contingency learning but did reduce subjective awareness when $P(o|a) > P(o|\sim a)$. It could be that vmPFC is not important for translating changes in A-O contingency to performances but is critical in the understanding of it.

The human studies had pointed out the possible implications of mPFC, mOFC and anteromedial CN, but not the vmPFC in instrumental A-O contingencies. However, lesion studies encompassed relatively large areas in the mPFC, OFC or vmPFC and often these

terms include over-lapping PFC sub-regions (also see chapter 1.2.2). For instance, area 14 could be considered as part of the larger vmPFC or as part of the mOFC (Figure 1.2), but is it involved in A-O contingencies? Similarly, parts of area 32 could be in the vmPFC or the mPFC (Figure 1.2). Additionally, human imaging studies imply correlation but not causation. Thus, more studies drawing on causality between brain and behaviour with more precise targeting of these PFC sub-regions, such as area 32, 14, 25 and 24 (Figure 1.2), are needed in the literature to tease out their functions.

1.3.1b. Value comparison and updating

The OFC has long been implicated in the integration of sensory information, outcome valuation, value-based decision-making and stimulus-outcome associations (Jones and Mishkin, 1972; Rolls and Baylis, 1994; Baxter et al., 2000; Rolls, 2000; Izquierdo et al., 2004; Rudebeck et al., 2008; Walton et al., 2010; Rudebeck and Murray, 2011; Wallis, 2011; Gremel and Costa, 2013; Izquierdo, 2017). One theory postulates that the OFC uses a cognitive map to guide decision-making, where it integrates sensory information, learned stimulus and associated outcomes to optimise behaviour (Wilson et al., 2014). A plethora of human imaging studies have reported the role of OFC in affective processing in somatosensory (Francis et al., 1999; Rolls et al., 2003b), gustatory (O'Doherty et al., 2002; Small et al., 2003) and abstract rewarding stimuli, such as money (Knutson et al., 2001; O'Doherty et al., 2001).

Valentin et al. (2007) conducted an outcome devaluation task in human subjects to discern the neural correlates involved. Two stimuli were presented on the screen at one time, where each stimulus was associated with various probabilities of juice delivery. The high probability stimulus could result in the delivery of a neutral juice (not to be devalued or valued), water or juice that was to be devalued or valued; the low probability stimulus resulted in the delivery of only the neutral juice and water. Subjects were sated with the devalued juice after they were trained on the stimulus-outcome associations. They then completed the outcome devaluation task under extinction, to dissociate stimulus-response-outcome learning from stimulus-response learning. mOFC, central OFC and lOFC activity decreased when subjects chose the high probability stimulus in the devalued condition and increased in the valued condition, which indicated these regions

are sensitive to the changes in the incentive values of the outcome and how these changes relate to instrumental actions. In agreement with Valentin et al. (2007), Gläscher et al. (2008) showed that vmPFC (including bits of mOFC) activity encoded value representation in both stimulus and action-outcome associations. Two reversal tasks were used: one stimulus- and another action-based. In the stimulus-based task, two different stimuli were presented simultaneously to signal the human subjects to press a left or right button to obtain outcomes; in the action-based task, only one stimulus was presented and subjects needed to choose between two different actions to obtain outcomes. OFC/vmPFC activity was found to correlate with the value of the chosen outcome in both situations. These findings are contradictory to studies that showed OFC/vmPFC activity was often implicated in stimulus-outcome and not action-outcome value representation. For instance, Wunderlich et al. (2010) designed a task where two stimuli were presented simultaneously, followed by the presentation of an additional stimulus to signal which action to take to obtain the reward. Thus, the human subjects can register the reward value with the initial stimulus presentation, before the subsequent stimulus-action pairing was revealed. The OFC/vmPFC signal indicating the value chosen was observed during stimulus presentation but not when actions could be made. Gottfried et al. (2003) used a pavlovian outcome devaluation task, where two distinct stimuli were paired with two different odours. One of the odours was devalued via satiation of food that had the same odour. Mid-central OFC activity decreased from pre- to post-devaluation towards the devalued stimulus, which indicated that OFC activity tracked changes in the value of the pavlovian-conditioned stimulus.

The different findings where OFC/vmPFC were implicated in stimulus- and/or action-outcome associations could be due to the different OFC regions involved. In studies where OFC/vmPFC were implicated in instrumental learning, the OFC regions were more medial, and in stimulus-related situations, the OFC/vmPFC regions were more mid-lateral. This was true for Valentin et al. (2007), Gläscher et al. (2008) and Gottfried et al. (2003), but not Wunderlich et al. (2010), where the OFC/vmPFC involved in stimulus-response was more medial. There seems to be a mid-lateral functional dissociation in the OFC/vmPFC. Nevertheless, these OFC and vmPFC activities included relatively large brain regions. Thus, more precise targeting might be needed to differentiate the functional difference between the OFC sub-regions. Indeed, functional differences were observed

within the OFC/vmPFC sub-regions in non-human primates and rodent studies in value-based decision-making, which will be discussed in Chapter 1.3.2c.

The anterior caudate nucleus is involved in goal-directed actions that require value updating, but as the behaviour becomes more habitual, the neural signal shifts away from the more anterior caudate nucleus towards the posterior caudate nucleus and the putamen. In an outcome devaluation task in humans, one group of subjects was not over-trained (1-day) and another group was over-trained (3-days) (Tricomi et al., 2009). The subjects responded by pressing the indicated buttons in a free-operant manner and rewards were delivered under a variable interval schedule to promote habit formation (Dickinson and Nicholas, 1983). After training, the subjects were sated on one of the two rewards until it was unpleasant. They were then tested under extinction to determine whether their behaviour became habitual. The 1-day group still maintained goal-directed behaviour and responded less to the devalued reward when compared to the non-devalued reward; the 3-day group became more habitual, where they did not decrease their responding in the devalued condition. When comparing neural activity in the last two sessions of training in the 3-day group and the first two sessions, there was an increase of activity in the right posterior putamen/globus pallidus. Valentin et al. (2007) also conducted outcome devaluation via selective satiation in humans. Although the effect was weak and bordering significance, they found that posterior caudate activity was related to habit learning. The human imaging literature has thus provided strong evidence for a gradient of functional specialisation in the dorsal striatum, where goal-directed behaviour was initially linked with anterior caudate and putamen and as behaviour became more habitual, the posterior caudate and putamen became more active.

1.3.2. The role of PFC and dorsal-striatum in goal-directed behaviour: non-human primates and rodents

1.3.2a. Action-outcome contingencies

The mPFC is implicated in contingency learning. Common marmosets, following excitotoxic (fibre-sparing) lesions of area 32, became insensitive to action-outcome associations in a contingency degradation task that contained pavlovian elements

(Jackson et al., 2016). The task examined contingency acquisition and the lesion additionally included a small region of perigenual anterior cingulate cortex, area 24ab. In rodents, PL has been proposed to be homologous to primate area 32 (Vogt et al., 2013; Heilbronner et al., 2016). Lesions of the PL blunted the animals' sensitivity to the acquisition of instrumental contingency (Balleine and Dickinson, 1998; Corbit and Balleine, 2003). There seems to be an agreement between the rodent and non-human primate literature that area 32 and PL are needed for instrumental contingency learning, but none of the literature has investigated area 32/PL's role in the expression of instrumental contingencies.

The anterior cingulate cortex (ACC) is often implicated in the mediation of decision-making using action-outcome associations (McCoy and Platt, 2005; Kennerley et al., 2006; Wallis and Kennerley, 2011). In human functional magnetic resonance imaging (fMRI) studies, ACC activity has been related to relevant parameters of decision-making such as reward size and probability (Brown and Braver, 2005; Knutson et al., 2005). Neurophysiological studies have shown that ACC neurons encode decision-making variables and that damage to the ACC caused impairment in decision-making (Walton et al., 2003; Williams et al., 2004; Kennerley et al., 2006). ACC neurons encode signals of whether or not an outcome is expected (Hayden et al., 2011) and responding to outcomes that one's action is not responsible for, and record consistencies or inconsistencies of expectations during outcome delivery (Hayden et al., 2009). The ACC has been suggested to use positive or erroneous action-reinforcement history to guide current behaviour and is critical in decision-making that is action-driven rather than stimulus-driven (Bush et al., 2002; Kennerley et al., 2006; Rushworth et al., 2007; Rudebeck et al., 2008). Macaques with lesions of the ACC sulcus also have difficulties interpreting the changing probabilities of the outcome associated with rewarded or non-rewarded actions (Kennerley et al., 2006). Rudebeck et al. (2008) found that the same ACC sulcus lesioned rhesus macaques had impaired action-outcome associations in reward guided behaviour when trained to move a joystick in both deterministic and stochastic action-reward situations. The lesion of mPFC in the common marmoset by Jackson et al. (2016) also encompassed a small part of area 24 that sits anterior to the genu of the corpus callosum. This medial PFC lesion impaired the learning of contingency degradation. The ACC in non-human primates is thus implicated in voluntary action-outcome selection. However, scarcely any studies in the

literature have investigated the role of ACC in A-O contingencies, and few have considered its role in the expression of contingency knowledge. Additionally, none of the studies in the literature has used the contingency degradation task to examine the role of area 24.

When examining the OFC, excitotoxic lesions of area 11 in the common marmoset impaired the acquisition of contingency degradation that contained pavlovian elements (Jackson et al., 2016). In rodents, Ostlund and Balleine (2007) used a purely pavlovian contingency degradation task, where animals associated a tone with pellet reward delivery and noise with sucrose delivery (counter-balanced). Each conditioned stimulus (CS) was presented the same amount of time in each session, in which the corresponding reward was delivered on a random time schedule during each CS presentation. In the contingency degradation sessions, one of the outcomes was delivered non-contingently to degrade the relationship between the CS and the outcome. Lateral OFC lesioned animals were insensitive to this degraded relationship compared to sham-lesioned animals. Thus, it seems that the OFC is crucial in learning pavlovian, that is, stimulus-outcome contingencies. However, the role of area 11, the more lateral part of OFC, in the learning and expression of instrumental A-O contingencies is still unclear. Anterior mOFC, on the other hand, was found to not be involved in instrumental contingency degradation learning Bradfield et al. (2015), but its role in the expression is not known.

Investigations in rodents have consistently implicated the dorsomedial striatum (DMS), which is thought to be homologous to the primate caudate nucleus (CN), in mediating A-O contingencies. Pre-training lesion of the rodent posterior DMS (pDMS) but not the anterior DMS (antDMS) impaired instrumental contingency degradation (Yin et al., 2005b). Pre- and post-training lesion in the pDMS impaired animals' performance in the contingency degradation tasks. Additionally, inactivation of pDMS also impaired the expression of learned A-O contingencies. Thus, pDMS is essential for the learning and expression of goal-directed actions that involve A-O contingencies. However, whether or not the CN in non-human primates is important for the learning and the expression of A-O contingencies is unknown.

To investigate the correlation of neuronal activation within the mPFC and dorsal striatum, Fitoussi et al. (2018) used Zif268 (immediate early gene) immunoreactivity. They divided the rats into a 'flexible' group and 'non-flexible' group on a rat 'gambling' task, where they found that flexible rats showed sensitivity to the action-outcome

contingencies by decreasing their response rate in the presence of free reward while the inflexible group did not. Animals with high Zif268 expression in the PFC region had low expression in the striatum. They observed that rats with poor sensitivity to A-O contingencies had lower expression of Zif268 in the dorsal striatum (eg. pDMS, antDLS, pDLS) and higher expression of Zif268 in the mPFC (PL and IL) than rats who were more sensitive to A-O contingencies. They postulated that imbalanced activation between the medial PFC and dorsal striatum may be indicative of maladaptive habit formation such as those seen in the inflexible rats who were also insensitive to A-O contingencies.

To conclude, evidence in the literature has indicated the mPFC (area 32), OFC (area 11), dorsal striatum and potentially the ACC (area 24) in the learning of A-O contingencies. However, there is a lack of studies in rodents and non-human primates in examining the neural substrates that affect the expression of A-O contingencies. That is, which brain region maintains and utilises A-O contingency knowledge once it was learned? Are the brain regions that are critical in the learning of A-O contingencies still critical in maintaining that knowledge? We know the dorsal striatum is important in both the learning and expression of A-O contingencies, but which PFC sub-regions might work with the dorsal striatum to achieve this is still unknown.

1.3.2b. Value comparison and updating

More studies have used the outcome devaluation task ('wanting') than the contingency degradation task ('belief') to investigate goal-directed behaviour. Rats with PL lesions were unable to reduce responding to devalued outcomes when compared to valued outcomes in the acquisition of outcome devaluation using a sensory-specific satiation procedure (Killcross and Coutureau, 2003; Ostlund and Balleine, 2005). Ostlund and Balleine (2005) also tested the expression of outcome devaluation but the PL lesion did not have any effect. It seems that PL is important in the acquisition but not in the expression of instrumental outcome value comparison and updating. However, a non-human primate study that also investigated the role of mPFC in instrumental outcome valuation might indicate contradictory results. Aspiration lesions of the prelimbic cortex (location corresponding to area 32) in rhesus macaques yielded equivocal results, in the learning of a sensory-specific instrumental outcome devaluation task (Rhodes and Murray,

2013). Two identical stimuli were presented concurrently either on the left or the right side of the touchscreen. The animals performed either a tap or hold action that corresponded to the left or the right stimulus to obtain a specific reward associated with the specific action. The outcome was devalued using selective satiation and the devaluation task itself was performed under extinction. Upon visual inspection of the behavioural performance the animals responded similarly compared to control; the authors concluded the results were equivocal because there was no significant difference between devalued and non-devalued responding after PL lesion, although the p value was 0.051. To note, the PL lesion only encompassed 55-83% of area 32 and the aspiration lesion itself also must have destroyed white matter tract within or passing through the area. Nonetheless, it seems that area 32 lesion in the rhesus macaques did not impair the learning of outcome devaluation but PL lesion in the rodents did, which could call into question the functional homology between area 32 in non-human primates and PL in rodents. Thus, it is ever more important to use a non-human primate to further examine and clarify the role of area 32 in goal-directed behaviour.

A cortico-striatal 'loop' has consistently been implicated in controlling goal-directed actions. pDMS inactivation via NMDA antagonism, which blocked excitatory input into the pDMS, impaired the learning of outcome devaluation (Yin et al., 2005a). Hart et al. (2018a) used a lesion and pharmacological disconnection procedure to disconnect the PL and pDMS, which resulted in the impairment of outcome devaluation in rats. Using immunohistochemistry, they first verified that by lesioning the anterior corpus callosum (antCC), the PL in one hemisphere could only project to the ipsilateral but not to the contralateral pDMS. They then unilaterally disrupted input from PL into the pDMS by lesioning the antCC and PL in one hemisphere. Thus, only the pDMS in the hemisphere contralateral to the lesioned PL was able to receive input from the intact PL. Rats were divided into group “instrumental”, where their responses led to a specific outcome, and group “yoked”, where they received the same amount of reward, but the reward delivery was not contingent upon their responses. In group instrumental, the pDMS that received PL input had a higher level of phosphorylated extracellular signal-related kinase/mitogen activated protein kinase (pERK/pMAPK), a factor important in learning and memory, than the pDMS not receiving PL input in the yoked group. This was relevant because Hart and Balleine (2016) had reported that instrumental training

induced phosphorylation of ERK and MAPK in the pDMS and that preventing phosphorylation in the PL impaired goal-directed learning. They continued to conduct a disconnection procedure whereby the antCC and the contralateral PL and pDMS were lesioned so that the PL and pDMS that were left intact could not communicate with each other. Only those animals undergoing the disconnection procedure were impaired in the learning of outcome devaluation. They replicated the result with the same procedure, but inactivated pDMS using APV (NMDA-receptor antagonist) to block excitatory input instead of a lesion. As lesioning the pDMS prevented input from other PFC sub-regions and not only the PL, to specifically silence PL neurons projecting to pDMS, Hart et al. (2018b) used Cre-dependent Gi-coupled designer receptors exclusively activated by designer drugs (DREADDs). Cre-dependent hM4Di-DREADDS was bilaterally infused into the PL and adeno-associated virus (AAV)-Cre bilaterally into the pDMS or the nucleus accumbens (NAc). Bilateral silencing of PL-pDMS but not PL-NAc projecting neurons impaired the learning of outcome devaluation. They then determined that the bilateral presence of one of the two PL projection neuron types, the intra-telencephalic neurons, were necessary for the learning of value updating because only silencing the bilateral PL projection to pDMS impaired performance. Thus, in rodents, PL projection to pDMS is critical in the learning of goal-directed actions as measured by outcome devaluation.

OFC is consistently implicated in value-coding and value-based decision-making. Neurophysiological studies in non-human primates have revealed that OFC neurons code for flavour, appearance and texture of food (Rolls, 2005) and respond to affective characteristics (reward versus aversion) as well as sensory characteristics of the stimuli. Neurons that are involved in value-based decision-making, where decisions in a given context were made based on the value associated with the outcome, are abundant in the OFC (Schoenbaum et al., 1998; Tremblay and Schultz, 1999; Wallis and Miller, 2003; Padoa-Schioppa and Assad, 2006; Padoa-Schioppa and Assad, 2008). For instance, OFC neurons code for valuation and variables involved in choice processes and the value of current choices relative to the recent history of choices (Kennerley et al., 2011); neuronal activity in OFC correlates with the value of offered and chosen outcomes, regardless of spatial location and motor responses (Padoa-Schioppa and Assad, 2006).

Rhesus macaques with OFC excitotoxic lesions became insensitive to goal devaluation in an outcome devaluation task using selective satiation procedures

(Rudebeck and Murray, 2011; Rhodes and Murray, 2013). The former study lesioned Walker's area 11 and 13 in a pavlovian task and the latter study lesioned Walker's area 11, 13 and 14 in an instrumental task. As mentioned in Chapter 1.3.1b, the more lateral OFC (area 11 and 13) could be involved in pavlovian-related situations, and the more medial OFC (area 14) could be involved in instrumental-related situations. The impairment observed after area 11, 13 and 14 lesions in the instrumental task could be due to the area 14 lesion and not area 11 and 13.

In rodents, changes in the magnitude of OFC neural activity after changes in outcome value correlated positively with the level of goal-oriented behaviour (Gremel and Costa, 2013). Chemogenetic inhibition of OFC impaired goal-directed actions, whereas optogenetic activation of OFC increased goal-directed lever pressing in an outcome devaluation task (Gremel and Costa, 2013). Mice were trained in a random interval (RI) schedule in one context, and the random ratio (RR) schedule in another context. During the outcome devaluation task using selective satiation, in the RI trained context, mice displayed habitual behaviour and became impaired in outcome devaluation (pressing lever similarly to both devalued and valued lever). In the RR trained context, mice displayed goal-directed behaviour, where they pressed less to devalued lever compared to the valued lever. The authors demonstrated that OFC activity in mice is essential for goal-directed value updating through neural recordings, chemogenetic and optogenetic manipulations of OFC activity. While Gremel and Costa (2013) showed the importance of OFC in instrumental goal-directed actions, Ostlund and Balleine (2007) showed contrarily that the OFC is not involved in instrumental-based conditioning but is involved in pavlovian-based conditioning. In an outcome devaluation task, where the value of an outcome associated with an instrumental action (lever pressing) was reduced and the value of the other outcome was not reduced, both pre- and post-training OFC lesion did not impair the rats' sensitivity to changes in outcome value associated with instrumental action. This might be because the two studies described here were conducted in two different rodent species, or that they used different task designs (this will be further explored in the General Discussion, Chapter 5.3).

1.3.2c. Differentiating OFC sub-regions

Although in all animal species, the OFC appears to be crucial for learning goal-directed actions that require the understanding of outcome valuation and action-outcome contingencies, different sub-regions of OFC have subtly different roles. Most of the previous literature has consisted of human fMRI and animal lesion studies, which have been focused on manipulating a large part if not all of OFC, even though the OFC is composed of anatomically and functionally unique sub-regions. Recent literature has shifted to investigate the functions of different OFC sub-regions in reward-guided behaviour and goal-directed/habitual systems. For example, Izquierdo (2017) reviewed the various sub-divisions of the functional heterogeneity of rat OFC in various behavioural tasks such as reversal learning, delay discounting, reinforcement devaluation and pavlovian-instrumental transfer (Figure 1.6) (also see Turner and Parkes (2020)).

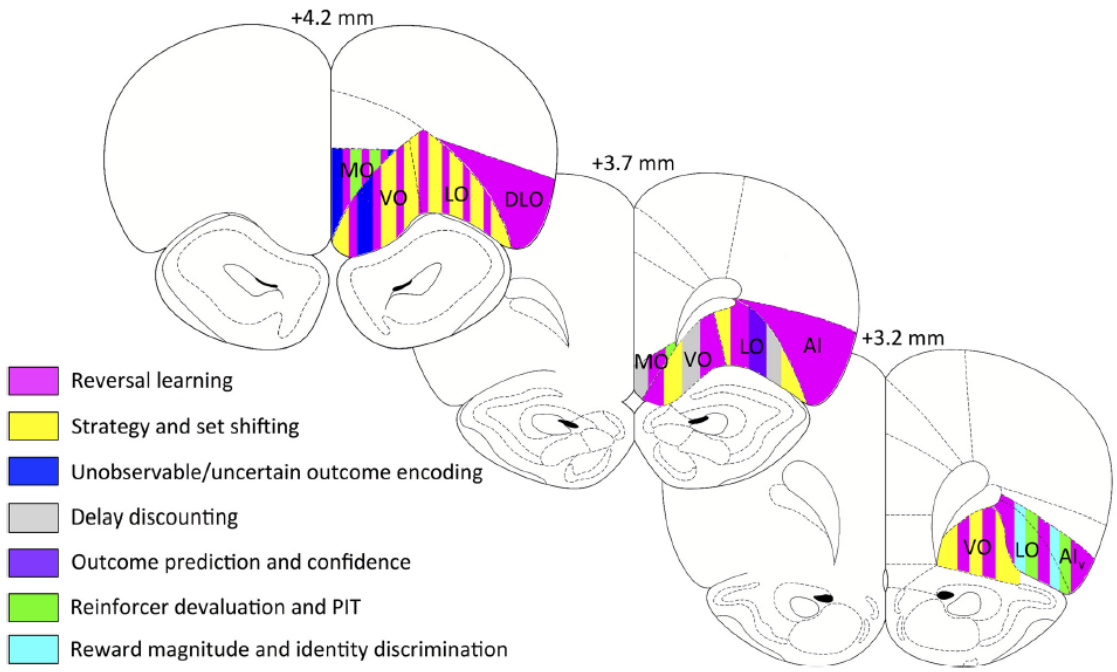

Figure 1.6. The rat OFC and localised functions of each OFC sub-region.

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Non-human animal studies could target a very specific sub-region of the OFC to investigate possible causality between the brain and behaviour. Common marmosets with the lesioned area 11 (anterior lateral OFC) did not learn the association between action and outcome in a contingency degradation task (Jackson et al., 2016). The lesion extended to the medial area 14, although a substantial proportion of the lesion was within area 11. In rats, lOFC lesion impaired pavlovian contingency degradation (Ostlund and Balleine, 2007) and mOFC lesion did not impair an instrumental form of contingency degradation (Bradfield et al., 2015). It seems that lOFC is involved in contingency degradation learning but mOFC is not. lOFC lesions (Ostlund and Balleine, 2007) and DREADDs inactivation (Parkes et al., 2017) in rats did not impair instrumental outcome devaluation, whereas mOFC lesion and chemogenetic inactivation did (Bradfield et al., 2015). Furthermore, Bradfield et al. (2018) separately lesioned the anterior or posterior mOFC, finding that only the anterior mOFC lesion, and not the more posterior mOFC lesion, impaired instrumental outcome devaluation. Rudebeck and Murray (2011), using a pavlovian form of specific satiety outcome devaluation, showed that area 11/13 lesions, but not area 14 excitotoxic lesion in macaques impaired value updating of outcomes. These studies indicated that the more lateral OFC is involved in the pavlovian form of learning while anterior mOFC is important in instrumental learning.

Such functional specialisation within the medial vs. lateral, and anterior vs. posterior OFC was further demonstrated in Panayi and Killcross (2018). They showed the lOFC lesions in rats impaired performance in a pavlovian but not instrumental outcome devaluation procedure using taste aversion, where the devaluation was achieved via pairing the reward with nausea induced by the injection of lithium chloride. They found that both anterior and posterior LO lesions impaired performance on the pavlovian outcome devaluation task, while only the posterior LO lesion impaired reversal learning and sign-tracking. For sign-tracking, the rewarded CS+ and non-rewarded CS- were accompanied by lever insertion on either the left or the right of the food magazine, but pressing the lever had no consequences. Some animals nevertheless approach and touch the lever associated with the CS+, so-called "sign-tracking", which presumably arises from pavlovian approach tendencies to the CS+. Sign-tracking is to be contrasted with goal-tracking when animals direct their behaviour instead to the reward delivering magazine.

When compared to sham and anterior LO lesioned animals, posterior LO lesioned animals showed less sign-tracking behaviour and thus more goal-tracking behaviour.

Murray et al. (2015) differentiated the role of lateral OFC in the non-human primates further, by targeting area 11 and 13 individually and manipulating each area at different stages of outcome devaluation. The animals started to learn to discriminate 60 pairs of stimuli on a touchscreen. One of the stimuli in each pair was rewarded (S+) and the other one was non-rewarded (S-). Half of the S+ led to one food, the other half led to another food. In the probe session, only S+ was presented. Animals had to choose between the two S+ and the food reward associated with the S+ was delivered. The authors inactivated area 11 and 13 at the selective satiation stage, where one of the food rewards was freely available for the animals to decrease outcome value, and at the choice stage, where animals must choose between devalued or non-devalued options. Inactivation of area 11 during the selective satiation stage did not affect animals' choice at the subsequent outcome devaluation task, but inactivation of area 11 during the choice stage did impair their ability to use the updated outcome value to guide behaviour. On the other hand, area 13 inactivation during the selective satiation stage did impair animals' choice at outcome devaluation but did not impair animals' performance when it was inactivated during the choice stage. They concluded that area 11 is critical for using the updated value information to make goal-based decisions, but is not needed during the value-updating, whereas area 13 is not necessary to make the decision but is critical in outcome value updating.

The literature has indicated that the OFC sub-regions indeed are functionally different. For instance, the lateral OFC is essential for pavlovian, stimulus-response learning and acquisition of contingency degradation, while the anterior mOFC is for the learning of instrumental value-updating but not instrumental contingency degradation. However, there is a lack of studies in the current literature that have investigated the expression of instrumental goal-directed actions while at the same time specifically targeting specific OFC sub-regions.

1.3.3. Summary

A plethora of human, non-human primate and rodent studies implicated the medial and orbital areas of PFC and dorsomedial striatum in controlling goal-directed behaviour as measured by contingency degradation ('belief') and outcome devaluation ('wanting'). However, several puzzling gaps are also present in the literature. Firstly, as mentioned in Chapter 1.3.1a, the human lesion studies usually include large areas in the mPFC, OFC and vmPFC. The more precisely targeted sub-regions within these areas were shown in non-human animal studies to have overlapping but distinctive functions. Thus, a non-human primate, with the closest evolutionary relation and PFC anatomy to humans, is an ideal candidate to investigate functions of PFC sub-regions. Additionally, compared to human imaging studies, non-human primate studies could conclude causality rather than correlation. Secondly, potentially contradictory results were observed with rodent PL and primate area 32 in outcome devaluation learning, which called into question the functional homology between rodent and primate mPFC. This would require further investigations, ideally using non-human primates. Thirdly, the literature had focused overwhelmingly on outcome devaluation but not enough on contingency degradation. This is especially true in studies using rhesus macaques, where they focused on the investigation of value-based decision-making and changes in the incentive value of the outcome. Fourthly, studies mostly examined the learning and not the expression of goal-directed behaviour. Although learning is a very important aspect of everyday life, expression of learned knowledge is critical in understanding chronic behavioural impairments seen in neuropsychiatric disorders. Despite the current trend in the non-human animal literature to differentiate the PFC sub-regions, no study to date has investigated, using a purely instrumental task, the role of area 32 (mPFC), area 24 (perigenual ACC), area 11 (anterior lateral OFC), area 14 (mOFC) and caudate nucleus in the expression of A-O contingencies using contingency degradation.

1.4 Neuropsychiatric disorders related to an impairment in the 'balance' between goal-directed and habit systems

1.4.1. Obsessive-compulsive disorder

Obsessive-compulsive disorder (OCD) is a severe and disabling neuropsychiatric disorder that affects roughly 2-3% of the population (Sasson et al., 1997; Ruscio et al., 2010). It is characterised by persistent, reoccurring obsessive thoughts and repetitive, compulsive behaviour that when performed, does not lead to meaningful consequences. It imposes tremendous social and economic burden in society and results in bizarre behaviour in individuals, which seriously hinders everyday life. Some classic examples are compulsive hand-washing even though the hands are clean, and excessive checking of the door to confirm it is locked. Interaction between different psychological factors has been suggested to underlie OCD: cognitive inflexibility, emotional vulnerability and an imbalance in the habit and goal-directed system, where the system tips towards the former (Robbins et al., 2019).

Many of the neural substrates implicated in OCD patients overlap with brain regions and circuits that affect the goal-directed and habit system, such as OFC, ACC and the striatum. The most consistent imaging finding is putative hyperactivity in the head of the caudate nucleus, mOFC, ACC and perhaps lOFC, in both adults and paediatrics (Baxter et al., 1988; Whiteside et al., 2004; Maia et al., 2008; Menzies et al., 2008; Fitzgerald et al., 2011; Gillan et al., 2014b; Pauls et al., 2014). Using resting-state fMRI, Harrison et al. (2009) found that OCD patients had significantly increased functional connectivity between anterior OFC (and its surrounding areas) and ventral caudate/nucleus accumbens regions. The strength of this connectivity also predicted symptom severity. In children with OCD, Fitzgerald et al. (2011) found a significant increase in functional connectivity (resting-state fMRI) between the dorsal striatum and vmPFC and decreased functional connectivity between the dorsal striatum and the rostral ACC. Hyperactivity in the ACC of OCD patients in decision-making tasks, such as the Stroop task, was observed in incongruent conditions when compared to congruent conditions (Schlosser et al., 2010). Hyperactivation of ACC in resting-state and symptom provocation was also documented

(Swedo et al., 1989; Perani et al., 1995; Breiter and Rauch, 1996; Nakao et al., 2005a; Koch et al., 2012).

Studies with functional imaging or neuropsychological components also revealed OCD patients' behavioural impairments in tasks that require a dominant goal-directed system and abnormalities in brain regions that are important in goal-directed and habit systems. Gillan et al. (2015) conducted a habit avoidance task in healthy controls and OCD patients. Patients and control subjects were instructed to avoid a shock that could be applied to either one of the wrists, in which the shock arrival was signalled by different CSs. After the subjects were over-trained on this avoidance paradigm, they were told that one of the electrodes attached to one of the wrists was disconnected and they could no longer be shocked on that wrist (devalued), but they should keep avoiding shocks from the other wrists (non-devalued). Behaviourally, about half of the OCD subjects developed more habitual behaviour than healthy subjects. In terms of brain imaging, OCD patients showed hyperactivation in the mOFC and caudate nucleus during the acquisition of habitual avoidance and then reductions in activation with overtraining. This hyperactivity was associated with subjective ratings of an increased urge to perform habits. They also found abnormal connectivity between caudate and subgenual anterior cingulate cortex (area 25) in avoidance from OCD patients. Vaghi et al. (2019) also developed a contingency degradation task for healthy and OCD patients. A session of 12 blocks of 2 minutes each was presented and each block contained 120 1 second bins. There were three conditions: positive contingency, where the probability of an outcome given an action [$P(o|a)$] was greater than the probability of an outcome in the absence of that action [$P(o|\sim a)$], degraded contingency, where $P(o|a) = P(o|\sim a)$], and negative contingency, where $P(o|a)] < P(o|\sim a)$]. The participants were presented with a white triangle and could decide whether to respond to it for the possibility of receiving a monetary reward. The reward could also be delivered noncontingently to the response according to different contingency probabilities. The participants were asked at the end of each block to subjectively rate the causality of their action and the outcome. OCD patients responded more in the low contingency condition when compared to healthy controls, which indicated the patients' impaired sensitivity to instrumental contingency. Interestingly, they expressed the same causality judgement when compared to healthy controls, which indicated a discrepancy between impaired performance but intact internal awareness, a

possible deficit in 'meta-cognition'. This deficit might be related to the abnormal activities found in the ACC of OCD patients. ACC was active in cognitive control tasks that require 'meta-cognition', such as conflict monitoring, prospective judgement and appropriate performance adjustment (Modirrousta and Fellows, 2008; Fleming and Dolan, 2012; Paul et al., 2015; Qiu et al., 2018).

Overall, hyperactivity in the vmPFC, OFC, ACC and the head of the caudate nucleus was observed in OCD patients compared to healthy subjects. Additionally, OCD patients were impaired in their performance in the contingency degradation task. Since most non-human animal studies have focused on lesion and inactivation of brain regions, it would be beneficial to investigate the effect of activating PFC sub-regions on behaviour, which would mimic the hyperactivity detected in OCD patients.

1.4.2. Other disorders

Several other mental health disorders also have been shown to have an impaired 'balance' between the goal-directed and habit systems. Gillan et al. (2016) used an online platform to recruit a large sample of the general population to identify a reduction in goal-directed control that was associated with compulsive behaivur. A reduction in goal-directed control was seen in OCD, binge-eating disorder and alcohol addiction subjects. Indeed, studies had shown that in both OCD and binge eating disorder subjects, reduced grey matter volume in the caudate nucleus was associated with a bias towards habit formation (Voon et al., 2015). The mPFC, OFC and the striatum associated with goal-directed and habit formation have also been implicated in compulsive drug-seeking behaviour (Everitt and Robbins, 2005; Everitt et al., 2008; Ersche et al., 2013; Ersche et al., 2016). Thus, a reduction in goal-directed control is present in multiple mental health disorders, which signifies the need to understand better the goal-directed and habit systems, together with their neural basis.

1.5 Rationale and Aims

The current thesis aims to address the neural underpinnings of goal-directed actions as measured by detecting causality between the action performed and the outcome. A novel, touchscreen-based contingency degradation task was used to measure the subjects' expression of the behavioural output of action-outcome contingencies, which is critical in goal-directed performance. The subjects used in this thesis are common marmosets, a critical experimental species that bridges rodent and human studies. This study focuses on the prefrontal cortex and the striatum, investigating a total of six specifically targeted brain regions that are highly implicated in controlling goal-directed behaviour.

Although the general direction of the research scene is moving towards clearer labels used to address the function of PFC sub-regions, the confusing terms still used across species might contribute to a lack of translational studies using similar behavioural tasks and targeting similar brain regions to replicate results consistently. For instance, rodent studies focus more on regions such as the mPFC and human and non-human primate studies utilise nomenclature such as dlPFC, OFC and cognition more compared to rodent (Laubach et al., 2018). Therefore, it is ever pertinent as a field to have studies that use similar behavioural tasks across species and targeting PFC sub-regions that are homologous. Thus, more precise manipulations are required to address the differential contributions of PFC sub-regions. None of the existing literature has addressed, in combination, the direct manipulation of specific prefrontal sub-regions in the primate, and the expression and maintenance of learned action-outcome associations that do not depend on reward value but causal predictions of an action and its consequences. There is currently a large void in the literature on causally identifying the precise brain region that integrates, stores and maintains learned information from other brain regions on goal-directed actions. The current thesis is designed to fill this gap in the current literature.

The common marmosets are an appropriate species to bridge rodent and human studies. Firstly, it is possible to precisely target and reversibly manipulate brain regions in the marmosets as well as in the rodents; however, as mentioned above in Chapter 1.2.2, it is exceedingly more difficult to draw homologies between rodent and humans than

between a non-human primate and humans. The marmosets could adapt to tasks similar to those in the rodent and humans and are easier to handle compared to macaque monkeys, whilst having a primate PFC for appropriate comparison with human studies. The current thesis also addresses issues not easily attainable in human studies. Human imaging studies only report on correlation but not causation, and lesion studies in humans incorporate large areas of the brain that are not precise enough to tease out functional dissociations. The within-subject design of the current study not only is statistically desirable but also is a demonstration of the application of the 3-Rs (Replacement, Reduction and Refinement) in animal research, in reducing subject numbers and refining animal welfare and reducing the economic burden as non-human primate studies are so expensive. Taken together, this thesis aims to investigate important issues in the field in the following ways: 1). Use of reversible, precise manipulations of PFC sub-regions and striatum 2). Provision of translational potential that is difficult to attain in rodent and human studies 3). Investigation of the expression of action-outcome contingencies that are understudied 4). Use of a within-subject design that investigates the function of multiple brain regions in one unifying task.

Chapter 2 of the thesis will describe a novel contingency degradation task used to investigate the expression of goal-directed action in terms of understanding the association between action and outcomes. In the contingency degradation task, animals need to maintain their learned knowledge to decrease their responding to the degraded outcome. Chapter 2 will validate the pharmacological methods used in this thesis to manipulate PFC and striatum. Inactivation in PFC will use muscimol/baclofen (mus/bac; $GABA_A$ and $GABA_B$ agonist, respectively) and activation using dihydrokainate acid (DHK; glutamate reuptake inhibitor). I chose to activate the PFC sub-regions because as reviewed in Chapter 1.4.1, neuroimaging studies of OCD patients had consistently implicated abnormal over-activity of PFC regions, such as the medial and lateral OFC, ACC and mPFC. For Inactivation in the caudate nucleus, I will use CNQX (AMPA receptor antagonist) to block excitatory input into the caudate nucleus. This is because the caudate nucleus is dominated by GABAergic neurons (interneurons, medium spiny neurons), which could make the effect of mus/bac uncertain. More validations of the drug DHK and CNQX will be presented in chapter 2. Additionally, chapter 2 will demonstrate the anatomical connectivity between area 24 and the caudate nucleus (both targeted in this

thesis), which are pertinent in the cortico-striatal loop that is implicated in goal-directed action.

Chapter 3 will investigate the different roles of PFC sub-regions in contingency degradation: perigenual anterior cingulate cortex (area 24), anterior orbitofrontal cortex (area 11) and ventromedial PFC (area 14-25). Chapter 4 will continue the investigation to include more PFC sub-regions (area 14 and area 32) and the caudate nucleus. All brain regions will be reversibly inactivated and activated to explore their role in the expression of goal-directed action, except for the caudate nucleus, which will only be inactivated. Additional experiments and analysis, such as the use of the progressive ratio schedule and juice preference, were also conducted to control possible effects of motivation and to further understand and interpret the results generated from the main contingency degradation experiments.

Chapter 5 will synthesise all the data together from Chapter 2-4 across 6 brain regions in a speculative discussion of theories of the specialised role of PFC and striatum in goal-directed control and its implications. Possible limitations of the methods used as well as possible future directions and studies will also be addressed.

Chapter 2

General Methods and Neurobiological Validation

2.1. Subjects and housing

Twelve common marmosets (*Callithrix jacchus*; four males and eight females) were used in this thesis. One animal had died prematurely during the experiments before the commencing of manipulations, thus was excluded from the twelve animals and the thesis. They were housed and bred-on-site in a conventional barrier facility in the University of Cambridge Marmoset Breeding Colony. Experimental animals were housed in male-female pairs in custom-made housing (Tecniplast UK Ltd., Kettering, UK). Not all animals in this thesis were housed with a partner who is also used in the studies from the

current thesis. The rooms were constantly kept at 24° C and relative humidity at 55%. The rooms were illuminated gradually from 7:00 am to 7:30 am and dimmed from 7:00 pm to 7:30 pm to simulate day/night cycle. The marmosets were tested 4-5 days per week and not at the weekends. All monkeys were fed 20 g of MP.E1 primate diet (Special Diet Services) and sliced carrots 5 days a week after the daily behavioural testing session, with simultaneous free access to water for 2 hours. On weekends, their diet was supplemented with fruit, rusk, malt loaf, eggs, bread, and treats, and they had free access to water. The male marmosets were vasectomised to prevent pregnancy of their female partners. Their home cages were filled with environmental enrichment such as ropes, ladders, swinging cardboard boxes. All animals were carefully monitored by the unit Named Animal Care and Welfare Officer (NACWO), researchers, Named Veterinary Surgeon (NVS) and animal technicians. The projects were conducted under Home Office Project Licences 70/7618 and P09631465, and all studies were verified and authorised by the unit NACWO. The projects were regulated under the Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 following ethical review by the University of Cambridge Animal Welfare and Ethical Review Body (AWERB).

2.2 Testing Apparatus

Testing took place using an automated touch-screen apparatus (Biotronix, Cambridge, UK). Marmosets were transferred from their home cages to the testing apparatus via a Perspex box, which is designed to be inserted directly into the testing apparatus for the duration of testing. The marmoset can move freely within the box and is not otherwise restrained. One side of the box was opened to enable the marmosets to interact with computer-controlled stimuli presented on the touchscreen (Campden Instruments, Longhborough, UK) through a set of vertical metal bars that separate the touchscreen from the marmosets. They received liquid reinforcements from a spout that was suspended centrally in front of the touchscreen. The spout contains four tubes and liquid reinforcement was moved through the tubes by pumps (Autoclude, Essex, UK), allowing the delivery of up to four different liquid rewards. An infrared beam sits at the mouth of the sprout and detects licking of reward. A speaker at the back of the testing

box (not seen by the animals) played birdsong to signal the delivery of rewards. The experiments were monitored and could be recorded by mounted cameras in the testing chamber. The MonkeyCantab programme (R.N. Cardinal) controls the touchscreen, pumps, sprout and speakers through the Whisker control system (Cardinal and Aitken 2010).

2.3. Pre-touchscreen training

The animals went through pre-touchscreen training and touchscreen training before going onto the contingency degradation task (Figure 2.1). The main food reinforcer (banana milkshake, Nesquik) was initially introduced into the marmosets' home cages for them to become familiar with the reward. They were trained to get into the carrying box willingly by providing marshmallow reward for compliance. They were transferred to the testing apparatus to enable them to become acclimatised to the testing procedures and equipment. A syringe filled with milkshake was introduced to them while they were sitting inside the box in the testing apparatus. They were shaped to approach the licking spout using the syringe with milkshake and marshmallow. Once animals confidently approached the spout without experimenter guidance, the reward was delivered freely through the licking spout in the testing apparatus according to a fixed schedule: 8-sec reward with 8-sec inter-trial intervals (ITIs). During reward delivery, birdsong was also played to act as a conditioned reinforcer in subsequent touchscreen training sessions.

2.4. Touchscreen training

Three training phases were conducted and each phase was completed in separate training sessions (Table 2.1). In the initial phase of touchscreen training, marshmallows were taped onto a horizontal green bar spanning across the touchscreen to encourage animals to respond to the bar (Figure. 2.2A). A touch on the green bar generated the birdsong that signalled the availability of reward; a touch outside the green bar had no consequences. The banana milkshake was delivered as a reward for 8 seconds. In the

second phase, animals responded to a small green square centred on the touchscreen (Figure 2.2B). In the final training phase, a green square that was randomly and alternately presented to the left and right of the touchscreen (Figure. 2.2C). The size of the marshmallows taped onto the screen in the position, where the stimulus was presented, decreased over time as animals associated the touching of the stimulus with the delivery of the reward. The animals were also moved to a "lick contingent" condition, where the birdsong signalled reward delivery, but the animals had to lick the spout to have the reward delivered rather than the reward being delivered automatically. After a fixed ratio 1 schedule, where each response generated reward delivery, animals were switched to a variable ratio (VR) 3 schedule, where they received a reward after every 2-4 responses. They then moved to a VR 6 schedule, and eventually a VR 10 (range 5-15 responses per reward) schedule. Following stable performance (3 consecutive sessions of consistent responding), the banana milkshake was replaced with blackcurrant, strawberry, or summerfruit juice. Each animal was assigned a pair of juices, with one juice always associated with the left, and the other the right, stimulus (counterbalanced between subjects). After another 3 stable sessions of performance, animals were transferred to the final contingency schedule (described in detail below) in which the green squares were replaced with compound, multi-coloured stimuli (Maltese Cross; Figure 2.2D). The sequence of training is summarised below in table 2.1.

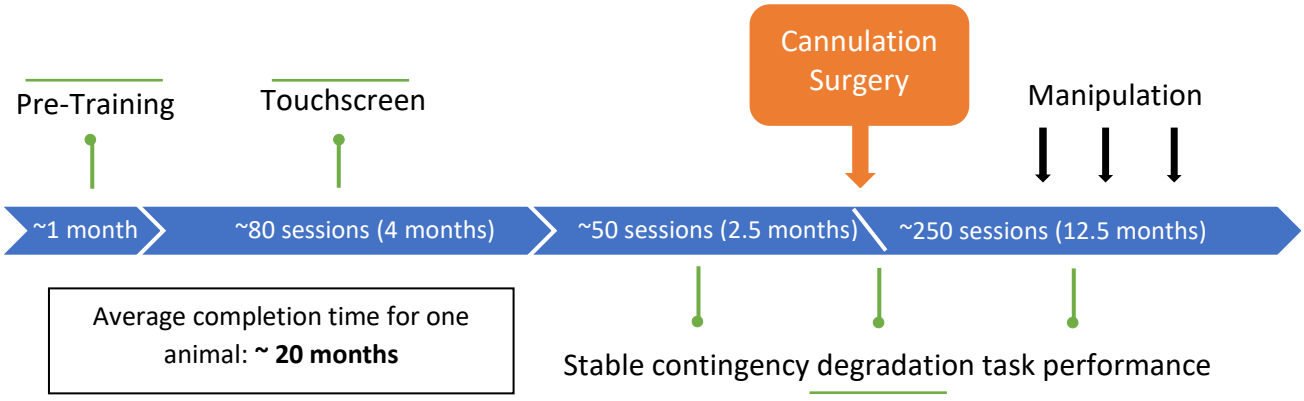


Figure 2.1. Timeline of the experiment, with the average length (in months) for one animal to finish each stage. All the animals started with pre-training, moved on to touchscreen training, received cannulation surgery when stable contingency degradation task performance was established, and then manipulations after surgery. On average, one animal took 20 months to finish the experiment.

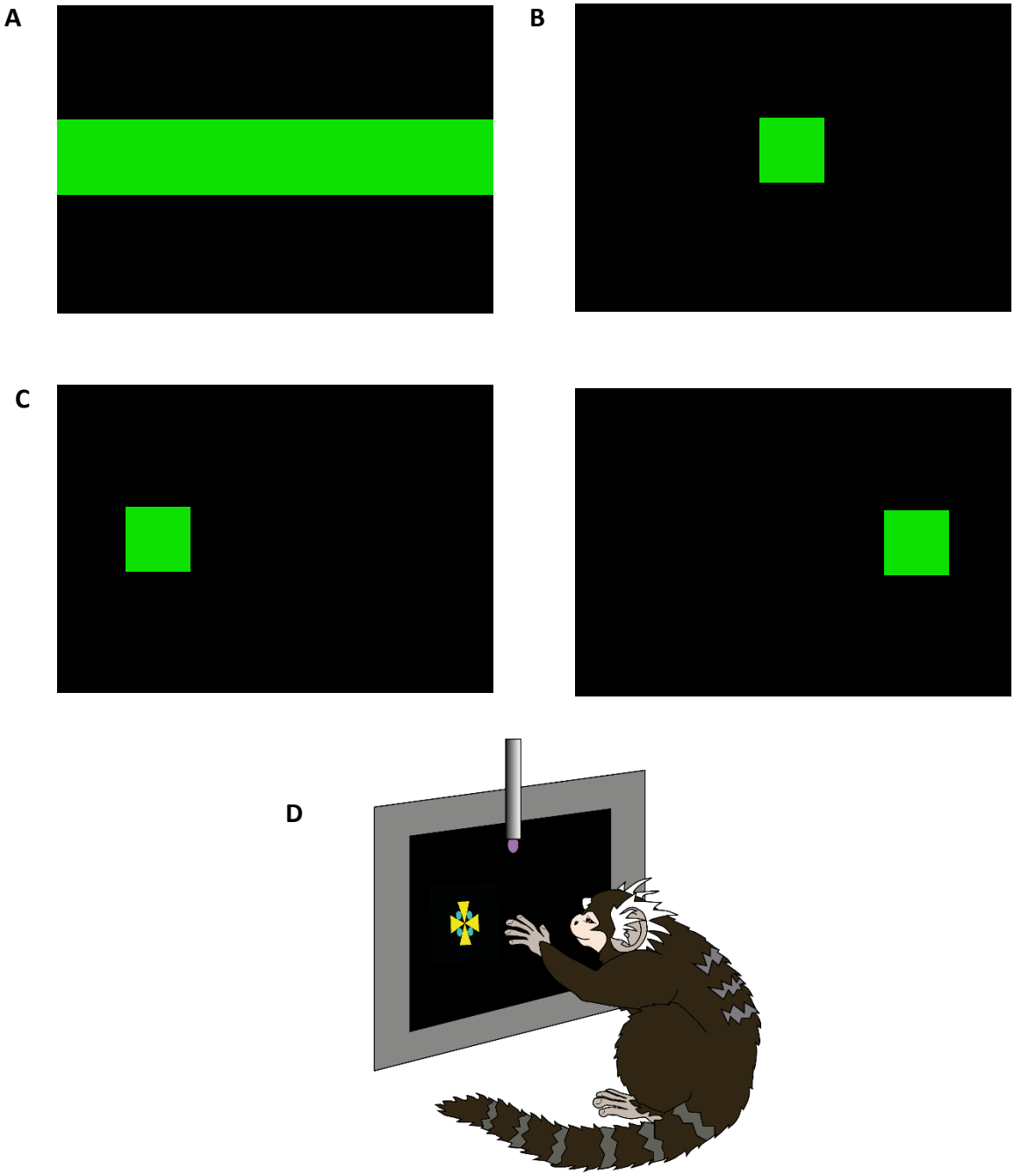


Figure 2.1. Three distinct phases of training that occurred in separate sessions. For each stage, the marmoset needed to touch the stimulus in order to obtain a reward delivered from the spout. A). First phase. The green horizontal bar. B). Second Phase. After good responding to the green bar the stimulus changed to a green square at the centre of the touchscreen. C). Third phase. After good responding to the central green square, marmosets respond to the green square presented one at a time rotating between left and the right of the touchscreen. D). Third phase. Green square was changed to the Maltese cross. Marmosets touch the stimulus and a type of juice reward will drop from the liqueur sprout hanging from above the touchscreen.

Table 2.1. Touchscreen training schedule.

Schedule	Training Phase	Stimulus	Juice	Reward Length	ITI
FT	1	green bar	Banana milkshake	8 sec	1 sec
FT	2	green square centre	Banana milkshake	8 sec	1 sec
FT	3	green square L/R side	Banana milkshake	8 -> 5 sec	1 -> 3sec
FR1	3	green square L/R side	Banana milkshake	5 sec	-
VR 3	3	green square L/R side	Banana milkshake	5 sec	-
VR 6	3	green square L/R side	Banana milkshake	7.5 sec	-
VR 10	3	green square L/R side	Banana milkshake	10 sec	-
VR 10	3	green square L/R side	Juice	10 sec	-
Contingency	3	green square L/R side	Juice	10 sec	-
Contingency	3	Maltese cross L/R side	Juice	10 sec	-

2.5. Contingency degradation task

The contingency degradation task measures goal-directed behaviour (action-outcome associations). A four-day contingency degradation block consisted of two control sessions followed by two contingency probe sessions (Figure 2.3). Any pharmacological manipulations of the brain occurred only in the probe sessions. On the first two control sessions, animals responded to one of the stimuli (left or right location) for contingent reward in the first session and the other stimulus on the opposite location for a different contingent reward in the second session. The two stimuli are identical and only differ in their display location (i.e. either on the left or the right of the touchscreen, never displayed concurrently). Performance across these sessions provided a control baseline for comparison against the subsequent two probe sessions that would follow. Whether or not an outcome (o) is contingent upon an action (a) depends not only on the probability of the outcome occurring with the action [$P\ (o|a)$] but also on the probability of the outcome occurring in the absence of that action [$P\ (o|{\sim}a)$]. To determine the animals' sensitivity to contingent action-outcome associations, two probe sessions were presented.

In each probe session, the non-contingent reward was introduced. In one condition, the non-contingent reward was the same as the contingent reward, resulting in contingency degradation (degraded). In the second condition, the non-contingent reward was the alternative reward not contingently available in that session, thus maintaining action-outcome associations for the first reward (non-degraded). Hence, if the animal was responding in a goal-directed fashion, he or she would only reduce responses in the degraded sessions in which the response-outcome associations had been weakened (degradation effect). This contingency degradation effect was first observed by Rescorla (1966, 1968). To implement these schedules, each 12-minute session was divided into 1-sec bins (Figure 2.3). The probability of receiving the contingent reward was p = 0.10, i.e. an average of 10 responses would yield reward (VR 10, range 5-15). The probability of receiving the non-contingent reward varied according to each animal. We customised the non-contingent reward probability for each animal because of the large individual variance in response rate between marmosets. For example, if p = 0.067, for every 1-sec bin when the animals did not respond, the probability of non-contingent reward delivery was 0.067 (1 reward delivered on average every 15 sec of non-responding, range 10-20). The most appropriate non-contingent probability for each animal was determined to ensure that they would detect the free rewards but not so many rewards as to produce satiety and result in cessation of responding. As soon as marmosets exhibited the degradation effect, they were cannulated.

I also conducted baseline sessions, separate from the contingency degradation task, to examine the effect of pharmacological manipulations on animals' responding. The baseline sessions also consisted of four days block. The first two days were the same as the contingency degradation task, and the last two days were a repeat of the first two days but with pharmacological manipulations in the brain.

On average, animals took 20 months to finish the entire experiment (Figure 2.1). The experiment was designed to investigate three brain regions, with three manipulations (control, inactivation, activation) in each brain regions and four different session conditions for each manipulation (non-degraded session, degraded session, baseline degraded juice, baseline non-degraded juice), all of which were conducted within-subject. Three of a total of six brain regions were examined in Chapter 3, and the remaining three in Chapter 4.

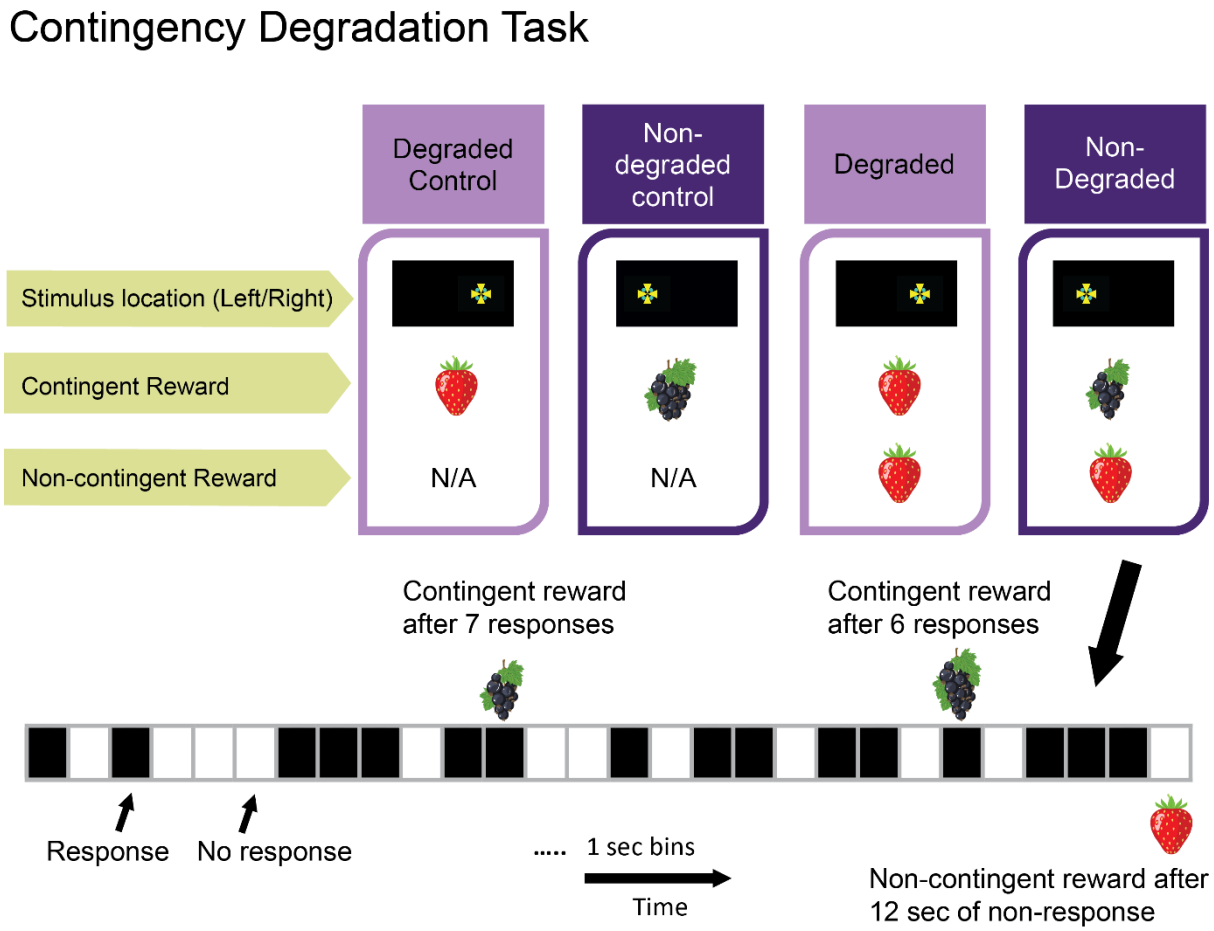


Figure 2.3. The contingency degradation task. In this figure, the degraded reward was strawberry juice and the non-degraded reward was blackcurrant juice. There was no non-contingent reward in the two control sessions. In the degraded session, if animals responded to the stimulus, the contingent reward (strawberry juice) was delivered (probability = 0.1). If animals did not respond to the stimulus, strawberry juice was also delivered (probability = 0.067). In the non-degraded session, if animals responded to the stimulus, the contingent reward (blackcurrant juice) was delivered (probability = 0.1). If animals did not respond to the stimulus, non-contingent strawberry juice was delivered (probability = 0.067). The 12-min session was divided into 1 sec bins. Black boxes indicated a response and white boxes a non-response within that 1 sec bin.

2.6. Behavioural measures

The main behavioural measure was the contingency degradation index. This was calculated as follows:

$$Contingency\ degradation\ index = \%\ of\ control\ session = \left(\frac{response\ rate\ in\ degraded\ or\ non-degraded\ degradation\ session}{response\ rate\ in\ degraded\ or\ non-degraded\ control\ session}\right) * 100$$

This percentage of control session approach is important due to the animals' individual variability in response rate.

To take into account the additional time animals spent drinking in degradation probe sessions with additional (free) reward compared to control sessions, the above index compared response rates rather than response numbers as the primary behavioural measure.

Response rate (responses per min.) in control sessions is calculated as follows:

$$Response\ rate = \frac{Response\ number\ of\ contingent\ reward}{(720 - number\ of\ contingent\ reward * 10\ )/60}$$

Where 720 is session length in seconds and 10 is reward duration in seconds.

The response rate in degradation sessions:

$$Response\ rate = \frac{Response\ number\ of\ contingent\ reward}{[720 - (number\ of\ contingent\ and\ noncontingent\ reward) * 10\ ]/60}$$

Lick latency and the number of licks per reward were measured as indicators for primary motivation. Lick latency was the time (sec.) between the onset of birdsong that signals the delivery of reward and the first lick at the spout for the reward. The median of lick latencies was taken for each animal and averaged across animals for each session type.

The number of licks per reward was calculated for each animal as follows:

$$Lick\ per\ reward = \frac{Total\ number\ of\ licks\ in\ one\ session}{Total\ number\ of\ rewards\ delivered\ in\ one\ session}$$

Due to the individual variability of each animal, percentage of control, calculated in the same way as contingency degradation index, was used instead of absolute values of lick latency and lick per reward.

2.7. Cannulation surgery

2.7.1. Pre-surgery

All surgical instruments and consumables were autoclaved in autoclave pouches and laid out on a disinfected bench. Stereotaxic equipment with cannula and depth probe arm holders were prepared and disinfected with Anistel. Anaesthetic machines, monitoring equipment, oxygen tanks and saline bags were checked to be full and functional at least one day before the surgery. Marmosets were weighed before the cannulation surgery to ensure their weight had not declined by more than 5% from the day before, or else the surgery was postponed until the regaining of lost weight. The nest boxes for the marmosets were modified to include a flapping door so that the implanted marmoset did not damage their cannula by contact with the nest box. No food was given to the marmoset at least 12 hours before the surgery to prevent vomiting during surgery.

On the day of surgery, marmosets were conveyed to the pre-surgery room and premedicated with ketamine hydrochloride (Vetalar; 0.05 mL of a 100 mg/mL solution, i.m.; Amersham Biosciences and Upjohn, Crawley, UK) and then given a long-lasting nonsteroidal, anti-inflammatory analgesic (Carprieve; 0.03 mL of 50 mg/mL carprofen, s.c; Pfizer, Kent, UK) and placed in an incubator (Vetario S20 Intensive Care Unit, Brinsea Products Ltd., Summerset, UK). After they were sedated, they were placed onto a heat mat to keep them warm, as marmosets could become hypothermic when under anaesthetic. The top of their head was shaved for access to the brain and their hands/feet shaved for attachment of monitoring equipment.

2.7.2. Intubation and maintaining anaesthesia

Marmosets were transferred with the heat mat to the surgical table and kept under anaesthesia using a facemask with 4% isoflurane in 0.7L/min O_2. When they were unconscious and with reduced muscle tension, they were intubated. One researcher held the head of the marmoset (by their cheekbones) and the upper jaw open, while the second researcher held the lower jaw open to apply a topical anaesthetic (Intubeaze 20mg/ml lidocaine hydrochloride spray, Dechra Veterinary Products Ltd., Shropshire, UK)

using a cotton bud to the back of the marmoset's mouth. The second researcher then moved the intubation tube down the marmoset's throat and attached the intubation tube onto the anaesthetics machine (Compact Anaesthesia Systems, VetTech Solutions Ltd.), now without the face mask connected. The marmoset's breathing was checked by the coordinated movement of their chest and a balloon attached to the tubing. Following intubation, they were placed into a stereotaxic frame modified for the marmoset (David Kopf, Tujanga, CA) and maintained on 2.0–2.5% isoflurane in 0.3 L/min O_2 throughout the surgery. Pulse-rate, O_2 saturation, breathing rate, and CO_2 saturation were all monitored by pulse oximetry and capnography (Microcap Handheld Capnograph, Oridion Capnography Inc., MA, USA) while the core body temperature was monitored by a rectal thermometer (TES-1319 K-type digital thermometer, TES Electrical Electronic Corp., Taipei, Taiwan) covered with a disposable sheath (Temperature Probe Cover pre-lubricated, SA Instruments Inc., New York, USA).

2.7.3. Cannulation

The marmoset was secured in the stereotaxic frame by ear, mouth (roof of the mouth) and eye bars (supraorbital foramen of the eye sockets). To prevent dryness and irritation of eyes, ophthalmic ointment (Lacri-lube, or Viscotears, Allergan Inc., CA, USA) was applied. A surgical lamp (Brandon Medical, Leeds, UK) illuminated the cranium and provided an additional heating source for the marmoset. Chloraprep (SEPP applicators, BD, Berkshire, UK) was applied to the head and an antimicrobial incise drape (Ioban 2 Antimicrobial Incise Drape, 3M, Minnesota, USA) covered the head. A sterile polythene operating cover (Buster Cover-Op, VetTech Solutions, Ltd.) was placed over the marmoset's body. Autoclaved instruments were carefully emptied from the sterile pouches onto a sterile drape placed within reach of the surgeon. After a midline incision was made on the head using a sterile scalpel, a metal tissue spreader (eye speculum) was placed to hold the skin and muscle back to expose the skull. Sterile (1ml of 0.9% saline) was administered subcutaneously every hour to ensure hydration of the animal.

A stereotactic coordinate system was used to determine the location of targeted brain regions. The superior sagittal sinus at anteroposterior (AP) 17.5 was the zero

coordinate for the lateromedial (LM) direction and the interaural line at the ear bar was the zero coordinate for the anteroposterior direction (more positive towards the anterior side). To account for the varied brain size of marmosets, Dias et al. (1997) first described using "depth check" in surgery. At AP 17.5 and LM -1.5, the surgeon used a dental drill (Dental Unit Polisher/Drill Unit II, Eickemeyer, Tuttlingen, Germany) with attached burr (Ash Steel Burs, Dentsply Ash Instruments, Surrey, UK) to drill a hole on the skull. A probe (Smooth Dental Broach, Micro-Mega, Besancon, France) on a stereotaxic arm was lowered to measure the distance between the surface of the brain and the base of the skull. This measurement was used to standardise further stereotaxic coordinates. If the depth fell in between 5.8-6.8mm, no adjustments were made; if the depth was <5.8mm, the probe was moved in increments of 0.5mm posteriorly along the AP axis until the depth fell within the required range; if the depth was >6.8mm, the probe was moved in increments of 0.5mm anteriorly along the AP axis. If the coordinates were adjusted due to depth checks, the coordinates for subsequent cannula placements were adjusted either anteriorly or posteriorly by the same amount that the probe was adjusted to reach the desired depth (5.8-6.8mm). The surgeon used a movable microscope (S5 Opmi-MD microscope, Carl Zeiss Ltd, Cambridge, UK) to monitor the entire surgical process whenever necessary.

The surgeon drilled holes in the skull where the targeted cannula placements were located with newly adjusted coordinates. Steel skull screws (Plastic One, Virginia, USA) were screwed into the skull in appropriate places around the cannula holes. A layer of adhesive (Super-Bond C&B, Sun Medical Co. Ltd., Shiga, Japan) was applied onto the skull around the cannula holes. Together with the skull screws, the adhesives help to adhere the dental cement onto the skull, where the cement was used to hold the cannula securely in place without movement. Cannulae (Plastics One) were lowered bilaterally into desired brain regions (eg. Area 11, area 24, area 32 and caudate nucleus) using the stereotaxic arm. Dental cement was then applied to hold the cannula firmly in place. Dummy cannulae of the appropriate length were inserted into the guide cannulae, which were protected with metal or plastic dust caps. The co-ordinates for each brain region are listed in experimental Chapters 3 and 4. Cannulae locations were evaluated post-mortem (for further details, see Chapter 3 and 4). Each animal received bilateral cannulae in two target regions; access to area 14-25 or area 14 was via extended injectors through cannulae in

area 24 or area 32, respectively. The opened skin was sutured at the front and back of the dental acrylic head mount (Coated VICRYL (polyglactin 910) Suture, Ethicon, Puerto Rico, USA) and the animal was slowly brought back to conscious awakening by lowering the isoflurane flow. To prevent tissue inflammation, 0.18-mL dexamethasone phosphate i.m. were injected (3.8mg/ml, Aspen Pharma, Berkshire, UK), with 0.09 mL in each leg.

2.7.4. Post-operative care

The anaesthesia machine was turned off, marmoset de-intubated and the temperature probe removed. Oxygen continued to be supplied to the marmoset and their vital functions closely monitored. When the marmosets were able to maintain a high oxygen saturation level (95% or above) without the oxygen supply, they were placed into the incubator to recover. They were closely monitored by researchers and signs such as awareness and limb movements and coordination were carefully observed. Water or food was given gradually after one to two hours following surgery. After postoperative recovery, all monkeys were returned to their home cages and received the analgesic meloxicam (0.1 mL of a 1.5 mg/mL oral suspension; Boehringer Ingelheim, Germany) for the next 3 days as well as at least a full 7 days of "weekend diet" and water *ad libitum* to ensure complete recovery before returning to testing. The implants were cleaned with 70% ethanol during every infusion and at least once every week (and caps and cannula dummies changed) to ensure the cannula site remained free from infection.

2.8. Intracerebral drug infusion

Infusions were delivered through injectors that extended beyond guide cannulae into the brain region of interest. This way, the cannulae is positioned immediately above the targeted brain region to minimise cannula track damage into the brain and reduced the likelihood of fluid tracking back into the guide cannula rather than spreading to the surrounding tissue. The lengths of the injectors varied according to each targeted brain region and sometimes between animals, but not within animals.

The infusion procedure was described by Clarke et al. (2015) using aseptic procedures. The injectors were connected to PTFE tubing (inner diameter 0.3mm, VWR International Ltd., UK) attached to 10 mL Hamilton Syringes (Sigma-Aldrich, Missouri, USA), which were mounted on an infusion pump (Kd scientific, Massachusetts, USA). The two ends of PTFE tubing were connected to the injectors and syringe via solva tubing (inner diameter 0.38mm, Pulse Instrumentation, Wisconsin, USA). All syringes, tubing, injectors were sterile and assembled on a sterile surgical drape. Hamilton Syringes, tubing and injectors were filled with sterile saline solution (sodium chloride 0.9% w/v, Hameln Pharmaceuticals Ltd., Gloucester, UK). A small air bubble was created in the tubing by drawing the syringe plunger back a small distance, then drawing up additional saline solution or drug from a sterile Eppendorf. This way, the movement of the drug could be tracked by marking the bubble, in addition to separating saline and the drug.

The marmoset was diverted to the top right corner of their home cage where a researcher besides the experimenter handled him/her using suitable gloves. The marmoset was held in a restraining but comfortable position and brought to the minor procedures room where the infusion took place. The experimenter removed the dust caps and dummy cannulae, cleaned the guide cannulae with 70% ethanol wipes and with sterile gloves placed the injectors into the guide cannulae. To inactivate prefrontal cortical regions, a mixture of 0.1 mM muscimol ($GABA_A$ receptor agonist) and 1.0 mM baclofen ($GABA_B$ receptor agonist) was infused at a rate of 0.25 µL/min for 2 minutes (mus/bac; dissolved in PBS or saline). To inactivate the caudate nucleus (CN), 1.0 mM of CNQX (potent selective AMPA/Kainate receptor antagonist) was infused at a rate of 0.3 µL/min for 1 minute (CNQX dissolved in saline). To activate prefrontal regions, 6.25 nmol/µL

dihydrokainic acid (DHK; dissolved in PBS or saline) was infused at a rate of 0.5 µL/min for 2 minutes. DHK increases extracellular glutamate levels via inhibiting the excitatory amino acid transporter-2 (EAAT2/GLT-1) mediating glutamate re-uptake in neighbouring astrocytes, thereby increasing local activation. Please see chapters 3 and 4 for more details of the drugs used. The injector was left in place for one additional minute for drugs to diffuse. All drugs were pre-prepared, aliquoted, and stored at -20° C. On the day of the infusion, the drug was taken out of the freezer, defrosted and vortexed before use.

2.9. Statistical analysis

Data were analysed using a mixed-model ANOVA with the free programming language R for Windows GUI version 3.5.1 (R core team, 2019). I used the Lme4 package to conduct linear mixed-effects models with Type III analysis of variance with Satterthwaite's method for degrees of freedom (Bates et al., 2015). Bartlett's test was used to determine the homogeneity of variance. Each significant main effect ($p<0.05$) was further examined using pair-wise comparisons of least square means (lsmeans package in R) for specified factors in linear or mixed models. Data from drug manipulations on baseline sessions underwent the same analysis. All graphs were first completed in GraphPad Prism version 7 for Windows (GraphPad Software, La Jolla, California, USA, www.graphpad.com). The graphs were then transferred to Adobe Illustrator CS6 (Abobe Inc., San Jose, California, USA) for further modification.

2.10. Histology

At the end of the experiment, all monkeys were sedated with ketamine hydrochloride (Pharmacia and Upjohn, 0.05 mL of a 100 mg/mL solution, i.m.) and humanely euthanised with Euthatal (1 mL of a 200 mg/mL solution, pentobarbital sodium; Merial Animal Health Ltd; i.v.) before being perfused transcardially with 400 mL of 0.1 M

PBS, followed by 400 mL of 4% paraformaldehyde fixative solution over approximately 15 minutes. The cannulae and dental cement were carefully removed. After the brain was removed it was left in the 4% paraformaldehyde fixative solution overnight, before being transferred to 0.01M PBS-azide solution for at least 48 hours and then transferred to 30% sucrose solution for further 48 hours for cryoprotection. Brains were sectioned on a freezing microtome (coronal sections; 40-60mm), mounted on slides and stained with Cresyl Violet. The sections were viewed under a Leitz DMRD microscope (Leica Microsystems, Wetzlar, Germany). The cannula locations for each animal were represented on schematised coronal sections of the marmoset brain (see chapter 3 and 4 for details). Before euthanasia, some animals underwent infusions of drugs for *c-fos* verification and/or track tracing studies (see Chapter 2.11).

2.11 Neurobiological Validation

As described in Chapters 1.5 and 2.8, three drugs were used to either inactivate or activate the different brain regions: muscimol/baclofen ($GABA_A$/$GABA_B$ antagonists), DHK (EAAT2/GLT-1 mediated glutamate reuptake inhibitor) and CNQX (AMPA receptor antagonist). Additionally, this thesis aimed to understand the contribution of the frontostriatal circuit in goal-directed actions, especially the connectivity between area 24 and CN. This is because it became clear during the experimental work to be described in Chapter 3 that area 24 is likely the most important region of those investigated for mediating action-outcome contingencies. Thus, it became pertinent to understand where in the CN area 24 projects for the studies in Chapter 4, so I focused on examining this issue after the experiments were completed in Chapter 3. This section aims to 1) validate methodologically the effectiveness of the three drugs used in the marmoset and 2) validate the anatomical connectivity between area 24 and CN in the marmoset. These forms of validation will be supported with behavioural, anatomical and immunohistochemical evidence.

2.11.1. Validation of neuropharmacological manipulations

2.11.1a Dihydrokainate acid (DHK)

To increase neuronal activity and thus to activate the brain, I infused DHK into the PFC regions. DHK is an inhibitor of astrocytic excitatory amino acid transporter 2 (EAA2/GLT-1) (Arriza et al., 1994; Anderson and Swanson, 2000). Glutamate is the major excitatory amino acid neurotransmitter in the central nervous system, and its release and clearance are tightly regulated. The role of EAA2 is to down-regulate glutamate levels in the synapse so that glutamate receptors are not over-stimulated, which could potentially lead to adverse effects such as epileptic seizures. EAA2 is found throughout the brain and primarily in astrocytes. It is responsible for >90% of glutamate uptake (Maragakis and Rothstein, 2004; Lauriat and McInnes, 2007; Sheldon and Robinson, 2007). As an inhibitor of EAA2, DHK blocks the clearance of extracellular glutamate (microdialysis: (Fallgren and Paulsen, 1996)). This blockage was shown to increase the excitability of the neuronal population and post-synaptic action (Munoz et al., 1987). Electrophysiology data showed that DHK increased miniature IPSCs in the presynaptic terminals in hippocampal neurons (Mathews and Diamond, 2003). Behaviourally, intracerebroventricular injection of DHK in rats induced signs of anhedonia (reduced intracranial self-stimulation) and impairment in spatial memory (Bechtholt-Gompf et al., 2010). The authors also showed increased neuronal activity after DHK injection in the IL of rats via increased *c-fos* expression. A subsequent study where the authors infused DHK into the PFC of rats also produced signs of anhedonia (John et al., 2012). In the marmoset, Alexander et al. (2019) not only demonstrated that DHK infusion into area 25 produced anhedonia-like behaviour, but also used 18-FDG PET imaging to show increased FDG uptake after DHK infusion into area 25 when compared to saline infusion. To further validate the activation effect of DHK in the marmoset brain and in the PFC region that is critical to this study, I conducted immunohistochemistry to characterise neuronal activity in area 11 after DHK infusion.

Immunohistochemistry protocol

Area 11 in the left hemisphere of monkey M1 was infused with saline and the right hemisphere area 11 was infused with DHK (6.25 nmol/µL), one hour before terminal perfusion (perfusion methods described in Chapter 2.10). The rate of infusion of DHK solution was 0.5 µL/min. for 1 minute, with 1 minute of wait time for the drug to settle, the same procedure as used for DHK infusions in the main experiments (described in Chapter 2.8). Monkey M1 was perfused and the brain was processed and sectioned as described in Chapter 2.10. The section was 60 µm thick and one in every five sections was taken for *c-fos* immunohistochemistry. I chose to use *c-fos*, the immediate early gene, as a measure for neuronal activation and conducted all the immunohistochemistry protocols. On day 1, the brain sections were put into well plates to wash three times for 10 minutes each in 0.01M phosphate-buffered saline (PBS). The PBS was changed between each wash in all situations. The sections were quenched to prevent endogenous peroxidase activity (which may lead to high background staining) in 10% methanol and 10% H_2O_2 (H1009-500ml, Sigma-Aldrich) mixed solution for 10 minutes. The sections were then washed again three times for 10 minutes each in 0.01M PBS. The sections were blocked in pre-prepared blocking buffer for one hour in room temperature on a rocker. The blocking buffer was made of 3% normal goat serum (NGS; s-1000, Vector Labs) and 0.3% of Triton X-100 (X100-500ml, Sigma-Aldrich) in 0.01M PBS. The sections were incubated overnight at 4°C in blocking buffer with 1:2000 Rabbit polyclonal to *c-fos* primary antibody (ab190289-100ul, Abcam). On day 2, the brain sections were washed three times for 10 minutes each in 0.01M PBS. They were then incubated for two hours in room temperature on a rocker, in blocking buffer with 1:500 Goat Anti-Rabbit IgG H&L (Biotin) secondary antibody (ab6720-1mg, Abcam). The brain sections were washed three times for 10 minutes each in 0.01M PBS. They were incubated for 30 minutes in room temperature on a rocker with avidin-biotin complex (ABC, ready-to-use, PK7100, Vector Labs). The brain sections were washed three times for 10 minutes each in 0.01M PBS. The sections were reacted with 3,3'-Diaminobenzidine (DAB), using the ImmPactDAB horseradish peroxidase (HRP) Substrate Kit (SK-4105, Vector Labs). The reaction time inside DAB was determined empirically under the microscope. Once the desired staining was achieved, the section was immediately transferred to ice-cold 0.01M PBS to terminate the DAB reaction. The

brain sections were mounted on gelatin-coated slides and dried overnight at room temperature. They were then dehydrated for 2 minutes each in solutions in the following order: 100% ddH_2O, 25% ddH_2O/75% ETOH, 100% ETOH, 50% ETOH/50% Xylene, 100% Xylene. The slides were coverslipped with DPX.

Image analysis

Images were acquired under bright-field using a stereomicroscope (M205 FA; Leica, Wetzlar, Germany). Cell counting for c-*fos* expression was conducted automatically using ilastik (version 1.3.3) (Berg et al., 2019) and FIJI (Fiji is just ImageJ) (Schindelin et al., 2012). The sections were 60 µm thick and one in every five sections was taken for *c-fos* immunohistochemistry. Therefore, between each *c-fos* section analysed, 60 x 5 = 300 µm of distance was covered. Four sections of area 11 slices were used for cell counting analysis, covering a total of 1.2 mm of the distance around the infusion site. Because the infusion site of area 11 is very close to the base of the skull, I could not take one complete section of the brain area to conduct image analysis (Figure 2.3A). Therefore, I segmented all four area 11 brain slices into smaller regions of interests for more accurate analysis (Figure 2.3B). The areas of the segmented regions were equal across the two hemispheres for each brain slices and the total areas of the brain slices were equal between the right and the left hemispheres.

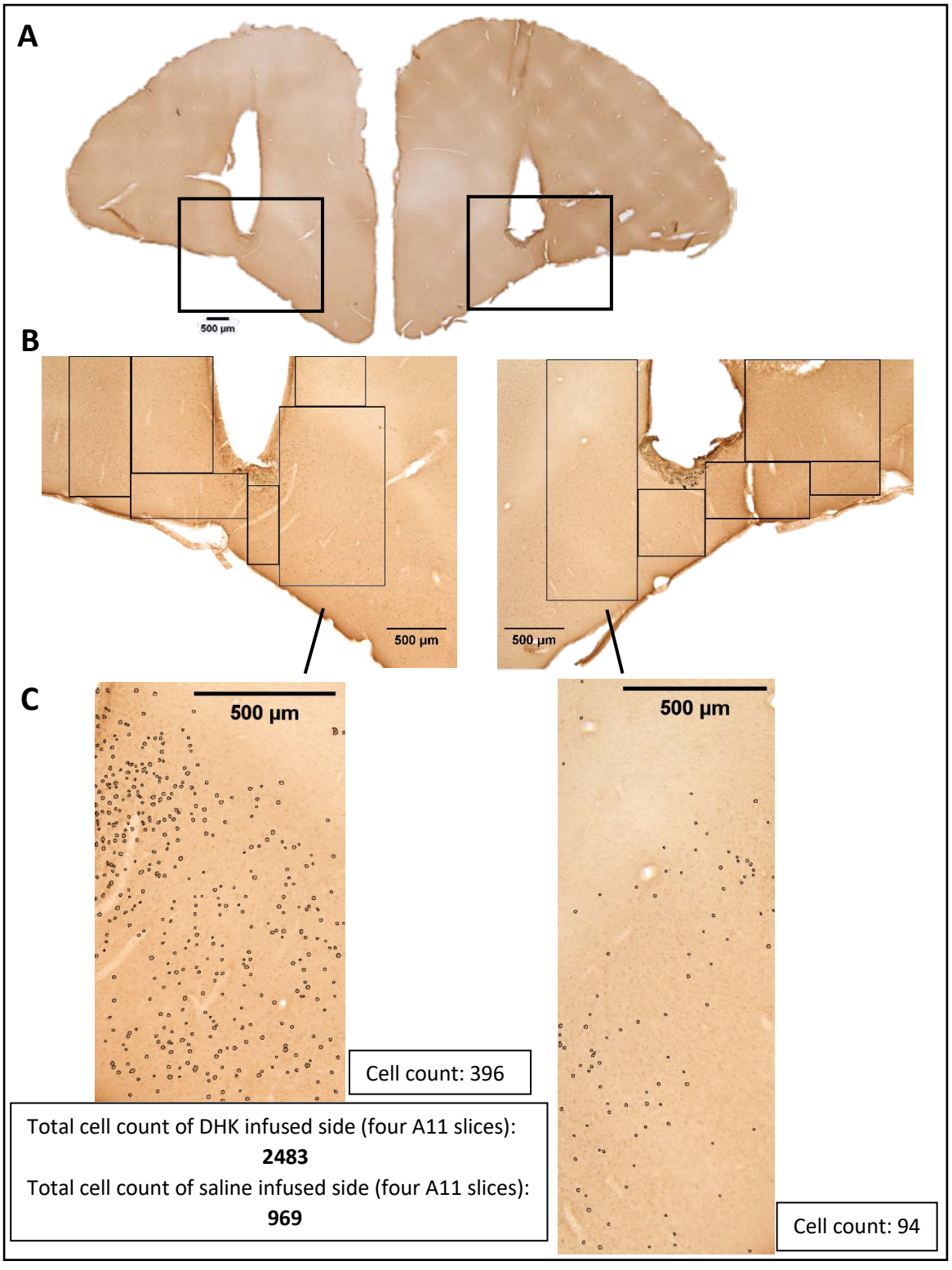


Figure 2.4. Image analysis of *c-fos* expression after DHK infusion into the left side and saline infusion into the right side of area 11. A). whole brain view of area 11 cannulae placements with region of interests highlighted. B). area 11 is segmented into smaller sections for improved automated cell counting analysis. C). Examples of sections from the two hemispheres. The DHK infused site has more cells expressing *c-fos* and the saline infused site has fewer cells that express *c-fos*. *c-fos* expressing cells were highlighted for better visualisation.

DHK induced neuronal activation as visualised via *c-fos* expression

When compared to the saline-infused side, the DHK-infused side had higher levels of *c-fos* expression. The total cell counts that express *c-fos* in the DHK infused hemisphere across the four area 11 slices were 2483, whereas the cell counts were 969 in the saline-infused site. Therefore, DHK infusion did cause an increase in neuronal activity, as measured in the form of *c-fos* expression.

2.11.1b CNQX

CNQX was used to inactivate the CN instead of muscimol/baclofen. I chose to use CNQX for several reasons. Firstly, previous preliminary data using the contingency degradation paradigm had yielded equivocal results after infusion of muscimol into the CN. The response rate in the non-degraded sessions of the two animals was very variable, with a standard error of means of 42%. One reason for this could be that, after histological analysis, one of the animal's caudate placements was elevated with the result that the cannulae targeted the corpus callosum. Secondly, another reason for our previous equivocal caudate result was that it was unclear that GABA agonism would inactivate the CN (as distinct from the neocortex where it is effective). Muscimol/baclofen acts on GABA receptors. The striatum not only contains a variety of neurotransmitter systems and cell types, but it is also dominated by GABAergic medium spiny neurons (MSNs), which are projection neurons that contain either D1 or D2 receptors. Muscimol has been used in the rodent literature to inactivate dorsal striatum. However, there are differences between the striatum of the primate and rodent. In monkeys, about 77% of MSNs are present in the striatum as compared with rodents (over 95%) (Graveland and DiFiglia, 1985; Haber, 2016). To complicate the situation further, monkeys have fewer MSNs in the caudate than putamen, a difference not observed in rodents (Graveland and DiFiglia, 1985). However, whether or not the overall activity of striatum would be inactivated through muscimol is unclear. Therefore, since I was interested in how the excitatory inputs from the PFC affect the CN, I decided to block glutamatergic input into the CN using a glutamate receptor antagonist. Since a commonly used glutamate receptor antagonist CNQX has not previously been used in marmosets, I needed to validate its effect. Here I will provide

evidence from the literature in other species, as well as the behavioural evidence I obtained in this thesis.

To prevent excitatory input into the CN, I used CNQX, a potent α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptor antagonist. In mice, CNQX infusion into the rostral part of dorsal striatum blocked the local field potential (LFP), a representation of the summed synaptic activity in the area surrounding the recording electrode, and almost completely blocked the striatal field response to cortical stimulation in the PL (Galinanes et al., 2011), a region thought to be equivalent to area 32/24 in the primates and the region of interest. In rhesus macaques, the large-amplitude fluctuations of LFP generated by $GABA_A$ antagonist (gabazine) were blocked by a combination of CNQX and MK-801 (NDMA receptor antagonist) (Darbin and Wichmann, 2008). Behaviourally, CNQX infusion into the dorsolateral striatum caused rats to become less sensitive to outcome devaluation, a task used to measure goal-directed behaviour (Furlong et al., 2014).

Validation experiment: unilateral CNQX infusion into the caudate nucleus and disconnection between the caudate nucleus and OFC induced motoric bias

In the CNQX validation experiment, I tested for potential motoric bias following unilateral CN inactivation via CNQX and also a disconnection between OFC and CN. Rats showed an ipsilesional response bias after unilateral lesion of DMS (Brown and Robbins, 1989) and contra-lesional neglect after ipsilateral lesions of mPFC and medial caudate-putamen (Christakou et al., 2005). Thus, I predicted that with unilateral inactivation of CN, the animal would decrease responding to the stimulus presented contralateral (left or right) to the side (left or right) of striatal inactivation. Four sets of manipulations were conducted after monkey M1 completed his main experiments (Figure 2.5). In all the manipulations, M1 was presented with a single Maltese Cross stimulus (same as the main experiments) either on the left or the right of the touchscreen. Under a variable ratio schedule of 10, M1 received a juice reward associated with the side of the stimulus in an average of 10 responses. In each set of the manipulations, the left or the right side of the brain region was infused in two separate sessions for counterbalancing purposes. In the first manipulation, the stimulus was presented contralaterally to the side of CNQX infusion

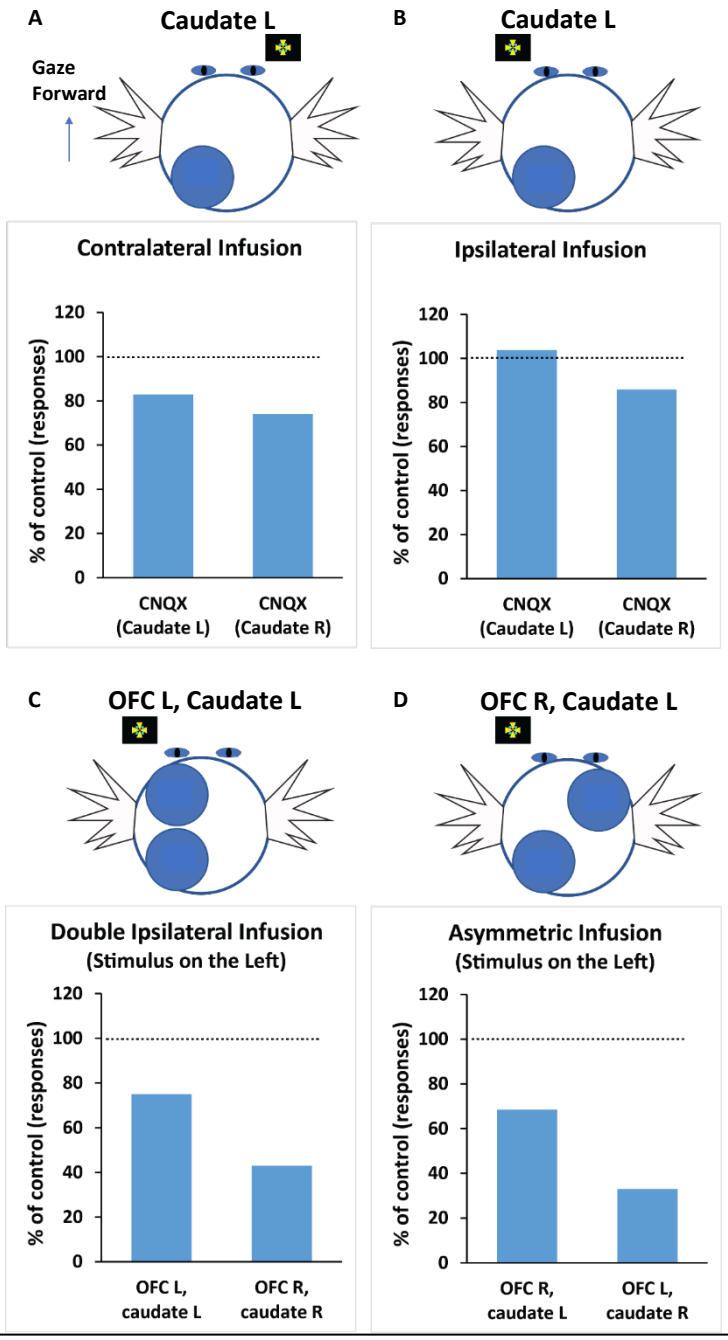


Fig 2.5. CNQX infusion into the CN and/or OFC, represented as the percentage of control session responses. The diagrams above each graph gave an example of the manipulation situation, with a view from above the animal's head where he was facing the stimulus on the touchscreen. The blue-filled circle indicated infusion site(s). A). When the stimulus was presented contralaterally to the unilateral CN infusion side (left or right), Monkey M1 decreased responding compared to the control session where no drug was infused. B). When the stimulus was presented ipsilaterally to the unilateral CN infusion side, M1 did not decrease responding after CNQX infusion into the left side CN. C). OFC and CN were infused unilaterally on the same side. M1 decreased responding when the infusion was on the same side as the stimulus, but even more when the infusion was contralateral to the stimulus side . D). OFC and CN were infused unilaterally in opposite hemispheres. M1 decreased responding markedly more when the infusion side (right) of CN was contralateral to the stimulus side (left).

into the CN, i.e. if CNQX was infused into the left CN, the stimulus was presented on the right side of the touchscreen (Figure 2.5A). In the second manipulation, the stimulus was presented ipsilaterally to the side of CNQX infusion into the CN, i.e. if CNQX was infused into the left CN, the stimulus was presented on the left side of the touchscreen (Figure 2.5B). In the third manipulation, the stimulus was always presented on the left side of the touchscreen, with the infusion of CNQX into CN and ipsilateral infusion of muscimol/baclofen into the OFC, i.e. infusion into CN and OFC always occurred in the same hemisphere (Figure 2.5C). In the fourth manipulation, the stimulus was always presented on the left side of the touchscreen, with the infusion of CNQX into CN and contralateral infusion of muscimol/baclofen into the OFC, i.e. infusion into CN and OFC always occurred contralaterally to each other (Figure 2.5D).

The results indicated that monkey M1 did decrease responding whenever the stimulus was presented contralaterally to the side of the CN CNQX infusion (Figure 2.5A, C, D). When the stimulus was presented to the same side of the CN infusion, the animal did not decrease his responding (104% of control; Figure 2.5B, caudate L) or did not decrease responding as much (86% of control; Figure 2.5B, caudate R) when compared to the contralateral stimulus presentation (74% of control; Figure 2.5A, caudate R). Unilateral OFC inactivation decreased animals' responding regardless of whether the OFC inactivation was contralateral or ipsilateral to the stimulus (Figure 2.5C, D). This is because while monkey M1 did not decrease responding when the stimulus was presented ipsilaterally to the side of CN inactivation (Figure 2.5B, caudate L), with the addition of OFC inactivation, he did reduce responding (Figure 2.5C, OFC L; Figure 2.5D, OFC R). When OFC and CN were inactivated together with the CN inactivation being contralateral to the stimulus presented, a larger decline in responding was observed (Figure 2.5C & D, caudate R), compared to when the CN inactivation was ipsilateral to the stimulus presented (Figure 2.5C & D, caudate L). In the double ipsilateral infusion (Figure 2.5C), the drop was from 75% to 42% of control; in the asymmetric infusion (Figure 2.5D), the drop was from 69% to 33% of control. Thus, three conclusions could be drawn from these behavioural observations: 1). Unilateral CN inactivation using CNQX, which was contralateral to the side of stimulus presentation, led to a decrease in responding. 2). Unilateral OFC inactivation using muscimol/baclofen decreased responding irrespective of the side of stimulus presentation. 3). Unilateral OFC inactivation, irrespective of the side of stimulus

presentation, together with unilateral CN inactivation that was contralateral to the side of stimulus presentation, led to the largest reductions in responding. Given the behavioural results, I could conclude that CNQX at least did affect behaviour similarly to lesions of the rodent DMS (Brown and Robbins, 1989; Christakou et al., 2005). Additionally, the CN data to be presented in Chapter 4 of this thesis was similar to the effects of posterior dorsomedial striatum (pDMS) lesion and inactivation results from rodent studies that measure goal-directed action (Yin et al., 2005b). In conclusion, there is strong behavioural evidence to support the inactivating effect of CNQX in CN.

2.11.1c Muscimol and Baclofen

Muscimol and baclofen are well-established in combination as a means for decreasing neuronal activity via activation of GABA receptors and thus to produce reversible inactivation effects in the neocortex in behavioural studies in both rodents and primates. For instance, muscimol ($GABA_A$ agonist) depressed spontaneous and evoked neuronal firing (Johnston et al., 1968) while binding to the $GABA_A$ receptor in the rat brain (Beaumont et al., 1978). Martin (1991) used autoradiography to measure the intracortical spread of muscimol and changes in [1-^{14}C] glucose uptake after infusion of muscimol into the cerebral cortex of rats. They found that glucose uptake was reduced maximally for a region of 1.0 mm in radius and a smaller reduction in the surrounding area (up to 3.0 mm in radius). Edeline et al. (2002) also obtained electrophysiological and autoradiographic results that demonstrated the inactivation effect and spread of muscimol in rat cerebral cortex. They observed decreases in neuronal activity of up to 3mm from the infusion site. Using labelled [3H]muscimol, they showed the spread of muscimol was between 5.25 mm^2 to 12 mm^2 depending on infusion volume and rats' survival time after infusion. Muscimol has also been used extensively to reversibly inactivate brain regions in behavioural studies in rodents and monkeys (Lomber, 1999; Martin and Ghez, 1999; Majchrzak and Di Scala, 2000; West et al., 2011; Jackson et al., 2016; Jackson et al., 2019). Baclofen ($GABA_B$ agonist) also decreases neurotransmitter release from presynaptic terminals and mediates synaptic inhibition (Bowery, 1989; Bowery and Pratt, 1992; Bowery, 1993). Consequently, a mixture of muscimol and baclofen has also been used with the aim of blocking all of the GABA receptors in the PFC of rodents (Takahashi et al.,

2009; St Onge and Floresco, 2010) and marmosets (Clarke et al., 2015). Given the abundance of electrophysiological, radiological and behavioural evidence on the reversible inactivation effect of muscimol and baclofen, I proceeded with the reasonable assumption that these agents would be effective in producing reversible inactivation in the PFC subregions investigated in Chapters 3 and 4.

2.11.2 Neuroanatomical connectivity between area 24 and caudate nucleus

In this thesis, I aimed to investigate the importance of frontal-striatal circuits in goal-directed actions. Thus, I needed to determine the neuroanatomical connectivity between critical PFC regions and CN. After identifying area 24 as the critical region controlling action-outcome contingencies (results presented in Chapter 3), I needed to identify the region of the CN to which area 24 projects. Human, rodent and macaque tracing studies had indicated that medial PFC and dorsal ACC project to the anterior dorsal striatum (see Chapter 1.2). However, tracing studies and the marmoset brain atlas project had not investigated connectivity of area 24a, i.e. the perigenual anterior cingulate cortex that this thesis targeted, with other brain regions (Roberts et al., 2007; Majka et al., 2020) (also see http://www.marmosetbrain.org/). Therefore, to validate area 24's connectivity with CN, I conducted retrograde tracing from our targeted region of the CN, to confirm those PFC regions projecting to the targeted CN.

2.11.2a Tracer infusion and immunohistochemistry protocol

The left CN of monkey M13 was infused with the retrograde tracer choleratoxin B subunit (C9903, Sigma-Aldrich) via guide cannulae. The rate of infusion was 0.1 µL/min. for 2 minutes, with 25 minutes of wait time for the drug to diffuse (infusion procedure described in Chapter 2.8). M13 was perfused after 10 days and the brain was processed and sectioned as described in Chapter 2.10. The section was 40 um thick and one in every five sections was taken for immunohistochemistry. I conducted all the immunohistochemistry protocols. On day 1, the brain sections were put into well plates to wash three times for 10 minutes each in 0.1M Tris-NaCl (pre-made the day before, pH adjusted to 7.4; Tris-base, T4661-100g, Sigma-Aldrich; NaCl – S7653-1Kg, Sigma-Aldrich).

The washes happened in room temperature and the wells were placed on a rocker. The 0.1M Tris-NaCl was changed between each wash in all situations. The sections were quenched to prevent endogenous peroxidase activity in 10% methanol and 10% H_2O_2 mixed solution for 5 minutes. The sections were then washed again three times for 10 minutes each in 0.1M Tris-NaCl. The sections were blocked in 0.1M Tris-NaCl with 0.2% Triton X-100 and 1% normal swine serum (S-4000, VectorLabs) for one hour in room temperature on a rocker. The sections were incubated overnight in room temperature, placed on a rocker, immersed in 0.1M Tris-NaCl with 0.2% Triton X-100, 1% normal swine serum and 1:2000 goat anti-choleragenoid primary antibody (703, Quadratech). On day 2, the brain sections were washed three times for 10 minutes each in 0.1M Tris-NaCl. They were then incubated for two hours in room temperature on a rocker, in 0.1M Tris-NaCl with 0.2% Triton X-100 and 1:200 biotinylated donkey anti-goat secondary antibody (bs-0294D-Biotin-BSS, Stratech). The brain sections were washed again three times for 10 minutes each in 0.1M Tris-NaCl. They were incubated for 90 minutes in room temperature on a rocker with ready-to-use avidin-biotin complex, which afterwards were washed three times for 10 minutes each in 0.1M Tris-NaCl. The sections were reacted with 3,3'-Diaminobenzidine (DAB), using the ImmPactDAB horseradish peroxidase (HRP) Substrate Kit (SK-4100, Vector Labs). The reaction time inside DAB was determined empirically under the microscope. Once the desired staining was achieved, the section was immediately transferred to ice-cold 0.01M PBS to terminate the DAB reaction. The brain sections were mounted on gelatin-coated slides and dried overnight at room temperature. They were then dehydrated for 2 minutes each in solutions in the following order: 100% ddH_2O, 25% ddH_2O/75% ETOH, 100% ETOH, 50% ETOH/50% Xylene, 100% Xylene. The slides were coverslipped with DPX.

2.11.2b Image analysis

Images were acquired under bright-field using a stereomicroscope (M205 FA; Leica, Wetzlar, Germany). Cell counting was conducted automatically using ilastik (version 1.3.3) (Berg et al., 2019) and FIJI (Schindelin et al., 2012). The sections were 40 µm thick and one in every five sections was taken for immunohistochemistry.

2.11.2c Connectivity validation: area 24 projects to the anterior caudate nucleus

The area 24 (perigenual anterior cingulate cortex) that was targeted for this thesis projects to anterior CN (Figure 2.6). The choleratoxin B subunit retrograde tracer was successfully injected into the anterior CN (Figure 2.6A). Bilateral projections from area 24 were observed, with more projections from the ipsilateral side (Figure 2.6B). Besides area 24, the anterior CN also receives PFC projections from area 32 and area 8. It would have been possible to analyse projections from the entire brain into the CN, but it would have been outside of the scope of the current thesis.

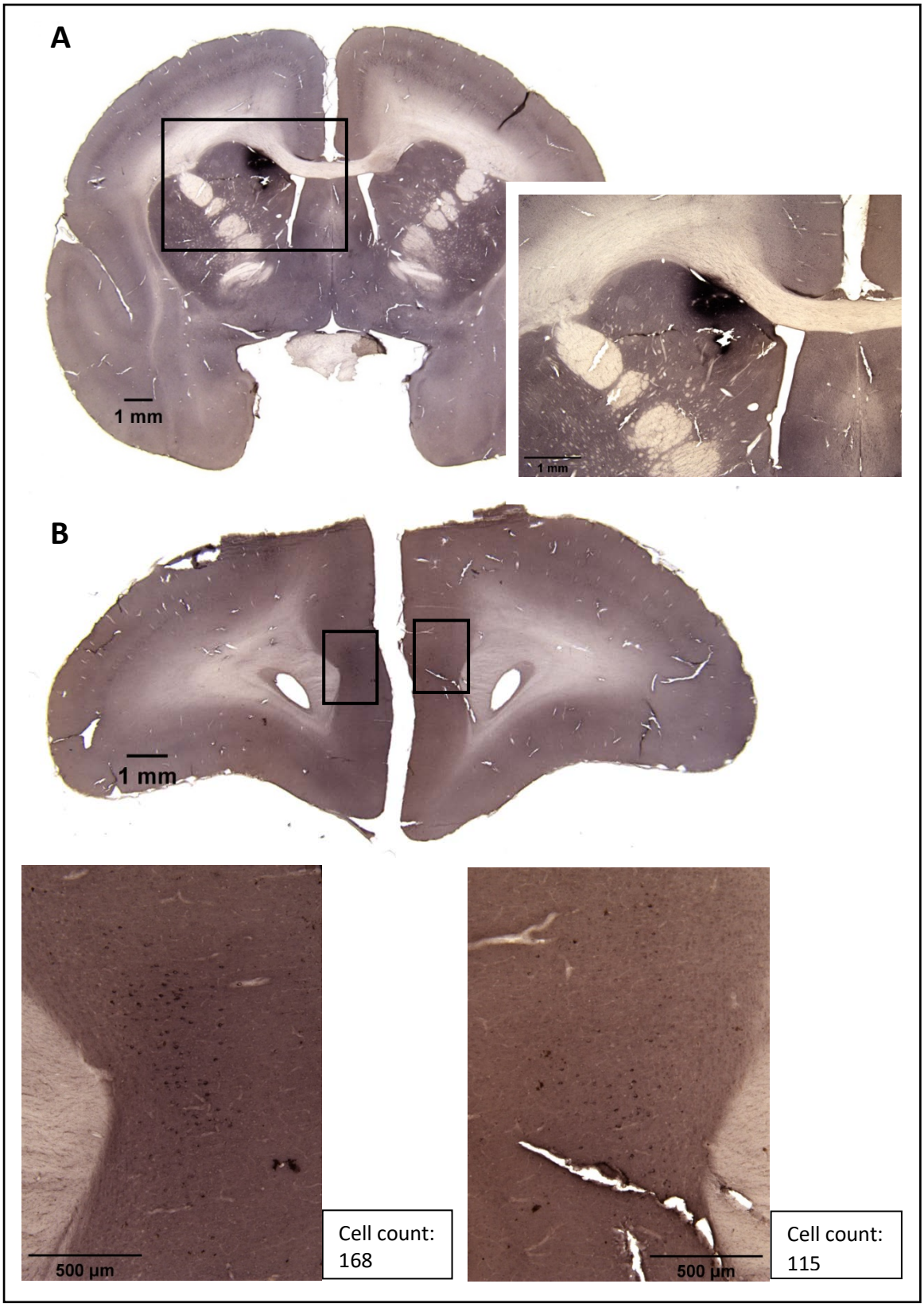
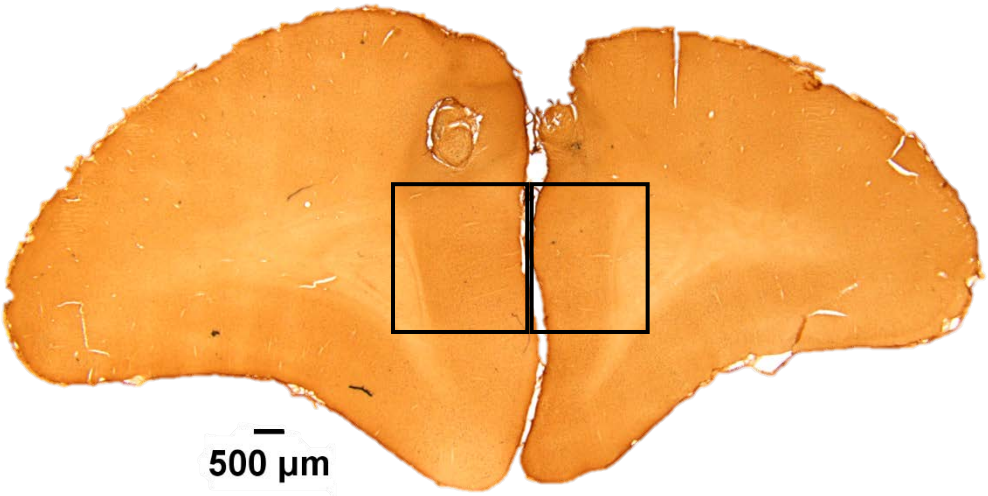
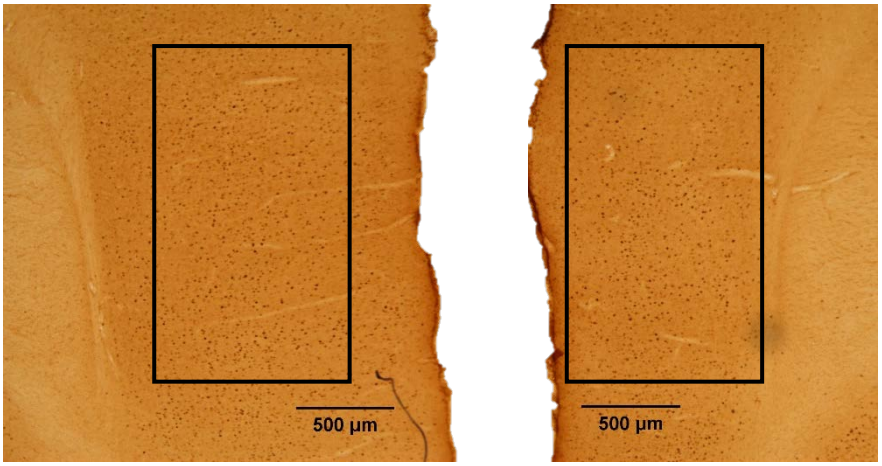


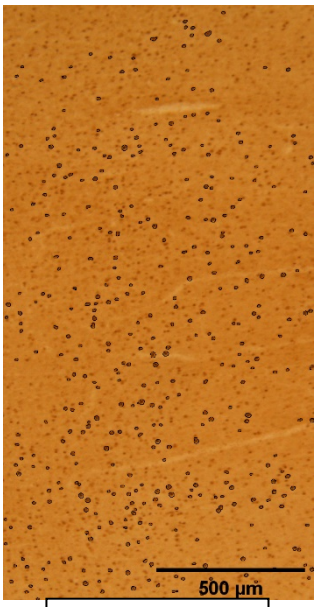
Figure 2.6. Retrograde tracing that verified the projection from area 24 to anterior CN. A). The retrograde tracer choleratoxin B subunit seen in the left anterior CN. B). Cell bodies of CN projecting neurons within the area 24 at the approximate placement used in this thesis. Ipsilateral projection from area 24 to CN is more than that from the contralateral projection.

2.11.3 *C-fos* expression in area 24

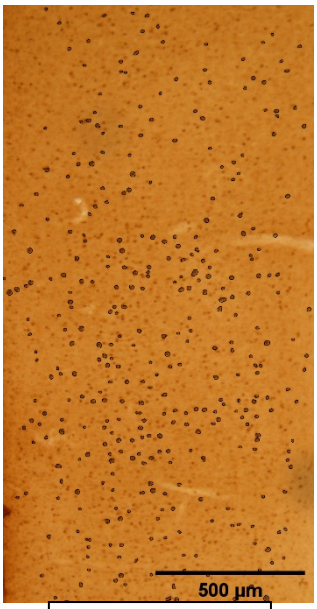
The same procedures were conducted as for area 24 DHK infusion, with the exception that the brain sections were 40 µm thick instead of 60 µm. No differences in numbers of cells that express *c-fos* were observed across the two hemispheres (DHK infused side: 1488, saline-infused site: 1438). Examples of the sections are provided in Figure 2.7. Similar results were observed in another set of rostral dorsal ACC slides, which also received activation to examine *c-fos* expression. The *c-fos* immunohistochemistry was completed by another experimenter in the laboratory in one of their animals but the images have not yet been analysed. The infusion site was slightly posterior to the area 24 as defined in this thesis, and mGluR (metabotropic glutamate receptors) agonists were infused instead of DHK.







Cell count: 384



Cell count: 336

Total cell count across four slices of area 24:

DHK side: 1488

Saline side: 1438

Figure 2.7. DHK infusion into the left area 24 and saline infusion into the right area 24. No difference in c-Fos expression was found between hemispheres.

Chapter 3

The role of perigenual anterior cingulate cortex, anterior orbitofrontal cortex and subgenual anterior cingulate cortex in goal-directed behaviour measured by contingency degradation

3.1. Introduction

As described in Chapter 1, it has been suggested that the compulsive behaviour seen in OCD patients, may represent a shift away from goal-directed towards habitual control (Graybiel and Rauch, 2000; Gillan et al., 2011; Gillan and Robbins, 2014; Robbins

et al., 2019). Such imbalance between goal-directed actions and habits may arise from a deficit in goal-directed behaviour, namely a failure to recognise the causal relationship between a specific action and a specific consequence resulting from that action. The assumption is that these two systems compete (Balleine and O'Doherty, 2010) and thus a weakening of the goal-directed system will result in stronger habitual control. One method for investigating this balance between instrumental and habitual control is to implement contingency degradation (Rescorla, 1966; Hammond, 1980; Dickinson and Weiskrantz, 1985; Balleine and Dickinson, 1998). This method involves systematically varying the relationship between actions and outcomes, mainly by presenting outcomes uncorrelated with actions. When the probability of an outcome given an action [$P(a|o)$] is degraded to values of P=0.50 or less it is clear that instrumental responding is no longer required. However, the action may continue to be performed even though it is ineffective, presumably as a consequence of habitual control.

Evidence from human, non-human primate and rodent studies have pointed to possible candidates within the prefrontal cortex, such as the ACC, OFC and vmPFC, to control A-O contingencies. In human fMRI studies, as detailed in Chapter 1.3., Tanaka et al. (2008) and Liljeholm et al. (2011) investigated brain activity using fMRI in healthy subjects when they performed tasks requiring them to assess the contingent relationship between their actions and outcomes. Tanaka et al. (2008) showed that part of ventromedial PFC, medial OFC and anterior medial caudate nucleus (CN) were more active when the subjects' actions were highly predictive of the outcome than when their actions did not predict the outcome well. The mPFC is the only region of these that tracks the contingency changes over time. mPFC and lateral OFC activity were also found to correlate with subjective contingency judgements. Liljeholm et al. (2011) included the additional non-contingent outcome situation [$P(a|\sim o)$]. They found that activity in mPFC and anterior CN correlated with the probability of response-contingent reward delivery [$P(a|o)$], whereas activities in the inferior frontal cortex and posterior CN correlated with the probability of non-contingent reward delivery [$P(a|\sim o)$]. In a subsequent study using a task assessing response-outcome and stimulus-response associations, Liljeholm et al. (2015) showed that in response-outcome conditions vmPFC exhibited greater activity. vmPFC lesioned subjects can learn A-O contingencies but have reduced awareness of such relationships when the changes in contingency are positive (i.e. the performance of an

action increases the probability of outcome) when compared to lateral PFC lesioned and elderly healthy subjects. All of these groups showed impaired sensitivity when changes in contingency were negative (i.e. the performance of an action decreases the probability of an outcome) (O'Callaghan et al., 2019).

Rodent studies examined the role of mPFC and OFC in A-O contingencies. PL lesioned rats are insensitive to contingency degradation (Balleine and Dickinson, 1998; Corbit and Balleine, 2003). lOFC lesion in rats impaired a pavlovian form of contingency degradation, whereas mOFC lesion in rats did not impair an instrumental form of contingency degradation (Ostlund and Balleine, 2007; Bradfield et al., 2015). Non-human primate studies that directly manipulate PFC sub-regions indicated the involvement of mPFC, OFC and ACC in A-O contingencies. As detailed in Chapter 1.3.2a, anterior cingulate lesions in macaques impaired their ability to adapt their actions to changes in outcome probabilities (Kennerley et al., 2006; Rudebeck et al., 2008; Chudasama et al., 2013). Area 32 (mPFC) or area 11 (OFC) lesions in marmosets impaired acquisition of A-O contingencies (Jackson et al., 2016). The task used in Jackson et al. (2016) and the one employed in this thesis share several similarities but also have has several subtle but important differences. The current task is designed to maximise the A-O component, minimise the stimulus-outcome component and make it amenable to repeated and reversible brain manipulation procedures (drug infusions). For instance, the current task uses the same stimuli presented in different positions to distinguish between the two A-O associations, whereas Jackson et al. (2016) used different stimuli presented in different positions, thus making the current task less stimulus-driven.

I decided to apply the contingency degradation task because, despite its powerful translational potential, it has not been used extensively compared with other methods of measuring goal-directed behaviour, such as outcome devaluation. There is a lack of evidence in the current literature that uses a within-in subject design to differentiate the causal role of primate PFC sub-regions in goal-directed behaviour. Based on evidence from the rodent, non-human primates and human studies, I chose three PFC sub-regions to investigate in this chapter. I chose the ACC because its involvement in A-O associations was implicated in macaques lesion studies (Chudasama et al., 2013), but has not been investigated in rodents nor marmosets using tasks that measure the expression of A-O contingencies. Area 11 was chosen because OFC activity was associated with A-O

knowledge in human imaging studies (Liljeholm et al., 2011). While lateral OFC lesions impaired pavlovian contingency degradation learning in both rodents and marmosets (Ostlund and Balleine, 2007; Jackson et al., 2016), its role in instrumental contingency degradation expression is not known(Bradfield and Hart, 2020). vmPFC is a large region in the PFC that was consistently found to correlate with goal-directed behaviour in human imaging studies(Balleine and O'Doherty, 2010); in this thesis, I decided to narrow the targeted vmPFC areas to area 14-25. The current chapter aims to explore the causal role of specific prefrontal cortex sub-regions (area 24, area 11 and area 14-25), using reversible pharmacological manipulations (muscimol/baclofen to inactivate, dihydrokainic acid to activate), in the expression of action-outcome associations as measured by contingency degradation. Furthermore, this chapter included control experiments, such as progressive ratio and reward licking analysis, to examine possible motivational dysfunction that might affect the results in the main contingency experiments.

3.2. Methods

3.2.1. Subjects

Five common marmosets (three females, two males) were used in this study. For details on the marmosets' housing facility, husbandry, testing apparatus, pre-training, touchscreen training, the contingency degradation task itself, behaviour measures, surgical procedures, intracerebral infusions and histology, refer to the appropriate General Methods section (Chapter 2). Any additional procedures and details on the training and manipulations for individual animals that are specific to this study are described below.

3.2.2. Touchscreen Training and Contingency Degradation Task

The contingency degradation task was explained in detail in chapter 2. Animals received the degraded sessions and the non-degraded sessions, and the degraded juice and the non-degraded juice in a counter-balanced manner (Table 3.1)[1].

Table 3.1. Degraded vs. non-degraded session order and juice assignment

	Degraded	**Non-degraded**	**Degraded or non-degraded session first**
M1	Blackcurrant	Strawberry	Nondegraded
M2	Blackcurrant	Strawberry	Degraded
M3	Strawberry	Blackcurrant	Nondegraded
M5	Strawberry	Blackcurrant	Degraded
M4	Strawberry -> Summerfruit	Blackcurrant -> Brand change	Nondegraded

[1] For M4, the strawberry juice (Ribena, Suntory, UK) was changed to summerfruit juice (Tesco high juice, UK) and the blackcurrant juice's brand was changed from Ribena to Tesco high juice in the spring of 2018. This unforeseeable change was due to the introduction of sugary drinks tax in the UK and Ribena changed their long-standing formula in April 2018 with much lower sugar content and added artificial sweeteners. The marmosets did not like the new Ribena recipe. Thus, my experiments were delayed for at least a month or two to test and find new juice brands with enough sugar content that the marmosets found them rewarding and were motivated to work for the reward.

3.2.3. Cannulation placement

Tables 3.2 and 3.3 show the cannulation co-ordinates and target brain areas for each animal. The animals were cannulated in two brain regions during surgery. However, if the cannulation angle of area 24 was vertical (perpendicular to the brain surface), a third more ventral brain region (area 14-25) could be reached during drug infusions via extended injectors through the area 24 cannula (Figure 3.1 E, F). Data for the CN were collected in just two subjects and only in one subject was the cannula placement in the desired location. The other animal's cannulae location was in the corpus callosum, above the desired location that was the anterior CN. Thus, the data for the CN is not discussed further.

Table 3.2. Cannulation co-ordinates and angle of cannulae placement.

Area	AP co-ordinate (mm)	LM co-ordinate (mm)	Depth (mm)
Area 11	+17.0	+/- 3.0	1.7 (from base)
Area 24*	+15.4	+/- 1.0	2.5 (from surface)

AP: anteroposterior; LM: lateromedial; *Area 14-25 and area 14 were reached by extending the injectors via the area 24 and area 32 guide cannulae, respecively.

Table 3.3. Cannulation locations of each subjects.

	CANNULATION AREAS		
Subject	Area 11	Area 24	Area 14-25
M1	√		
M2	√	√	√
M3	√	√	√
M4	√	√	√
M5		√	

3.2.4. Intracerebral infusions

Animals received saline, muscimol/baclofen and dihydrokainic acid (DHK) infusions into their designated sites and muscimol infusions into the CN. The rates, dosage and vehicles of each drug and the procedures of the infusions are described in chapter 2. In one case (M4), 6.25 nmol/µL DHK infusion into A14-25 caused non-specific behavioural disruption such that the animal failed to respond in the testing apparatus. Therefore, the DHK dose was reduced to 3.0 nmol/µL. The injectors were placed into the guide cannulae, extending 1.0mm below the cannulae for areas 24, 1.0mm for area 11 and 4.5mm for area 14-25.

3.2.5. Motivational control experiment: progressive ratio task

I modified the contingency degradation task using a progressive ratio (PR) schedule for the contingent responses to assess the motivation of some of the animals. The task remained the same as the contingency degradation task, except that a PR schedule replaced the usual VR schedule for the contingent responses. The progressive ratio schedule was similar to that used by Pryce et al. (2004) and had been used in another study in this laboratory (Alexander et al., 2019). The first trial requires one response to receive a juice reward, then the response required increases with an increment of 1, but the increment doubles every eight trials. For instance, trials 1-8 need 1-8 responses, respectively, but for trial 9-16, animals need to respond 10, 12, 14...to 24 times, respectively for each trial, to obtain one reward. The maximum increment is +8. The session was terminated after two minutes of inactivity or reached the 30 minutes session length, whichever came first. The breakpoint is the total number of responses animals made before termination of the session.

As a pilot study, only M4 underwent the PR schedule with saline and muscimol/baclofen infusions into area 11. He was trained under the PR schedule of the contingency degradation task after data collection in the main contingency degradation task. In the two control baseline sessions (without free reward), he trained under the PR schedule instead of the VR schedule. In his degradation sessions (with free reward), the non-contingent free reward was delivered the same way as the contingency degradation

task, the contingent reward was delivered based on his responding under the PR schedule. Only the non-degraded condition was tested, that is, the contingent and non-contingent free reward were unmatched. He received saline and mus/bac infusions into area 11 after degradation and baseline sessions.

3.2.6. Histology and cannulae placement verification

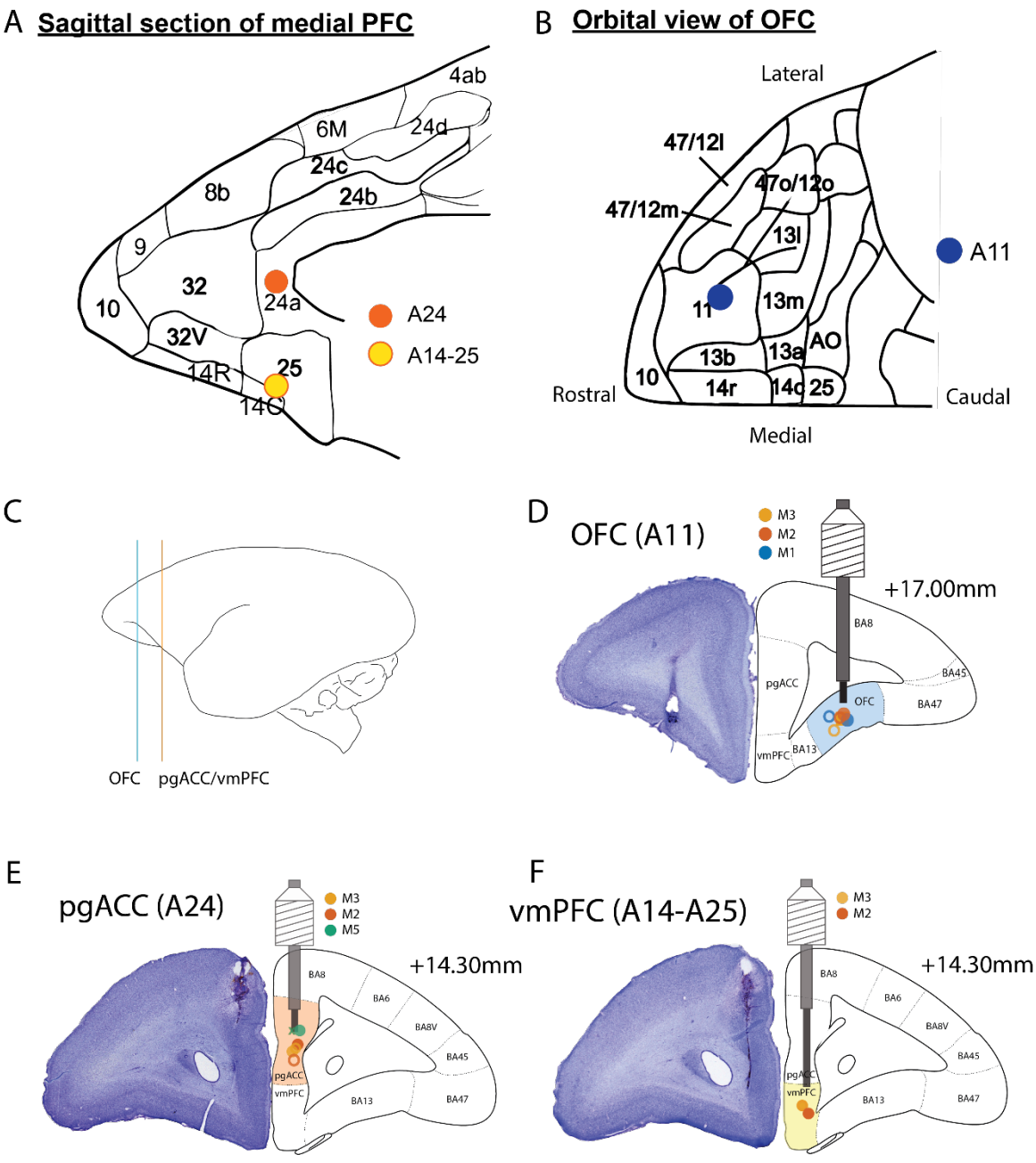


Figure 3.1. Schematic diagrams of cannulae placements in the PFC regions. A). Sagittal section of the medial PFC, with area 24 and area 14-25 cannulae target location. B). Orbital view of area 11 and its cannulae target location. C). The locations of area 11, area 24 and area 14-25 cannulae in the whole brain sagittal view. D-F). The actual cannulae placements of M3, M2, M1 and M5 in area 11, 24 and 14-25. Area 14-25 was reached by vertically extending the area 24 injector, thus targeting both area 24 and 14-25 via the same cannula guide. M4 is still alive thus no histology is available.

3.2.7. Statistical analysis

Data were analysed using a mixed-model ANOVA with the free programming language R for Windows GUI version 3.5.1 (R core team, 2019). I used Lme4 package to conduct linear mixed-effects models with Type III analysis of variance with Satterthwaite's method for determining degrees of freedom (Bates et al., 2015). Bartlett's test was used to determine the homogeneity of variance. Each significant main effect ($p<0.05$) was further examined using pair-wise comparisons of least square means (lsmeans package in R) for specified factors in linear or mixed models. Fixed factors were the between-subject factor infusion area (Region; A11, A24, A14-25) and the within-subject factors were infusion types (treatment; saline, mus/bac, DHK) and degradation types (degradation; degraded vs. non-degraded). The subject was a random factor. To account for individual variabilities in response rate, the dependent variable was the contingency degradation index (described in chapter 2.6). Data for all brain regions on degradation sessions were square-root transformed but the data presented in graphs are not transformed for clarity. Data from drug manipulations on baseline sessions underwent the same analysis.

Data from control and degradation sessions in the absence of drugs were square-root transformed to avoid violations of the assumptions of ANOVA and analysed using within-subject repeated measures ANOVA in R (afex package; R Core Team, 2019). However, for clarity, the data presented in the graphs were not transformed. Factors for the response rate data (Figure 3.2A) include two within-group factors of degradation (degraded vs. non-degraded) and free juice (presence vs. absence). The factor for the contingency degradation index data (Figure 3.2B) has two levels, degraded vs. non-degraded).

Lick latency (time between the onset of birdsong that signals the reward and the time animals start to lick from the spout) and lick per reward (average number of licks an animal does for the duration of each reward delivery) data in contingency degradation and baseline sessions for all brain regions were measured using linear-mixed model ANOVA in R. They were analysed to examine whether pharmacological manipulations in brain regions resulted in motivational dysfunction. Factors include two within-group factors of degradation (degraded vs. non-degraded) and treatment (saline vs. mus/bac vs. DHK).

Only one animal (M4) was tested in the progressive ratio version of the contingency degradation task, so no statistical analysis was conducted.

3.3 Results

3.3.1. Validation of contingency degradation effect

All animals prior to drug infusions showed evidence of sensitivity to contingency degradation as illustrated in Figure 3.2. In the final contingency degradation block that preceded the first infusion, animals showed a reduction in responding in the degraded condition (free and contingent reward matched) compared to non-degraded sessions (free and contingent reward unmatched) and control sessions (no free reward; Figure 3.2A). Within-subject repeated measures analysis of variance revealed a significant interaction between free juice (presence vs. absence) and degradation (degraded vs. non-degraded; $F_{1, 4} = 12.744$, $p = 0.0234$) on response rate, and a main effect of free juice ($F_{1, 4} = 15.512$, $p = 0.0170$). Post-hoc analysis showed a reduction in the response rate in the degraded session when compared to non-degraded session under free juice condition ($t_{6.62} = -3.578$, $p = 0.0383$) and no difference in response rate between the two control sessions in the absence of free reward ($t_{6.62} = 0.142$, $p = 0.999$). There was also a significant reduction in response rate in degraded sessions (free juice) when compared to degraded control (absence of free juice; $t_{7.98} = -5.301$, $p = 0.0032$) and non-degraded control sessions (absence of free juice; $t_{6.42} = -3.719$ $p = 0.0337$). There was no significant difference when comparing non-degraded session with degraded control session ($t_{6.42} = -0.237$ $p = 0.995$) and non-degraded control session ($t_{7.98} = -0.128$ $p = 0.999$).

Animals exhibited individual variations of response rate; for instance, M1's response rate shown in Figure 3.2A was higher than the other subjects. To account for individual variations in response rate across sessions and animals, I decided to use the percentage of animals' response rate in degradation sessions compared to control sessions instead of raw response rate numbers (Figure 3.2B). I define this as the Contingency Degradation Index (CDI), which is also described in detail in the General Methods section (Chapter 2.6). Before any drug manipulations, animals showed a decrease in responding to the degraded session compared to the non-degraded session. Within-subject repeated measures analysis of variance revealed a main effect of degradation ($F_{1, 4} = 12.559$, $p = 0.02393$). A *post hoc* t-test on the least squared means

revealed that animals significantly reduced their responding in the degraded compared with the non-degraded condition (t_4 = -3.462, p = 0.0258).

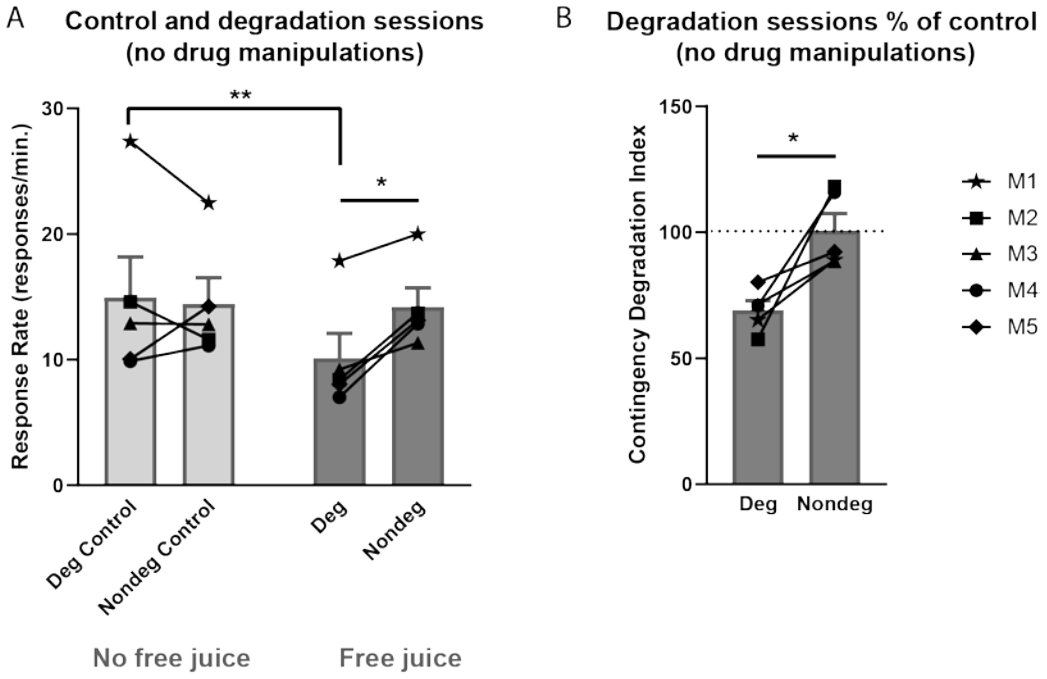


Figure 3.2. The last degradation session before drug manipulations demonstrated animals' sensitivity to contingency degradation pre-treatment. A). Animals decreased responding in the degraded session compared to non-degraded session and control sessions. There was no difference in response rate between control sessions and nondegraded sessions. B). Degradation effect demonstrated using the contingency degradation index measurement. Animals showed decreased response in the degraded compared to non-degraded sessions. *: $p < 0.05$, **: $p < 0.01$.

3.3.2. Areas 24, 11 and 14-25 contribute differentially to the control of action contingencies

Linear mixed model analysis of the full degradation sessions dataset with all brain regions revealed a significant three-way interaction between region, drug, and session ($F_{4, 39.68}$ = 5.6866, p = 0.00103). Subsequent analyses of the effects of drug on session type were carried out within each brain region independently (Fig. 3.3).

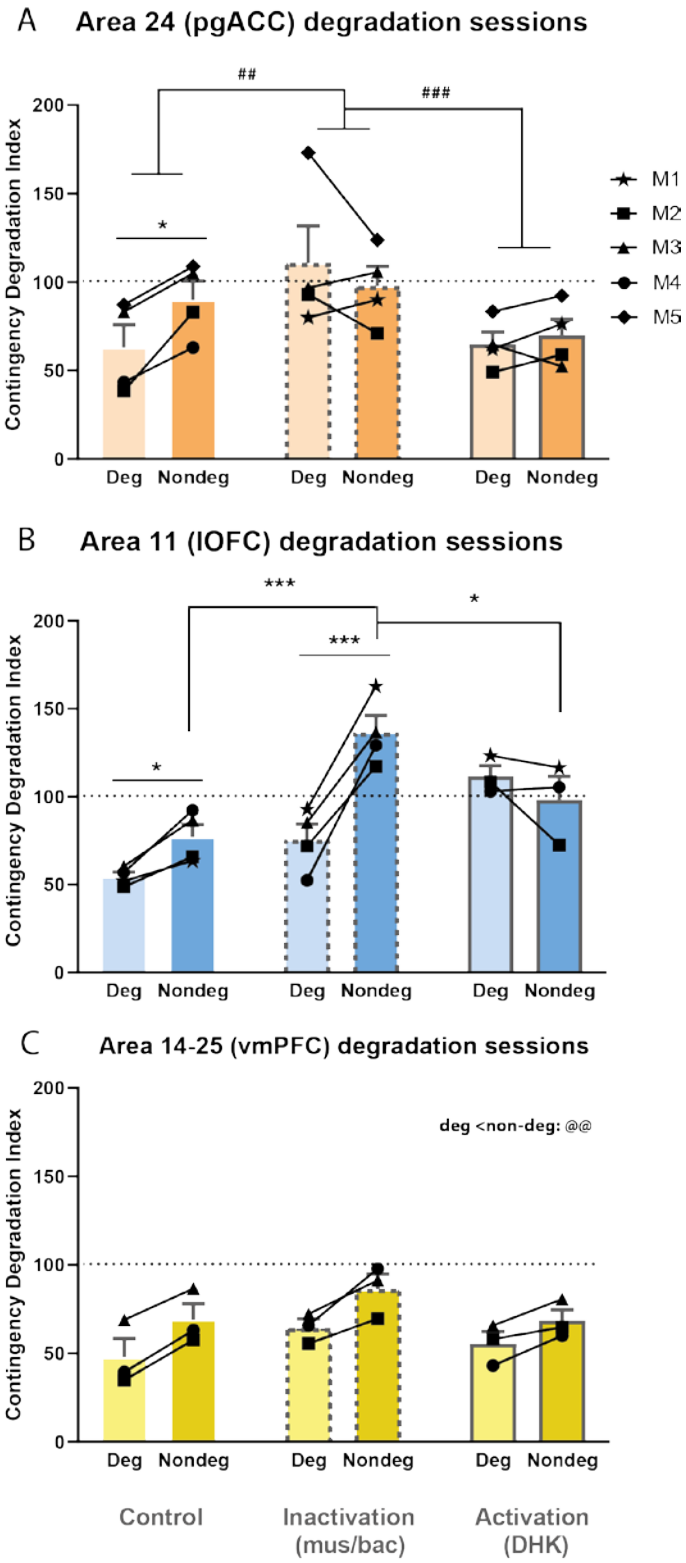


Figure 3.3. Effects of activation or inactivation of critical PFC regions in contingency degradation. A). Animals with Area 24 inactivation and activation were insensitive to contingency degradation. Although animals significantly decreased their response rate after activation when compared to inactivation. B). Area 11 inactivation apparently enhanced animals' sensitivity to contingency degradation, while activation impaired animals' sensitivity. Only the response rate in the non-degraded sessions after inactivation was significantly different from that of control sessions, but no significant difference was observed in the degraded sessions after inactivation and control. C). Despite the implications of vmPFC's involvement in goal-directed behaviour, inactivation and activation of Area 14-25 did not affect animals' performance. The area seems to not be involved in the expression of A-O associations.

* indicates significant effect of degradation x treatment interaction, # indicates significant effect of treatments, @ indicates significant effect of degradations. */#/@: $p < 0.05$, **/##/@@: $p < 0.01$, ***/###/@@@: $p< 0.001$.

3.3.2a. Area 24 inactivation impaired sensitivity to contingency degradation

Both area 24 inactivation and activation blocked the normal reduction in response rates in the degraded sessions when compared to non-degraded sessions (Fig 3.3A). Linear mixed-model analysis of variance revealed a two-way interaction of drug x degradation ($F_{2,10}$ = 4.131, p = 0.0492), a main effect of drug ($F_{2,10}$ = 12.370, p = 0.00198) and no main effect of degradation ($F_{1,10}$ = 0.588, p = 0.461). Subsequent post-hoc analysis revealed a significant difference between degraded and non-degraded sessions only following saline infusion (t_{10} = -2.604, p = 0.0263). In contrast, there were no differences between degraded and non-degraded sessions after inactivation (t_{10} = 1.430, p = 0.183) or activation (t_{10} = -0.153, p = 0.881). This lack of difference after inactivation occurred due to selective increase in responding in degraded sessions when compared to saline (t_{10} = 4.085, p = 0.0057), whereas there was no change of responding in non-degraded session compared to saline (t_{10} = 0.051, p = 0.999). Although both inactivation and activation blunted animals' sensitivity to contingency degradation, their effects on overall responding differed. Responding across degraded and non-degraded sessions following inactivation was greater than that of both activation (t_{10} = -4.947, p = 0.0015) and saline (t_{10} = 2.925, p = 0.037), while activation and saline did not differ (t_{10} = -2.022, p = 0.157). Overall, both inactivating and activating area 24 blunted sensitivity to contingency degradation, but in slightly different ways.

3.3.2b. Area 11 inactivation enhanced response rate in non-degraded sessions only and activation abolished differential responding towards degraded and non-degraded sessions

There was a greater difference in response rate between degraded and non-degraded sessions following inactivation of area 11 compared to saline infusion data, whereas area 11 activation abolished this difference (Figure 3.3B). Linear mixed-model analysis of variance revealed a two-way drug x degradation interaction ($F_{2,14.945}$ = 9.036, p = 0.00268), as well as main effects of drug ($F_{2,15.94}$ = 14.236, p = 0.000284) and degradation ($F_{1,14.935}$ = 13.1, p = 0.00254). Subsequent least squared means post-hoc analysis on the interaction indicated that responding in degraded sessions was significantly reduced compared to non-degraded sessions under both saline ($t_{15.1}$ = -2.516, p = 0.0237) and

inactivation infusions ($t_{15.1}$ = -5.320, p = 0.0001), meaning that animals showed degradation effects in both cases (Fig 3B). In contrast, activation of area 11 abolished the degradation effect ($t_{15.1}$ = 1.007, p = 0.330). Further analysis of area 11 inactivation and saline effects revealed a significant increase in the difference in responding between degraded and non-degraded conditions after inactivation when compared to saline infusion ($t_{6.29}$ = 5.487, p = 0.0032). This effect was driven by a significant increase in responding in the non-degraded condition after inactivation when compared to saline ($t_{15.36}$ = 5.095, p = 0.0003) but not in the degraded condition ($t_{15.36}$ = 1.888, p = 0.1755). In summary, whereas activation of Area 11 abolished the degradation effect, inactivation apparently enhanced it.

3.3.2c. Area 14-25 is not involved in mediating contingency degradation expression

Area 14-25 inactivation or activation did not affect responding of animals when compared to saline (Fig. 3.3C). Linear mixed-model analysis of variance revealed a main effect of degradation ($F_{1,11.943}$ = 9.718, p = 0.00243), but not of drug ($F_{2,12.02}$ = 3.126, p = 0.0807) nor interaction of drug x degradation ($F_{2,11.943}$ = 0.229, p = 0.799). Further post-hoc analysis showed that responding in non-degraded sessions was significantly greater than that of degraded sessions across all drug conditions (t_{12} = -3.117, p = 0.0089). Therefore, area 14-25 seemed to not be involved in the use of previously acquired response-outcome contingencies to guide responding following contingency degradation.

3.3.3. Pharmacological manipulations in baseline sessions without degradation did not affect animals' responding except during area 24 activation

Linear mixed model analysis of the full baseline sessions dataset with all brain regions revealed a significant two-way interaction between drug and region ($F_{4, 34.604}$ = 5.390, p = 0.00176). Subsequent analyses of the effects of drug were carried out within each brain region independently.

3.3.3a. DHK infusion into area 24 decreased baseline responding

Activation of area 24 via DHK depressed responding of animals in baseline sessions when compared to saline or inactivation (Fig. 3.4A). Linear mixed-model analysis of variance revealed a main effect of drug ($F_{2,10}$ = 12.180, p = 0.00209), but not of degradation juice conditions ($F_{1,10}$ = 0.0118, p = 0.916). Post-hoc analysis of drug revealed a significant decrease in animals' responding across degraded and non-degraded juice conditions after activation, when compared to inactivation (t_{10} = -4.322, p = 0.0039) or saline (t_{10} = -4.225, p = 0.0045).

3.3.3b. Drug infusions into area 11 or area 14-25 did not affect baseline responding

Saline, inactivation and activation of area 11 or area 14-25 did not alter responding of animals in baseline sessions (Fig. 3.4B, C). Linear mixed model analysis of the area 11 or area 14-25 baseline sessions revealed no main effects of degradation juice conditions (area 11: $F_{1, 13.12}$ = 0.185, p = 0.674; area 14-25: $F_{1, 10}$ = 0.217, p = 0.651) or drug (area 11: $F_{2, 13.723}$ = 0.185, p = 0.106; area 14-25: $F_{2, 10}$ = 0.300, p = 0.747).

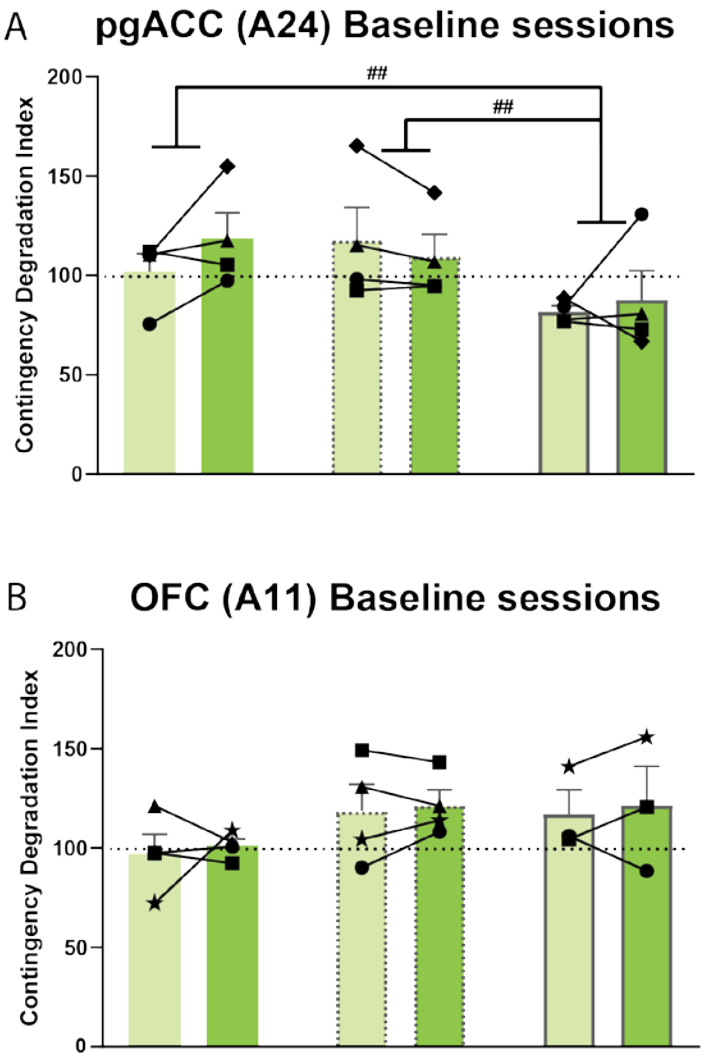


Figure 3.4. Effects of control, activation or inactivation of critical PFC regions in baseline sessions. A). Animals with Area 24 activation decreased their response rate across sessions when compared to control and inactivation. B-C). No effect of drug treatments in area 11 and area 14-25 on baseline session performance.
# indicates significant effect between treatments, @ indicates significant effect between degradations. ##: $p < 0.01$

3.3.4. Control experiments for dysregulation of motivation

3.3.4a. Area 11 inactivation did not affect the animal's performance in contingency degradation task under a progressive ratio schedule

One hypothesis to explain the increase in the responding of animals in the non-degraded session after area 11 inactivation is dysregulation of primary motivation. One way of testing whether area 11 inactivation causes the animals to become more motivated in the presence of a free reward is to make the animals respond under a progressive ratio (PR) schedule. The probe sessions involved free-reward, using the same parameters as the contingency degradation sessions, but the contingent reward was delivered under a PR schedule instead of a variable ratio schedule. Because only one animal (M4) had undergone this procedure, no detailed statistical analysis was done. Although M4 deceased his breakpoint in the probe session compared to baseline sessions, most likely due to the presence of free reward, there were no differences between his breakpoint neither after control or inactivation of area 11 in the probe sessions nor the baseline sessions (Figure 3.5).

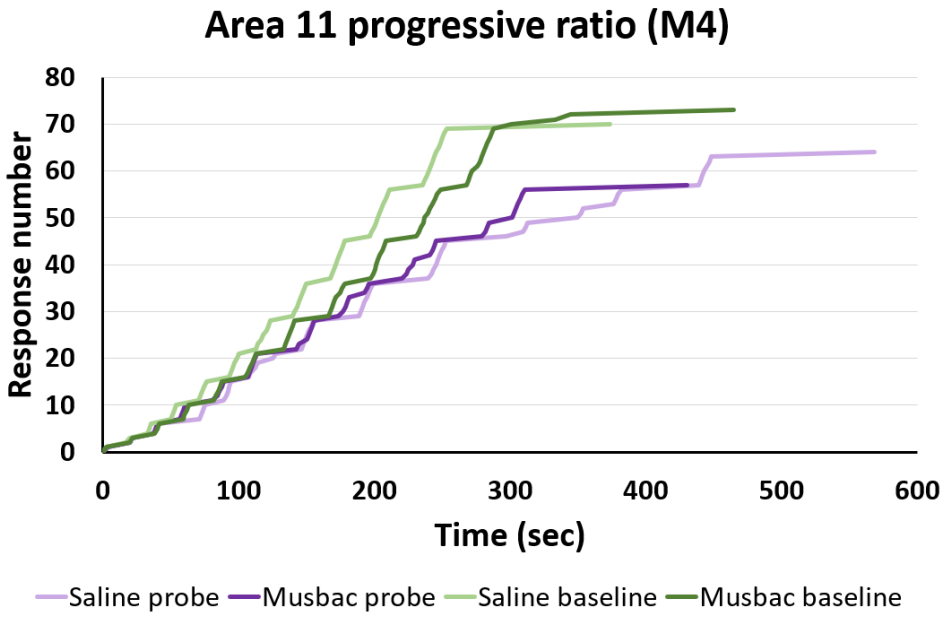


Figure 3.5. No difference in break point was observed after area 11 inactivation and control manipulations in baseline session (no free reward present) and probe sessions (contingent and non-contingent free reward were unmatched) with a progressive ratio schedule. M4 decreased his break point slightly in the probe session, but the decrease was observed in a similar magnitude after both control and inactivation manipulations.

3.3.4b. Analysis of lick latency and lick per reward

The lick data were analysed to examine the effect of motivation on the decrease in responding after activation of area 24 in the baseline sessions and the enhanced sensitivity of contingency degradation after area 11 inactivation. In degradation sessions, area 11 inactivation did not affect animals' lick latency (Figure 3.6C) but increased the number of licks per reward across sessions when compared to control (Figure 3.7C). In baseline sessions, area 24 activation did not affect animals' lick latency (Figure 3.6B) nor the number of licks per reward when compared to control (Figure 3.7B).

Area 11

Linear-mixed model analysis of variance on lick latency in degradation sessions after area 11 manipulations did not revealed any main effects (Treatment: $F_{2, 13.256} = 2.822$, $p = 0.0953$, Degradation: $F_{1, 13.021} = 0.289$, $p = 0.600$, Treatment x Degradation: $F_{2, 13.021} = 2.692$, $p = 0.105$; Figure 3.5C). Linear mixed-model analysis of variance on lick per reward in degradation sessions after area 11 manipulations revealed a main effect of treatment ($F_{2, 13.594} = 4.841$, $p = 0.0259$; Figure 3.6C). Post-hoc analysis showed a significant increase in licking per reward after area 11 inactivation when compared to saline control infusions ($t_{13} = 2.849$, $p = 0.0342$) and a trend of increase in licking per reward after activation when compared to saline control ($t_{13.7} = 2.361$, $p = 0.0803$).

Area 24

Linear mixed-model analysis of variance on area 24 lick latency in baseline sessions revealed a main effect of degradation x treatment interaction ($F_{2, 12} = 4.072$, $p = 0.0447$; Figure 3.5B). Post-hoc analysis showed that lick latency after area 24 activation in the degraded juice sessions was longer than that of the non-degraded juice sessions ($t_{10} = 2.449$, $p = 0.0343$) and a trend for longer lick latency in degraded session compared to control ($t_{10} = 2.4$, $p = 0.087$). Linear mixed-model analysis of variance on area 24 lick per reward in baseline sessions did not revealed any main effects (Treatment: $F_{2, 10} = 1.574$, $p = 0.255$, Degradation: $F_{1, 10} = 2.401$, $p = 0.152$, Treatment x Degradation: $F_{2, 10} = 0.0829$, $p = 0.921$; Figure 3.6B). Post-hoc analysis showed that lick latency after area 24 activation in the degraded juice sessions was longer than that of the non-degraded juice sessions (t_{10}

= 2.449, p = 0.0343) and a trend for longer lick latency in degraded session compared to control (t_{10} = 2.4, p = 0.087). Linear mixed-model analysis of variance on area 24 lick per reward data in degradation sessions revealed a main effect of treatment ($F_{2, 10}$ = 5.758, p = 0.0217; Figure 3.6A). Post-hoc analysis indicated higher lick per reward after area 24 inactivation when compared to control (t_{10} = 2.93, p = 0.0367) and activation (t_{10} = 2.947, p = 0.0357).

No effects were observed in area 14-25. For the full analysis, please see Appendix section A.

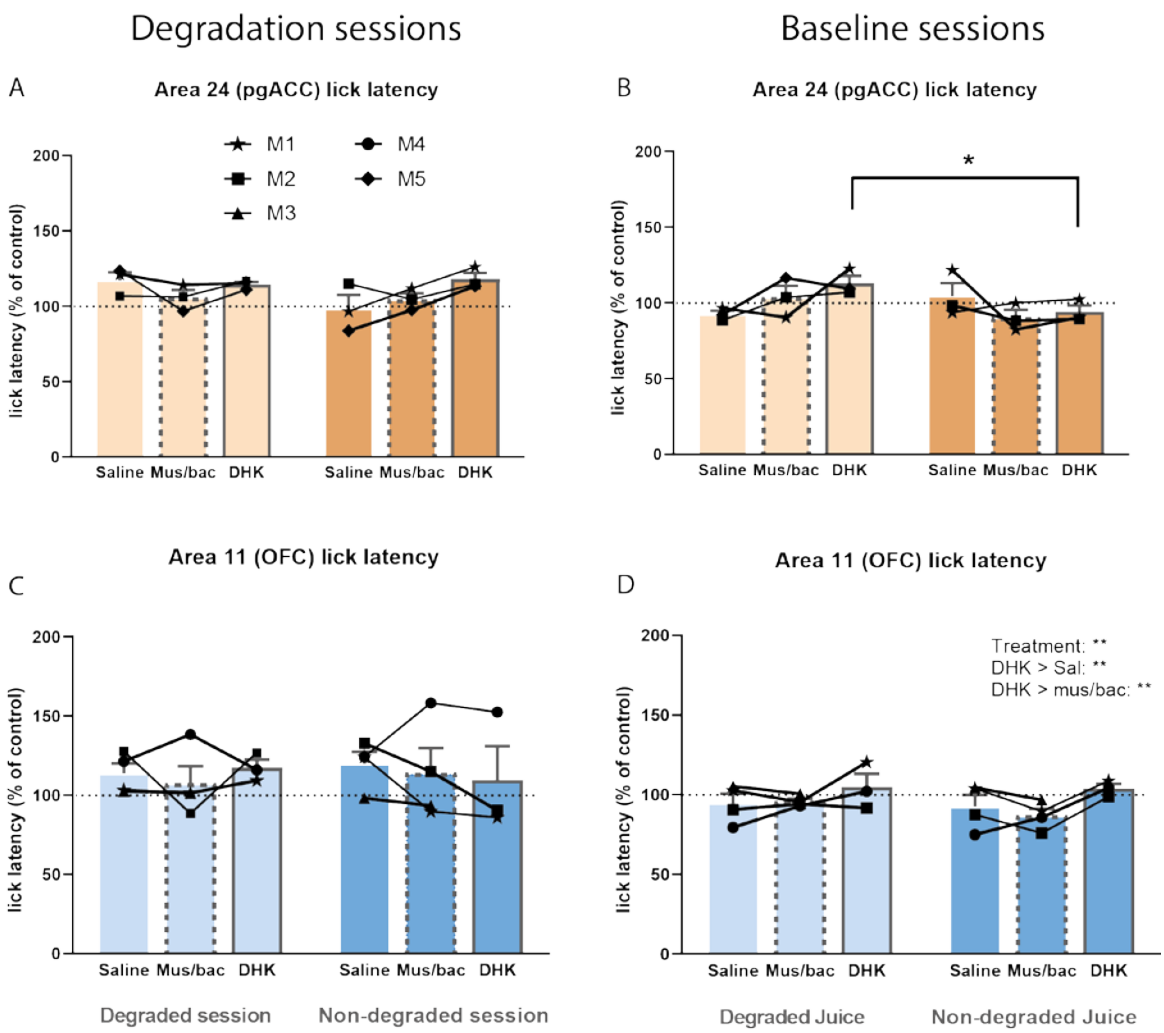


Figure 3.6. Lick latency of animals after drug manipulations of each PFC regions. Lick latency is the time between the onset of birdsong that signals the reward and the time animals start to lick from the spout. A). No significant difference in lick latency in the degradation session after area 24 manipulations. B). In baseline sessions, animals initiated licking significantly slower after area 24 activation in the degraded juice condition when compared to the non-degraded juice condition. C). No significant differences in lick latency in the degradation sessions after area 11 manipulations. D). In baseline sessions, animals initiated licking significantly slower after area 11 activation in both degraded and non-degraded sessions compared to inactivation and control.
*: $p < 0.05$, **: $p < 0.01$.

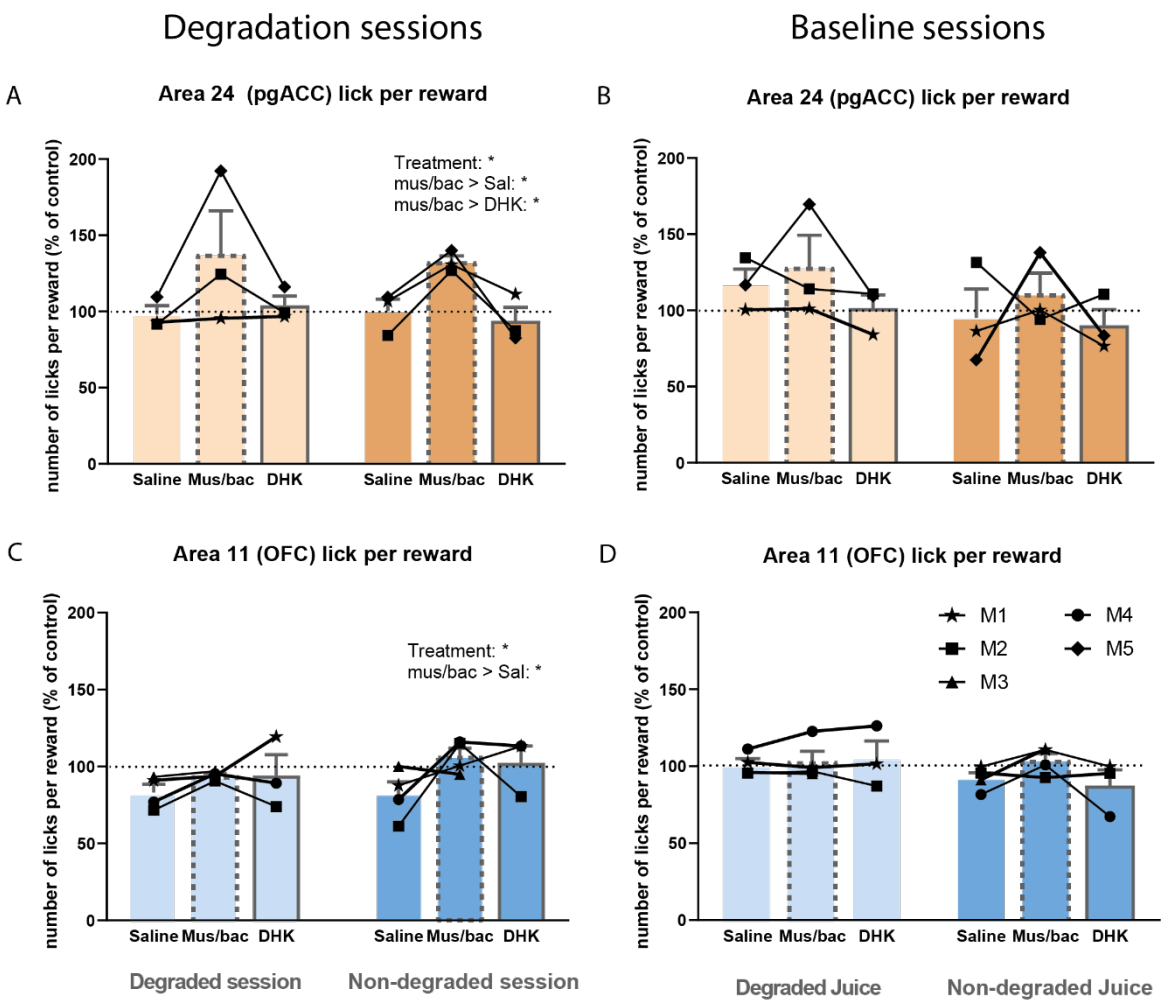


Figure 3.7. Animals' lick per reward after each PFC region's manipulations. Lick per reward is the average number of licks an animal does for the duration of each reward delivery. A). Animals produced significantly more licks per reward after area 24 inactivation when compared to activation and control. B). No significant difference in lick per reward was observed in baseline sessions after area 24 manipulations. C). Animals significantly licked more per reward after area 11 inactivation across degraded and non-degraded session when compared to control. D). No significant difference was observed after area 11 manipulations in baseline sessions.
*: $p < 0.05$.

3.4. Discussion

This study used a novel version of the contingency degradation task to investigate which of the precisely-targeted PFC sub-regions, when inactivated or activated, affect the expression of goal-directed actions. Out of the three PFC regions tested, only inactivation of area 24 during task performance impaired animals' sensitivity to contingency degradation. Thus, I identified area 24 (pgACC) as the critical PFC sub-region necessary for maintaining learned action-outcome (A-O) associations, but not area 11 (anterior OFC) or area 14-25 (vmPFC). Both activation and inactivation of area 24 impaired contingency degradation, which signified the need to have optimal activity in area 24 to maintain goal-directed actions. Inactivation of area 11 apparently enhanced animals' sensitivity to A-O associations, making them respond more in the non-degraded session after mus/bac infusion when compared to saline control. This might be due to interference between pavlovian-based and instrumental-based elements of the task. A more detailed analysis will be presented in the discussion of area 11 results (Chapter 3.4.2). Manipulations of area 14-25 did not affect animals' sensitivity to contingency degradation, suggesting that this region of the vmPFC is not necessary for expressing goal-directed actions. None of the existing literature has addressed the causal role of specific primate PFC sub-regions in the expression and maintenance of learned A-O associations that do not depend on changing reward value but on causality between an action and its consequences. My study has thus filled this gap in the literature and provided a valuable bridge between rodent, macaques and human studies in understanding the causal role of PFC sub-regions in maintaining goal-directed behaviour.

3.4.1. The expression of goal-directed actions requires optimal levels of activity in area 24

A normally functioning area 24 is required for animals to manifest their learned goal-directed behaviour, as evidenced by an impairment in their behaviour after both inactivation and activation of area 24. After inactivation of area 24, animals maintained their response rate in the non-degraded session but did not decrease their response rate in the degraded session. After activation of area 24, animals did not differentiate between

degraded and non-degraded sessions but decreased responding across the two sessions when compared to saline control.

This study's result is consistent with the literature that area 24 (anterior cingulate cortex) is involved in A-O associations and high-level reward-guided decision making (Holroyd and Coles, 2002; Rushworth et al., 2004; Rushworth et al., 2007; Rushworth and Behrens, 2008; Hayden et al., 2009; Hayden and Platt, 2010; Wallis and Kennerley, 2011; Holroyd and Yeung, 2012). The contingency degradation task requires the animal to understand and remember which A-O associations they have learned in previous control sessions and to adjust their responding to the now changed A-O contingencies in the degradation sessions. This means that to be goal-oriented in this task, the animals need to be fully aware of their response history and the outcome associated with their actions to adjust their responses in real-time with the help of previously learned A-O contingencies. Thus, instead of encoding the outcome of a single action, the animals need to have a holistic understanding of their current A-O contingency and how this contingency relates to previous ones within the same session and across sessions. In the current task, they need to maintain knowledge about two sets of A-O relationships and additionally in degradation sessions, the violation of one set of those relationships due to the presentation of matched non-contingent rewards being associated with both action and non-action.

The ACC seems to be important for selecting and maintaining learned task information across time and sessions (Amiez et al., 2006; Kennerley et al., 2006; Seo and Lee, 2007). For instance, Seo and Lee (2007) used a two-player zero-sum game (matching pennies) (Barraclough et al., 2004; Lee et al., 2004) in macaques, where ACC neurons encode the rate of rewards in previous trials or to current reward choice that is modulated by previous choices. Therefore, one possible explanation for the animals' impairment in the contingency degradation task could be their inability to track previous A-O information when performing actions. Animals were unable to decrease their responding in the degraded condition because, with an inactivated area 24, they could not utilise learned knowledge from previous sessions and were unable to track the consequences of their previous responses within the session. Their inability to evaluate the meaning of their actions drove them to become more habit-like, i.e., they kept on performing the action even though it was less effective.

ACC seems to be important for more complex tasks such as the disambiguation of choices that share similar elements, such as sensory modalities, colour, shape and context (Bussey et al., 1997a; Bussey et al., 1997b; Cardinal et al., 2003; Schweimer and Hauber, 2005). Cardinal et al. (2003) showed that peri- and post-genual ACC lesioned rats were impaired in autoshaping and two-stimulus discrimination approach task, but not in pavlovian-to-instrumental-transfer (PIT) or conditioned reinforcement. At first, the results seem to be contradictory, in that all the tasks involve pavlovian conditioning. However, when examined closely, ACC lesions only impaired animals' performance in tasks that are more complex (more than one stimulus present) and had stimuli that share common features that require disambiguation. The autoshaping and two-stimulus discrimination approach task both used a very similar stimulus; on the other hand, the PIT task and the conditioned reinforcement task used distinctive and simple stimuli. This is relevant to interpreting the findings in the current thesis, in that the contingency degradation task required disambiguation. One difference between the current contingency degradation task and Jackson et al. (2016) is that the two touchscreen stimuli used were the same (compound coloured Maltese crosses). Moreover, the same CS (birdsong) signalled the delivery of both rewards, which were delivered from the same licking spout. The only element an animal could rely on to distinguish the rewards was the position of the two stimuli, one on the right and one on the left of the touchscreen, each associated with a specific reward. Thus, the sensory modalities, shapes, colour and context that the animal could use to distinguish between the two A-O associations were very similar. Much more instrumental in nature rather than being pavlovian driven, the contingency degradation task I used, with its identical stimuli and reward delivery signal, required the help of ACC to disambiguate similar choices and guide instrumental actions mediated by reward outcomes.

Area 24 activation also caused the animals to behave in a less goal-directed manner. It might seem paradoxical that both inactivation and activation caused impairment. However, one could explain this result with an inverted U function, where both too little and too much activity cause deficits in behaviour. For the animals to use the learned A-O associations, area 24 evidently requires an optimal level of activity.

The impairment after area 24 activation was however qualitatively different from that of inactivation. The average response rate between non-degraded and degraded

sessions after activation was significantly lower than after inactivation, i.e. while animals did not show that they could differentiate between degraded vs. non-degraded session, they decreased their response rate overall across the two sessions. This decrease in response rate was also seen in the baseline control sessions, where drug infusions were made when there was no free reward involved. These control baseline sessions investigated the effect of the drug on animals' response rate; activation of area 24 via increasing extracellular glutamate consistently lowered animals' responding. However, this effect was not likely due to motivation. In the baseline sessions, lick latency and the number of licks per reward after area 24 activation was not significantly different from that of saline.

The current study on perigenual anterior cingulate cortex (area 24) has perhaps filled a void in the literature for establishing a causal relationship between this region and the utilization or implementation of A-O associations. One point to note is that area 24 in this study is located at the most dorsal edge of the genu of the corpus callosum, the most rostral part of dorsal anterior cingulate cortex, very close to the area 32 and 24 boundaries (Figure 3.1A). Although it is often difficult to interpret cross-species neuroanatomy and homologies, many of the macaque electrophysiological studies recorded from dorsal ACC that is posterior to the genu of the corpus callosum; whereas macaque lesion studies often ablated a relatively large amount of dorsal ACC tissue. The ACC areas studied in the human neuroimaging or lesion literature were often too large, thus lacking anatomical specificity and making interpretation difficult. Rodent studies on goal-directed behaviour focus on PFC regions that are anterior to ACC and largely on the acquisition and not on the expression of A-O associations. As ACC in the general sense is a relatively large brain area, we should consider the exact locations of the ACC targeted and the terminologies used when comparing findings between and within species.

Like any other brain area, area 24 does not work alone. Anatomically, it is located between the sensory and motor PFC regions and has extensive connectivity with them. Tang et al. (2019) suggested pregenual area 24 to be the central hub for integrating information from motor planning and executive decision-making areas and sensory, limbic regions, through anatomical connectivity evidence in both macaques and humans. For animals to understand and successfully execute goal-directed behaviour in the current contingency degradation task, they need to be able to combine complex sensory

information and motor execution functions. Pregenual area 24 is necessary for this integrative function. Area 24's relationships with other brain regions will be detailed further in Chapter 5 (General Discussion), which will provide a more systematic and integrated description of the role they play together in controlling goal-directed actions, once all of the data collected in this thesis from multiple brain regions have been presented.

3.4.2. Inactivation of area 11 may enhance action-outcome associations via decreased interference from pavlovian-driven cues

Contrary to expectations, area 11 inactivation did not impair animals' sensitivity to contingency degradation, but instead actually appeared to enhance animals' ability to discern A-O associations; the animals increased their response rate significantly only in the non-degraded session but not in the degraded session after inactivation when compared to control. Activation of area 11 did impair animals' performance. Evidently, the engagement of area 11 to express goal-directed actions that are instrumentally driven is not required. One of the interesting findings after area 11 inactivation was the significant increase in the response rate only seen in the non-degraded session. To explain this specific increase in response rate and the intact sensitivity of A-O associations after area 11 inactivation, I am proposing three alternative possibilities: (i) an energising or invigorating effect of elevated arousal on response output; (ii) a dysregulation of motivation or (iii) interference between instrumental-driven vs. pavlovian-driven systems. The available evidence appears to support the third proposition.

3.4.2a. Area 11 is important for pavlovian encoding

The most plausible hypothesis for explaining the area 11 inactivation results is that it is involved in pavlovian encoding. Inactivation of area 11 did not impair animals' performance in this largely instrumental-based task but made them more focused on using action-driven cues and not stimulus-driven cues. This is apparently contrary to this laboratory's previous finding, that area 11 lesion impaired contingency degradation learning (Jackson et al., 2016). However, there are subtle but crucial differences between

the current task and Jackson et al. (2016). The current contingency degradation task was re-designed to minimise its pavlovian components and to encourage animals to use instrumentally-driven behaviour. The previous contingency degradation task used in this laboratory (Jackson et al., 2016) utilised different stimuli in different positions to associate with a specific reward, whereas the current task had the same stimuli in different positions for each juice reward delivered. This way, in our current task, animals used the location of the stimuli and their actions but not the stimulus itself to distinguish specific rewards, while in Jackson et al. (2016) the animals could also use different stimuli to distinguish the rewards. Moreover, our current task used a variable ratio schedule instead of Jackson et al.'s variable interval schedule, in which the former is associated with generating goal-directed actions and the latter with habitual actions (Dickinson and Nicholas, 1983; Rossi and Yin, 2012). Because the marmosets were lesioned before the contingency training, Jackson et al. (2016) investigated the acquisition of the A-O associations as opposed to the current thesis' focus on the expression of such relationships. Thus, the finding from Jackson et al. (2016) that area 11 lesion resulted in impaired contingency degradation and the current thesis' finding that area 11 inactivation did not, might be due to area 11's involvement in pavlovian-driven learning (Jackson et al., 2016) rather than instrumental-driven expression (this thesis).

Other sources of evidence from the literature also strongly implicate the role of area 11 (anterior OFC) in acquiring, updating and learning new information when the tasks have strong pavlovian components and are stimulus-response driven (Murray et al., 2007; Rushworth et al., 2007; Rudebeck et al., 2008). Neural activity in the OFC was shown to correlate with the value of reward expectation on visual stimulus presentation (Tremblay and Schultz, 1999; Wallis and Miller, 2003). Many OFC impairments have been observed using stimulus-reinforcement learning tasks (Rolls, 2004; Murray et al., 2007). In a pavlovian version of the contingency degradation task, rats associated different CSs with different reward outcomes and did not need to perform actions to obtain rewards (Ostlund and Balleine, 2007). Lateral OFC lesions impaired the learning of this pavlovian contingency degradation paradigm, whereas both pre- and post-training lesions failed to affect an instrumental form of outcome devaluation. Panayi and Killcross (2018) showed that lateral orbital (LO) lesions impaired outcome devaluation in pavlovian but not instrumental procedures; they also showed that both anterior and posterior LO lesions

impaired pavlovian outcome devaluation. Thus, it could be concluded that the lateral OFC is not involved in the learning and expression of more instrumental-based goal-directed tasks but is pertinent for learning the pavlovian versions.

As suggested in the ACC section (Chapter 3.4.1.), ACC is more engaged by A-O association tasks, such as the one in this thesis, and OFC is recruited in stimulus-outcome, value-updating based tasks. The current contingency degradation task has the same CS, stimulus and reward delivery location across the two degradation conditions. The only way animals could distinguish degraded vs. non-degraded situations is through their action of touching the same stimulus in different locations of the touchscreen. Whilst usually differentiating stimuli could aid the animal in associating their responses with reward, in this task the identical CSs and stimulus might make the goal to establish correct A-O associations more difficult for the animals. Therefore, both ACC and OFC are in a sense competing with each other, the ACC using the more informative instrumental component and the OFC using the weaker and potentially confusing pavlovian component to complete the task. When OFC is inactivated, ACC could more completely take over control of performance and help to focus the animals' attention on the instrumental aspect of the task, with some likely beneficial effects on performance. Therefore, we observed an increase in response rate to the only A-O association that is not degraded ('meaningful' action), but not to the A-O association that is degraded ('not meaningful' action). The competition between instrumental vs. pavlovian components could also perhaps explain the impairment of A-O associations after area 11 activation. If area 11 but not area 24 activity is pharmacologically enhanced, animals could shift away from using instrumental cues towards using pavlovian cues to perform the task. This would not aid animals to perform well in the current task because it requires instrumental knowledge and the use of pavlovian cues would only lead to possible confusion.

How did the animals still show the degradation effect in the control condition when both ACC and OFC are hypothetically competing with each other? It could be that animals learn goal-directed behaviour predominately through A-O rather than S-R associations. Fisher et al. (2020) had designed an outcome devaluation task where two levers on either side of the testing apparatus have a different CS presented above each lever to help animals to learn. There was a congruent situation, where the learned CS-lever-outcome relationship is intact, and an incongruent situation, where the CS

associated with one lever is now presented together with the other previously not associated lever. The animals still learned outcome devaluation in the incongruent situation, indicating that they can learn goal-directed behaviours predominantly via A-O rather than stimulus-response based mechanisms. Interestingly, the number of responses the animal made to the non-devalued lever did decrease somewhat in the incongruent situation, which suggests even though animals could still learn via action-based strategies, the presence of incongruent CSs did slightly hinder their performance. This is compatible with the findings of the current contingency degradation task in that by removing the brain region responsible for processing stimulus-outcome information, animals maintained the learned action-driven information and stayed more focused in performing goal-directed behaviour.

3.4.2b. Arousal effect unlikely to cause the enhancement of A-O contingencies after area 11 inactivation

At first glance, the enhancement of response rate after area 11 inactivation could be due to an arousal effect caused by the availability of free rewards in degradation sessions. OFC has been shown to track the subjective pleasure of food in humans (Small et al., 2001; Kringelbach et al., 2003; Kringelbach, 2010; Berridge and Kringelbach, 2015). Although there was no difference between degraded and non-degraded sessions in the total number of juice deliveries in one session, animals do gain more juice delivery when comparing degradation sessions (with free reward) with baseline sessions (without free reward). Thus, it might be considered that the increase in response rate after area 11 inactivation is because animals were motivationally aroused by free juice availability. This putative arousal effect can be seen in paradigms such as the generalised form of pavlovian-to-Instrumental Transfer (PIT). A typical version of generalised PIT occurs when a CS (A) that has never been associated with any instrumental actions (but has been associated with reward delivery), elicits generalised instrumental responses associated with other CSs (B or C). Animals readily respond because they are aroused by the CS (A) that they link with reward delivery. Unfortunately, there have been few publications that have investigated the relationship between generalised PIT and the OFC. However, I do not believe an arousal effect as seen in generalised PIT would explain the current area 11

inactivation result. All animals indeed showed a significant increase in their average response rate across the degraded and non-degraded sessions after area 11 inactivation when compared to saline control. However, the driver for this effect was specific: only the response rate in the non-degraded session was increased significantly after area 11 inactivation, but not the response rate in the degraded session. If generalised arousal had indeed being involved, then there should have been observed a significant increase in the degraded session response rate as well, which was however absent. Additionally, we did not observe an elevated response rate after area 11 inactivation in the baseline sessions, showing that the inactivating drug by itself did not arouse the animals sufficiently to increase their response rate.

3.4.2c. Dysregulation of motivation unlikely to cause the enhancement of A-O contingencies after area 11 inactivation

The only difference between non-degraded and degraded sessions is that in the non-degraded session, the free reward is different from that the animals are working to obtain, whereas, in the degraded session, both rewards are the same. Animals might increase their responding because their motivation is enhanced by this variety: two types of juices being available instead of one. OFC neurons have been implicated in sensory-specific satiety, the decline of appetite for the continuous consumption of a type of food (Rolls et al., 1981; Rolls et al., 1984; Rolls et al., 1986; Critchley and Rolls, 1996; Kringelbach et al., 2003; Delamater, 2007; Pritchard et al., 2008). Thus, it has been shown that if one is sated with one food, it could lead to increased consumption of other types of food if variety is present (Rolls et al., 1981; Rolls et al., 1984). Varieties of food also enhance food intake and contribute to obesity development in rats (Rolls et al., 1983). OFC neurons were shown to inhibit the response to sated food (Critchley and Rolls, 1996). However, the outcome devaluation task, which relies on sensory-specific satiety, was not impaired after lOFC, or ventral orbital (VO) + LO lesion in rats (Ostlund and Balleine, 2007; Balleine et al., 2011). Additionally, the animals in the current task were unlikely to have been sated, as they continued to drink the juices until the end of the sessions, and the amount of juice they were capable of drinking in one setting far exceeded the volume of juices available in a testing session.

Although explaining the current result using sensory-specific satiety is problematic, the animals could still have received an incentive motivational boost to respond more in the non-degraded session due to the two types of juice being present. One method for investigating motivation is the progressive ratio schedule. Effortful aspects of motivation are measured using the breakpoint, i.e. the highest ratio completed, which is thought to measure how much effort an animal is willing to spend to obtain a reward (Hodos, 1961; Bowman and Brown, 1998; Baunez et al., 2002). The modified contingency degradation task using such a PR schedule is described in detail in section (3.2.5). In short, the variable ratio schedule that dictates the number of responses required for one reward is replaced by a PR schedule. If area 11 inactivation indeed increases animals' motivation via the presence of food variety, we should have observed a higher breakpoint after inactivation when compared to saline infusion. However, the results for a single marmoset (presented in section (3.3.7a) indicated that there was no change in breakpoint after area 11 inactivation when compared saline in the probe and baseline sessions, making a motivation-driven theory of response rate enhancement unlikely.

The reward licking data analysis also indicated the unlikeliness of the motivation-driven hypothesis. In the degradation sessions, lick latency was not affected by area 11 inactivation. Area 11 inactivation increased the number of licks per reward when compared to saline. However, this increase was a generalised effect across the degraded and non-degraded session and not specific to the non-degraded session.

3.4.2d. Summary

To conclude, the theory of competition between instrumental vs. pavlovian system in performing goal-directed behaviour, in which area 11 mediates the pavlovian components, seems to be the most plausible for explaining the enhanced A-O association after area 11 inactivation and impairment after area 11 activation. Because the current task is designed for the animals to use instrumental components to perform the task successfully, inactivation of area 11 made the animals' use of instrumental cues stronger whilst area 11 activation perhaps, somewhat speculatively, made them more focused on pavlovian cues. Areas 24 and 11 seem to work to keep the balance between instrumental

and pavlovian components in goal-oriented tasks, which will be explored further in the General Discussion (Chapter 5).

3.4.3. Area 14-25 is not involved in the expression of action-outcome associations

Animals still maintained their level of goal-directed behaviour after inactivation and activation of area 14-25, which indicated that this region is not important in the expression of A-O associations. This is perhaps a little surprising given the implication of vmPFC and mOFC in OCD-related (Milad and Rauch, 2012; Gillan and Robbins, 2014; Gillan et al., 2015; Robbins et al., 2019) and goal-directed behaviour imaging studies in healthy human volunteers (Valentin et al., 2007; de Wit et al., 2009; de Wit et al., 2012; McNamee et al., 2015; Reber et al., 2017). Since the current cannulation target comprised the boundary between area 14 and 25, and area 14 proper was specifically targeted in the next study (Chapter 4), the possible role of area 14 (mOFC) will be discussed in the next chapter and area 25 will be discussed here.

The current study's finding of area 14-25's apparent non-involvement in goal-directed action might be due to the area targeted, the task itself and brain homologies across species. The vmPFC in human imaging studies is implicated in goal-directed behaviour. In an outcome devaluation task, vmPFC and the adjacent mOFC showed changes in activity when contrasting non-devalued and devalued conditions (Valentin et al., 2007). Additionally, studies using the contingency degradation task that have already been described in detail in chapter 1, also implicated involvement of the vmPFC in tracking the contingent relationship between an action and an outcome (Tanaka et al., 2008; Liljeholm et al., 2011). The term vmPFC as used in human imaging studies is however inconsistent across studies. It could refer to a large portion of the medial wall of PFC, most of the medial OFC and sometimes all tissue that is both ventral and medial in the PFC. Because the area 14-25 region targeted in the current study is very localised, other brain regions such as area 24 or different parts of OFC, which might all be included in the vmPFC signals in imaging studies, are sufficient to support the performance of the marmosets for the current contingency degradation task [but see O'Callaghan et al. (2019), where vmPFC lesioned patients were sensitive to A-O contingencies but impaired in subjective

judgement]. Thus, inactivating area 14-25, in this case, did not impair animals' performance. It is evidently perhaps more difficult for human studies to pin-point specific PFC sub-regions to investigate causality between actions and outcomes. To better facilitate translational studies across species, we could use brain regions in human imaging studies to identify potential brain regions to target in animal work and then use the causal findings from animal work to provide plausible ROIs in human studies.

One reason that the current study's area 14-25 manipulations did not affect animals' knowledge in goal-directed behaviour could be the involvement of area 25 in developing and maintaining habitual behaviour and not in goal-directed behaviour. Altered activity in area 25 has been found in OCD in avoidance behaviour. Gillan et al. (2015) measured abnormal brain activity using a habit avoidance paradigm and found abnormal connectivity between CN and subgenual anterior cingulate cortex (area 25) in avoidance in OCD patients who developed habitual behaviour compared with those who did not. Patients and control subjects were instructed to avoid a shock that could be applied to either one of the wrists, in which the shock arrival was signalled by different CSs. After the subjects were over-trained on this avoidance paradigm, they were told that one of the electrodes attached to one of the wrists is disconnected and they could no longer be shocked on that wrist (devalued), but they should keep avoiding shocks from the other wrists (non-devalued). This task is somewhat similar to the contingency degradation task in that it also involves extinction. Behaviourally, about half of the OCD subjects developed more habitual behaviour than healthy subjects. CN connectivity with area 25 was positively coupled in OCD patients who developed habit and negatively coupled in those who did not. The authors found over-activation of mOFC and CN but not putamen in OCD subjects compared to healthy subjects, thus postulating the aversive habit system was driven by CN rather than putamen. Given that the CN was over-active in OCD patients who exhibited habitual behaviour and CN connectivity was positively coupled with area 25 in the same patient group, area 25 should be over-active in OCD patients who developed habit. Therefore, area 25 activation seems to be critical in the development of avoidance habits.

Area 25's debatable rodent homologue, the infralimbic cortex (IL) (Heilbronner et al., 2016; Roberts and Clarke, 2019), has been linked with avoidance-behaviour and habit formation (Bravo-Rivera et al., 2014; Bravo-Rivera et al., 2015). In outcome devaluation

tasks, inactivation of IL reinstated goal-directed responding in overtrained rats (Coutureau and Killcross, 2003) and IL lesioned rats maintained goal-directed behaviour despite extensive response training that otherwise caused habits (Killcross and Coutureau, 2003). Haddon and Killcross (2011) trained rats in two instrumental bi-conditional discrimination tasks in two contexts and over-trained them on one discrimination. In control manipulations, rats responded more to over-trained (habitual) but context-inappropriate stimulus, whereas IL inactivated rats reduced such responding. Coutureau and Killcross (2003) suggested that IL is important when there is a competition between goal-directed and habitual systems and when the habitual system becomes dominant over the goal-directed system. Indeed, when an over-training component is not involved, IL inactivation did not affect animals' performance in goal-directed behaviour (Marquis et al., 2007). Our current contingency degradation task does not involve over-training and animals were checked throughout the study to make sure they maintain goal-directed behaviour. Thus, our task did not have a dominant habitual component. If IL is indeed homologous to area 25, then area 25 manipulations should not affect animals' performance, which is what our study showed. If this habit and goal-directed competition theory hold, then one could hypothesise that area 25 inactivation in an over-trained task where the habit system becomes dominant, would result in a shift towards the goal-directed system and possibly reverse the over-trained habitual behaviour.

3.4.5. Summary

Out of the three PFC regions tested, only perigenual anterior cingulate cortex (area 24) inactivation impaired animals' expression of A-O associations as measured by contingency degradation in common marmosets. Area 24 activation also impaired goal-directed behaviour, thus an optimal activity of area 24 is needed for animals to express their understanding of the causal relationship between their action (and non-action) to a specific outcome. The anterior orbitofrontal cortex (area 11) inactivation seemed to enhance animals' sensitivity to A-O associations whereas its activation impaired such sensitivity. Given the current task's emphasis on action-driven and minimal cue-driven elements, one explanation could be area 11's importance for learning and using the pavlovian components of tasks when both the pavlovian and instrumental elements are

present. Area 24 is necessary for expressing learned goal-directed actions with strong instrumental components, whereas area 11 focuses on processing pavlovian cues. Ventromedial PFC (area 14-25) is not involved in A-O association expression. Given the important role of human vmPFC and OFC in goal-directed actions, it is quite unexpected that both area 11 and area 14-25 inactivation did not impair goal-directed behaviour in the current study.

The human vmPFC and OFC regions encompass large areas in the PFC. To segregate and expand further on the specific PFC sub-regions in marmosets, in extensions of this analysis (Chapter 4), I decided to investigate the causal role of area 14 proper and another important mPFC region, area 32, in contingency degradation. Both brain regions' homologues in human and rodents are implicated in goal-directed actions. Furthermore, fronto-striatal activities and interactions are heavily involved in compulsivity, goal-directed and habitual system balance and A-O associations (Balleine and O'Doherty, 2010). After identifying one PFC regions, area 24, that is critical in maintaining goal-directed information, it would be crucial to know whether the striatum, specifically the CN, is also implicated, given the extensive anatomical projections from area 24 to the CN (Ferry et al., 2000; Heilbronner et al., 2016), confirmed in Chapter 2 of this thesis. In the next chapter, I will describe the roles of area 32, 14 and CN in goal-directed behaviour, expanding and pinpointing in a precise and causal manner the critical PFC sub-regions together with the striatum.

Chapter 4

The role of the medial prefrontal cortex, medial orbitofrontal cortex and caudate nucleus in goal-directed behaviour as measured by contingency degradation

4.1. Introduction

In the general introduction (Chapter 1), I established the need to investigate the potential roles of PFC sub-regions and dorsal striatum in goal-directed behaviour. In Chapter 3, I examined the roles of the perigenual anterior cingulate cortex (area 24), anterior orbitofrontal cortex (area 11) and ventromedial prefrontal cortex (area 14-25). From these three PFC sub-regions, I identified area 24 as one of the critical regions in maintaining the expression of action-outcome (A-O) contingencies. In this chapter, I explore additional PFC sub-regions and the caudate nucleus (CN) in their invovlement to control goal-directed actions.

As detailed in Chapter 1.3, the mPFC plays an important role in goal-directed actions, as shown using the contingency degradation paradigm, in humans (Tanaka et al., 2008; Liljeholm et al., 2011), non-human primates (Jackson et al., 2016) and rodents (Balleine and Dickinson, 1998; Corbit and Balleine, 2003). A previous study in this laboratory using the contingency degradation task in marmosets found that area 32 lesions impaired the learning of A-O associations. Lesion of PL in rats also impaired acquisition of A-O associations. In this chapter, I inactivated (muscimol/baclofen) and activated (DHK) area 32 to investigate its role in the expression of A-O contingencies, which has not previously been investigated, especially in non-human primates.

In Chapter 3, the anterior OFC (area 11) was found not to be necessary for maintaining A-O contingencies; however, the role of area 14, another OFC sub-region, was not clear. Area 14 sits on the medial-orbital surface of PFC. Bradfield et al. (2015) showed that anterior mOFC lesions did not affect rats' ability to learn contingency degradation. However, in human imaging studies, the vmPFC, which often included parts of mOFC, was demonstrated to be active when the action is contingent upon the outcome (Liljeholm et al., 2011) and in response-outcome conditions (Liljeholm et al., 2015). The mOFC is also consistently hyperactive in OCD patients, who were impaired in a contingency degradation task and were theorised to have imbalanced goal-directed and habit systems (Gillan and Robbins, 2014; Robbins et al., 2019; Vaghi et al., 2019). Although the human studies demonstrated area 14's potential function in contingency degradation and the rodent studies did not, the causal role of area 14 has not previously been investigated in the expression of A-O associations. Additionally, although manipulations of area 14-25 did not affect animals' sensitivity to contingency degradation (Chapter 3), the targeted region included both caudal area 14 and rostral area 25. Thus, the current chapter aims to precisely target area 14 more specifically (anterior) in order to resolve its function in maintaining A-O contingencies, via inactivation (muscimol/baclofen) and activation (DHK).

This thesis also aims to understand the contribution of frontal-striatal circuits in the expression of A-O contingencies. The dorsal striatum, as part of the frontal-striatal circuity (detailed in Chapter 1.3), was consistently found to be important in both the learning and expression of A-O contingencies. In human imaging studies, caudate nucleus (CN) activity was related to perceiving the relationship between actions and outcomes (Tricomi et al., 2004; Tanaka et al., 2008; Liljeholm et al., 2011). The head of the CN was

also hyperactive in OCD patients in neuroimaging studies (Whiteside et al., 2004; Gillan and Robbins, 2014; Pauls et al., 2014). In rodent studies, both pre- and post-training lesions of the posterior dorsal medial striatum (pDMS), which is thought to be homologous to the primate CN, and inactivation of pDMS had abolished the sensitivity to contingency degradation in rats (Yin et al., 2005b). Non-human primate studies have not previously used the contingency degradation task to investigate the role of CN in goal-directed actions. To understand the causal role of the CN and its relationship with the PFC in controlling goal-directed actions, the current chapter aimed to target the anterior CN region that receives projections from area 24, which was found to be necessary for expressing A-O contingency in Chapter 3. I have, in fact, already demonstrated in Chapter 2.11.2 that the targeted CN in this chapter indeed receives neuronal projections from the region of area 24 that was targeted in Chapter 3. To block excitatory PFC projections into the anterior CN, I infused CNQX to decrease AMPA receptor activity in the CN (for the rationale and validation of this pharmacological approach, please see Chapter 2.11.1b).

Given the evidence from the literature and expanding on the regions of interest considered by this thesis, this chapter intends to understand the specific roles, if any, of area 32 (mPFC), and area 14 (mOFC), as well as how the anterior CN interacts with critical PFC regions, in the expression of A-O contingencies. I also included some motivational control experiments in certain animals using the progressive ratio and reward licking analysis, as before, and an additional juice preference test.

4.2. Methods

4.2.1. Subjects

Five common marmosets (three females, two males) were used in this study. For details on the marmosets' housing facility, husbandry, testing apparatus, pre-training, touchscreen training, the contingency degradation task itself, behaviour measures, surgical procedures, intracerebral infusions and histology, refer to the appropriate General Methods sections (Chapter 2). Any additional procedures and details on the training and manipulations for individual animals that are specific to this chapter are described below.

4.2.2. Touchscreen Training and Contingency Degradation Task

The contingency degradation task was explained in detail in Chapter 2. Animals received the degraded sessions and the non-degraded sessions in a counter-balanced manner (Table 4.1).

Table 4.1. Degraded vs. non-degraded session order and juice assignment

	Degraded	**Non-degraded**	**Degraded or non-degraded session first**
M6	Summerfruit	Blackcurrant	Nondegraded -> Degraded
M7	Summerfruit	Blackcurrant	Degraded
M8	Summerfruit -> Applemango	Applemango -> Summerfruit	Nondegraded
M9	Summerfruit	Blackcurrant	Degraded
M10	Summerfruit	Blackcurrant	Nondegraded

Table 4.2. Cannulation co-ordinates and angle of cannulae placement

Area	AP co-ordinate (mm)	LM co-ordinate (mm)	Depth (mm)
Area 11	+17.0	+/- 3.0	1.7 (from base)
Area 24*	+15.4	+/- 1.0	2.5 (from surface)
Area 32*	+16.8	+/- 1.0	1.5 (from surface)
Caudate	+11.0	+/- 2.2^	5.0^ (from surface)

^the caudate nucleus guide cannula was at 10 degrees angle away from the inter-aural line. Therefore, the LM of the guide entering the brain surface is +/- 3.2mm, whereas the actual targeted location inside the caudate nucleus is +/- 2.2mm and 5.0mm vertically from the brain surface.

4.2.3. Cannulation placement

Tables 4.2 shows the cannulation co-ordinates and target brain areas for each animal. All animals were cannulated in area 32, 14 and CN. The animals were cannulated in two of the brain regions in a single surgery. However, a third brain region (area 14) could be reached via extended injectors through area 32 cannulae (Figure 4.2D).

4.2.4. Intracerebral infusions

Animals received saline, muscimol/baclofen and dihydrokainic acid (DHK) infusions into area 32 and 14, and CNQX infusions into the CN. The rates, dosage and vehicles of each drug and the procedures for the infusions are described in Chapter 2. The injectors were placed into the guide cannulae, extending 1.5mm below the cannulae for areas 32, 1.0mm for the caudate nucleus, and 3.5mm for area 14.

4.2.5. Control experiments for potential motivational influences

The progressive ratio (PR) task and juice preference test were used to investigate possible dysfunction of motivation as a result of the manipulations of a brain region. The effect of area 14 activation (via DHK) on motivation and reward preference was examined with the PR and juice preference task, respectively. Area 14 activation is of interest because, as will be described later in Chapter 4.3.3b, this manipulation specifically depressed responding in the non-degraded juice condition, but not in the degraded juice condition, in the baseline sessions.

4.2.5a. Progressive Ratio Task

Monkey M7 and M9 underwent the PR task after they finished the main contingency degradation experiments. Due to time constraints, the current thesis did not have time to obtain data from M10. M7 and M9 had two control sessions (without free reward) and two probe sessions (with free reward) as described in Chapter 3.2.5. They received DHK and saline infusions into area 14.

4.2.5b. Juice preference test

Monkey M7 and M9 received juice preference tests after the main experiments. The animals underwent habituation days before the actual infusion days. During habituation days, the animal was restricted alone to the top right quadrant of its home cage and was presented with two bottles that contained different juices. The juices were the same type and concentration as the ones they received during the main contingency degradation testing. The bottles were presented for a total of 30 minutes. The weight of each bottle was recorded at the beginning, 15 minutes and the end of testing. This was to measure how much juice they drank from each bottle, which was used to establish their preference for each type of juice. The position of the bottles (left or right) was switched after 15 minutes, and the position of the bottle at the beginning of the test was counterbalanced. After four days of habituation, the animal received DHK or saline

infusion into area 14 and was returned to the sectioned quadrant of their home cage for juice preference testing.

4.2.6. Histology and cannulae placement verification

One out of the five subjects (M6) used in this study was available at the time of this thesis's completion. For details on the histology protocols, please see Chapter 2.10.

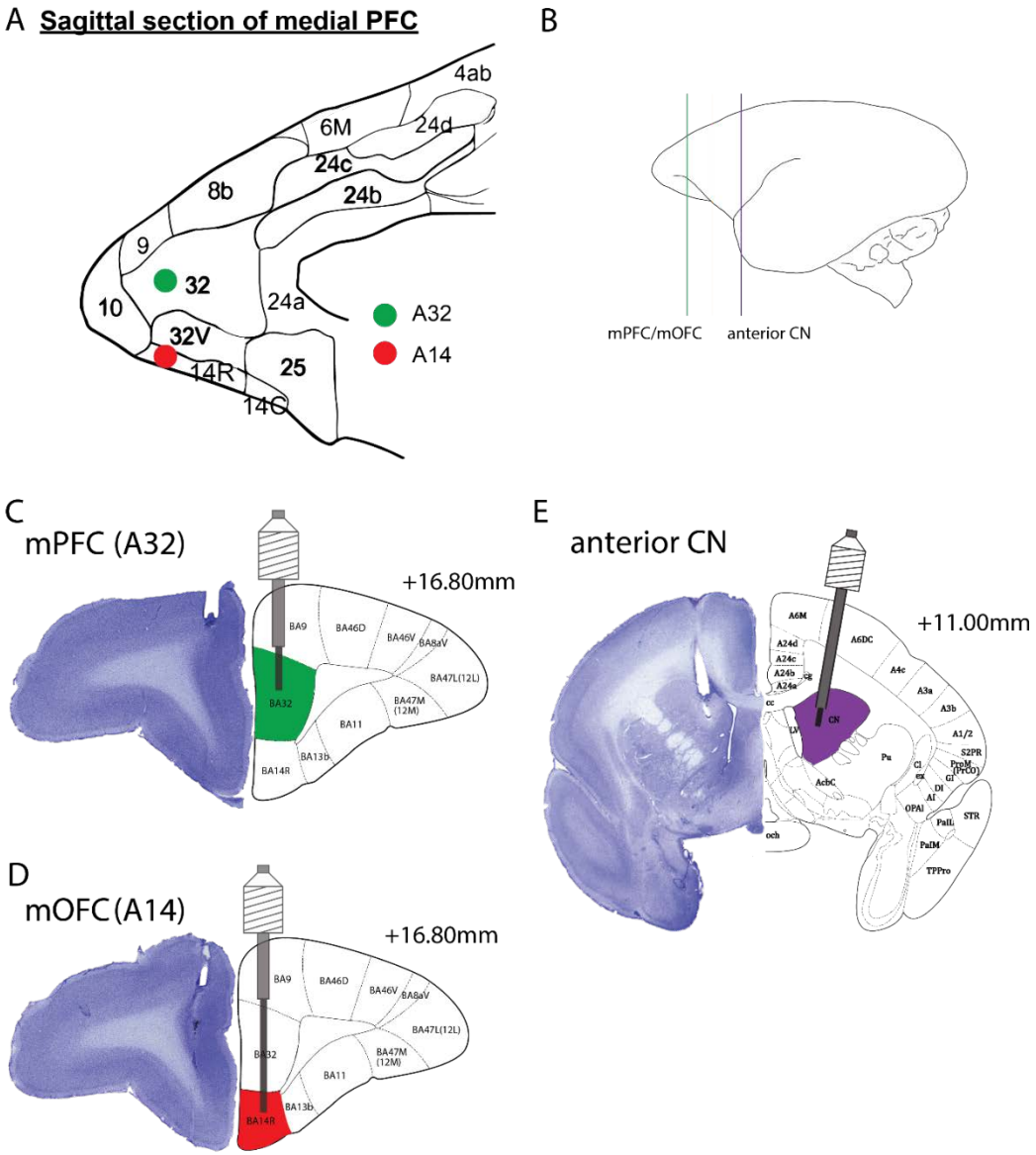


Figure 4.2. Schematic diagrams of cannulae placements A). Sagittal section of the medial PFC, with area 32 and area 14 cannulae target location. B). locations of area 32, area 14 and anterior CN cannulae in the whole brain sagittal view. C-E). The cannulae placements in area 32, 14 and anterior CN with Cresyl violet stained sections. Area 14 was reached by vertically extending the area 32 injector, thus targeting both areas 32 and 14 via the same cannula guide.

4.2.7. Statistical analysis

Data were analysed using a mixed-model ANOVA with the free programming language R for Windows GUI version 3.5.1 (R core team, 2020). I used the Lme4 package to conduct linear mixed-effects models with Type III analysis of variance with Satterthwaite's method for determining degrees of freedom (Bates et al., 2015). Bartlett's test was used to determine the homogeneity of variance. Each significant main effect ($p < 0.05$) was further examined using pair-wise comparisons of least square means (lsmeans package in R) for specified factors in linear or mixed models. Fixed factors were the between-subject factor infusion area (Region; A32, A14, caudate nucleus) and the within-subject factors were infusion types (treatment; saline, mus/bac and DHK for area 32 and area 14, saline and CNQX for CN) and degradation types (degradation; degraded vs. non-degraded). The subject was a random factor. To account for individual variabilities in response rate, the dependent variable was the contingency degradation index (described in Chapter 2.6). Data from drug manipulations on baseline sessions underwent the same analysis.

Data from control and degradation sessions in the absence of drugs were analysed using linear mixed model ANOVA in R. However, for clarity, the data presented in the graphs were not transformed. Factors for the response rate data (Figure 4.3A) include two within-group factors of degradation (degraded vs. non-degraded) and free juice (presence vs. absence). The factor for the contingency degradation index data (Figure 4.3B) has two levels, degraded vs. non-degraded).

Lick analysis data in contingency degradation and baseline sessions for all brain regions were square-root transformed to avoid violations of the assumptions of ANOVA and analysed using linear mixed model ANOVA in R. However, for clarity the data presented in the graphs were not transformed. Measurements included lick latency (time between the onset of birdsong that signals the reward and the time animals start to lick from the spout), lick per reward (average number of licks an animal does for the duration of each reward delivery, the total number of licks during reward delivery and the total number of licks outside of reward delivery. They were analysed to examine whether pharmacological manipulations in brain regions resulted in motivational dysfunction.

Factors include two within-group factors of degradation (degraded vs. non-degraded) and treatment (saline vs. mus/bac vs. DHK for area 32 and area 14; saline vs. CNQX for CN).

Two animals (M7 and M9) were tested in the progressive ratio version of the contingency degradation task and juice preference test, so no statistical analysis was conducted.

4.3. Results

4.3.1. Validation of contingency degradation effect

All animals prior to drug infusions showed evidence of sensitivity to contingency degradation as illustrated in Figure 4.3. In the final contingency degradation block that preceded the first infusion, animals reduced responding in the degraded session (free and contingent reward matched) compared to the non-degraded session (free and contingent reward unmatched) and control session (no free reward; Figure 4.3A). Within-subject repeated measures analysis of variance revealed a significant interaction between free juice (presence vs. absence) and degradation (degraded vs. non-degraded; $F_{1, 4} = 16.415$, $p = 0.0155$) on response rate, and a main effect of degradation ($F_{1, 4} = 8.306$, $p = 0.0449$). Post-hoc analysis showed a reduction in the response rate in the degraded session when compared to a non-degraded session under the free juice condition ($t_{7.60} = -4.775$, $p = 0.0069$) and no difference in response rate between the two control sessions in the absence of free reward ($t_{7.60} = 0.257$, $p = 0.994$). There was also a significant reduction in response rate in degraded sessions (free juice) when compared to degraded control (absence of free juice; $t_{7.70} = -4.557$, $p = 0.0087$) and non-degraded control sessions (absence of free juice; $t_{6.86} = -3.640$ $p = 0.0337$). There was no significant difference when comparing the non-degraded session with the degraded control session ($t_{6.86} = 1.195$ $p = 0.649$) and the non-degraded control session ($t_{7.70} = 0.257$ $p = 0.380$).

When measured using the Contingency Degradation Index (CDI), animals showed a decrease in responding in the degraded session compared to the non-degraded session before any drug manipulations (Figure 4.3B). Within-subject repeated measures analysis of variance revealed a main effect of degradation ($F_{1, 4} = 14.836$, $p = 0.0183$). A *post hoc* t-test on the least squared means revealed that animals significantly reduced their responding in the degraded compared with the non-degraded condition ($t_4 = -3.852$, $p = 0.0183$).

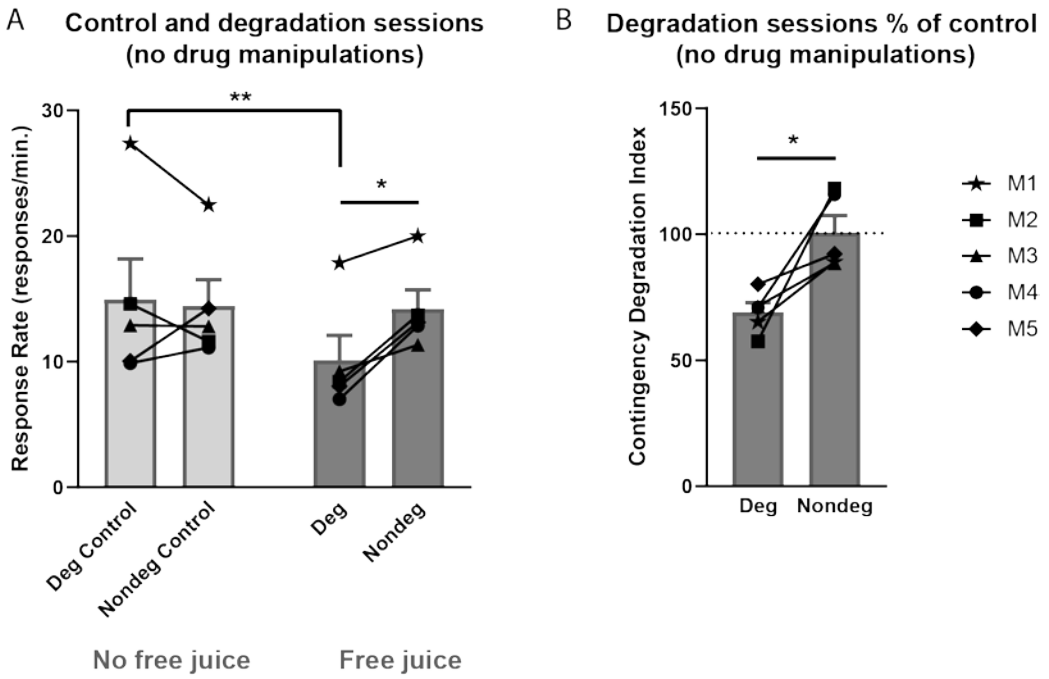


Figure 4.3. Animals are sensitive to contingency degradation before drug manipulations. A). Animals decreased responding in the degraded session (free juice) compared to non-degraded session (free juice) and control sessions (no free juice). There was no difference in response rate between the control sessions. B). Degradation effect demonstrated using the contingency degradation index measure. Animals showed decreased responding in the degraded compared to non-degraded sessions. *: $p < 0.05$, **: $p < 0.01$.

4.3.2. Areas 32, 14 and caudate nucleus contribute differentially to the control of action contingencies

Linear mixed model analysis of the full degradation sessions dataset revealed a main effect of session ($F_{1,\ 42.004} = 13.8196$, $p = 0.000589$) and significant interactions between drug and degradation ($F_{3,\ 42.004} = 3.4243$, $p = 0.0256$). Linear mixed model analysis of the full degradation sessions dataset with all brain regions did not reveal a significant three-way interaction between brain region, drug, and degradation ($F_{2,\ 42.004} = 0.6572$, $p = 0.524$). However, to tease apart how different brain region was affected by infusions and contingency degradation sessions types, I continued to analyse the effects of the infused drugs on session types within each brain region.

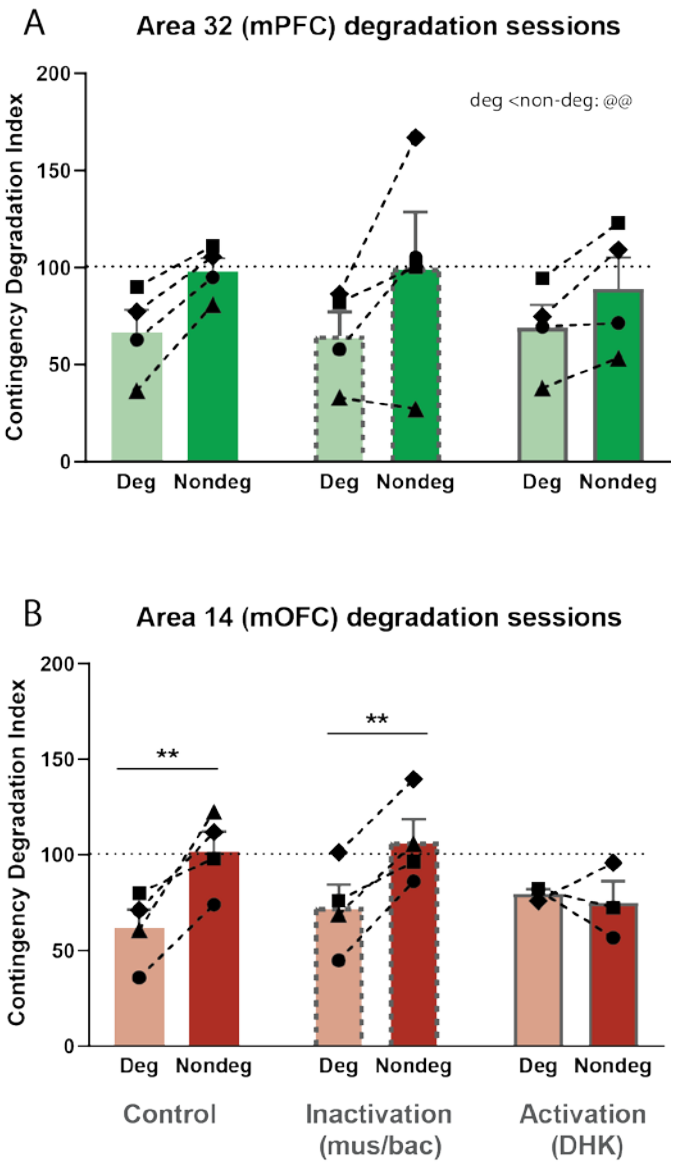


Figure 4.4. Effects of activation or inactivation of critical fronto-striatal regions in contingency degradation. A). In area 32, no differences were found between degraded and non-degraded sessions across drug manipulations. B). Inactivation of area 14 did not affect animals' sensitivity of contingency degradation, whereas activation made them insensitive. C). Inactivation of the caudate nucleus using CNQX made animals insensitive to contingency degradation.
* indicates significant effect of degradation x treatment interaction, # indicates significant effect between treatments, @ indicates significant effect between degradations. */@: $p < 0.05$, **/@@: $p < 0.01$

4.3.2a. Area 32 is not involved in mediating contingency degradation expression

Area 32 inactivation or activation did not affect responding of animals when compared to saline (Fig. 4.4A). Linear mixed-model analysis of variance revealed a main effect of degradation ($F_{1,15}$ = 13.958, p = 0.00199), but not of drug ($F_{2,15}$ = 0.0766, p = 0.927) nor interaction of drug x degradation ($F_{2,15}$ = 0.347, p = 0.713). Further post-hoc analysis showed that responding in non-degraded sessions was significantly greater than that of degraded sessions across all drug conditions (t_{15} = -3.736, p = 0.002). Therefore, area 32 appeared not to contribute to the expression of contingency degradation.

4.3.2b. Area 14 inactivation did not impair sensitivity to contingency degradation

Area 14 inactivation did not block the normal reduction in response rates in the degraded sessions when compared to non-degraded sessions, whereas activation did (Fig 4.4B). Linear mixed-model analysis of variance revealed a two-way interaction of drug x degradation ($F_{2,13.11}$ = 5.229, p = 0.0214), a main effect of degradation ($F_{1,13.11}$ = 15.368, p = 0.00173) and no main effect of drug ($F_{2,13.302}$ = 1.288, p = 0.308). Subsequent post-hoc analysis revealed a significant difference between degraded and non-degraded sessions following saline (t_{13} = -4.124, p = 0.0012) and mus/bac infusions (t_{13} = -3.543, p = 0.0036). In contrast, there were no differences between degraded and non-degraded sessions after activation (t_{13} = 0.441, p = 0.666). Responding during the non-degraded session after activation was significantly lower than that after inactivation ($t_{13.2}$ = -2.912, p = 0.0302) with a trend to be lower than that of saline ($t_{13.2}$ = -2.416, p = 0.0742). Conversely, the responding of degraded session after activation was not significantly different from that of inactivation ($t_{13.2}$ = 0.769, p = 0.728) and saline ($t_{13.2}$ = 1.792, p = 0.210). Thus, the blunting effect observed after activation might not be due to animals actually losing their sensitivity to contingency degradation but a specific drug effect, which will be explored more in the Discussion section (Chapter 4.4) together with other data presented in the current chapter. Overall, inactivating area 14 did not blunt animals' sensitivity to contingency degradation, whereas activation did.

4.3.2c. Caudate nucleus inactivation impaired sensitivity to contingency degradation

Caudate nucleus (CN) inactivation using CNQX blocked the normal reduction in response rates in the degraded sessions when compared to non-degraded sessions (Fig 4.4C). Linear mixed-model analysis of variance revealed a two-way interaction of drug x degradation ($F_{1,9}$ = 5.514, p = 0.0434). Subsequent post-hoc analysis revealed a significant difference between degraded and non-degraded sessions only following saline infusion (t_9 = -2.732, p = 0.0231). There were no differences between degraded and non-degraded sessions after inactivation (t_9 = 0.588, p = 0.571).

4.3.3. Pharmacological manipulations in baseline sessions without degradation affected responding differentially in each brain region

Linear mixed model analysis of the full baseline sessions dataset with all brain regions revealed a significant two-way interaction between drug and degradation juice conditions (degraded juice vs. non-degraded juice) ($F_{3, 43.379}$ = 2.987, p = 0.0413) and a trend of a two-way interaction between degradation juice conditions and region ($F_{2, 43.379}$ = 3.087, p = 0.0558). Subsequent analyses of the effects of the drug were carried out within each brain region independently.

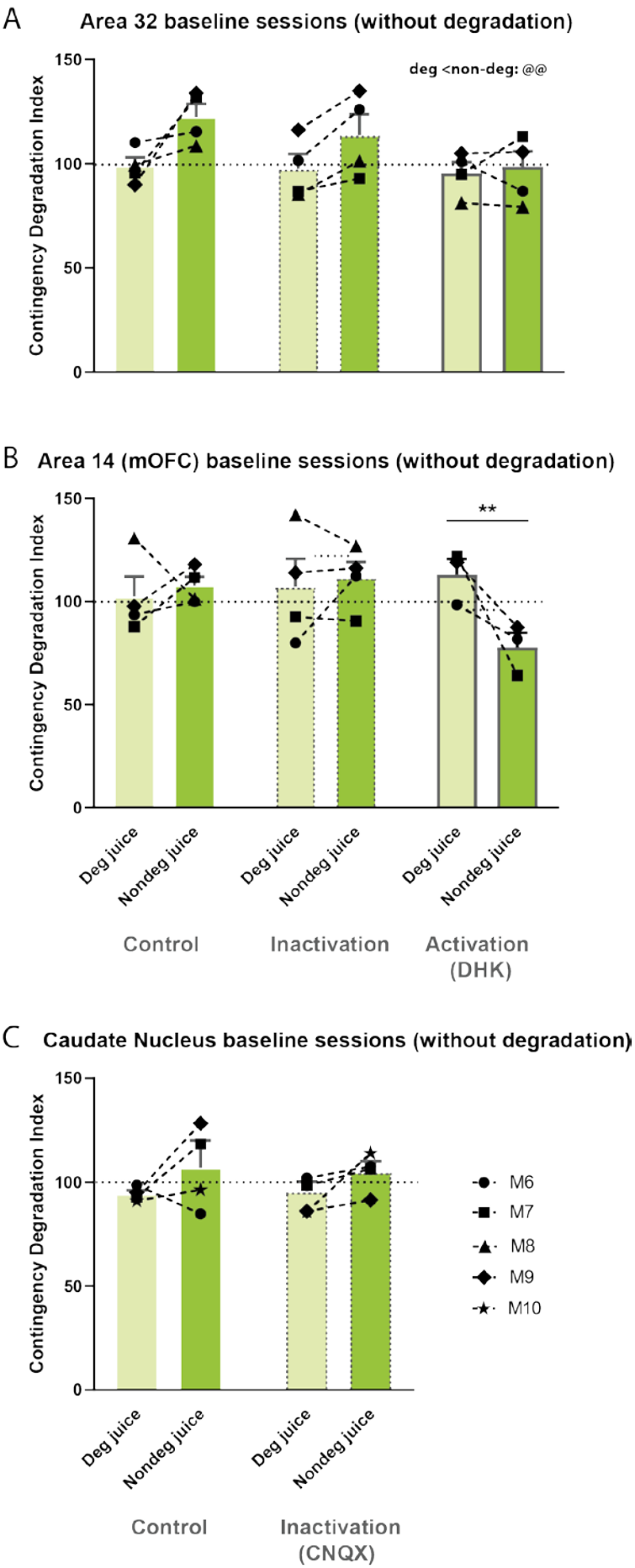


Figure 4.5. Effects of control, inactivation or activation of critical frontostriatal regions in baseline sessions. A). Animals who had area 32 manipulations decreased their response rate in the degraded juice condition compared to non-degraded juice condition across all drug manipulations. B). Area 14 activation specifically decreased responding in the non-degraded juice condition compared to the degraded juice condition. C). No significant effects were observed after CN manipulations.

* indicates significant effect of degradation juice conditions x treatment interaction, @ indicates significant effect between degradation juice conditions.

*/@: $p < 0.05$, **/@@: $p < 0.01$

4.3.3a. Responding in the degraded juice condition was significantly lower than responding in the non-degraded juice condition across all manipulations in area 32

In all drug manipulations, animals decreased their responding in the degraded juice condition compared to non-degraded juice condition (Fig. 4.5A). Linear mixed-model analysis of variance revealed a main effect of degradation juice conditions ($F_{1,14.999} = 9.730$, $p = 0.00704$) and a trend of drug ($F_{2,14.999} = 2.935$, $p = 0.0840$). Post-hoc analysis of degradation juice conditions revealed a significant decrease in animals' responding in degraded juice condition when compared to non-degraded juice condition across all drug manipulations ($t_{15} = -3.119$, $p = 0.0070$).

4.3.3b. DHK infusion into area 14 specifically decreased responding in the non-degraded juice condition

Saline infusions and inactivation of area 14 did not affect animals' responding in baseline sessions; activation of area 14 via DHK specifically depressed responding in the non-degraded juice condition, but not in the degraded juice condition (Fig. 4.5B). Linear mixed-model analysis of variance revealed a two-way interaction of drug x degradation juice conditions ($F_{2,12.815} = 5.673$, $p = 0.0172$). Post-hoc analysis revealed that only after DHK infusion, responding in the non-degraded juice condition was significantly higher than that of the degraded juice condition ($t_{13} = 3.335$, $p = 0.0054$). The responding of non-degraded juice condition after DHK infusion was significantly lower than that of saline ($t_{13.2} = -2.894$, $p = 0.0312$) and mus/bac infusion ($t_{13.2} = -2.848$, $p = 0.034$). Conversely, the responding during the degraded juice condition after DHK infusion was not significantly lower than that of saline ($t_{13.2} = 1.487$, $p = 0.328$) and mus/bac infusions ($t_{13.2} = 0.992$, $p = 0.595$). This specific decrease in the responding of the non-degraded juice condition after DHK infusion will be explored further in later sections of this chapter.

4.3.3c. No effects were observed after caudate nucleus manipulations

Saline and inactivation of CN did not alter responding of animals in baseline sessions (Fig. 4.5C). Linear mixed model analysis of CN baseline sessions revealed no main

effects of degradation juice conditions ($F_{1,12}$ = 4.084, p = 0.0662) or treatments ($F_{1,12}$ = 0.0696, p = 0.796).

4.3.4. Motivational control measures (area 14)

The control experiments were mainly conducted to examine possible motivational causes of the specific decrease of responding in the non-degraded juice condition but not the degraded juice condition after area 14 activation, in the baseline sessions (Figure 4.5B). Saline infusion and inactivation of area 14 in the baseline sessions did not affect the animal's responding (Figure 4.5B). In the main degradation experiment, area 14 activation did not alter degraded session responding but decreased non-degraded responding, thus impairing animals' sensitivity to contingency degradation (Figure 4.4B). However, this might be due to the specific decrease in responding in the non-degraded juice condition as observed in the baseline session (without degradation). Therefore, motivational control experiments were conducted to access potential motivational dysfunction after area 14 activation. The PR task was used to investigate the possible effect of area 14 activation on how much effort the animals were willing to invest to obtain rewards (see Chapter 3.2.5 for the rationale of using PR task). Animals were responding to their preferred (non-degraded) reward in the PR task. Thus, the results in the PR task would not fully explain why there was no decrease in the animals' response rate in the *degraded* (less-preferred) juice condition after area 14 activation. Therefore, I conducted a juice preference task to investigate whether area 14 activation affected animals' preference for juices when the animals were presented with the non-degraded (preferred) juice versus the degraded juice simultaneously. Because vmPFC/mOFC, which often includes area 14, was implicated in reward preferences (Tremblay and Schultz, 1999; O'Doherty, 2011), the fact that animals reduced responding towards one juice condition and not the other might be caused by a shift in their reward preference. Furthermore, analysis was performed on reward licking in the main degradation and baseline sessions.

4.3.4.a. Area 14 activation decreased breakpoint in the progressive ratio task in the probe session

In probe sessions, both animals decreased the breakpoint (BP) after area 14 activation via DHK infusion when compared to saline (Figure 4.6). For M7, in baseline control sessions, the BP after DHK infusion was also lower than that of saline infusion (Figure 4.6A). BP after saline infusion in the probe session decreased when compared to saline infusion in the baseline session, presumably due to the availability of free reward in the probe session. At the beginning of all the sessions, DHK infusion in the probe session had a depressed response rate compared to other manipulations. This response rate increased to match the other sessions around 600 seconds. For M9, all the sessions started with a similar response rate (Figure 4.6B). In the probe session, DHK drastically reduced the BP when compared to the saline. There was no difference in BP between saline and DHK infusion in the baseline sessions.

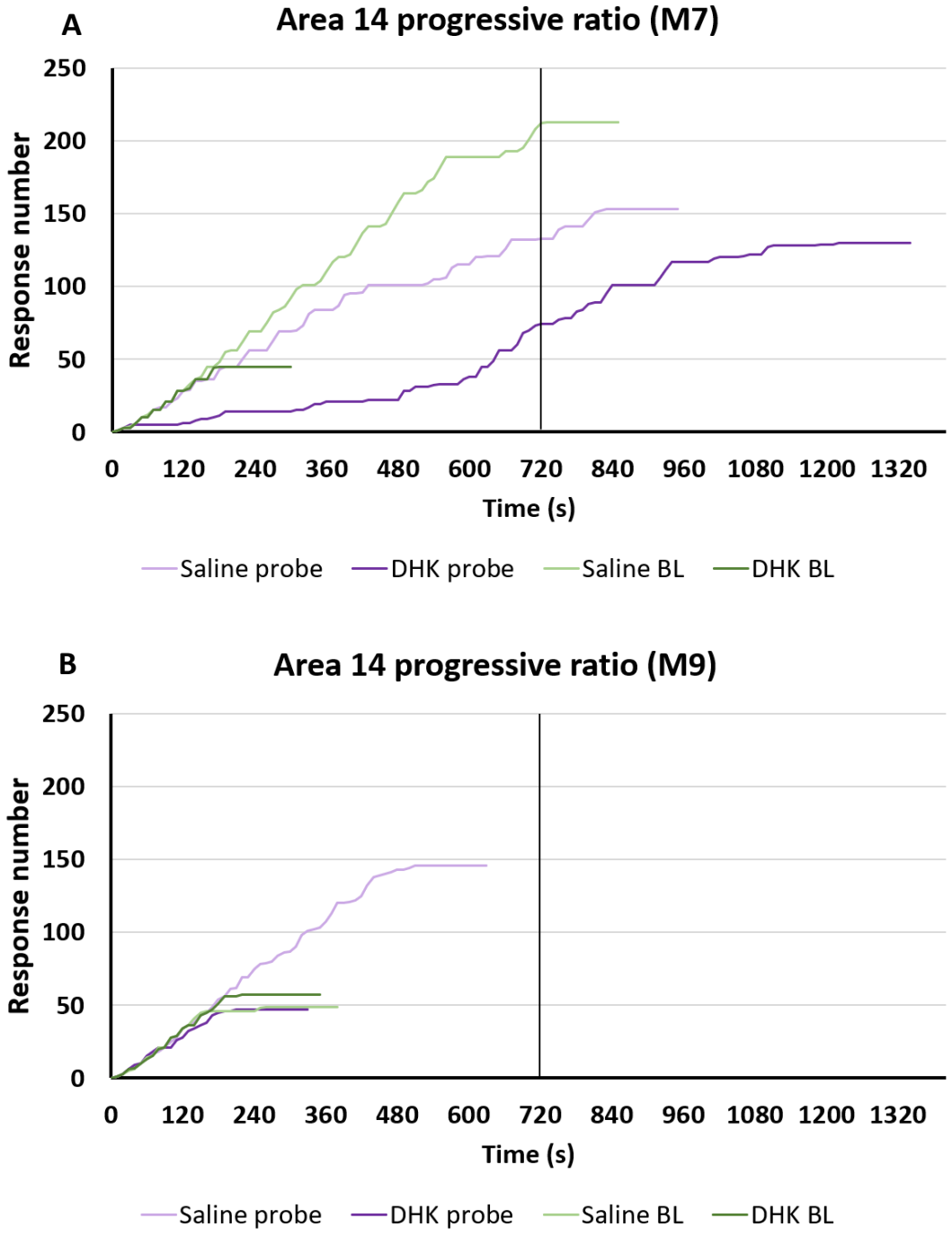


Figure 4.6. In the probe sessions, area 14 activation decreased animals' BP compared to saline. The vertical black line at 720 seconds indicated the 12 minutes mark, which was the session length of the main degradation task. A). Progressive ratio performance of M7. In the probe and baseline sessions, area 14 activation decreased BP compared to saline. B). Progressive ratio performance of M9. Area 14 activation in the probe session decreased BP. There was no difference between the BP of area 14 activation and saline in the baseline sessions.

4.3.4.b. Area 14 activation had inconsistent effects on juice preference

Area 14 activation increased M7's consumption of less preferred juice and decreased M7's consumption of preferred juice, whereas area 14 activation did not change M9's preference of juice (Figure 4.7). M7's preferred juice was blackcurrant, as indicated by the higher consumption of blackcurrant juice compared to summerfruit juice in the saline session. In the DHK session, however, M7 decreased consumption of the more preferred blackcurrant juice and increased consumption of the less preferred summerfruit juice (Figure 4.7A). M9's more preferred juice was summerfruit, as its overall consumption was higher than that of the blackcurrant juice in the saline session. When compared to saline infusion, M9's overall consumption of blackcurrant and summerfruit juice was slightly increased in a similar manner after DHK infusion. Thus, area 14 activation did not affect Monkey M9's preference for juices (Figure 4.7B).

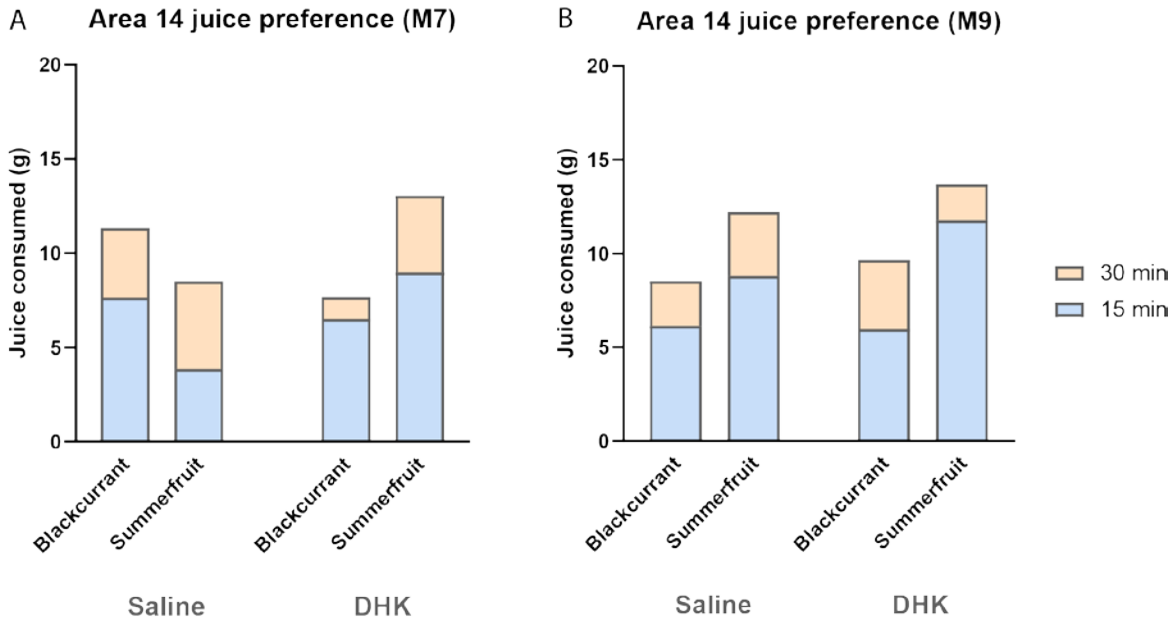


Figure 4.7. Blackcurrant and summerfruit juice consumed in the 30 minutes juice preference tests, after saline and DHK manipulations in area 14. A). After area 14 activation, M7 decreased the consumption of blackcurrant juice (preferred juice) and increased summerfruit juice consumption when compared to saline. B). After area 14 activation, M9 increased consumption of both juices similarly when compared to saline.

4.3.4.c. Area 14 activation decreased licks per reward on the baseline

On baseline sessions, area 14 activation decreased the number of licks per reward when compared to control. No difference was observed between activation and control in lick latency and the number of licks outside of reward delivery. Linear mixed-model analysis of variance on licks per reward in area 14 baseline sessions revealed a main effect of treatment ($F_{2, 13.106} = 5.645$, $p = 0.0171$; Figure 4.8D). Post-hoc analysis showed that the number of licks per reward across sessions was significantly lowered after area 14 activation when compared to control ($t_{13.6} = -2.954$, $p = 0.0271$). Linear mixed-model analysis of variance on lick latency in area 14 degradation sessions revealed an interaction of treatment x degradation ($F_{2, 13.268} = 3.866$, $p = 0.0475$; Figure 4.8C). However, post-hoc analysis did not reveal any significant results but three trends. In the degraded session, lick latency of saline was higher than that of DHK ($t_{13.6} = -2.553$, $p = 0.0571$); the degraded session after DHK infusion was lower than that of non-degraded session ($t_{13.1} = -1.857$, $p = 0.0859$); the degraded session after saline infusion was higher than that of non-degraded session ($t_{13.1} = 2.101$, $p = 0.0556$).

Analysis of the total number of licks during reward delivery for area 14 can be found in Appendix B. Analysis of lick latency, licks per reward, licks during reward delivery and licks outside of reward delivery for area 32 and CN, can be found in Appendix C. In baseline sessions, lick latency was significantly lowered after area 32 inactivation when compared to saline and activation in the non-degraded session, and inactivation in the degraded session. On degradation sessions, CN saline infusion in the non-degraded session resulted in a significantly higher number of licks outside of reward delivery when compared to inactivation in the non-degraded session and saline in the degraded session.

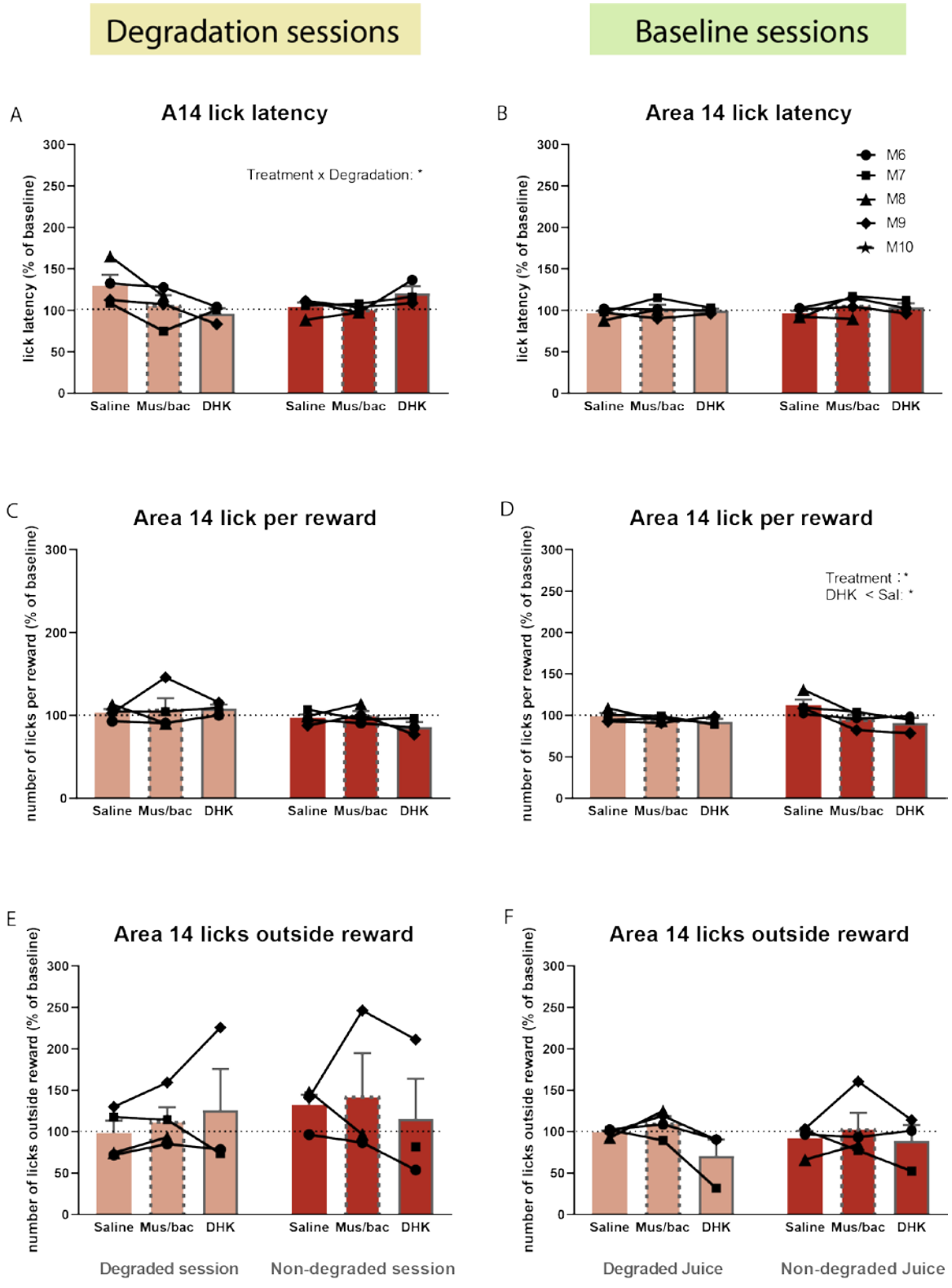


Figure 4.8. Analysis of number of licks per reward after area 32, area 14 and CN manipulations in degradation and baseline sessions. D). Number of licks per reward across sessions was significantly lowered after area 14 activation than that of control. A-C, E-F). No significant effects were observed.
*: $p < 0.05$

4.4. Discussion

The current study completed the present investigation of the role of frontostriatal brain regions in the expression of goal-directed behaviour. The cortical regions, area 32 and area 14 were not required for the expression of learned knowledge in contingency degradation; conversely, CN was essential in maintaining such information. Inactivation and activation of area 32 and inactivation of area 14 did not affect the animals' performance and so we conclude that these regions, unlike area 24 (Chapter 3) are not implicated in the expression of instrumental contingencies. However, activation of area 14 did impair the sensitivity to contingency degradation, so this apparent paradox is considered below (see section 4.4.2 for further analysis). Altogether, this study has further extended our understanding of how different PFC sub-regions and the striatum contribute to the expression of the causal relationship between actions and outcomes.

4.4.1. Area 32 is not involved in the expression of action-outcome associations

Perhaps surprisingly, inactivation and activation of area 32 did not affect animals' sensitivity to contingency degradation. As discussed in Chapter 1.3 and Chapter 4.1., area 32 (mPFC) is an important PFC region that is consistently implicated in controlling goal-directed behaviour in humans (Tanaka et al., 2008; Liljeholm et al., 2011), non-human primates (Jackson et al., 2016) and rodents (Balleine and Dickinson, 1998; Corbit and Balleine, 2003). The non-involvement of area 32 in the current study may be due to area 32's involvement in the acquisition of A-O associations and not their expression. Indeed, area 32 lesions in marmosets impaired the acquisition of contingency degradation (Jackson et al., 2016) but not the expression (current thesis); in rodents, PL lesions impaired the acquisition of contingency degradation (Corbit and Balleine, 2003). Of note, the PL lesion in Corbit and Balleine (2003) encompassed a small portion of PL that was renamed to area 24a in the most recent rodent brain atlas (Paxinos and Watson, 2017), where the posterior portion of the PL is designated as area 24a and the anterior portion of the PL as area 32. This PL lesion location was comparable to the marmoset lesion from Jackson et al. (2016), which was also at the posterior area 32, bordering area 24a. It seems

that in terms of area 32/PL's role in the acquisition of A-O contingencies, the rodent and marmoset literature are in agreement, although, the contingency degradation task in Jackson et al. (2016) has stronger pavlovian-driven elements (see Chapter 3.4.2). None of the studies in the literature have hitherto investigated the expression of contingency degradation, thus the current study fills this important gap in knowledge to suggest the involvement of area 32 in the acquisition, but not the expression of contingency degradation effects on instrumental behaviour. How area 32 may work together with other brain regions to influence A-O contingencies will be explored in the General Discussion (Chapter 5).

4.4.2. Area 14 is not involved in the expression of action-outcome associations, but its activation resulted in decreased motivation to work for rewards

Area 14 (anterior mOFC) inactivation did not impair animals' sensitivity to contingency degradation but area 14 activation did. Area 14's inactivation result was consistent with rodent anterior mOFC lesion studies. Bradfield et al. (2015) investigated the effect of mOFC lesion in the acquisition of contingency degradation and outcome devaluation, in which mOFC lesions impaired the latter but not the former condition. They concluded that this opposite effect in task performance was because the reward variables in the contingency degradation paradigm are observable during the task whereas they are not in outcome devaluation. Lesion of anterior but not posterior mOFC impaired outcome devaluation (Bradfield et al., 2018). This provided further evidence that outcome devaluation and contingency degradation are measuring different aspects of goal-directed behaviour and PFC sub-regions serve differential roles in the computation of instrumental knowledge.

Another possible reason for area 14's non-involvement in contingency degradation was that the mOFC tracks the intrinsic representations of action-associated outcome value and contrasts values of alternative choices (Padoa-Schioppa and Assad, 2006; Valentin et al., 2007; Noonan et al., 2010; Rudebeck and Murray, 2011; Wallis, 2011; Stalnaker et al., 2015). Unlike the outcome devaluation task, where the incentive value of the rewards was changed, the contingency degradation task only changed the relationship

between the action and the outcome. Thus, animals did not need area 14's ability to track changes in outcome *values* to perform contingency degradation, which required animals to track the changes in A-O *contingencies*.

Contrary to the effects of inactivation, Area 14 activation did impair animals' performance in the contingency degradation test. The degraded session after area 14 activation was not significantly different from that of saline and inactivation, whereas the response rate in the non-degraded session after area 14 activation was significantly lower than the response rate after inactivation, showing a trend to be lower than that of saline (Figure 4.4B, $p = 0.0742$). Thus, animals' insensitivity to contingency degradation after area 14 activation was caused by a decrease in the response rate in the non-degraded session. In the baseline sessions, area 14 activation also caused animals to significantly decrease their response rate in the non-degraded juice condition when compared to control and degraded juice condition. This suggested possible non-specific behavioural effects interfering with performance. That is, the insensitivity observed after area 14 activation in the degradation sessions might be due to the effect of DHK itself on response rate rather than a blunted sensitivity to contingency degradation. I hypothesised that this could be due to area 14's role in controlling motivation. To understand how motivation affected this decrease in response rate, I conducted several tests; a progressive ratio schedule, a juice preference choice test and analysis of reward licking data.

4.4.2a. The possible role of area 14 in motivational control of instrumental performance

A progressive ratio (PR) task, a juice preference test and an analysis of licking for rewards were conducted to investigate possible motivational causes for the decrease in response rate (only in the non-degraded juice condition) after area 14 activation in the baseline sessions. These conditions were chosen to explore the involvement of motivation in affecting animals' behaviour.

Progressive ratio task

In both animals tested, area 14 activation decreased the breakpoint (BP) in the probe session when compared to saline; in one animal, area 14 activation decreased the

BP in the baseline session (no free reward present; Figure 4.6). Although only two animals were able to undergo the PR schedule, area 14 activation did cause animals to be much less motivated to work for rewards with an increasing amount of required effort to obtain the rewards, especially in the presence of free reward. This result was consistent with the findings of Gourley et al., (2016), where excitatory DREADDs applied to the mOFC of mice decreased BP in a PR task. Since in the PR task from the current thesis the animals only responded to their preferred (non-degraded) juice, the results from the task alone did not fully explain why the response rate in the degraded session (less preferred juice) was not decreased after area 14 activation. Nonetheless, the current thesis's observation that animals decreased their responding in the non-degraded juice condition of the baseline session after area 14 activation could be due to a decrease in animals' motivation to work for their preferred rewards.

Juice preference

All animals had a slight preference for one of the two rewarded juices if they were presented with the two juices simultaneously. One of the two animals tested in the juice preference task reversed its juice preference after area 14 activation, but the other animal did not change its preference (Figure 4.7). Thus, no decisive conclusions can be drawn from the juice preference data as more animals need to be tested. OFC and vmPFC, which usually include area 14, are found to compute the relative value of available outcomes and relative reward preference (Tremblay and Schultz, 1999; O'Doherty, 2011). In humans, mOFC activity was positively correlated with subjective ratings of taste pleasantness (de Araujo et al., 2003; Rolls et al., 2003a). Lesions in the vmPFC of non-human primates altered their food preferences compared to controls and made them less consistent in their food choices (Baylis and Gaffan, 1991). Similarly, Rudebeck and Murray (2011) conducted an object preference test in area 11/13 and area 14 lesioned (excitotoxic) rhesus macaques, for which in each trial they presented animals with two objects to choose from. They measured the inconsistency of monkeys' object choice, without the learning of object-food association. The area 14 lesion group performed less consistently than the area 11/13 lesion group and control group, whereas no difference was observed between the latter two groups. Similarly to decreasing area 14 activity, over-activity in area 14 could result in a change or inconsistency in reward preference (inverted-U, in

which too much or too little activity both impair performance). This could explain why animals only decreased their responding in the preferred juice condition after area 14 activation in the baseline session (Figure 4.5B). Although the present observations in a limited number of monkeys are suggestive, the findings from the juice preference task were not sufficiently consistent to draw any firm conclusions.

Reward licking analysis

On the baseline sessions of the main experiment, the number of licks per reward was significantly decreased across juice conditions after area 14 activation when compared to saline (Figure 4.8D). This could possibly indicate a decreased willingness to consume the juice rewards, but such decreases only occurred in the baseline sessions and were not specific to the non-degraded juice condition. On both degradation and baseline sessions, area 14 activation did not affect animals' latency to approach the reward (Figure 4.8A, B) and the number of licks outside of reward delivery (Figure 4.8E, F).

Summary

The specific decrease in response rate observed in the non-degraded juice condition of the baseline session after area 14 activation, was likely caused by a decrease in some aspects of the motivation to work for rewards. Preliminary results indicated that area 14 activation did indeed decrease the animals' willingness to work for reward when efforts to obtain such rewards were increased. Additionally, Area 14 activation may cause reductions in consumption of preferred reward and increases in consumption in the non-preferred reward (i.e. changes in juice preference). Area 14 activation may decrease animals' eagerness to consume the reward (licks per reward) but did not affect animals' motivation to approach reward (lick latency). Therefore, it can be concluded that the effects on degradation after activation (via DHK) of area 14 were also accompanied (and probably confounded) by changes in aspects of motivation (San-Galli et al., 2018).

In general, the findings are compatible with a possible function of area 14 in representing comparative reward values and effort-based motivation (Tremblay and Schultz, 1999; O'Doherty, 2011; San-Galli et al., 2018) in contrast to contingency knowledge (Bradfield et al., 2015); although in the current thesis, the evidence for a lack of effect in A-O contingencies was greater than that of a deficit in value comparison. More

of area 14's relationship with other PFC regions to aid goal-directed behaviour will be discussed in Chapter 5 (General Discussion).

4.4.3. The caudate nucleus is critical for the expression of action-outcome associations

As predicted, inactivation of the anterior caudate nucleus (CN) blunted animals' sensitivity to express learned contingency degradation. This is consistent with the current literature where CN activity is critical to understand A-O contingencies in humans (Tricomi et al., 2004; Tanaka et al., 2008; Liljeholm et al., 2011). In rodents, the primate homologue of the posterior dorsomedial striatum (pDMS) was necessary for both the acquisition and expression of contingency degradation (Yin et al., 2005b). The current study achieved CN inactivation by reducing the glutamatergic input into the CN via AMPA-receptor antagonism (CNQX). CN receives projection from the cortex, thalamus and the brain stem. As described in Chapter 1.3, cortico-striatal connectivity plays a critical role in goal-directed behaviour and A-O associations. Disrupting the communication between mPFC and pDMS in rodents via disconnection, DREADDS and NMDA-antagonism in the pDMS all impaired goal-directed behaviour in rodents (Hart et al., 2018a; Hart et al., 2018b). CNQX administration almost completely blocked the striatal field response to PL stimulation (Galinanes et al., 2011). Thus, CNQX infusion in the current study could have caused a blockade of excitatory input from the PFC, which led to an impairment in expressing A-O associations.

Thalamic nuclei and their connectivity with pDMS have also been implicated in goal-directed behaviour. The parafascicular (Pf) nuclei in the thalamus project to the associative and limbic dorsal striatal areas in primates and DMS in rats (Bradfield et al., 2013; Gonzales and Smith, 2015). Lesion of the Pf and disconnection between Pf and pDMS caused rats' insensitivity to the learning of A-O contingencies (Bradfield et al., 2013). Reducing the excitatory input from Pf to the cholinergic interneurons in the pDMS also decreased the firing rate of cholinergic interneurons in the pDMS (Bradfield et al., 2013). Excitatory inputs from the PFC and thalamus both control the CN in learning and expressing A-O contingencies; severing this connectivity would thus result in impairments in goal-directed behaviour. More of how CN's connectivity with area 24 and other PFC

regions might affect goal-directed behaviour will be discussed in Chapter 5 (General Discussion).

4.4.4. Summary

Reducing the activity in area 32 and area 14 did not affect animals' performance in expressing goal-directed behaviour as measured by contingency degradation, whereas reducing the excitatory input into the CN did. While activation of area 32 did not change behaviour, area 14 activation blunted sensitivity to contingency degradation and reduced the motivation to work for their preferred rewards. Additionally, area 14 activation did not affect animals' latency to approach the reward. In the General Discussion (Chapter 5), I will attempt to synthesize the results obtained from Chapters 2, 3 and 4 into an integrated proposal to explain how mPFC, lOFC, mOFC, ACC, vmPFC and CN relate to one another to control goal-directed behaviour.

Chapter 5

General Discussion

5.1. Results Summary

The current thesis provided, for the first time in the literature and across species, causal evidence that only the PFC sub-region of area 24 (perigenual anterior cingulate cortex), and not area 32 (anterior mPFC), area 11 (anterior lateral OFC), area 14 (anterior medial OFC) or area 14-25 (vmPFC), is necessary for the expression of instrumental action-outcome (A-O) contingencies (Table 5.1, Figure 5.1). Although only the inactivation of area 24 and caudate nucleus (CN) impaired A-O contingency knowledge, inactivation and activation of each PFC sub-regions affected animals' performance differently. Both inactivation and activation of area 24 impaired contingency knowledge, which indicated an optimal level of area 24 activity was required. Impaired performance resulting from

reducing the excitatory input into CN signified the potential involvement of other brain regions, such as area 24, that work together with the CN. Area 32 inactivation and activation did not affect the current task. Given the previous literature, this could be because area 32 is needed during the learning of the A-O contingencies, but is no longer required once the information was acquired. Area 11 may be critical for the learning of contingency degradation where pavlovian elements (stimulus-response) predominate (Jackson et al., 2016); however, when instrumental elements were more dominant, as in the studies reported in this thesis, an active area 11 appears to interfere with the expression of A-O contingencies. By reducing the activity of area 11, such interference is hypothetically reduced and thus the expression of A-O contingencies enhanced. Area 14 was not involved in the expression of A-O contingencies but affected effort-based motivation and value-based decision-making (Table 5.2, 5.3). Area 25 was not involved at all in instrumental performance in this task. In the following sections, I will explain in detail how these brain regions may differentially contribute to the acquisition and expression of goal-directed behaviour, outline possible limitations of the thesis and propose future directions to address outstanding issues.

Table 5.1. Summary of results from the **main contingency degradation** experiments.

	Degradation		**Baseline**	
	Inactivation	**Activation**	**Inactivation**	**Activation**
Area 24 (perigenual ACC)	Blunted	Blunted	No effect	Decreased across juice conditions
Area 11 (anterior lOFC)	Enhanced	Blunted	No effect	No effect
Area 14-25 (vmPFC)	No effect	No effect	No effect	No effect
Area 32 (mPFC)	No effect	No effect	Degraded juice < non-degraded juice	
Area 14 (anterior mOFC)	No effect	Blunted**	No effect	Decreased in non-degraded juice
Caudate Nucleus (anterior ventromedial)	Blunted	N/A	No effect	N/A

** This blunt in the sensitivity to contingency degradation might be due to the decrease in the response rate in the non-degraded juice condition on the baseline session (see Chapter 4.4.2).

Tables 5.2. Summary of relevant results from the **motivational control** experiments.

Progressive Ratio

	M5		M7		M9	
	Inactivation		Activation		Activation	
	Probe	Baseline	Probe	Baseline	Probe	Baseline
Area 11	No effect	No effect	N/A	N/A	N/A	N/A
Area 14	N/A	N/A	Decreased BP	Decreased BP	Decreased BP	No effect

BP: breakpoint

Juice preference

	M7	M9
	Activation	
Area 14	Reversed preference	No effect

Lick data analysis

	Lick latency		Lick per reward		Lick outside reward	
	Degradation	Baseline	Degradation	Baseline	Degradation	Baseline
Area 24	No effect	Non-deg juice DHK < deg juice DHK	Sal < mus/bac; DHK < mus/bac	No effect	N/A	N/A
Area 14	Treatment x Degradation	No effect	No effect	DHK < Sal	No effect	No effect

For more analysis on the lick data please see the Appendix.

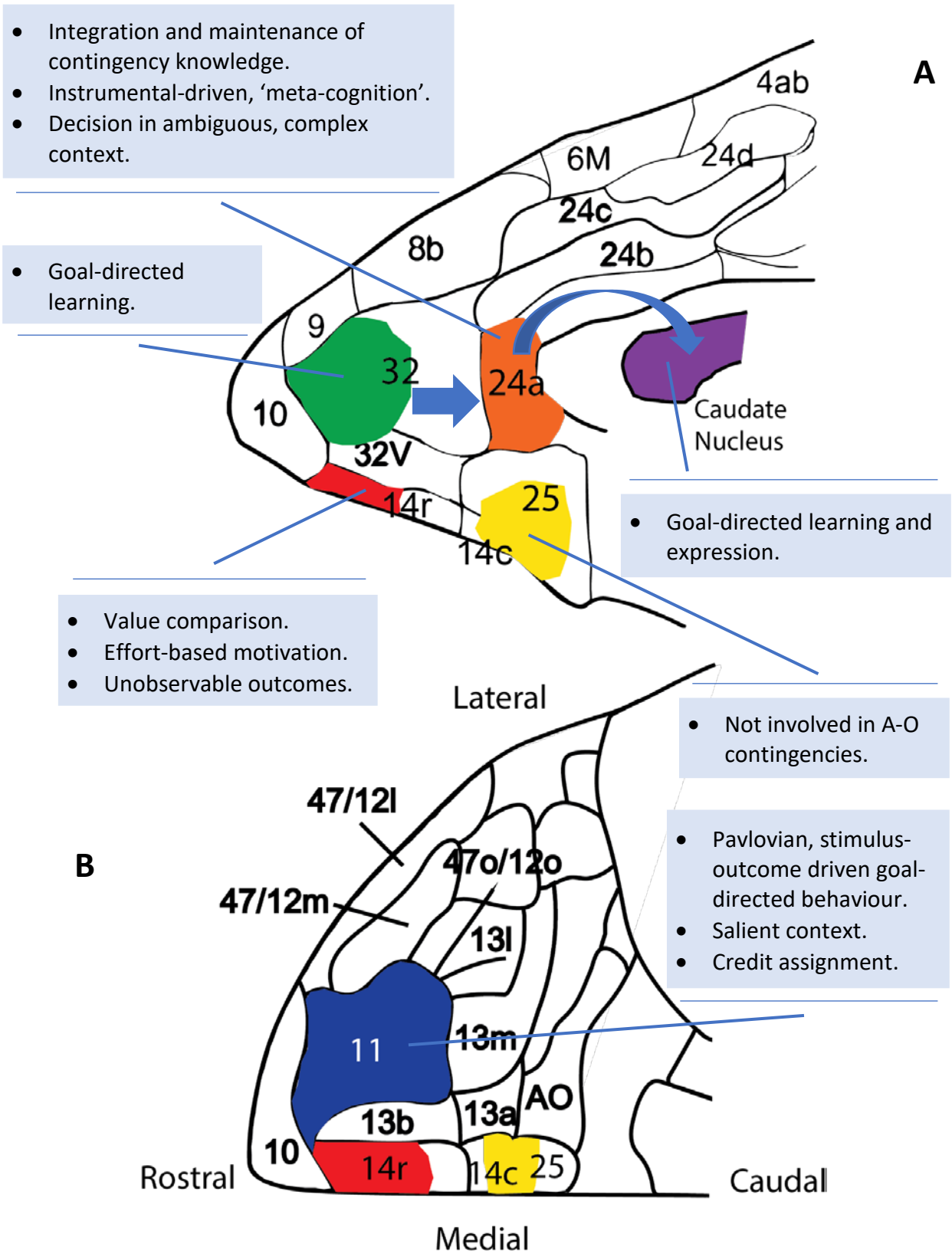


Figure 5.1. Different roles of PFC sub-regions and CN in goal-directed behaviour, and their anatomical location in the brain. A). Sagittal view of the medial surface of the PFC. There is the possibility that area 32 transfers learned goal-directed knowledge to area 24 for further integration and maintenance. Excitatory connectivity from area 24 to CN might be important for the expression of A-O contingencies. B). Orbital view of the various sub-regions of the OFC.

5.2. Area 24 is important for the expression, whilst area 32 is important for the acquisition, of A-O contingencies

Area 24 is the only PFC sub-region studied in this thesis that, when inactivated, affected animals' sensitivity to express their understanding of A-O contingencies (Table 5.1). As described in detail in Chapter 3.4.1, area 24 is critical in integrating, disambiguating, and maintaining learned knowledge and past behaviour in selecting the most appropriate action in an ambiguous context. (Cardinal et al., 2003; Kennerley et al., 2006; Seo and Lee, 2007; Rushworth and Behrens, 2008). This is exactly what the current task demands. The current study provides the first causal evidence, to my knowledge, that area 24 is the region that presumably stores but certainly utilises instrumental contingency knowledge. On the other hand, as described in Chapter 4.4.1, area 32's perhaps surprising non-involvement in the current contingency degradation task is most likely due to its involvement in the acquisition and not the expression of goal-directed behaviour. As there was no causal data in the literature that indicated the role of area 32/PL in the expression of A-O contingencies, this thesis provides the first-known causal evidence that area 32 was not involved in this function.

Given area 24's involvement and area 32's non-involvement in the expression of instrumental A-O association, it could be that when the animal started to learn goal-directed behaviour, they required area 32. When the information is learned, however, they do not need the participation of area 32 and instead require area 24 to use the learned information. There hence seems to be an anterior to posterior transfer of information in the medial PFC (Figure 5.1.). Indeed, Tang et al. (2019) suggested the boundary area of pregenual area 24 and area 32 (2/3 area 24 and 1/3 area 32) to be the central hub for integrating information from sensory, motoric, limbic and executive decision-making regions, through anatomical connectivity evidence in macaques. They also found a similar perigenual ACC region in humans.

In the other major paradigm that measures goal-directed behaviour, the outcome devaluation task, PL in the rodent is also involved in its acquisition but not the expression. PL lesions in rats impaired the acquisition but not the expression of instrumental outcome devaluation (Ostlund and Balleine, 2005; Tran-Tu-Yen et al., 2009). In both studies, most

of the extent of the PL lesions were within the area 32 defined in the most recent rodent atlas (Paxinos and Watson, 2017), but a small posterior part of the lesion was in area 24a. In the macaque monkey, however, area 32 aspiration lesions (non-fibre sparing) seemed not to affect the animals' ability to learn instrumental outcome devaluation ($p = 0.051$). Indeed, they decreased responding to the devalued outcome and had higher responding to the non-devalued outcome. Thus, functional homology between area 32 in primate and PL in rodent for the expression of instrumental value-updating is still questionable. On the other hand, anterior cingulate cortex lesions (i.e. of areas 24 a, b, c) in macaques did not affect the pavlovian and instrumental version of outcome devaluation (Chudasama et al., 2013). Thus, at least in the non-human primate, area 32 and area 24 seem not to be involved in goal-directed actions that require learning the changes in outcome values.

To conclude, this thesis has provided causal evidence to address the following hitherto unanswered question in the literature: which PFC sub-region(s) is responsible for integrating and maintaining knowledge in instrumental contingencies? Of the five PFC sub-regions tested, I found that only area 24 emerged as essential. Area 32/PL affects the acquisition but not the expression of A-O contingencies. Thus, I speculate that area 32 is one of the brain regions that transfer learned contingency knowledge to area 24 for integration, storage and retrieval to guide behaviour (probably via a cingulate-caudate interaction, see the next section).

5.3. Excitatory connections from area 24 to the CN may affect the expression of A-O contingencies

This thesis has identified area 24 as the critical PFC sub-region and anteromedial CN as the critical striatal region in a frontostriatal circuit that is critical for the expression of A-O contingencies. Were the area 24 and the CN region targeted in this thesis connected anatomically in the marmoset? Since no prior studies had verified this, in Chapter 2.11.2, I used retrograde tracing in the CN to map its connectivity with the PFC. Indeed, as shown in Chapter 2.11.2c, the anterior CN that was targeted receives projections from area 24 also targeted for this thesis. Across species, area 32 and area 24 projections overlap in the anteromedial CN (Heilbronner et al., 2016). I speculate that area 32 and anteromedial CN connectivity is necessary for the acquisition of A-O contingencies, while area 24 projection to the same area of CN is necessary for their expression (Figure 5.1). In rodents, disconnection lesions and DREADDs inactivation in rats have demonstrated that PL – pDMS connectivity, but not PL – nucleus accumbens connectivity, is essential for the acquisition of outcome devaluation (Hart et al., 2018a; Hart et al., 2018b). However, one could argue that this effect might be because the PL region they targeted was sufficiently posterior to be considered bordering the cingulate cortex and thus close to the area 24 targeted in the current thesis (Paxinos and Watson, 2017). Additionally, Heilbronner et al. (2016) have speculated that parts of the rodent PL are homologous to parts of primate area 24, according to their cortico-striatal projection meta-analysis. However, this is not, in fact, the case. The PL targeted in the above rodent studies, even with the most recent version of the rodent atlas, is situated in area 32 and not in area 24, although the most posterior extent of the lesion is near the boundary between area 32 and 24.

The current thesis taps into area 24 – CN connectivity indirectly through the use of CNQX to inactivate CN activity. This is because CNQX is an AMPA-receptor antagonist, which effectively silences excitatory input to the striatum from the cortex and the parafascicular (Pf)/centromedian nuclei in the thalamus (Galinanes et al., 2011; Bradfield et al., 2013; Gonzales and Smith, 2015) (see Chapter 4.4.3). In an electrophysiological study, Kita (1996) demonstrated that the response of striatal spiny neurons after cortical

stimulation was mainly mediated by AMPA-receptors. In situ hybridisation and immunohistochemistry studies had revealed the expression of AMPA-receptors subunits throughout the striatum in humans, rats and rhesus macaques (Tallaksen-Greene and Albin, 1994; Bernard et al., 1996; Ghasemzadeh et al., 1996; Deng et al., 2010). Together with CNQX's ability to almost completely block the striatal field response to PL stimulation (Galinanes et al., 2011), it could be said that in the current study, CNQX infusion in the CN most likely blocked excitatory signals from area 24. Without the CN to execute its command, area 24 alone is insufficient to maintain learned A-O associations. Likewise, without the overarching, integrative role of area 24, CN alone is not enough to support contingency expression.

5.4. OFC sub-regions complement each other and area 24 in affecting different aspects of goal-directed behaviour: potential double-dissociation between pavlovian vs. instrumental and value vs. contingency-based decision-making

Surprisingly, none of the OFC sub-regions or the vmPFC was necessary to maintain animals' sensitivity to instrumental A-O contingencies, although area 11 inactivation enhanced and activation impaired such sensitivity. Drawing from the data obtained in this thesis and the literature, area 11 is theorised to be in control of the stimulus-response and pavlovian-driven system (see Chapter 3.4.2 for a more detailed analysis), and in assigning values to stimuli in very salient contexts (Noonan et al., 2010; Walton et al., 2010). However, it is not essential for instrumental-driven behaviours and potentially not in effort-based motivation (Table 5.3). Despite its non-involvement in instrumental contingencies, area 14 was implicated to have an impact on comparing the value of the outcome (unobservable) and how much effort is allocated to reach the desired outcome value (see Chapter 4.4.2 for more analysis; Table 5.3). On the other hand, area 24 is critical in instrumental contingencies and has a 'meta-cognitive' role in integrating and utilising stored information to facilitate decisions when variables are ambiguous and similar in nature. However, it is not essential for pavlovian-driven or value/effort-based behaviours (Table 5.3). Therefore, area 24 is important for the 'belief' and area 14 is important for the 'wanting' in goal-directed actions, while area 11 uses salient stimulus-response information to help make optimal decisions and reach the desired goals. Area 24, area 11 and area 14 seem to have slightly overlapping, but largely distinctive roles (Table 5.3, Figure 5.1, 5.2). The following paragraphs will discuss supporting evidence for these speculative hypotheses.

		mPFC		ACC		lOFC		mOFC		Dorsal Striatum	
		Area 32	PL	Area 24	Cg	Area 11	LO/VLO*	Area 14	MO	CN	pDMS
Instrumental, A-O contingencies	Acquisition		1, 11				^, 6, 7, 8		3		4
	Expression	#		#		#		#		#	4
Pavlovian, S-R contingencies	Acquisition	2				2	5				
	Expression										
Instrumental, Outcome devaluation	Acquisition	9	10, 1, 12	13			^, 14, 15, 16, 17		3, 18 (ant)	?	4, 28
	Expression		12				14				4
Pavlovian, Outcome devaluation	Acquisition			13		19 (choice)	15	20		?	28
	Expression										
Effort-based motivation (progressive-ratio)			27	?	21,22	# (n=1)	23	# (n=2)	24, 25	?	26

Table 5.3. Possible implications of area 24, area 11 and area 14 in different tasks. The lists are potentially not exhaustive.

Green: implicated; **Orange**: not implicated; **Yellow**: no direct evidence, speculative, or unknown
Ant: anterior
#: current thesis.
*: Price (2007) considered LO/VLO as homologous to area 13 in macaques.
^: See 5.3.1 and 5.3.2 for more discussion on these studies with current thesis' theory.

1: Corbit and Balleine (2003); 2: Jackson et al. (2016); 3: Bradfield et al. (2015); 4: Yin et al. (2005b); 5: Ostlund and Balleine (2007); 6: Zimmermann et al. (2017); 7: Zimmermann et al. (2018) 8: (Balleine and Dickinson, 1998); Whyte et al. (2019); 9: Rhodes and Murray (2013); 10: Corbit and Balleine (2003); 11: Balleine and Dickinson (1998); 12: Ostlund and Balleine (2005); 13: Chudasama et al. (2013); 14: Ostlund and Balleine (2007); 15: Panayi and Killcross (2018); 16: Parkes et al. (2017); 17: Gremel and Costa (2013); 18: Bradfield et al. (2018); 19: Murray et al. (2015); 20: Rudebeck and Murray (2011); 21: Schweimer and Hauber (2005); 22: Hart et al. (2020); 23: Gourley et al. (2010); 24: Gourley et al. (2016); 25: (Walton et al., 2010); Munster and Hauber (2018); 26: Eagle et al. (1999); (Walton et al., 2010); 27: Caballero et al. (2019); 28: Corbit and Janak (2010)

5.4.1. Instrumental contingency degradation

Only area 24, and not area 11, area 14 or area 14-25, was involved in the expression of instrumental contingencies, based on results from the current thesis. The current thesis targeted anterior area 14/mOFC. Consistently, rodent studies that lesioned the anterior mOFC indicated that this region is not necessary for instrumental contingency learning (Bradfield et al., 2015). Area 14/14-25 are often included in human studies as vmPFC. Our results were consistent with a human lesion study examining A-O contingencies, in which patients with vmPFC lesion can learn A-O contingencies but were impaired in their subjective awareness in situations where their performance of an action increased the probability of an outcome (O'Callaghan et al., 2019). Thus, it might be that vmPFC, including area 14, is not essential in the learning or the expression of instrumental contingencies, but is critical in the subjective judgement of contingency learning. Indeed, Tanaka et al. (2008) found mPFC activity (in the ventral region) to increase linearly with increasing causality judgement. Of course, it is impossible to ask monkeys their opinion on the task; thus, the current thesis could only conclude that are 14/14-25 are not involved in instrumental contingency expression and not about subjective judgement.

The lateral OFC/area 11, was also found to not be necessary for the acquisition (Zimmermann et al., 2018) [chemogenetic inactivation of ventrolateral OFC (VLO) in mice] and expression of instrumental contingency (current thesis, area 11 in marmoset). The current thesis's finding that area 11 is not necessary for instrumental contingency in the marmoset challenges Bradfield and Hart (2020)'s prediction: because lOFC is important for representing an animal's initial state within a task space, lOFC inactivation will impair both the learning and performance of contingency degradation. Thus, the current thesis theorised that in addition to representing initial states, lOFC is critical in situations where pavlovian, salient and distinctive cues are important for goal-directed actions. This explains why area 11 lesion impaired contingency learning in the task with pavlovian elements (Jackson et al., 2016); it also explains how area 11 inactivation did not impair, but activation did, performance in the current task where the contingency expression is measured in a purely instrumental way (more details see chapter 3.4.2). Currently, there is no study in the literature, besides the current thesis, that investigated the role of lOFC

using a purely instrumental contingency task that does not involve extinction (Bradfield and Hart, 2020). Therefore, comparisons with other animal species are difficult. Additionally, inter-species homologies still need to be taken into account; for instance, which rodent OFC sub-region is homologous to which primate ones (see Chapter 1.2 for more discussion). Nonetheless, the current thesis has not only provided evidence for a role of area 11 in instrumental contingency that had never been previously investigated but has also proposed theories for possible area 11 functions.

In mice, *Bdnf* knockdown in the lateral OFC and ventral OFC impaired instrumental contingency acquisition (Zimmermann et al., 2017), and chemogenetic inhibition in the VLO immediately after the task impaired instrumental contingency degradation performance in the following day (Zimmermann et al., 2018). Additionally, a recent study by the same group also found that chemogenetic inactivation of VLO impaired contingency learning (Whyte et al., 2019). The contradictory finding of Zimmermann et al. (2017) when compared to the current thesis's theory, which hypothesised that lateral OFC is not involved in instrumental behaviour, might be due to the questionable translational power of inter-species homologies of the OFC or heterogenous roles within the OFC sub-regions (eg. anterior vs. posterior). For instance, in Jackson et al. (2016) and the current study, area 11 is in the anterior lateral OFC, whereas Zimmermann et al.'s VLO is considered to be homologous to primate's area 13 (Price, 2007). Although Jackson et al. (2016) did employ a response-outcome based contingency learning task, there were still strong pavlovian components present. Thus, one cannot rule out the possibility that the impairment found in Jackson et al. (2016) is due to area 11's role in pavlovian-based learning. Additionally, different mechanisms were involved in inactivation versus *Bdnf* knockdown of a brain region. Moreover, the contingency degradation task design used in Gourley's group (Zimmermann et al., 2017; Zimmermann et al., 2018; Whyte et al., 2019) is slightly different from the marmoset studies and rat studies (Balleine and O'Doherty, 2010). In the 'degraded' session training from Gourley's group, mice cannot access the reward via nose poke. That is, only the 'non-contingent' (reward freely available) situation was present and not the 'contingent' situation (animal needs to respond to get reward; methods see Chapter 2.5). Additionally, the impairment observed might be due to motivational, extinction-related or pavlovian-related deficits rather than to contingency degradation [for comments on Zimmermann et al. (2017), see Robbins (2017)]. Thus,

more studies still need to be conducted and ideally will be in non-human primate using a purely instrumental task.

Not many studies have examined PFC's role in pavlovian contingency. The most consistent results have been in the lateral OFC, where damage impaired pavlovian contingency learning (Chudasama and Robbins, 2003; Ostlund and Balleine, 2007; Jackson et al., 2016). Overall, area 24 was critical in using previously learned behaviour to disambiguate similar variables to make decisions based on A-O contingencies, while area 11 is important in assigning distinctive outcomes to distinctive actions or stimulus that have strong pavlovian elements. Area 14, on the other hand, is not involved in A-O contingencies (Figure 5.2).

5.4.2. Instrumental outcome devaluation

Manipulations of area 24 did not affect the acquisition of instrumental outcome devaluation task (Chudasama et al., 2013). Contrarily, anterior mOFC lesions (Bradfield et al., 2015; Bradfield et al., 2018), but not posterior mOFC in the rat (Bradfield et al., 2018; Munster and Hauber, 2018), impaired instrumental outcome devaluation. The current thesis targeted anterior mOFC (area 14). There seems to be a double dissociation between the role of area 24 and area 14 (Figure 5.2). It might be said that anterior mOFC (area 14) is not implicated in instrumental contingency but instrumental outcome devaluation; area 24 is the opposite. Area 14/mOFC is important for learning and establishing changes in the value of unobservable outcomes in rodents (Bradfield et al., 2015) and value comparison in macaques (Noonan et al., 2010) and humans (Noonan et al., 2017; Reber et al., 2017), while area 24 integrates, maintains and disambiguates observable contingencies that were accumulated from past behavioural histories to make decisions.

The majority of the studies in rodents demonstrated that lateral OFC manipulations did not affect instrumental devaluation (Ostlund and Balleine, 2007; Balleine et al., 2011; Parkes et al., 2017; Panayi and Killcross, 2018). This is consistent with the current thesis' theory, in which lOFC is important for differentiating salient contexts that are also pavlovian in nature. Indeed, LO lesions impaired pavlovian outcome

devaluation in rats (Ostlund and Balleine, 2007). Similarly, in outcome devaluation tasks that contain strong pavlovian components, inactivation (Murray et al., 2015) and lesion (Rudebeck and Murray, 2011) of the more lateral OFC (area 11 in the former, area 11/13 in the latter study) in macaques impaired devaluation. Murray et al. (2015) differentiated area 11 and area 13's role further in that the more anterior area 11 is necessary for choosing the more appropriate response based on changed outcome value, but not for the actual updating of the outcome value; area 13 had the opposite effect. This is further evidence to argue for the functional heterogeneity of lateral OFC and the role of area 11 in discriminating salient stimulus at the choice and not in actual value encoding.

In contrast, Gremel and Costa (2013) had demonstrated that chemogenetic inhibition of lateral OFC impaired *instrumental* outcome devaluation, whereas optogenetic activation of lateral OFC increased goal-directed pressing. This might seem contradictory to the theory that lOFC is important for pavlovian and not instrumental situations, and is also contradictory with the current thesis, where area 11/lateral OFC inactivation enhanced and activation impaired goal-directed actions. Nevertheless, the theory proposed by the current thesis might be able to explain this, due to the differences in task design. Gremel and Costa (2013) had tested and trained mice in two different contexts: one with a random interval schedule and another one in a random ratio schedule. Therefore, when the mice need to perform goal-directed actions in two separate contexts, interference between the contexts might occur. This could require the lOFC to discriminate between the two contexts and which action and outcome value relationship belonged to which. Therefore, lOFC inactivation impaired goal-directed actions and activation of this region perhaps made the contexts more salient, which led to better goal-directed lever pressing. In contrast, the animals were tested and trained in one context with identical stimuli or levers to respond to in the current thesis and in (Ostlund and Balleine, 2007; Balleine et al., 2011; Panayi and Killcross, 2018); thus, the contexts are not salient enough to require the lOFC.

To conclude, the current thesis postulates that area 24 is important for integrating A-O contingencies in ambiguous contexts but not in value comparison, whereas area 11 is important for choosing goal-directed actions in salient, pavlovian contexts using known knowledge, but not in value comparison. This could potentially be a double-dissociation between area 24 and area 11 (Figure 5.2). On the other hand, area 14 differs from both in

that it is not required for A-O contingencies (observable outcome) but in instrumental value comparison (unobservable outcome).

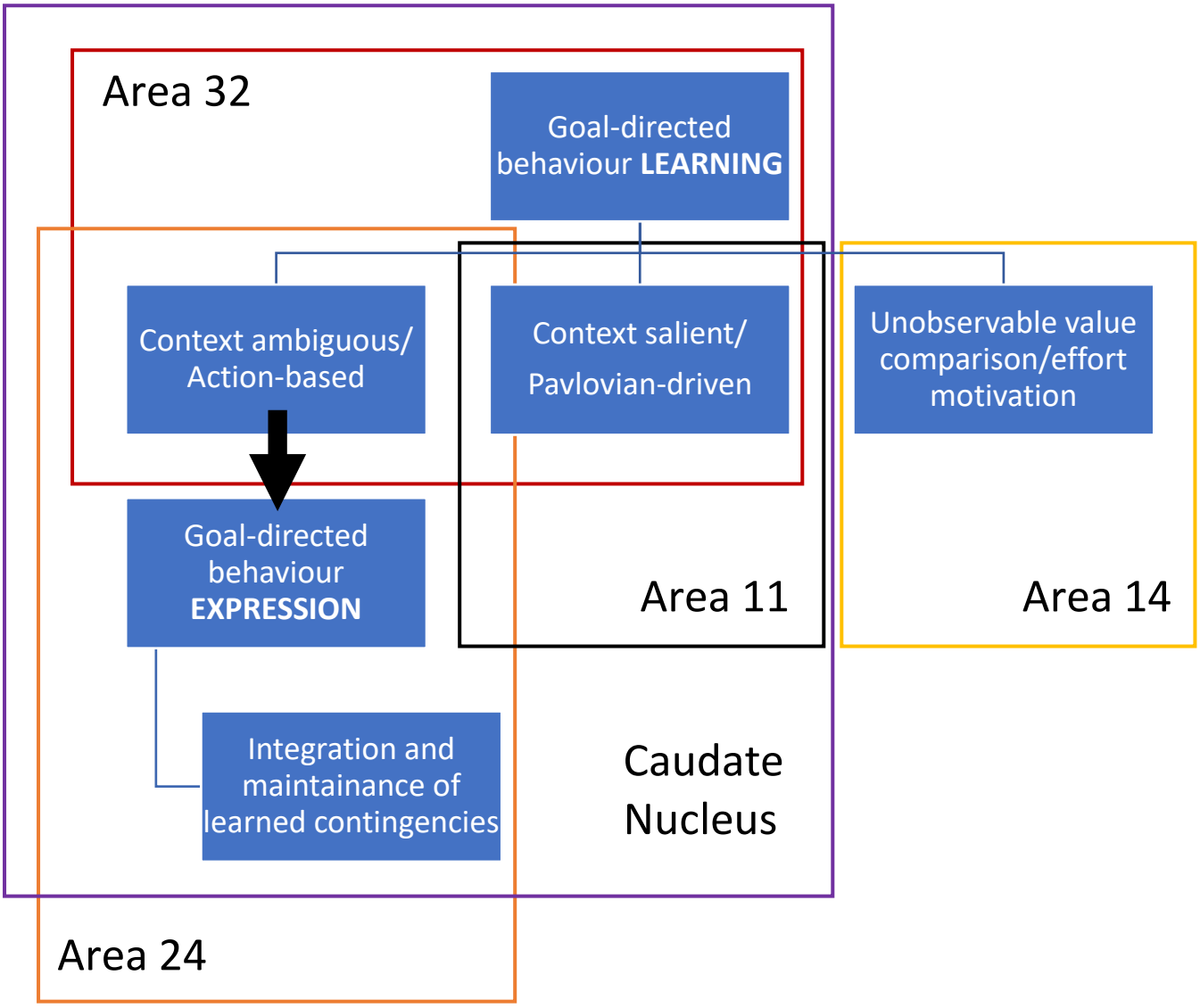


Figure 5.2. Distinct but overlapping roles of PFC sub-regions (speculative).

5.5. Conclusion

The PFC sub-regions within the mPFC, ACC and OFC have distinctive but overlapping functions in controlling goal-directed behaviour (Figure 5.1, 5.2 and Table 5.3). When learning goal-directed behaviours, area 32 and CN are recruited. In situations where the contexts are ambiguous, complex and action-based, area 24 exerts its "meta-cognitive" abilities to integrate and maintain learned contingency knowledge. On the other hand, in situations where the contexts are more salient and have pavlovian and stimulus-driven elements, area 11 is engaged. Meanwhile, the CN is active throughout all the above situations. Complementarily, area 14 is not involved in contingency learning or expression; instead, it is important when the outcome is unobservable, comparisons between outcome values are required and an optimal motivational effort is needed.

5.6. Limitations and Future Directions

There are several limitations in the current study: sample size, length of study and individual variability. Firstly, the subject size is small (n = 3 – 5 for each brain region). This is difficult to resolve because non-human primates take longer to reproduce, to reach adulthood, and require more care and attention than rodents. The current thesis adapted to the limited subject number by using a within-subject design and targeting multiple brain regions in one monkey. Therefore, fewer animals were needed to perform robust statistical analysis.

Secondly, since two to three brain regions were examined in each animal in addition to the complex task to study contingency expression, the time for each animal to finish the study was quite long (~ 20 months). This could render the interpretation of their performance impairment difficult. That is, the impairment could be due to the pharmacological manipulations or simply because the animals became more habitual in their responding via over-training. This thesis carefully controlled for this possibility. Saline control infusions in all conditions were conducted in close temporal proximity to each set of pharmacological manipulations. Additionally, each of the performance impairments observed was flanked by "contingency degradation check", where the animals underwent additional degradation sessions under control conditions. The pharmacological manipulation would only be considered valid if the animals' behaviour was still goal-directed in the "checks".

Thirdly, individual variability was observed. Two marmosets, who were excluded in the main experimental analysis, were unable to maintain their sensitivity to contingency degradation. After histological analysis, one of them was found to have an enlarged lateral ventricle in the right hemisphere, which could potentially cause her to behave more habitually. The other marmoset was her twin but was not observed to have anatomical abnormalities. Some marmosets are inherently more goal-directed and some are more habit-driven in nature. These two animals were excluded in this study and control measures, described in the previous graph, were implemented to ensure that all pharmacological manipulations were valid. This individual variability was not unique to

marmosets, as a rodent study using a modified contingency degradation task also found some rats were sensitive to the task and some were not (Fitoussi et al., 2018).

The current thesis only investigated the expression of instrumental A-O contingencies using the contingency degradation task; thus, it is still unknown whether or not area 24 (perigenual ACC) will affect the learning of this task. It is likely, because ACC (area 24 a, b, c) lesions impaired rhesus macaques' ability to learn pavlovian and instrumental A-O associations, as measured by object and action reversals (Chudasama et al., 2013). Future studies could investigate the role of area 24 in learning the contingency degradation task. This will shed light on whether area 24 is only required as a knowledge maintenance and integration centre for A-O contingencies, or is it also required at the initial learning stage.

The current thesis only offered indirect evidence that excitatory projection from area 24 to the CN might impair the expression of A-O contingencies. Future studies could manipulate the area 24-CN connection directly, using pharmacological disconnection or chemogenetic inactivation/activation. In the disconnection paradigm, one could reversibly inactivate area 24 with muscimol/baclofen and CN with CNQX. Each animal will undergo asymmetric and ipsilateral infusions, with the latter one acting as a control. In the disconnection manipulation, monkeys will receive an infusion in CN in one hemisphere and area 24 infusion in the opposite hemisphere, which disrupts the communication between these structures. In ipsilateral controls, monkeys will receive infusions in the same hemisphere, leaving the communications in the other hemisphere intact. If area 24-CN connection is indeed important for the expression of A-O contingencies, the animals will be impaired in the contingency degradation task after the asymmetric but not the ipsilateral manipulation.

Another possible direction to pursue is to target specific projections from area 24 to anteromedial CN using DREADDs. If CN is inactivated, it is unknown which projection from which brain areas are inactivated, because PFC sub-regions send overlapping projections to CN. In marmosets (Roberts et al., 2007), macaques (Averbeck et al., 2014) and humans (Draganski et al., 2008), mPFC and OFC projections to the dorsal striatum overlap extensively in the anteromedial CN. One way to disentangle the projections is to use "retro-DREADDs" (Marchant et al., 2016) by injecting DREADDs in area 24 and cre-recombinase based virus in CN. This way, only neurons projecting specifically from area

24 to CN will be over-activated or inactivated following the DREADDs ligand Clozapine N-Oxide (CNO). Over-activating the circuity using DREADDs also mimics the consistently observed effect of the over-active frontostriatal connectivity in OCD patients (Whiteside et al., 2004; Gillan and Robbins, 2014; Pauls et al., 2014). Manipulating the circuitry in such a precise manner with cutting-edge technology in non-human primates will provide tremendous insights into underlying functional mechanisms of the frontostriatal system in neuropsychiatric disorders.

Other brain regions and neurotransmitter systems are also involved A-O contingency. For instance, as reviewed in Chapter 4.4.3, parafascicular-pDMS connectivity is also critical for the learning of A-O contingencies (Bradfield et al., 2013). Rats with mediodorsal, but not anterior thalamus lesion were insensitive to contingency degradation and outcome devaluation (Corbit and Balleine, 2003). Parnaudeau et al. (2015) used DREADDs to decrease mediodorsal thalamic nuclei activity in mice, which also reduced sensitivity to contingency degradation.

The inferior parietal lobule also seems to be playing an important role in integrating contingency information. In human imaging studies, Liljeholm et al. (2011) found that vmPFC and right anterior CN activity indicated the probability of receiving a reward in the presence of an action [$P(o|a)$], and the activity in inferior frontal gyrus and left posterior CN varied with the probability of receiving the reward in the absence of an action [$P(o|\sim a)$]. More importantly, they found that the change in the instrumental contingency (ΔP), that is, the integration of the previous two probabilities [$\Delta P = P(o|a) - P(o|\sim a)$], was correlated negatively with activity in the left inferior and posterior partial lobules and the left middle frontal gyrus; these regions also track subjective causal judgements. The parietal lobules, similar to area 24, contribute to multimodal integration of sensory, motor and visual inputs to form a unified cognitive representation of the current situation and to direct optimal motoric actions (Gottlieb, 2007; Rizzolatti et al., 2014). It could be that the parietal lobule calculates the overall contingencies and area 24 retrieves this information for optimal decision-making. Future studies could investigate the causal role of parietal cortex in A-O contingencies via pharmacological manipulations or chemogenetic methods such as DREADDs.

The current study did not find any OFC sub-regions to be necessary for the expression of A-O contingencies. This is surprising, given that the OFC is consistently

implicated in goal-directed behaviour and A-O contingencies across species. Other OFC sub-regions that were not tested in the current thesis could be involved. Area 13, which is posterior to the primate area 11, is a likely candidate. There are very few non-human primates or rodent studies that distinguished between area 11/anterior OFC and area 13/posterior OFC. There is no study in the non-human primate that has investigated the role of area 13 in A-O contingencies. Non-human primate and rodent studies using the outcome devaluation task have shown that there are indeed differences in functionality between anterior/posterior sections of lateral and medial OFC (Murray et al., 2015; Bradfield et al., 2018; Panayi and Killcross, 2018). Thus, more studies investigating the role of OFC sub-regions are needed.

The current study did not directly examine cortico-cortical circuits. Given the distinctive but overlapping roles of the PFC sub-regions (Figure 5.2), functional connectivity within the PFC is critical for understanding goal-directed actions (Stolyarova, 2018). Although human imaging studies provide valuable information on PFC circuits, it is difficult to deliver *causal* evidence on how these PFC sub-regions interact (i.e. precise lesions in multiple PFC sub-regions). Therefore, it would be crucial to explore cortico-cortico circuitry in the non-human primates, which possess a more similar PFC to that of humans than rodents and can have multiple PFC sub-regions causally manipulated.

The rodent studies focused on the roles of PL and OFC in the acquisition of goal-directed actions but much less so on the role of ACC in the expression of goal-directed actions (Table 5.3). Furthermore, the rodent, and also the non-human primate, literature concentrated on the studying of value-based goal-directed behaviour (‘want’) and not on the contingent relationship between actions and outcomes (‘belief’). Therefore, there is a need for the literature to include more varied and precise PFC sub-regions in studying more varied elements that contribute to goal-directed behaviour.

5.7. Implications for Neuropsychiatric Disorders

The current study has demonstrated causal contributions of primate area 24 and the CN, via inactivation, in controlling the detection and expression of A-O contingencies in goal-directed behaviour. Additionally, over-activation in areas 24 and 11 impaired such detection and expression. These results have significant implications for the underlying etiology of neuropsychiatric disorders, such as obsessive-compulsive disorders (OCD) and schizophrenia, since impairments in goal-directed behaviour were observed in these disorders (Barch and Dowd, 2010; Gillan et al., 2014a; Morris et al., 2015). For instance, OCD patients were impaired in their performance in a contingency degradation task similar to the present study (Vaghi et al., 2019). Our findings concerning the over-activation of both areas 24 and 11 are consistent with the pathophysiology of OCD. The PFC, especially OFC and ACC, are overactive in OCD patients, (Baxter et al., 1988; Fitzgerald et al., 2011; Gillan and Robbins, 2014; Maia et al., 2008; Menzies et al., 2008; Pauls et al., 2014; Robbins et al., 2019; Whiteside et al., 2004), especially following symptom provocation (Rauch et al., 1994; Nakao et al., 2005b). Specifically, the impairment in behaviour observed in the present study after area 11 over-activation, which is a important area for pavlovian-stimulus control, may parallel overactivity seen in the OFC correlated with stimulus-realted sympotom provocation. Moreover, our results may also indicate a possible role for maladaptive Pavlovian-to-instrumental transfer effects (Bradfield et al., 2017). On the other hand, schizophrenia has been associated with a loss of GABA-ergic neurons in the anterior cingulate cortex (de Jonge et al., 2017); this might be associated with the impairments in goal-directed behavior seen in people with schizophrenia, which may underlie the ‘negative’ symptoms of schizophrenia (Morris et al., 2018). This thesis provided plausible PFC sub-regions as candidate for future human neuroimaging studies and potential targets for drug and treatment development in neuropsychiatric disorders with an impairment in goal-directed control.

Appendix

A. Reward licking analysis (area 14-25)

Lick Latency

Mixed-model analysis of variance on area 14-25 lick latency data in degradation sessions revealed a main effect of treatment x degradation interaction ($F_{2, 10} = 4.869$, $p = 0.0334$). Post-hoc analysis showed a higher lick latency in area 14-25 activation in the degraded session compared to non-degraded session ($t_{10} = 2.911$, $p = 0.0155$) and a trend of decreased lick latency in nondegraded session after activation when compared to control ($t_{10} = -2.398$, $p = 0.0873$).

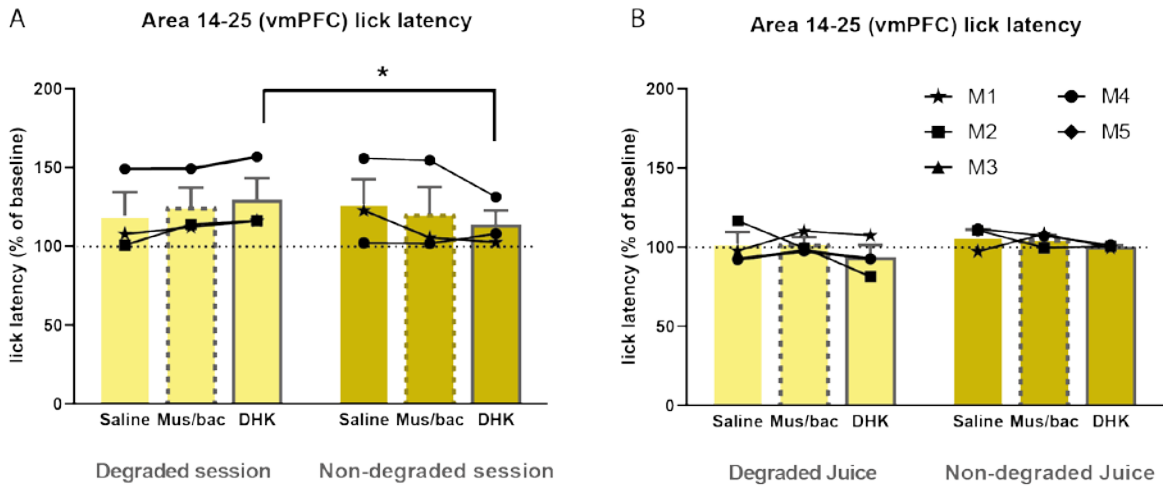


Figure A1. Lick latency of animals after drug manipulations of area 14-25. Lick latency is the time between the onset of birdsong that signals the reward and the time animals start to lick from the sprout. A). In degradation sessions, animals initiated licking significantly slower after area 14-25 activation in the degraded sessions compared to non-degraded sessions. B). In baseline sessions, no significant difference in lick latency was detected after area 14-25 manipulations.

*: $p < 0.05$

Licks per reward

Area 14-25 manipulations did not have any effect on the number of licks per reward.

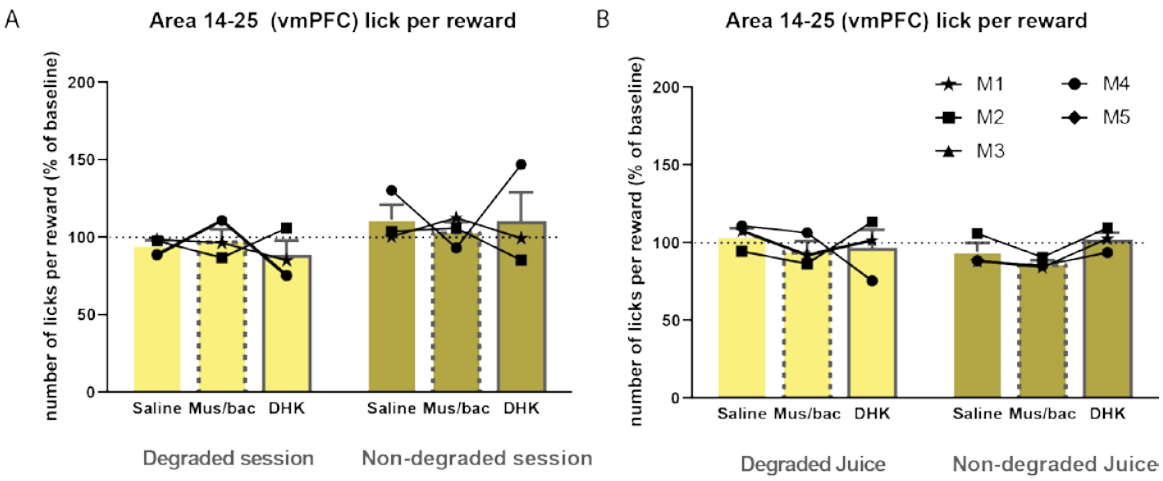


Figure A2. Animals' lick per reward after area 14-25 manipulations. Lick per reward is the average number of licks animal does for the duration of each reward delivery A-B). No significant difference was observed after area 14-25 manipulations in both baseline and degradation sessions.

B. Licks during reward delivery (area 14)

On baseline sessions, area 14 activation caused animals to reduce the number of licks during reward delivery in the non-degraded juice condition (Figure 4.8D). Additionally, this reduction was limited to the period of one to four minutes into the 12-minutes-long testing session (Figure 4.11). Linear mixed-model analysis of variance on the number of licks during reward delivery in area 14 baseline sessions revealed a main effect of treatment ($F_{2, 12.957} = 6.887$, $p = 0.00917$) and a main effect of treatment x degradation interaction ($F_{2, 12.782} = 12.489$, $p = 0.000985$; Figure 4.10D). Post-hoc analysis showed that the average number of licks across sessions was significantly lowered after area 14 activation when compared to saline control ($t_{13.3} = -3.665$, $p = 0.0073$). In the non-degraded juice session, area 14 activation significantly reduced the number of licks during reward delivery when compared to both inactivation ($t_{13.1} = -4.089$, $p = 0.0033$) and control ($t_{13.1} = -5.962$, $p = 0.0001$). Number of licks after area 14 activation in degraded juice session was not significantly different from that of control ($t_{13.1} = 0.683$, $p = 0.777$). Number of licks in the degraded juice session was higher than the non-degraded juice session after area 14 activation ($t_{13} = 4.058$, $p = 0.0014$); number of licks in the degraded juice session was lower than the non-degraded juice session in saline control ($t_{13} = -2.628$, $p = 0.0209$). Linear mixed-model analysis of variance on the number of licks during reward delivery in area 14 degradation sessions revealed a main effect of treatment x degradation interaction ($F_{2, 13.011} = 3.892$, $p = 0.0473$; Figure 4.10C). Post-hoc analysis showed that number of licks in the degraded juice session after area 14 activation were significantly higher than non-degraded juice session after area 14 activation ($t_{13} = 2.603$, $p = 0.0219$) and degraded juice session after saline control ($t_{13.1} = 2.778$, $p = 0.0388$).

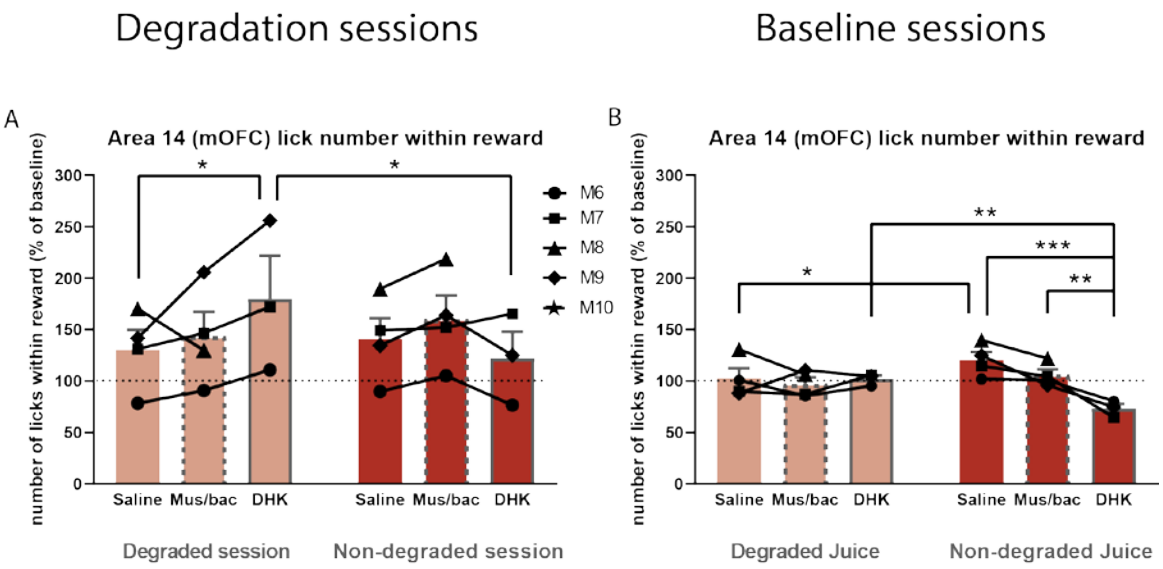


Figure B1. Analysis of number of licks during reward delivery after area 14 manipulations in degradation and baseline sessions. A). In degradation sessions, the number of licks after area 14 activation in the degraded session was significantly higher than that of area 14 activation in the non-degraded session and saline control in the degraded session. B). In baseline sessions, the number of licks after area 14 activation in the non-degraded juice session was significantly lower than that of inactivation and control in the the non-degraded juice session, and activation in the the degraded juice session. Saline control infusion into area 14 in the non-degraded juice session caused a significantly higher number of licks when compared to saline control in the degraded juice session.

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

In the baseline session, the total number of licks within the period of reward delivery was significantly decreased in the non-degraded juice condition after area 14 activation when compared to saline control and inactivation (Figure B1. B). However, this decrease in licking was only a secondary consequence of the decreased response rate. leading to a reduced number of rewards obtained within a 12-minute testing session. When the number of licks within each 30-second time frame was plotted across the 720-seconds long testing session (Figure. B2), a significant decrease in the number of licks was observed between 120-210 seconds into the session. This revealed that the decrease in licking occurred only during the first four minutes of the test session. In its last eight minutes, the DHK infused animal performed similarly when compared to the saline control animal.

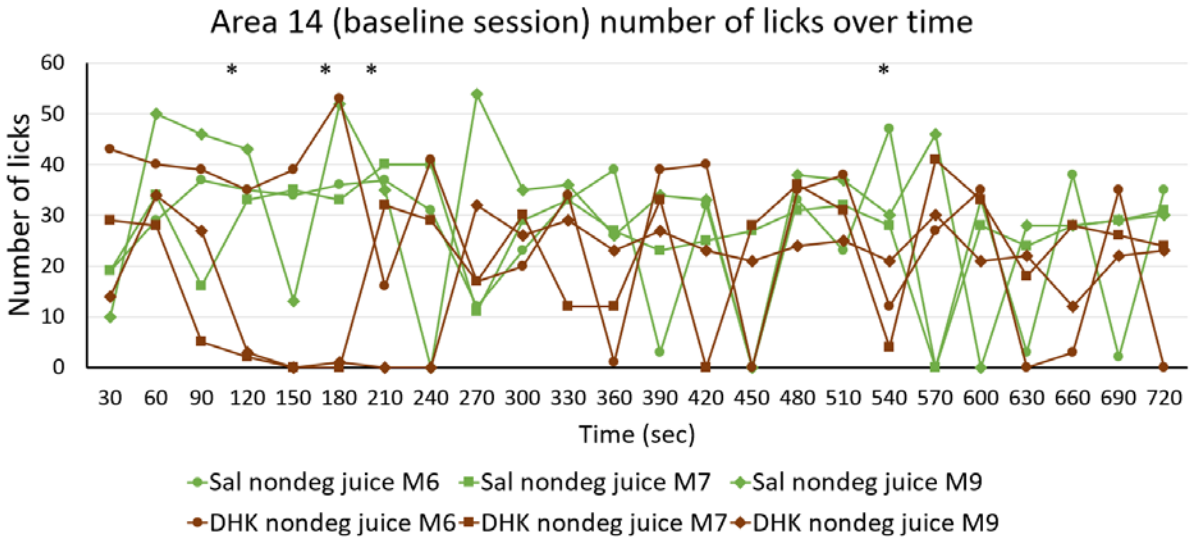


Figure B2. Number of licks over time was compared between area 14 saline control and activation (DHK) in the non-degraded juice condition of the baseline sessions. Each session lasted 720 seconds and lick number was measured every 30 seconds. Number of licks during saline infusion was significantly higher than that of DHK infusion at the 120 sec, 180 sec, 210 sec and 540 sec marks. M7 and M9 performed hardly any licking behaviour between 120 sec and 180 seconds.

*: $p < 0.05$

C. Reward licking analysis (area 32 and caudate nucleus)

Lick Latency

Linear mixed-model analysis of variance on lick latency in area 32 baseline sessions revealed a main effect of treatment ($F_{2, 15}$ = 7.855, p = 0.000464) and a treatment x degradation interaction ($F_{2, 15}$ = 13.870, p = 0.000389; Figure C1. B). Post-hoc analysis showed that average lick latency across sessions was significantly higher after area 32 activation when compared to saline control (t_{15} = 3.874, p = 0.0040), which in turn was higher than that of inactivation (t_{15} = -2.664, p = 0.0441). Lick latency was significantly lowered in the degraded juice session than the non-degraded juice session when area 32 was activated (t_{15} = -3.573, p = 0.0028). Lick latency was significantly higher in the degraded juice session than the non-degraded juice session when area 32 was inactivated (t_{15} = 3.871, p = 0.0015). Lick latency in the non-degraded juice session after area 32 activation was higher than that of inactivation (t_{15} = 6.461, p = <0.0001) and control (t_{15} = 2.604, p = 0.0494), and the control session had higher lick latency when compared to inactivation (t_{15} = -3.857, p = 0.0042).

Linear mixed-model analysis of variance on lick latency in CN degradation sessions revealed a main effect of treatment x degradation interaction ($F_{1, 9}$ = 5.169, p = 0.0491; Figure 4.8E). Post-hoc analysis showed that lick latency was significantly higher in the degraded juice session than the non-degraded juice session after saline infusion (t_9 = 2.431, p = 0.0379).

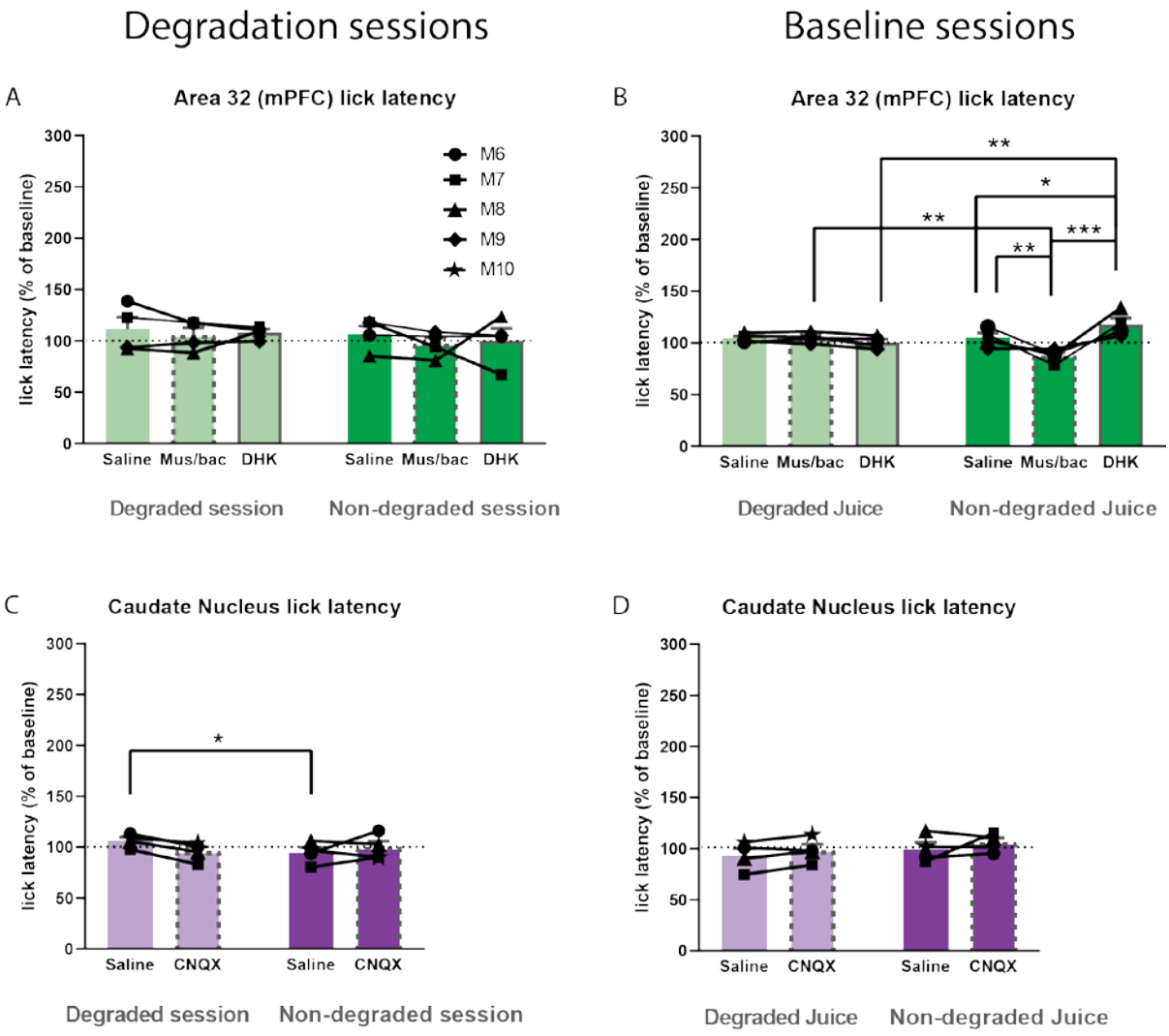


Figure C1. Analysis of lick latency (time between sound that signalled reward delivery and the start of licks to receive the reward) after area 32 and CN manipulations in degradation and baseline sessions. B). In baseline sessions, lick latency was significantly lowered after area 32 inactivation when compared to control and activation in the non-degraded session, and inactivation in the degraded session. Area 32 activation caused significant increase in lick latency in non-degraded juice session when compared to degraded juice session. C). CN saline infusion caused significant decrease of lick latency in the non-degraded session when compared to degraded session. A, D). No significant changes were observed.

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

Licks per reward

No significant effects were observed after area 32 and CN manipulations (Figure C2).

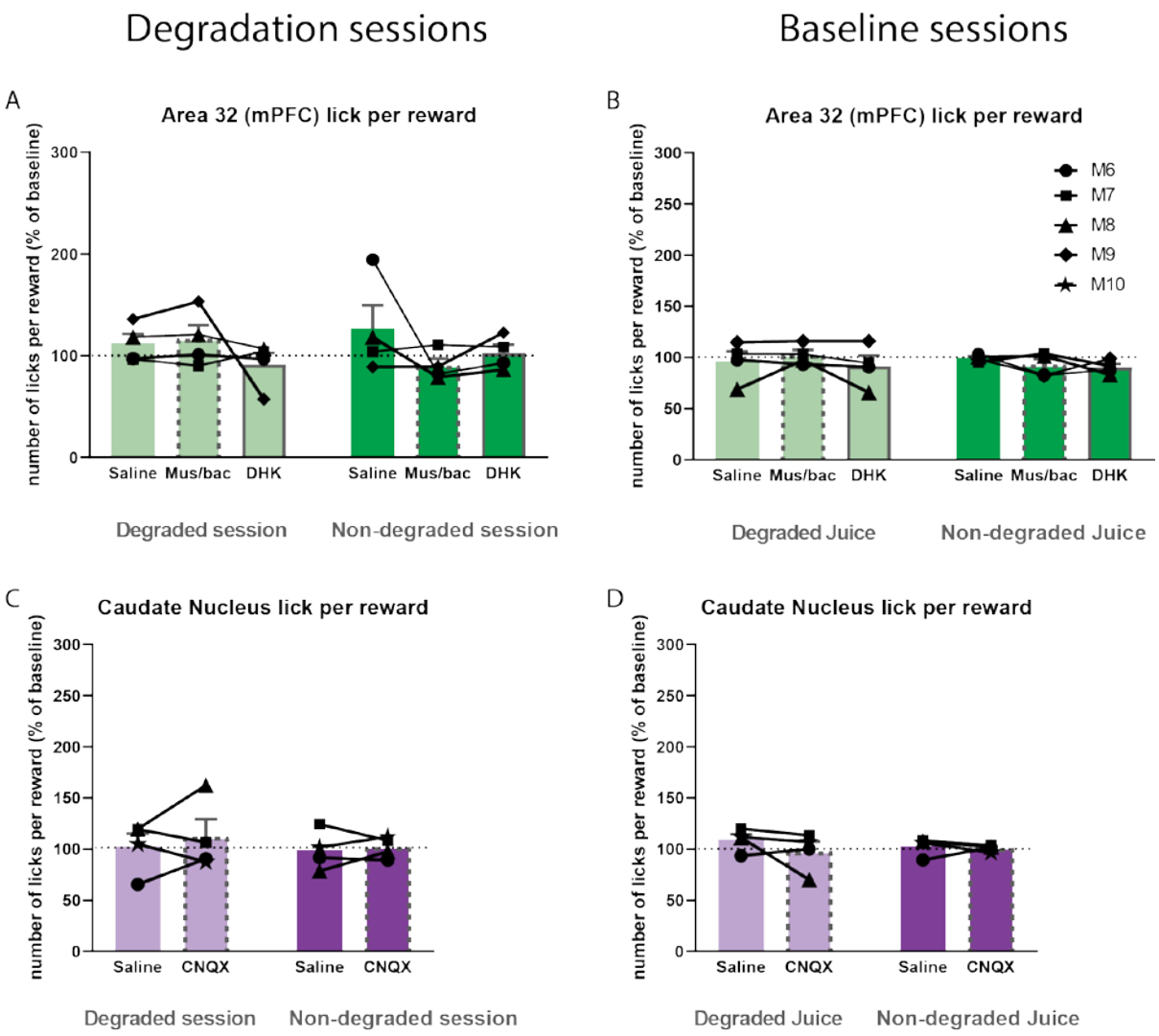


Figure C2. Analysis of number of licks per reward after area 32 and CN manipulations in degradation and baseline sessions. A-D). No significant effects were observed.

*: $p < 0.05$

Licks during reward delivery

No significant effects were observed after area 32 and CN manipulations (Figure C3).

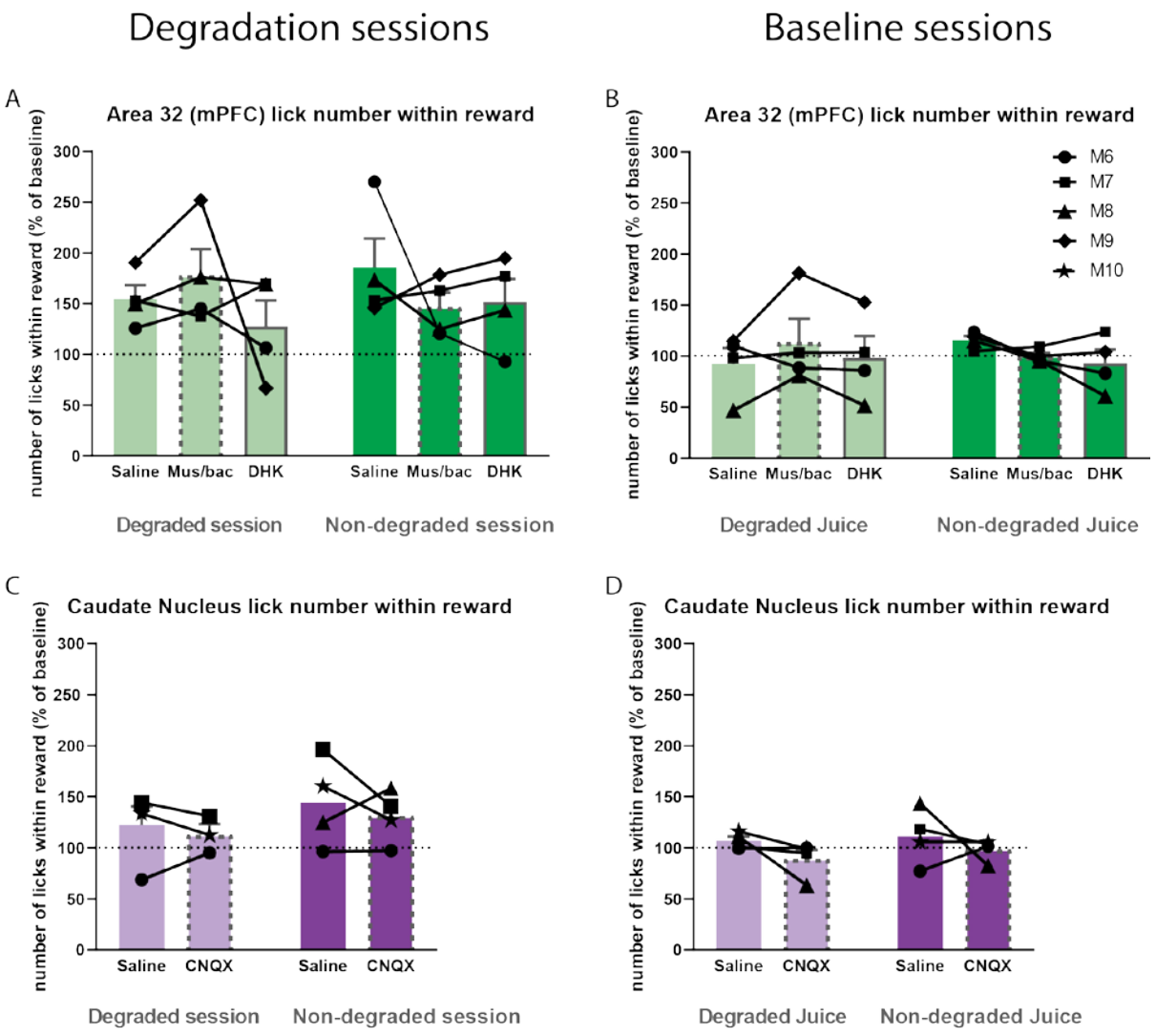


Figure C3. Analysis of number of licks during reward delivery after area 32 and CN manipulations in degradation and baseline sessions. A-D). No significant effects were observed.

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

Licks outside of reward delivery

Linear mixed-model analysis of variance on the number of licks outside of reward delivery in CN degradation sessions revealed a trend of treatment x degradation interaction ($F_{1, 9} = 4.558$, $p = 0.0615$; Figure C4. C). Post-hoc analysis showed that number of licks was significantly lower in the degraded juice session than the non-degraded juice session after saline infusion ($t_9 = 2.680$, $p = 0.0252$). CN inactivation caused the number of licks in non-degraded juice session to be significantly lower than that of control ($t_9 = 2.289$, $p = 0.0478$). Linear mixed-model analysis of variance on the number of licks outside of reward delivery in CN baseline sessions revealed a main effect of degradation ($F_{1, 12} = 5.394$, $p = 0.0386$; Figure C4. D). Post-hoc analysis showed that number of licks was significantly higher in the degraded juice session than the non-degraded juice session across drug manipulations ($t_9 = 2.323$, $p = 0.0453$).

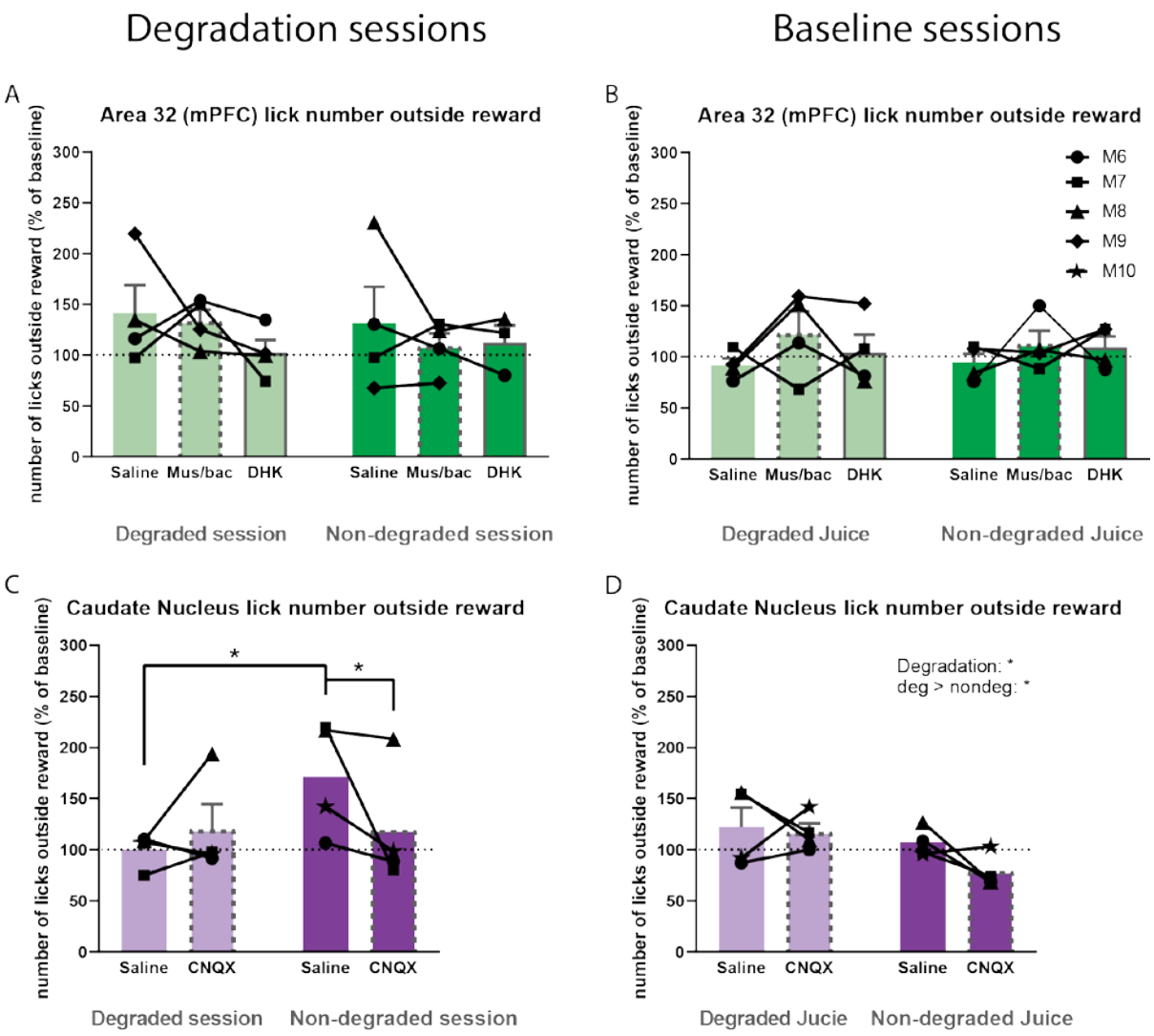


Figure C4. Analysis of number of licks outside reward delivery after area 32 and CN manipulations in degradation and baseline sessions. A-B). No significant effects were observed. C). On degradation sessions, CN saline control infusion in the non-degraded session resulted in a significantly higher number of licks when compared to inactivation in the non-degraded session and saline control in the degraded session. D). On baseline sessions, the number of licks was significantly higher in the degraded sessions when compared to non-degraded sessions.

*: $p < 0.05$

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