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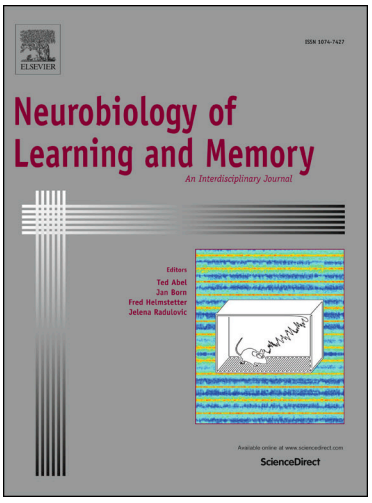

Inferring action-dependent outcome representations depends on anterior but not posterior medial orbitofrontal cortex

Laura A. Bradfield, Genevra Hart, Bernard W. Balleine

PII: S1074-7427(18)30230-2
DOI: https://doi.org/10.1016/j.nlm.2018.09.008
Reference: YNLME 6941

To appear in: *Neurobiology of Learning and Memory*

Received Date: 18 May 2018
Revised Date: 27 August 2018
Accepted Date: 19 September 2018

Please cite this article as: Bradfield, L.A., Hart, G., Balleine, B.W., Inferring action-dependent outcome representations depends on anterior but not posterior medial orbitofrontal cortex, *Neurobiology of Learning and Memory* (2018), doi: https://doi.org/10.1016/j.nlm.2018.09.008

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Inferring action-dependent outcome representations depends on anterior but not posterior medial orbitofrontal cortex.

Running title: Anterior mOFC mediates the retrieval of action outcomes

Authors: Dr. Laura A. Bradfield[1,2], Dr. Genevra Hart[1], and Prof. Bernard W. Balleine [1]

Affiliations: 1. School of Psychology, University of New South Wales, Australia, NSW 2052.

2. Centre for Neuroscience and Regenerative Medicine, University of Technology, Sydney, Australia, NSW, 2007.

Corresponding author:

Bernard W. Balleine

Decision Neuroscience Laboratory

Level 4 Mathews Building, UNSW Sydney

NSW, 2052, Australia

T +61 435659949

E bernard.balleine@unsw.edu.au

Conflict of interest: The authors declare no conflicts of interest.

Acknowledgements: The research reported in the manuscript was supported by grants to BWB and LAB from the NHMRC; GNT1087689 and GNT1148244. BWB is supported by a Senior Principal Research Fellowship from the NHMRC of Australia, GNT1079561.

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Abstract

Although studies examining orbitofrontal cortex (OFC) often treat it as though it were functionally homogeneous, recent evidence has questioned this assumption. Not only are the various subregions of OFC (lateral, ventral, and medial) hetereogeneous, but there is further evidence of heterogeneity within those subregions. For example, several studies in both humans and monkeys have revealed a functional subdivision along the anterior-posterior gradient of the medial OFC (mOFC). Given our previous findings suggesting that, in rats, the mOFC is responsible for inferring the likelihood of unobservable action outcomes (Bradfield et al., 2015), and given the anterior nature of the placements of our prior manipulations, we decided to assess whether the rat mOFC also differs in connection and function along its anteroposterior axis. We first used retrograde tracing to compare the density of efferents from mOFC to several structures known to contribute to goal-directed action: the mediodorsal thalamus, basolateral amygdala, posterior dorsomedial striatum, nucleus accumbens core and ventral tegmental area. We then compared the functional effects of anterior versus posterior mOFC excitotoxic lesions on tests of Pavlovian-instrumental transfer, instrumental outcome devaluation and outcome-specific reinstatement. We found evidence that the anterior mOFC had greater connectivity with the accumbens core and greater functional involvement in goal-directed action than the posterior mOFC. Consistent with previous findings across species, therefore, these results suggest that the anterior and posterior mOFC of the rat are indeed functionally distinct, and that it is the anterior mOFC that is particularly critical for inferring unobservable action outcomes.

Keywords: Goal-directed action; Pavlovian-instrumental transfer; outcome devaluation; outcome-specific reinstatement; nucleus accumbens core; mediodorsal thalamus.

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Introduction

The OFC has been argued to mediate a broad array of cognitive and behavioural functions in learning and decision-making, not all of which can be easily reconciled. This is due, in part, to the fact that the vast majority of studies examining the OFC have lacked specificity in detailing the particular subregion targeted (i.e. medial, ventral, or lateral OFC); something that is particularly true of studies into rodent OFC. One example is the well-established finding that OFC damage causes impairments in reversal learning (e.g. Izquierdo et al., 2004; Rolls et al., 1994; Schoenbaum et al., 2002, 2003). This effect was subsequently shown to be specific to lesions of the lateral portion of the OFC (lOFC), with medial OFC damage actually resulting in a facilitation rather than a deficit in reversal learning (Mar et al., 2011). Likewise, there have been several demonstrations of lOFC inactivation leaving instrumental outcome devaluation intact (Ostlund & Balleine, 2007; Parkes et al, 2017), whereas mOFC inactivation has been found to impair it (Bradfield et al., 2015). It is possible, therefore, that a number of seemingly inconsistent findings are in fact a result of functional heterogeneity across the OFC regions being manipulated. A recent review (Izquierdo, 2017) has added weight to this suggestion by detailing, with unprecedented specificity, the neuroanatomical placements described in studies of rodent OFC and how each subregion might be linked to its specific functions.

The impairment in outcome devaluation we observed as a result of mOFC inactivation was a part of a larger investigation into the function of the mOFC more generally (Bradfield et al., 2015). Specifically, we inactivated mOFC using both excitotoxic lesions and inhibitory (hM4Di) Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) in an instrumental choice situation where food outcomes (pellets or sucrose) were either observable or unobservable. Inactivating the mOFC selectively impaired performance during tasks in which outcomes were unobservable, i.e., in which they had to be recalled from memory, including outcome devaluation and specific-Pavlovian-instrumental-transfer (specific PIT). In contrast, performance on tasks in which the outcomes were presented and so observable, i.e., reinforced devaluation, outcome-selective reinstatement, and

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instrumental contingency degradation tests, was intact. Together, these results suggest that the mOFC is critical for inferring the occurrence of outcomes when they are unobservable as opposed to when they need merely to be recognised in the environment.

Beyond localising our placements in the medial subregion of OFC, however, we did not explore any differences of function along its anterior-posterior gradient. This could be significant because there are several lines of evidence suggesting that a further anterior-posterior distinction might exist. First, although we did not intentionally target the anterior mOFC, the placements in our 2015 study did tend to omit its posterior regions in an attempt to avoid overlap with prelimbic cortex. By contrast, in a recent study Munster and Hauber (2017) used more posterior (and from our inspection of their lesion image [Figure 2], more dorsal) mOFC lesion placements, and were unable to replicate the impairments we observed in outcome devaluation and specific PIT. Second, in her review spanning various rodent studies, Izquierdo (2017) suggested that the anterior and posterior regions of mOFC might be functionally distinct, proposing that unobservable outcome retrieval is restricted to anterior mOFC, whereas delay discounting involves the more posterior regions (Izquierdo, 2017; Figure 3). Finally, in other species and in humans especially, several studies have also suggested the existence of functional distinctions between anterior and posterior OFC (e.g. Kringelbach & Rolls, 2004; Mansouri et al., 2017; Smith et al., 2010). One particularly interesting finding from a meta-analysis of human neuroimaging studies suggests that activity in anterior but not posterior OFC correlates with representations of more complex or abstract reinforcers (Kringelbach & Rolls, 2004). Putting the question of homologies aside for a moment, this function appears to align closely with our proposal that the mOFC is necessary to infer action outcomes from memory.

Based on these findings, therefore, it might be reasonable to expect that the anterior and posterior regions of rodent mOFC carry out functionally distinct roles within dissociable neural circuits. This was the hypothesis investigated in the current study. First, we explored whether there were any observable differences in the density of output pathways from anterior versus posterior mOFC by

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placing retrograde tracers into the basolateral amygdala (BLA), posterior dorsomedial striatum (pDMS), nucleus accumbens core (NAc core), the mediodorsal thalamus (MD), and ventral tegmental area (VTA) and then quantifying the number of retrogradely labelled neurons in each region of the mOFC. We chose these structures because they have all been reported to receive some degree of input from mOFC (Hoover & Vertes, 2011), and they have all been previously identified as critical for various aspects of goal-directed action (see Hart et al., 2014). We next compared the performance of rats with specific excitotoxic lesions of either anterior or posterior mOFC on instrumental tasks in which action outcomes are absent on test, including specific PIT and instrumental outcome devaluation, and a further task for which outcomes are present on test: outcome-selective reinstatement. We predicted that rats with anterior but not posterior mOFC lesions would display deficits in both specific PIT and outcome devaluation, as these tasks require rats to infer absent outcomes, which we hypothesise relies on the anterior mOFC. We further predicted that all rats would demonstrate intact performance on a test of outcome-selective reinstatement, as the outcomes are presented during this test, can be directly recognised and, therefore, do not need to be inferred.

Material and Methods

Our first aim was to establish whether the output pathways of anterior vs. posterior mOFC to NAc core, pDMS, BLA, MD and VTA differed in density. Rats received unilateral injections of the retrograde tracer flurogold (FG) into the pDMS, NAc core, or VTA plus an injection of the retrograde tracer cholera toxin B (CTb) into the NAc core, BLA, MD or VTA. Ten days after surgery, rats were perfused and brains were processed for immunofluorescence identification of retrogradely labelled neurons in the anterior and posterior mOFC (Experiment 1). We then, in Experiment 2, compared the performance of rats with excitotoxic lesions of the anterior or posterior portion of mOFC to a

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control group with sham lesions in various decision-making paradigms: specific Pavlovian-instrumental transfer (specific PIT), outcome devaluation, and outcome-selective reinstatement.

Experiment 1. Comparison of afferents from anterior vs. posterior mOFC to pDMS, NAc core, BLA MD and VTA using retrograde tracing.

Animals

A total of 12 female and 11 male Long-Evans and rats weighing between 270-400g (post-natal day [PND] 120) at the beginning of the experiment were used as subjects. Rats were allowed access to unlimited chow and water throughout the experiment. Rats were housed in groups of 2-4 in transparent yellow-tinted plastic tubs (.5m^3) located in a temperature- and humidity- controlled vivarium. All procedures were approved by the University of New South Wales Ethics Committee and are in accordance with the guidelines set out by the American Psychological Association for the treatment of animals in research.

Surgery

Rats each received one injection of FG (pDMS, NAc core or VTA) and one of CTB into either NAc, BLA, MD or VTA in the contralateral hemisphere. Following surgeries, animals were left in their home cages for 2 weeks prior to brains being processed for immunofluorescence of retrogradely labelled projections from the anterior and posterior parts of the mOFC.

The stereotaxic surgery was conducted under isoflurane anesthesia (5% induction; 1-3% maintenance). Each rat was placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA), after which they received a 0.1ml subcutaneous injection of bupivicane hydrochloride at the incision site. An incision was made into the scalp to expose the skull surface, and the incisor bar was adjusted to place bregma and lambda in the same horizontal plane. Rats received an injection of 100 nl of 3% FG (Fluorochrome) solution diulted in 0.9% saline unilaterally into either the pDMS (in mm relative to bregma, anteroposterior, -0.1, mediolateral, +2.3, dorsoventral, -4.6) the NAc core (in mm

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relative to bregma, anteroposterior, +1.6, mediolateral, + 2.1, dorsoventral, -7.8) or the VTA (in mm relative to bregma, anteroposterior, -5, mediolateral, ±0.7, dorsoventral, -8.8). They then received a unilateral injection of 100nl of 0.1% CTB (List Biological Laboraties, CA) diluted in water into either the NAc or VTA at the same co-ordinates in the opposite hemisphere, or the BLA (in mm relative to bregma, anteroposterior, -2.7, mediolateral, ± 4.9, dorsoventral, -8.9) or the MD (in mm relative to bregma, anteroposterior, -2.9, mediolateral, ± 0.9, dorsoventral, -6). The infusion was conducted at a rate of 50nl/min, and needle was left in place for 2 min prior to removal to allow for diffusion. All rats received a subcutaneous injection of 0.1mL of carprofen and 0.3ml intraperitoneal injection of procaine penicillin solution (300mg/kg).

Tissue preparation and immunofluorescence

Two weeks after surgery, rats were rapidly and deeply anesthetized with sodium pentobarbital and perfused transcardially with cold 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were removed and postfixed in 4% paraformaldehyde overnight, before being sliced on a vibratome (Leica) into 40 µm coronal sections. Two anterior (A/P: +5.2mm) and two posterior (A/P: +4.2mm) mOFC sections from each rat was collected, along with 3 slices from each injection site, to be stained for CTB. Sections were rinsed 3 times for 10 min in 1X PBS, then submerged for 2hr in PBS with 0.5% triton and 10% NHS. Sections were then placed in 400ul of goat-anti-CTB (1:2000, List Biological Laboratories, Product #703) diluted in 0.2% triton and 2% NHS in PBS for 48hr at 4^{o}C. Sections were then rinsed 3 times for 10 min in PBS and then placed in 400uL of donkey-anti-goat Alexa 594 (Invitrogen) secondary at 1:1000 for 2hr in PBS with 0.2% triton and 2% NHS at room temperature. Sections were then rinsed twice more with PBS (10 min) and twice with PB (10 min) and mounted from PB using Fluoromount mounting medium. Locations and extent of injection sites were verified under a confocal microscope (Olympus BX16WI) and imaged using 4x and 10x air objectives.

For quantification of retrograde labelling in the mOFC, a single image was taken of the anterior and posterior mOFC per hemisphere of each slice (8 images in total per rat) on a confocal Microscope

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(Olympus BX16WI) using a 10x air objective. Images were quantified for FG and CTB labeled cells in the mOFC using imaging software (Fiji Cell Counter). The mean number of projections in each hemisphere (for each retrograde target) were averaged across rats and divided by the region area in each image to obtain a mean number of ipsilateral and contralateral projections per mm^2 within each pathway.

Experiment 2. The effect of excitotoxic lesions of anterior vs. posterior mOFC on specific PIT, instrumental outcome devaluation and outcome specific reinstatement.

Animals

A total of 30 Long-Evans rats (15 males and 15 females) weighing between 300-400g (PND 120-150) at the beginning of the experiment, were used as subjects. Each group (sham, ANT, and POST) consisted of 10 rats: 5 males and 5 females. During behavioural training and testing rats were maintained at ~ 85% of their free-feeding body weight by restricting their food intake to 10g of their maintenance diet (chow) per day.

Apparatus

Training and testing took place in 16 identical Med Associates (East Fairfield, VT) operant chambers enclosed in sound- and light-attenuating shells. Each chamber was equipped with a recessed food magazine, located at the base of one end wall, through which 20% sucrose solution (0.2 ml) and food pellets (45 mg; Bio-Serve, Frenchtown, NJ) could be delivered using a syringe pump and pellet dispenser, respectively. An infrared beam crossed the magazine to detect head entries. Each chamber also contained a pair of retractable levers that were located to the left or right of the food magazine. A house light (3 W, 24 V) located on the end wall opposite the magazine provided constant illumination, and an electric fan fixed in the shell enclosure provided background noise (≈70 dB) throughout training and testing. Two microcomputers running the Med-PC program (Med Associates) controlled all experimental events and recorded lever presses and magazine entries. The boxes also contained a white-noise generator, a sonalert that delivered a 3 kHz tone, and a solanoid

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that, when activated, delivered a 5 Hz clicker stimulus. All stimuli were adjusted to 80 dB in the presence of background noise of 60 dB provided by a ventilation fan. Outcome devaluation procedures took place in transparent plastic tubs that were smaller, but otherwise identical to the cages in which rats were housed.

Surgery

Stereotaxic surgery was conducted under isoflurane anesthesia (5% induction; 1-2% maintenance). Each rat was placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA), after which they received a 0.1ml subcutaneous injection of bupivicane hydrochloride at the incision site. An incision was made into the scalp to expose the skull surface, and the incisor bar was adjusted to place bregma and lambda in the same horizontal plane. Rats in Group ANT received lesions at the more anterior co-ordinates, for males: (in mm relative to bregma, anteroposterior, + 5, mediolateral, ± 0.6, dorsoventral, -4.6), and for females: (in mm relative to bregma, anteroposterior, + 5, mediolateral, ± 0.5, dorsoventral, -4.6) mm. Rats in Group POST received lesions at the more posterior co-ordinates, for males: (in mm relative to bregma, anteroposterior: + 3.8, mediolateral, ± 0.7, dorsovental, -5.7) for females: (in mm relative to bregma, anteroposterior: + 3.8, mediolateral, ± 0.6, dorsovental, -5.7). Excitotoxic lesions were made by infusing 0.3 µl of N-methyl-Daspertate (NMDA: 10mg/mL) in sterilised 0.1M phosphate buffered saline (PBS) pH 7.2 over 3 min. The needle was left in place for 2 min prior to removal to allow for diffusion. Sham-operated rats underwent the same procedures but no neurotoxin was infused. Half of the rats in Group SHAM received sham lesions (i.e. identical procedures with no neurotoxin infused) at the anterior coordinates and the other half received sham lesions at the posterior coordinates. All rats received a subcutaneous injection of 0.1mL of carprofen and 0.4ml intraperitoneal injection of procaine penicillin solution (300mg/kg). Rats were given 7 days to recover from surgery, after which they received 3 days of food deprivation prior to the commencement of experimentation. Rats were weighed and handled daily during this time.

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Behavioral procedures

Pavlovian training. For the first 8 days rats were placed in operant chambers for one, 60 min session of Pavlovian training per day, during which they received 2 min presentations of two conditioned stimuli (CS; white noise or clicker) paired with one of two outcomes (pellets or sucrose) on a random time 30 s schedule throughout the CS. Each CS was presented 4 times in pseudorandom order with a variable intertrial interval (ITI) that averaged to 5 min. Half of the rats received the white noise paired with pellets and clicker paired with sucrose solution, and the remaining half received the opposite CS-outcome contingencies. Magazine entries throughout the session were recorded and separated into a CS period and an interval before CS presentations of equal length (Pre-CS; 2 min).

Instrumental training. For the following 8 days, rats were trained to lever press on random ratio schedules of reinforcement. Each session lasted for a maximum of 50 min and consisted of two 10 min sessions on each lever (i.e. 20 min on left lever and 20 min on right lever in total) separated by a 2.5 min time-out period in which the levers were retracted and the house light was turned off. Alternately, if animals earned more than 20 outcomes on one lever during a 10 min session, the lever retracted and house light turned off immediately, and the 2.5 min time-out period began. Thus, rats could earn a maximum of 40 pellets and 40 deliveries of sucrose solution per session. The order of presentation of each lever was pseudorandom. For half of the animals in each group, the left lever earned pellets and the right lever earned the sucrose solution. The remaining animals were trained on the opposite action-outcome contingencies. For the first 2 days lever pressing was continuously reinforced. Rats were shifted to a random ratio (RR)-5 schedule for the next 3 days (i.e. each action delivered an outcome with a probability of .2), then to an RR-10 schedule (or a probability of .1) for the final 3 days.

Pavlovian-instrumental transfer. Following the last day of instrumental training rats were tested for specific Pavlovian-instrumental transfer. Throughout the test session both levers were extended and no outcomes were delivered. Responding on both levers was first extinguished for 8 min to reduce

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baseline performance. Subsequently, each CS was presented four times over the next 40 min in the following order: clicker-noise-noise-clicker-noise-clicker-clicker-noise. Each CS lasted 2 min and had a fixed ITI of 3 min. Magazine entries and lever pressing rates were recorded throughout the session and responses were separated into PreCS and CS periods (2 min each).

Devaluation extinction tests. Subsequent to the Pavlovian-instrumental transfer test rats were given one day of instrumental retraining on RR10 in the manner previously described. On the following day rats were given access *ad libitum* to either the pellets (25g place in a bowl in the devaluation cage) or the sucrose solution (100mL in a drinking bottle fixed to the top of the devaluation cage) for 1 hr. The aim of this prefeeding procedure was to satiate the animal specifically on one of the earned outcomes, thereby reducing its value relative to the alternative outcome (cf. Balleine and Dickinson, 1998). Rats were then placed in the operant chamber for a 10 min choice extinction test. During this test both levers were extended and lever presses recorded, but no outcomes were delivered. The next day a second devaluation test was conducted with the opposite outcome. That is, if rats were previously prefed pellets they now received sucrose, and if rats were previously prefed sucrose, they now received pellets. Rats were then placed back into the operant chambers for a second 10 min choice extinction test.

Outcome-induced reinstatement test. Subsequent to the two devaluation extinction tests, rats received instrumental retraining on an RR-10 schedule for one day. The next day an outcome-selective instrumental reinstatement test was conducted. The test session began with a 15 min period of extinction to lower the rats' rate of responding on both levers. They then received 4 reinstatement trials separated by 7 min each. Each reinstatement trial consisted of a single delivery of either the sucrose solution or the grain pellet. All rats received the same trial order: sucrose, pellet, pellet, sucrose. Responding was measured during the 2 min periods immediately before (Pre) and after (Post) each delivery.

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Tissue preparation for histological analysis

Subsequent to behavioral testing, rats received a lethal dose of sodium pentobarbital (300mg/kg i.p., Virbac Pty. Ltd., Australia). The brains were removed and sectioned coronally at 40µm through the mOFC using a cryostat. Every second section was collected on a slide and stained with cresyl violet. Slides were examined for placement and extent of the lesion: the latter was assessed by microscopically examining sections for areas of marked cell loss as well as general shrinkage of a region relative to controls, by a trained observer unaware of the subjects' group designations using the boundaries defined by the atlas of Paxinos and Watson (2014). Subjects for whom the lesions were too large or small, or the placements inaccurate, were excluded from the statistical analysis.

With regard to the atlas, It is important to note that for our prior study (Bradfield et al., 2015), as well as that of Munster and Hauber (2017), the schematic diagrams of neuroanatomical placements were based on the 4th edition of the rat brain atlas (Paxinos & Watson, 1998). This is relevant, because the posterior part (from 4.6mm to 3.7mm anterior to bregma) of what was considered to be mOFC in 1998 is considered to be anterior cingulate cortex area 32V in the most recent edition of the atlas (A32V; 7th edition, Paxinos & Watson, 2014). Therefore, the region we refer to in the current study as 'posterior mOFC' also encroaches on part of anterior A32V and these are the placements that are most closely in line with those described by Munster and Hauber (2017; as judged by the co-ordinates of lesions in Figures 2-3). By contrast, the area we have specified here to be the anterior mOFC extends from 5.6mm to 4.68mm anterior to bregma, which corresponds to mOFC in the 7th edition atlas, and posterior mOFC as extending from 4.2mm to 3.7mm anterior to bregma. However, we note that there are significant anatomical differences between each edition (for example, in the current addition the mOFC extends to +5.6mm, whereas in the 4th edition it did not extend beyond +5.2mm) hence, direct conversion between editions is somewhat compromised.

Placements in our current study are based on the most recent (2014) version of the atlas and should be highly accurate according to that version. It is further worth noting that because we are

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systematically targeting posterior mOFC for one of our groups, some damage to the ventral prelimbic cortex (PL) was inevitable. Nevertheless, this was expected to have little effect on behaviour; prior studies that have implicated PL in action-outcome encoding have tended to target its more dorsal region (e.g. Corbit & Balleine, 2003; Hart & Balleine, 2016).

Data analyses

All data were analyzed using orthogonal contrasts controlling the per-contrast error rate at $\alpha = .05$ according to the procedure described by Hays (1973). In particular, training data was analysed using contrasts examining linear trends and interactions with day, group, and CS period (where relevant). Animals in Group ANT were found to differ significantly in their acquisition of instrumental conditioning from Groups SHAM and POST (see below). To equate for this difference in responding, therefore, and in line with reporting of our previous data (e.g. Bradfield et al., 2015), outcome devaluation and reinstatement test data were reported both in their raw form and as a percentage of baseline responding, with baseline defined as the average left and right lever presses on the two days of instrumental training immediately preceding the test. One exception to this was Pavlovian-instrumental transfer testing, which was reported as lever presses per minute because the demonstration of transfer depends on a comparison between PreCS responding and responding during 'same' and 'different' stimulus presentations, such that responding during the PreCS provides an 'in-test' measure of baseline responding.

Test data were analysed using orthogonal complex contrasts, first establishing that there were no significant differences between Groups SHAM and POST, then averaging across these groups for comparison to Group ANT. We hypothesised these groups would differ during Pavlovian-instrumental transfer and outcome devaluation tests, but not reinstatement. If this analysis yielded significant interactions it was followed by simple-effects analyses. Data are presented as mean ± SEM and averaged across counterbalanced conditions.

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Results

Experiment 1. Comparison of afferents from anterior vs. posterior mOFC to pDMS, NAc core, BLA and MD using retrograde tracing.

Of the 46 retrograde injections in 23 rats that were conducted, 16 injections were excluded from the analysis due to misplacement or spread of the tracer beyond the boundaries of the target structure. This left 28 injections in 20 rats for the subsequent analysis; 6 in the NAc core, 6 in the pDMS, 6 in the BLA, 4 in the MD and 6 in the VTA. The results of tracing from these injections are shown in Figure 1. The top row (Fig 1A-E) shows examples of retrograde labelling in the anterior mOFC (+5.2mm anterior to bregma) as a result, injections of FG into NAc core (shown in Fig 1F), FG into pDMS (Fig 1G), CTB into BLA (Fig 1H, shown in red, with retrograde FG tracing to the NAc in green shown to identify structural border), CTB into MD (Fig 1I) and CTB into the VTA (Fig 1J). As can be seen in Figures 1 A-E, both the extent and distribution of projection neurons to each region differed substantially. In line with previous reports (Gabbott et al., 2005) projections from the mOFC to the NAc (Fig 1A) arise primarily from layer 5 with sparse ipsilateral projections arising from superficial layer 2, whereas projections from the mOFC to the BLA (Fig 1C) arise throughout layers, with substantial projections from superficial layers. Also consistent with previous reports (Gabbott et al., 2005), projections to the MD (Fig 1D) are dense, and located in layer 6, whereas projections to VTA (Fig 1E) are almost entirely confined to Layer 5 in the ipsilateral hemisphere. As can be seen from Figure 1B, there were very few projections from the mOFC to the pDMS in any layer.

The number of retrogradely-labelled neurons per mm^2 in each region of mOFC (+5.2mm for anterior and +4.2mm for posterior) in the hemisphere that was ipsilateral (blue bars) or contralateral (red bars) to the injection site are shown in the graphs in the bottom row of Figure 1 (Fig1 K-O). As shown in Figure 1K and 1M, projections from the mOFC to the NAc core (Fig 1K) and BLA (Fig 1M) were roughly equally ipsilateral and contralateral, and statistical comparison between ipsilateral and contralateral projections (averaged across anterior and posterior) confirmed that there was no

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significant difference in either mOFC-NAc ($F(1, 4) < 1.0$) or mOFC-BLA ($F(1, 5) < 1.0$). Projections to the MD and VTA (Figure 1N-O) were substantially stronger in the ipsilateral pathway, with very few contralateral mOFC-VTA projections in any rat. Statistical analysis confirmed that there were significantly more ipsilateral than contralateral mOFC-MD projections ($F(1, 3) = 22.2$, $p = 0.02$) and more ipsilateral mOFC-VTA projections ($F(1, 5) = 64.8$, $p < 0.01$). Likewise, the ipsilateral/contralateral pattern of projections was relatively preserved in both anterior and posterior mOFC in all regions. Figure 1L (mOFC-pDMS) shows projections from the mOFC to the pDMS and, as can be seen in Figure 1B, very few projections were observed in either hemisphere, and there were no statistically significant differences in the number of ipsilateral and contralateral projections ($F(1,5) = 1.5$).

There were clear differences in the overall density of labelling (i.e. averaged across ipsi and contralateral) from anterior vs. posterior mOFC to the NAc core (Figure 1K). Specifically, the anterior mOFC (+5.2) appears to project more densely than posterior mOFC (+4.2) to NAc core. Statistical analyses confirmed there were significantly more neurons per mm^2 projecting to the NAc from the anterior vs. posterior mOFC, averaged across ipsilateral and contralateral projections ($F(1, 5) = 17.9$, $p = 0.008$). Although the overall density of outputs from anterior and posterior mOFC to BLA (Figure 1M) appeared to be relatively similar, statistical comparison revealed there were significantly more projections from posterior mOFC-BLA than anterior mOFC-BLA (Means: Anterior mOFC Ipsi = 61.7, Contra = 60.9; Posterior mOFC Ipsi = 69.1, Contra = 63.4; $F(1, 5) = 62.8$, $p = 0.001$). There were no significant differences between density of projections between anterior and posterior mOFC to the MD ($F(1, 3) = 1.6$; just ipsilateral projections $F(1, 3) = 1.3$), or to the VTA ($F(1, 5) = 3.6$, just ipsilateral projections $F(1. 5) = 4.9$).

Together, these results suggest that the circuitry of anterior and posterior mOFC is similar but at least somewhat dissociable with respect to the density of projections, particularly to the NAc core and the BLA. Previous studies have shown that both of these structures are critical to goal-directed

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action, with the NAC core particularly important for performance following outcome devaluation and motivated action selection (Corbit et al., 2001; Ostlund & Balleine, 2008; Parkes & Balleine, 2013). Therefore, we suggest that the anterior mOFC and posterior mOFC differentially regulate performance in these tasks, and this is the hypothesis we tested in our subsequent study assessing the function of these mOFC subregions.

Experiment 2. The effect of excitotoxic lesions of anterior vs. posterior mOFC on specific PIT, instrumental outcome devaluation and outcome specific reinstatement.

Retrograde tracing revealed a greater density of projections from anterior mOFC relative to posterior mOFC to two regions that are critical to goal-directed action: the NAc core, and (to a lesser extent) the BLA. Based on these findings, the prediction that anterior mOFC is also more functionally relevant for goal-directed action than posterior mOFC was tested by comparing the performance of three naïve groups of rats: one with sham mOFC lesions (half anterior, half posterior, Group SHAM), one with anterior mOFC lesions (Group ANT), and one with posterior mOFC lesions (Group POST). It should also be noted that the animals in Experiment 1 were not food restricted, whereas animals used in behavioural studies were. Thus, it is possible that the anterior versus posterior differences observed in projection strength was affected by food restriction in Experiment 2, which could affect the results, although we find it unlikely that a stable measure of anatomical connectivity would be influenced by food restriction and are unaware of any published reports of food restriction altering anatomical connections. Tests included specific PIT, outcome devaluation, and outcome-selective reinstatement.

Histology

Figures 2D and 2G show representations of each overlapping placement of anterior mOFC (Group ANT, Figure 2D) and posterior mOFC (Group POST, Figure 2G). Figures 2A-C and 2G shows a sham

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anterior mOFC photomicrograph at +5.64mm from bregma, and and Figures 2B-C show excitotoxic lesions of anterior mOFC at +6.12mm and +5.64mm from bregma, respectively. Figure 2E shows a sham posterior mOFC micrograph at +3.72mm from bregma and Figure 2F shows an excitotoxic posterior mOFC lesion at +3.72mm from bregma. Lesions produced substantial cell loss which was restricted to within approximately a 1mm radius of the injection site. Seven rats were excluded because of incorrect lesion placement or size, and one rat was excluded for failing to acquire baseline levels of lever pressing responding, yielding the following group sizes: Group SHAM, n = 9, Group POST, n = 7, and Group ANT, n = 6. Therefore, a total of 22 rats were included in the experiment.

Behavioral results

Pavlovian conditioning: Figure 3B shows mean (±SEM) magazine entries for each group during acquisition of Pavlovian conditioning, averaged across stimuli. All rats acquired Pavlovian conditioning and acquisition did not differ between groups. There was a linear trend over days, $F(1,19) = 86.871$, $p = .00$, and this did not interact with group, $F(2,19) = .23$, $p = .797$. There was, however, a linear x CS Period (preCS vs. CS) interaction, $F(1,19) = 266.693$, $p = .00$, although this did not interact with group $F < 1$ (i.e. there was no linear x CS period x group interaction), suggesting that all groups increased responding linearly over days, specifically during the CS period relative to the preCS period.

Instrumental conditioning: Figure 3C shows mean (±SEM) lever presses for each group averaged across levers during the acquisition of Instrumental conditioning. Rats in Groups SHAM and POST responded at higher levels throughout instrumenal training than rats in Group ANT. There was no group main effect of SHAM versus POST, $F < 1$, but there was a group main effect of SHAM/POST (averaged) versus ANT, $F(1, 19) = 6.396$, $p = .02$. However, all groups including Group ANT did acquire instrumental responding, as there was a linear trend over days $F(1,19) = 42.582$, $p = .00$, and this did not interact with group, $F(1,19) = 2.624$, $p = .099$.

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Pavlovian-instrumental transfer test:

Predictions: Animals trained as above will typically demonstrate specific PIT when tested in the presence of both stimuli and levers together for the first time (design in Figure 2A). That is, the presentation of a stimulus will evoke responding on the lever associated with the same outcome as the stimulus. Importantly, because outcomes are not delivered on this test, animals must rely on their ability to infer the absent pellet and sucrose outcomes associated with their actions to perform this task appropriately. It was predicted, therefore, that animals in Group ANT (i.e. with anterior mOFC lesions) but not Groups SHAM or POST (posterior mOFC lesions) would display impaired performance on this task.

Results: Figure 3D shows mean (±SEM) lever presses for each group during the Pavlovian-instrumental transfer test. All groups responded more during CS presentations than during preCS baseline periods. There was a main effect of CS period, $F(1,19) = 97.708$, $p = .00$, and this did not interact with group, $F < 1$, indicating that all rats responded more during the CS presentations than during the 2 min preCS periods (and indeed the simple effects for all groups are significant, $p < .001$). However, although Groups SHAM and POST both demonstrated a robust transfer effect, selectively increasing responding on the lever that had been paired with the same outcome as each of the stimuli during training, Group ANT responded non-selectively, elevating performance on both levers relative to baseline. There was a main effect of CS identity ('same' versus 'different'), $F(1,19) = 26.920$, and although this did not interact with the SHAM vs. POST comparison, $F(1,19) < 1$, it did interact with the comparison between SHAM/POST (averaged) versus ANT, $F(1,19) = 6.224$, $p = .022$. This is supported by significant simple effects (same > different) for Groups SHAM, $F(1,19) = 23.552$, $p = .00$, and POST, $F(1,19) = 14.186$, $p = .001$, but not for Group ANT (same = different), $F(1,19) < 1$. Further, this is not likely to be a floor effect, because animals in all groups responded more during CS presentations than during the preCS periods (as evidenced by a significant main effect of CS period that did not interact with group). Rather, ANT mOFC lesions affected the selectivity of responding;

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i.e., animals in Groups SHAM and POST responded selectively (same > different) whereas animals in Group ANT responded equally on both levers (same = different).

Outcome Devaluation test:

Predictions. These same rats were subsequently given an outcome devaluation test during which they were first fed to satiety on one of the two outcomes to reduce its value, and then given a test in which they could choose between the lever associated with the still-valued outcome and that associated with the devalued outcome (design in Figure 4A). Typically, rats preferentially respond on the valued relative to the devalued lever. Again, because pellet and sucrose outcomes are not actually delivered on this test, devaluation performance relies on the rats' ability to infer the absent pellet and sucrose outcomes associated with their actions. As such, we again predicted that animals in Group ANT, but not Groups SHAM or POST, would be impaired on this task.

Results. Figure 4B shows mean presses (±SEM) lever presses during the outcome devaluation test, Figures 4D, E, and F, show the mean number of presses (±SEM) for Groups SHAM, POST, and ANT, respectively, plotted over the minutes of test, and Figure 4C shows the same data as a percentage of baseline responding. It is clear from these figures that, although groups SHAM and POST demonstrated robust devaluation effects, selectively responding on the valued relative to the devalued lever, group ANT did not and instead responded equally on both levers.

For the raw data (Figure 4B) a planned complex contrast revealed that Groups SHAM and POST (averaged) responded more than Group ANT, $F(1,19) = 7.52$, $p = .013$, but did not differ from each other, $F(1,19) = .255$, $p = .62$. Moreover, there was a main effect of devaluation, $F(1,19) = 20.98$, $p = .00$, which did not interact with the SHAM vs. POST comparison, $F < 1$, but did interact with the SHAM/POST vs. ANT contrast, $F(1,19) = 12.82$, $p = .002$. Follow-up simple effects analyses revealed a significant devaluation effect (valued > devalued) in Groups SHAM, $F(1,19) = 19.84$, $p = .00$, and POST, $F(1,19) = 18.84$, $p = .005$, but not Group ANT, $F < 1$.

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However, the fact that groups SHAM and POST demonstrated more overall responding than group ANT raises the possibility that the failure to show selective responding on test might have been due to a floor effect. We do not believe this to be the case for several reasons. First, t-tests comparing responding on the valued vs. devalued levers in each group during the first 2 mins of each test (averaged across tests), i.e., when responding was highest for each group, found significantly more responding on the valued than the devalued lever in Groups SHAM ($p = .039$, see Figure 4D) and POST ($p = .006$, See Figure 4E) but no difference in responding in Group ANT ($p = .3$, see Figure 4F). This suggests that even when responding was at its highest and so above floor no devaluation effect was observed in Group ANT.

Second, when responding on test was calculated as a percentage of baseline responding (Figure 4C), to remove group differences in overall responding the selectivity of responding in Groups SHAM and POST (valued > devalued) and the lack of selectivity in Group ANT (valued = devalued) remained. Specifically, there was no longer a significant difference in overall responding between Groups SHAM/POST (averaged) versus Group ANT, $F < 1$. However, there was a main effect of devaluation, $F (1,19) = 14.574$, $p = .001$, and this did not interact with the SHAM vs. POST comparison, $F < 1$, but did interact with the comparison (i.e. complex contrast) between SHAM/POST (averaged) versus ANT, $F (1,19) = 10.084$, $p = .005$. This is supported by significant simple effects (valued > devalued) for Groups SHAM, $F (1,19) = 19.572$, $p = .00$, and POST, $F (1,19) = 9.94$, $p = .005$, but not Group ANT, $F < 1$. Thus, the selectivity of responding was impaired in Group ANT even when baseline differences in responding were standardised across groups. Together, these results suggest that the anterior but not posterior mOFC plays a selective role in outcome devaluation.

Outcome-Selective Reinstatement test:

Predictions. In the test of outcome-selective reinstatement, animals were first given a period of extinction on both levers after which each outcome was presented twice with a 7 min interval between presentations (see design in Figure 5A). This typically reinstates responding on the lever

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associated with the presented outcome during training. Unlike specific PIT and outcome devaluation, reinstatement performance does not depend on inferring absent outcomes because they are actually delivered. We predicted, therefore, that performance would be intact for all animals.

Results. Figure 5B shows mean presses (±SEM), and Figure 5C shows the percent baseline (±SEM) lever presses for each group during the reinstatement test. It is clear from these figures that outcome delivery produced selective reinstatement in all groups, and that there were no differences in this effect. For Figure 5B, there were no group differences in overall responding, $Fs < 1.8$, $p > .2$. Rats in all groups did respond more in the 2 mins following outcome delivery relative to the 2 mins prior to it; there was a main effect of outcome delivery, $F(1,19) = 5.52$, $p = .03$, that did not interact with any group comparisons, $Fs < 1$. There was also a main effect of reinstatement (reinstated > nonreinstated lever), $F(1,19) = 14.644$, $p = .001$, that did not interact with group, $F < 1$.

Likewise, for the percent baseline data (Figure 5C) there were no group differences in overall responding, $Fs < 1.1$, $p > .3$. All groups responded more post-outcome delivery (relatively to the 2 mins prior); again, there was a main effect of outcome delivery, $F(1,19) = 5.1$, $p = .036$, and this did not interact with any group comparisons, $Fs < 1$. There was also a main effect of reinstatement (reinstated > nonreinstated lever), $F(1,19) = 25.69$ $p = .00$, that did not interact with group, $F < 1$.

Discussion

Taken together, the current findings demonstrate that the anterior and posterior subregions of the rodent mOFC can be dissociated both with regard to the density of their projections to specific target regions and with regard to their functions. First, we used retrograde tracing to assess the density of projections from anterior and posterior mOFC to the BLA, pDMS, NAc core, MD and VTA and found that, whereas projections to DMS, MD and VTA were relatively similar across anterior and posterior mOFC, anterior mOFC had a higher density of projections to the NAc core, whereas

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posterior mOFC had a slightly higher density of projections to the BLA. We then demonstrated that anterior but not posterior mOFC lesions impaired performance in specific PIT and instrumental outcome devaluation tests. In contrast, in the outcome-selective reinstatement test, during which outcomes were presented, performance was intact in all groups regardless of lesion placement. Thus, the performance of rats with anterior mOFC lesions in the current study directly replicates our prior findings (Bradfield et al., 2015), suggesting that it was damage to the anterior mOFC in particular that produced these previously observed effects and that damage to the posterior mOFC played no role. Indeed, the performance of Group POST did not differ from Group SHAM at any stage of any task. Importantly, this replication of our prior results provides strong evidence of the robust nature of these effects and for the role of the mOFC in inferring action-dependent outcome representations when these outcomes are absent or unobservable.

Although current results cannot speak to the function of posterior mOFC, the findings of Munster and Hauber (2017) suggested that it might regulate effort-related responding, and this is consistent with the conclusions of a recent review (Izquierdo, 2017) which argued for a possible role of posterior mOFC in delay discounting (based on the findings of Stopper et al, 2014). Finally, it is worth noting that although some of our posterior mOFC placements encroached on ventral prelimbic cortex (PL), the lack of effect on outcome devaluation dissociates our current findings from the abolition of outcome devaluation that is typically observed as a result of PL lesions (e.g. Corbit & Balleine, 2003; Hart et al., 2018), although those effects have been argued to involve dorsal rather than ventral PL. Nevertheless, the current study clearly demonstrates that the posterior mOFC is functionally distinct from both anterior mOFC and the PL, and that it is anterior mOFC in particular, that functions to infer the outcomes of goal-directed actions when they are unobservable.

One difference between the specific anterior mOFC lesions employed in the current study and the more general mOFC lesions employed in our previous study was, however, the depression in lever press acquisition observed in group ANT relative to groups SHAM and POST (Figure 3C). We

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suggest that this difference is likely the result of more focussed and pervasive damage to the anterior region in the current study which, given its functional importance, could have produced a more complete deficit in the ability of rats to generate outcomes from memory to guide responding. More specifically, in our prior study (Bradfield et al., 2015), the central point of our mOFC lesions was approximately +5.1mm from bregma, whereas the central point of lesions in the current study was located approximately +5.6mm from bregma. We suggest, therefore, that more of the neurons involved in inferring unobservable outcomes were affected by the lesions in the current study. From the perspective of the current hypothesis, therefore, because the outcome was delivered during acquisition, the performance of goal-directed actions in sham rats could have been guided both by feedback from outcome delivery and by their ability to retrieve or infer the outcome in its absence. In contrast, animals in Group ANT in the current study would have had to rely on direct feedback alone and to a greater extent than animals with more general mOFC lesions in our prior study. This is supported by the fact that group differences in responding during acquisition became more apparent over days as the ratio requirement increased, which in turn markedly increased the amount of time in which rats experienced the absence of the sucrose/pellet outcomes and their reliance on their ability to retrieve or infer the outcome to guide responding.

Nevertheless, despite the reduction in performance during acquisition in Group ANT, animals in this group showed intact outcome-selective reinstatement indicating that, although they were able to encode the association between the and action and outcome during training, they were only able to express this knowledge when tested in the presence of the outcomes. The fact that reinstatement was intact for Group ANT, coupled with the fact that Group ANT showed impaired selectivity of responding in specific PIT (even though overall levels of responding between groups was equivalent) and showed impaired selectivity of responding during the devaluation test (even during the first two minutes of the test when responding was highest and when baseline differences in responding were accounted for) is consistent with the conclusion that animals in Group ANT suffered a specific deficit in the ability to infer unobservable action outcomes, rather than a more

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general deficit in instrumental learning or performance.

It is of some interest that, although the anterior mOFC projects more heavily than posterior mOFC to NAC core (Figure 1K), the posterior mOFC appears to project more heavily to BLA. The heavier anterior mOFC→NAC core projections are not particularly surprising, given the functional role of anterior but not posterior mOFC in outcome devaluation (Figure 4), a task that is also mediated by NAC core (Corbit et al., 2003). It is possible, therefore, perhaps even likely, that the anterior mOFC → NACcore projection mediates outcome devaluation. However, the circuit that mediates the anterior but not posterior mOFC's role in specific PIT is less clear. The NAC core appears to play no role in specific PIT (Corbit et al, 2011), whereas, as Corbit et al (2001) also found, the NAC shell does, as does the BLA and MD (Ostlund & Balleine, 2008). Although the anterior and posterior mOFC projections to MD do not differ significantly and posterior mOFC was found to project more densely to BLA than anterior mOFC, the projections to the shell were not assessed here. Although the density of the projection may not reflect its functional significance, it is possible that efferent projections to NAC shell are critical for the effects observed here and it will be important to assess this possiblity in future studies.

The current results are also consistent with the finding that, in humans, it is the anterior part of the mOFC that is particularly critical for the representation of abstract outcomes (Kringelbach & Rolls, 2004). This implication of a functional homology supports findings published earlier this year suggesting that close, circuit-based, homologies of various OFC subregions exist between rats and primates (Heilbronner et al., 2017). Heilbronner et al., further argued that because homologies between the primate-human OFC are non-controversial, that rat OFC may also provide a useful homologue of human OFC. The current findings add to this argument, suggesting that there is a further functional homology with human mOFC, at least in the most anterior regions, which appears to support the representation of abstract or unobservable outcome representations in both species. This is significant because OFC dysfunction is heavily implicated across a number of psychiatric

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disorders including drug addiction (e.g. Volkow et al, 1991; Volkow et al, 1999) and obsessive-compulsive disorder (OCD: e.g. Bradfield et al., 2017; Graybiel & Rauch, 2000; Maia et al., 2008). With regards to OCD in particular, we have argued (Bradfield et al., 2017) that the abnormally high neural activity in mOFC observed in this disorder might result in the continual retrieval of action outcome representations and so the compulsive actions associative with the disorder. If correct, then rodent studies may become a powerful tool for revealing the underlying neurophysiological mechanisms of OCD and related psychiatric disorders and how to treat them.

Conclusions

Together with the findings of our previous study (Bradfield et al., 2015) and those of Munster and Hauber (2017), the current findings suggest that it is the anterior portion of the mOFC that is critical for animals to infer action-dependent outcomes when they are unobservable, whereas the posterior mOFC subserves a distinct function, perhaps related to response effort. Overall, these findings add to a growing trend within the literature of producing more specificity and consistency with regards to the neuroanatomy of the various functions of OFC subregions. Such specificity will be of critical importance when interpreting findings from future rodent studies of the OFC as well as from studies of homologous regions in primates and humans.

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Figure Captions

Figure 1. Retrograde tracing in anterior versus posterior mOFC. A-E) Retrogradely labelled neurons in anterior mOFC as a result of: FG injection into NAc core (A; inset shows imaged region in mOFC), FG injection into pDMS (B), CTB injection into BLA (C), CTB injection into MD (D), and CTB injection into VTA (E); scale bars = 300um. F-J show injection sites of: FG injection into NAc core (F; scale = 1mm), FG injection into pDMS (G; scale = 1mm), CTB injection into BLA (H; scale = 300um), CTB injection into MD (I; scale = 1mm) and CTB injection into VTA (J; scale = 1mm). I-L) Number of retrogradely-labelled neurons per mm^2 in ipsilateral and contralateral hemispheres of anterior and posterior mOFC as a result of injections into K) NAc core, L) pDMS, M) BLA, N) MD and O) VTA, scale bars represent 1 SEM. Abbreviations: FG = fluorogold, CTB = cholera toxin B, mOFC = medial orbitofrontal cortex, NAc core = nucleus accumbens core, pDMS = posterior dorsomedial striatum, BLA = basolateral amygdala, MD = mediodorsal thalamus, VTA = ventral tegmental area.

Figure 2. Lesion placements in anterior versus posterior mOFC. A-C) Photomicrographs of a sham anterior mOFC lesions (at +5.64mm) B-C) an excitotoxic anterior mOFC lesion at +6.12mm (B) and +5.64mm (C) from bregma. E-F) photomicrogrphas of a sham posterior mOFC lesion at +3.72mm (E), and an excitotoxic posterior mOFC lesion at +3.72mm (F). D & G) Representation of lesion placements, showing overlapping placements for anterior mOFC in coronal sections, mm from bregma, (D) and posterior mOFC (G).

Figure 3. Pavlovian-instrumental transfer. A) Specific Pavlovian-instrumental transfer design. B) Mean magazine entries per minute (± SEM) during acquisition of Palvovian associations, C) Mean lever presses per min (± SEM) during acquisition of instrumental contingenices, D) Mean lever presses per min (± SEM) during the specific Pavlovian-Instrumental transfer test. S1 = stimulus 1, S2 = stimulus 2, O1 = outcome 1, outcome 2 = O2, R1 = response 1, R2 = response 2.

Figure 4. Outcome devaluation. A) Outcome devaluation design. B) Mean presses per min (± SEM) during outcome devaluation testing, C) Mean percentage of baseline responding (± SEM) during outcome devaluation testing, D-F) Mean presses (± SEM) during outcome devaluation testing shown over 1 minute bins for Groups SHAM (D), POST (E), and ANT (F). O1 = outcome 1, outcome 2 = O2, R1 = response 1, R2 = response 2.

Figure 5. Outcome-selective reinstatement. A) Outcome-selective reinstatement design. B) Mean presses (± SEM) during reinstatement, C) Mean percentage of baseline responding (± SEM) during outcome-selective reinstatement testing. O1 = outcome 1, outcome 2 = O2, R1 = response 1, R2 = response 2.

Figure1

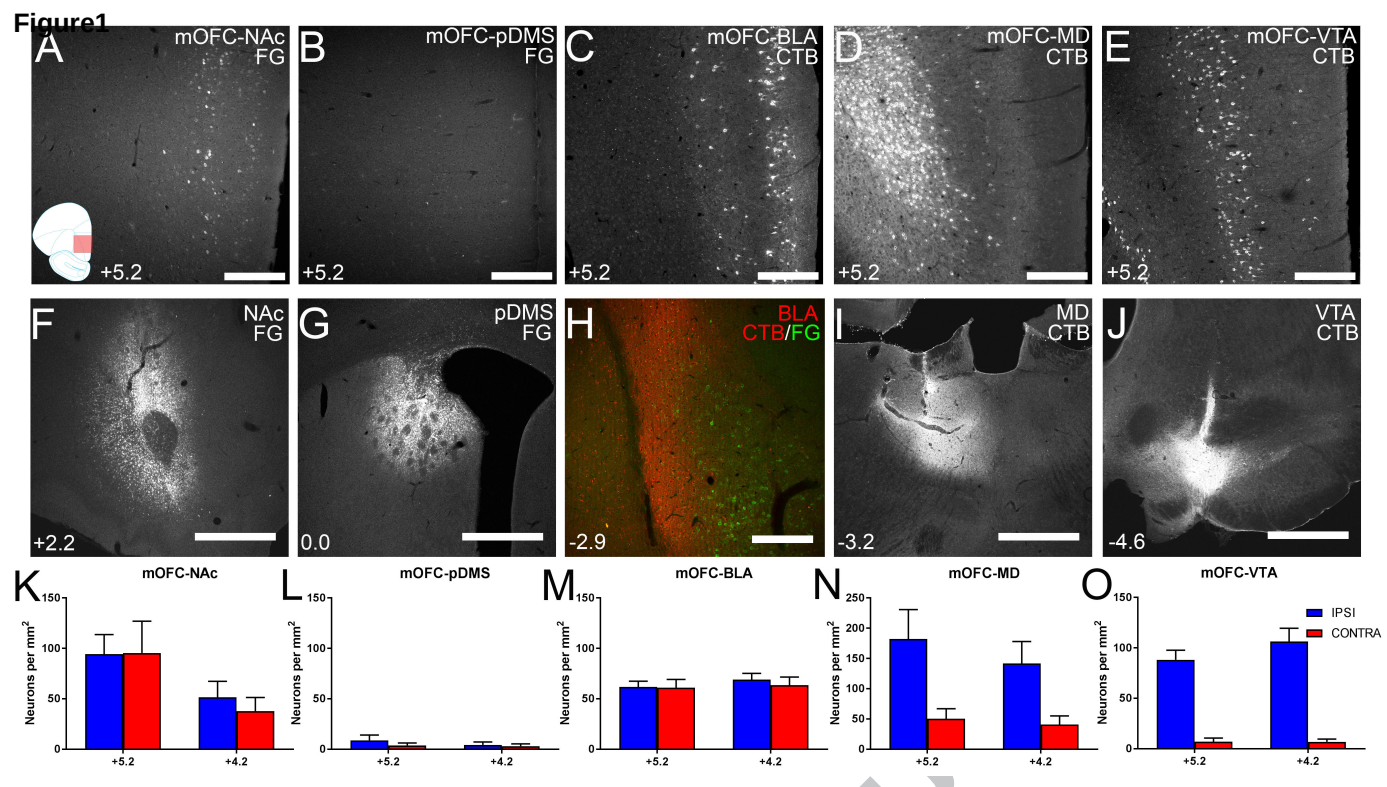
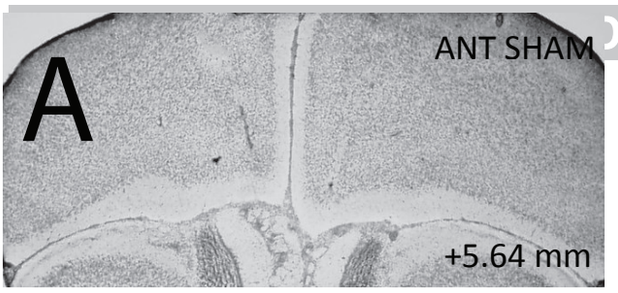
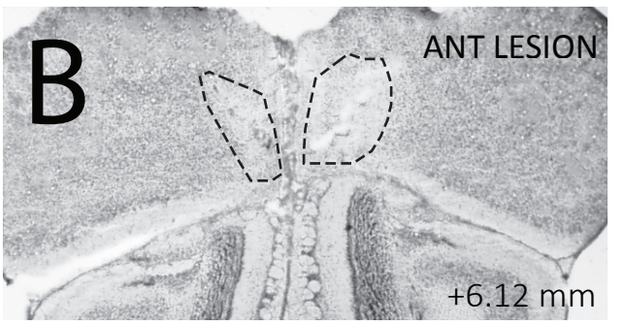
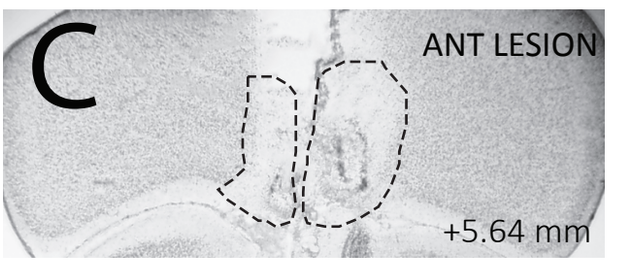

A
mOFC-NAc
FG
+5.2
B
mOFC-pDMS
FG
+5.2
C
mOFC-BLA
CTB
+5.2
D
mOFC-MD
CTB
+5.2
E
mOFC-VTA
CTB
+5.2
F
NAc
FG
+2.2
G
pDMS
FG
0.0
H
BLA
CTB/FG
-2.9
I
MD
CTB
-3.2
J
VTA
CTB
-4.6
K
mOFC-NAc
L
mOFC-pDMS
M
mOFC-BLA
N
mOFC-MD
O
mOFC-VTA
Neurons per mm2
+5.2
+4.2
IPSI
CONTRA

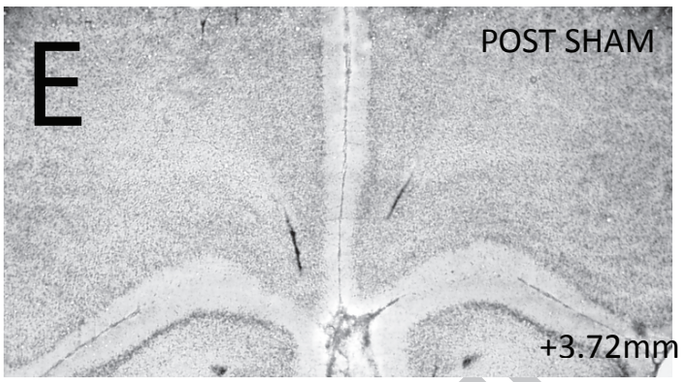
Figure2

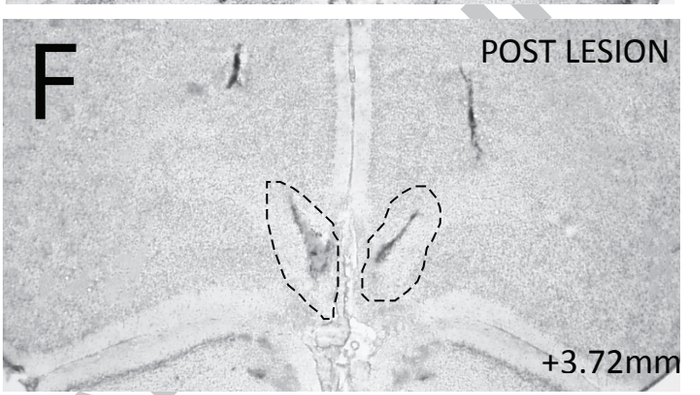
D MANUSCRIPT

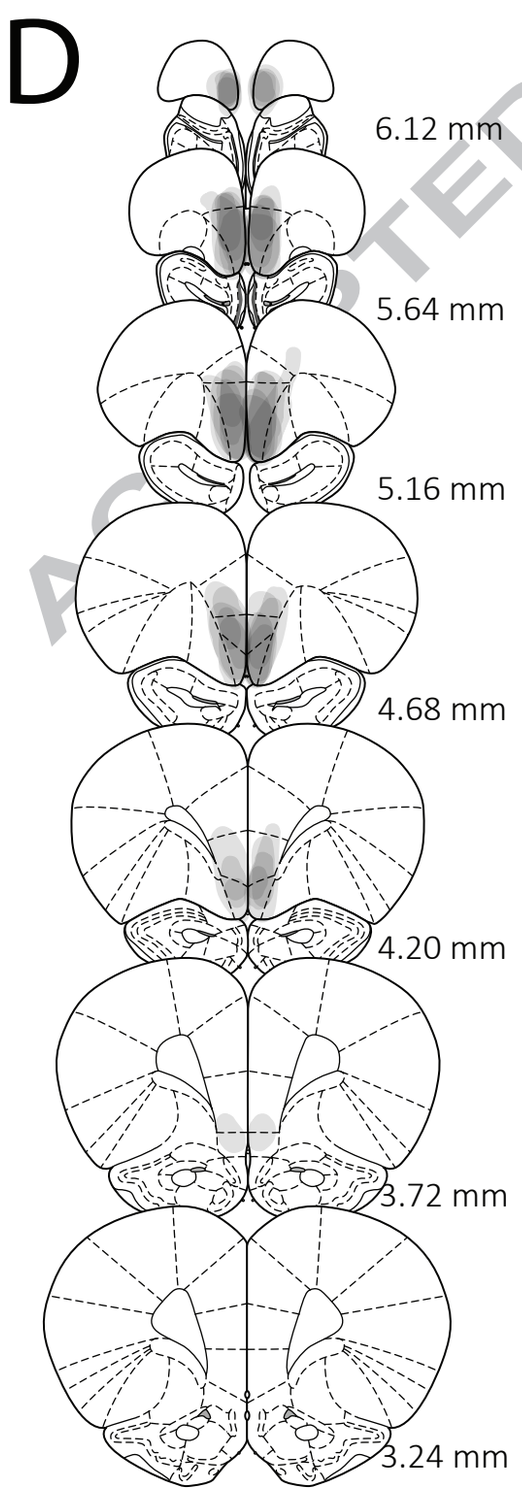


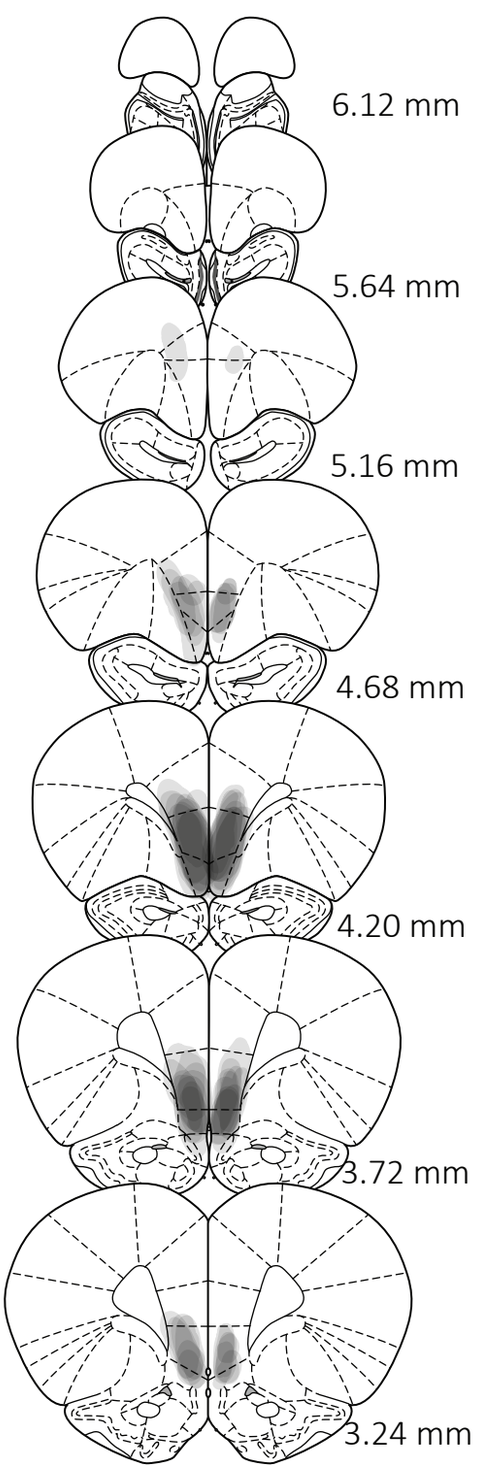












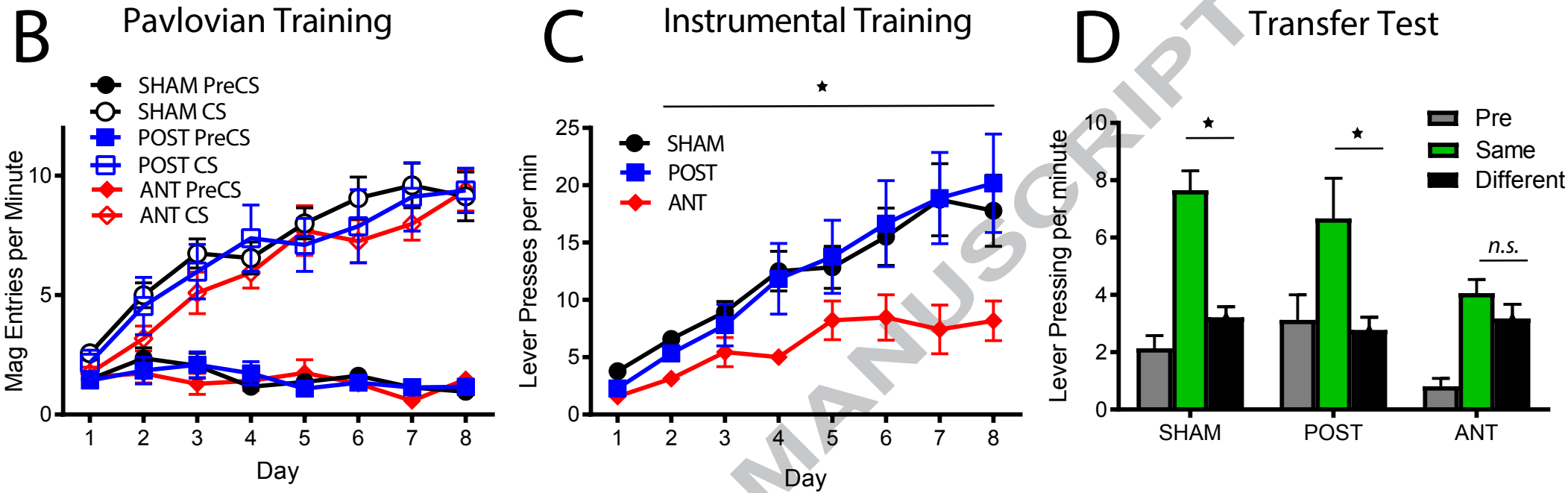
ACCEPTED MANUSCRIPT

Figure3

A

Pavlovian-instrumental transfer

Pavlovian	Instrumental	Transfer
S1 - O1 S2 - O2	R1 - O1 R2 - O2	S1: R1 vs. R2 S2: R2 vs. R1



ACCEPTED MANUSCRIPT

Figure4

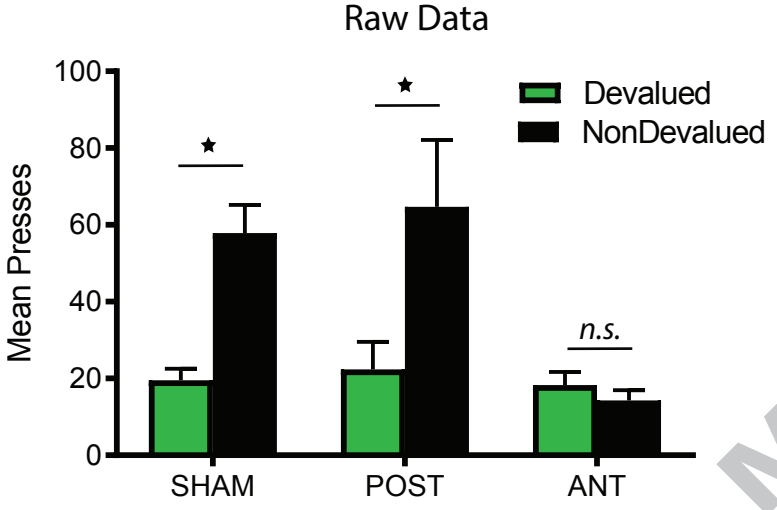
ACCEPTED MANUSCRIPT

A

Outcome Devaluation

Instrumental	Devaluation	Test
R1 - O1 R2 - O2	O1 (1 hr)	R1 vs. R2

B



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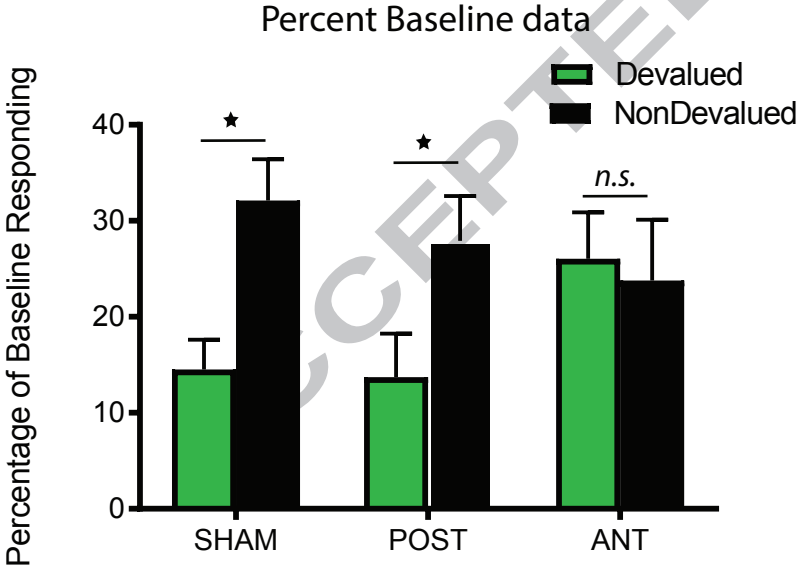

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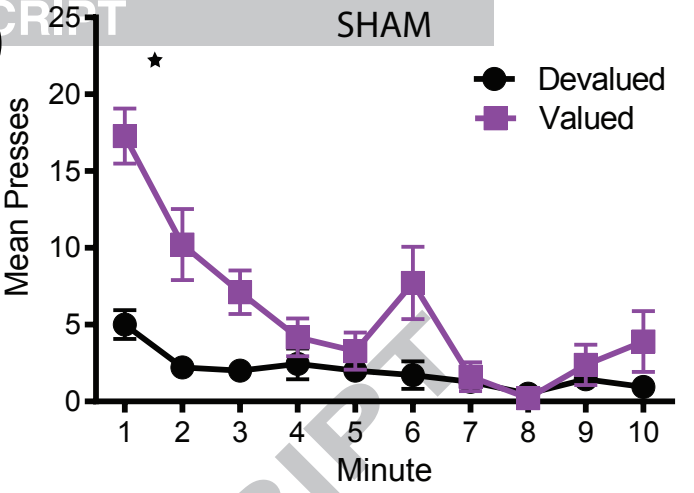

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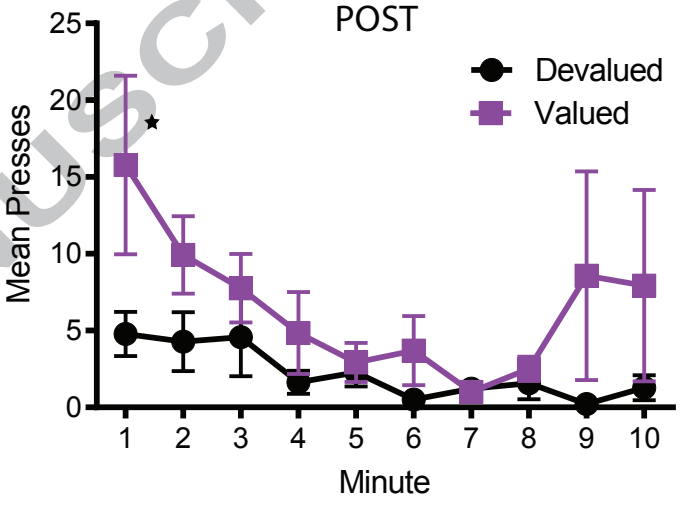

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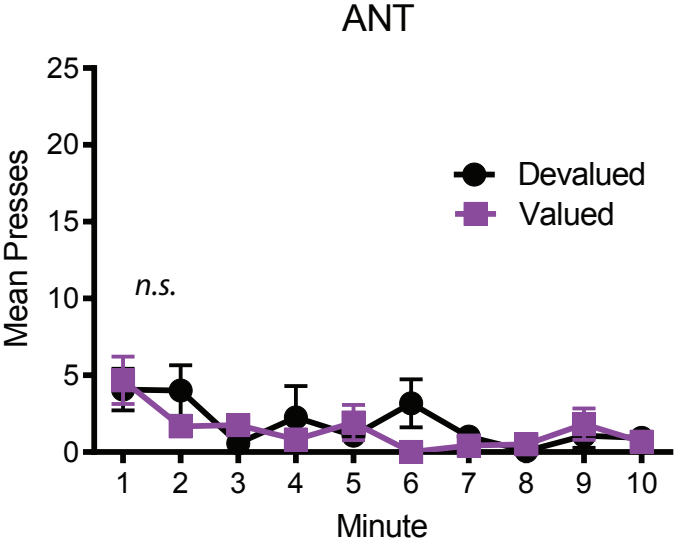


Figure 5

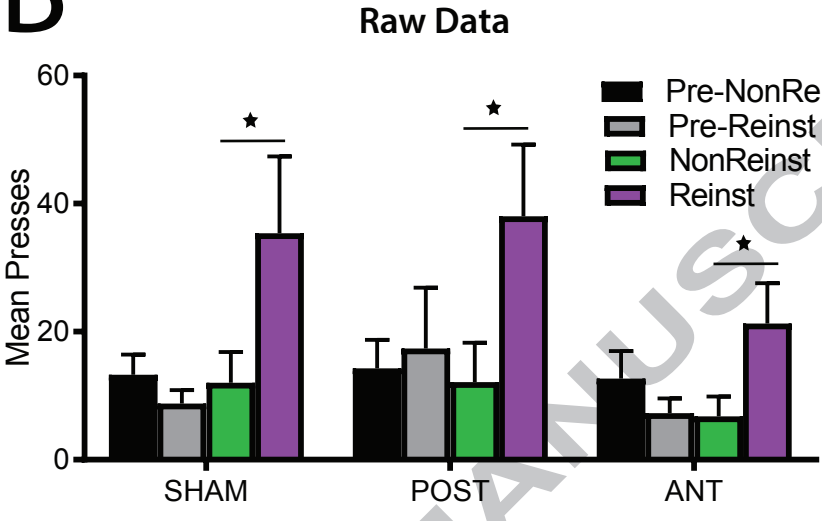
ACCEPTED MANUSCRIPT

A

Reinstatement

Instrumental	Extinction	Test
R1 - O1 R2 - O2	R1 - R2 -	O1: R1 vs. R2 O2: R1 vs. R2

B



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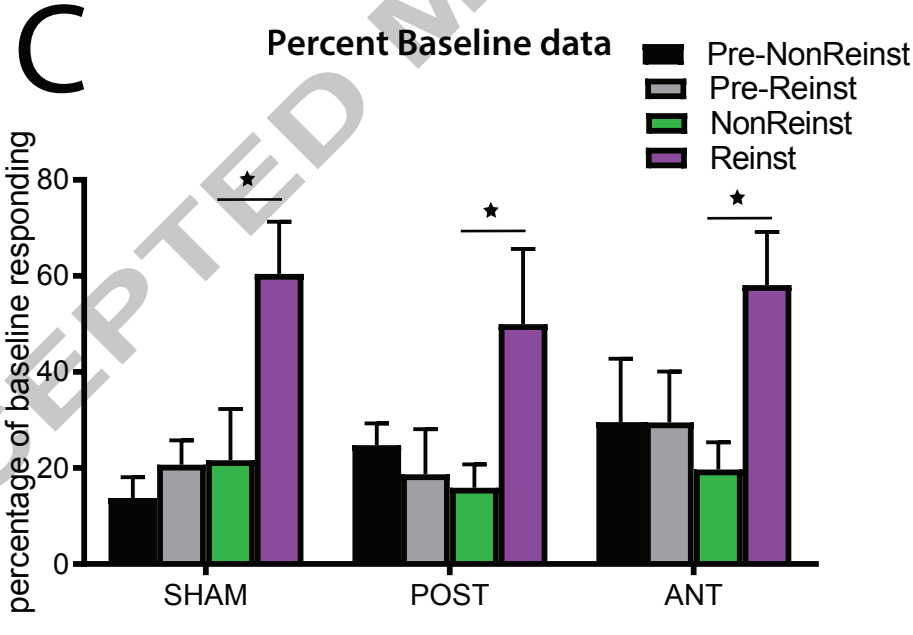

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Highlights

- The medial orbitofrontal cortex (mOFC) of the rat is functionally heterogeneous
- Anterior vs. posterior mOFC has stronger connections with the accumbens core
- Anterior vs. posterior mOFC is critical for inferring unobservable action outcomes
- Anterior vs. posterior mOFC is more directly involved in goal-directed action

ACCEPTED MANUSCRIPT