OFC Acquisition Stats

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**Acquisition**

In contrast to numerous reports that OFC lesions do not affect acquisition learning, OFC lesions significantly increased responding to the predictive cue relative to sham control animals (Figure 1A; lesions depicted in Figure 1-supplementary figure 1). Analysis of conditioned responding was conducted as a CS-PreCS difference score such that levels of responding reflected discriminative cue (CS) driven performance above baseline (PreCS). Acquisition of responding to the CS was significantly greater in the lesion group than the sham group (main effect of Group , , Block , , and Group x Block interaction , ). Follow up comparisons on each block revealed that responding in the lesion group was significantly higher than the sham group during the last 4 blocks (Block 1 , , Block 2 , , Block 3 , , Block 4 , , Block 5 , , Block 6 , , Block 7 , ). Given the ubiquity of non-significant effects of OFC lesions on acquisition learning in the literature, two independent replications of this novel effect were conducted (combined here; same pattern of statistical significance in both independent replications) to confirm the effect was robust.

**Locomotor activity**

The enhanced responding observed during the Pavlovian conditioning procedure in the OFC lesion group could simply reflect an enhancement of general locomotor activity, however locomotor activity (Figure 1C) did not differ between groups (main effect of Time , , but no significant effect of Group , , or Group x TimeBin interaction , ). Therefore the enhanced responding during acquisition is not simply due to OFC lesions inducing hyperactivity.

**Satiety**

To test whether the enhanced responding following OFC lesions was sensitive to levels of hunger or shifts in motivation, a subgroup of animals (subgroup 1) was tested when sated (Figure 1B). General satiety, following 24 hours access to home-cage food, did not affect the rate of responding in the sham group (Sham: Satiety vs Hungry , ) but significantly suppressed responding in the lesion group (Lesion: Satiety vs Hungry , )compared to subsequent testing 24 hours later when hungry again (no significant main effect of Group , , but a significant main effect of Hunger , , and Group x Hunger interaction , ). Since the satiety test session was rewarded, it is possible that OFC lesioned animals could learn that the reward was less valuable by direct experience with the reward, similar to incentive learning effects normally observed in instrumental conditioning (*REFS Balleine and Dickinson Chapter*). However, this possibility is unlikely as responding was comparable between groups on the first trial of the satiety test (, , Figure 1-figure supplement 2), before the reward was delivered. This suggests that animals with OFC lesions are sensitive to shifts in hunger motivation, and that the enhanced levels of responding observed when tested hungry are driven by an increased motivational control of magazine approach behaviour.

**Devaluation Test**

Traditionally, OFC lesions have been shown to cause deficits in outcome devaluation (*Refs*). Therefore, to test whether the present lesion manipulation was comparable to other reports we tested a subgroup of animals (subgroup 2) on Pavlovian outcome devaluation. First the sham and lesion animals were given novel acquisition training of two novel and unique cue-outcome relationship (Figure 1-figure supplement 3A). A specific taste aversion was then established by pairing consumption of one of the outcomes with illness (i.p. injection of lithium chloride; Devalued), and the value of the other outcome was left intact (Non-Devalued). Both groups learned the novel cue-outcome associations and acquired the specific taste aversion (Figure 1-figure supplement 3B).

Finally, during a devaluation test (Figure 1D), the two cues were presented in extinction. The sham group showed a significant devaluation effect, i.e. responding was lower to the devalued than non-devalued cue (, ). In contrast, the devaluation effect was abolished in the lesion group, and responding remained high to both the devalued and non-devalued cue (, ; Significant Group x Cue interaction , , but no main effect of Group , , or Cue , ). This finding successfully replicates the finding that both non-specific and focal OFC lesions abolish the outcome devaluation effect in rodents [REFS – Schoenbaum Series; Elife Panayi/Killcross].

**Figure 1 - Supplemental figure 3**

(**A**) All animals acquired responding to the novel cue-outcome pairs, and response levels were similar between cues (main effect of Block , , all remaining effects *F* < 0.98, *p* > .343). While responding in the lesion group was numerically higher, there was insufficient statistical evidence to support this difference. (**B**) All animals acquired a significant taste aversion to the outcome paired with post-consumption LiCl injections (significant Injection x Pairing interaction , , all remaining effects *F* < 2.03, *p* > .182), which did not significantly differ between lesion groups. Specifically, consumption of the outcome paired with LiCl significantly decreased between pairing 1 and 2 (LiCl: pairing 1 vs 2 , ), whereas consumption of the outcome paired with saline increased (saline: pairing 1 vs 2 , ).

**Post-training muscimol inactivation**

The enhanced Pavlovian responding observed following OFC lesions (Figure 1A) may be due to enhanced learning of the cue-outcome relationship in the OFC lesion group (Figure 2-figure supplement 1). This is consistent with a role for the OFC in representing outcome expectancy information. For example, incremental learning about a cue-outcome relationship is thought to depend upon prediction errors (REFS), i.e. the difference between the experience outcome value and the expected outcome value. The expected outcome value of a cue is incrementally updated until this prediction error discrepancy is minimised. If the OFC carries some aspect of outcome expectancy information (REFS), then OFC lesions might reduce the expected value of a cue which in turn would result in abnormally persistent prediction errors and enhanced learning. Therefore, disruption of OFC function should temporarily lower expected value, and enhance prediction errors and learning. We tested this hypothesis by inactivating the OFC after first successfully acquiring cue-outcome learning i.e. when expected value is high and prediction errors are low. If the OFC carries some aspect of the learned expected value, then inactivation of the OFC should enhance prediction errors, and responding should increase to reflect new learning. Following this, returning function to the OFC should result in an over-expectation of the value of the outcome, and performance should decrease to reflect the extinction of this over-expectation. Importantly, while this account is couched in terms prediction-error learning mechanisms, the prediction remains true for any account of OFC lesions enhancing learning (Figure 1A).

We tested this hypothesis by first training a new group of animals on the same simple Pavlovian task for 9 days, before implantation of bilateral cannulae targeting the OFC (Figure 2A, Days 1-9; significant main effect of Day , , but no main effect of Group , , or Group x Day interaction , ). Following post-operative recovery (histology depicted in *Figure 2-figure supplement 2*), and prior to infusion, response levels were similar in both groups (Figure 2A, Post; no significant differences between Groups , ).

Contrary to our prediction, intra-OFC muscimol infusions disrupted the further acquisition of responding relative to the saline group (Figure 2A, Infusion - Days 12-15; Significant Group x Day interaction , , but no main effect of Group , , or Day , ). Simple effects revealed significantly greater responding in the saline group on the last 2 days of infusions (Muscimol vs Saline: Day 12 , , Day 13 , , Day 14 , , Day 15 , ). Furthermore, the saline group increased responding across infusion days 12-15 (Saline: positive linear trend , ), whereas the muscimol group did not (Muscimol: no significant linear trend , ). Therefore, post-training inactivation of the OFC impaired acquisition.

Post-infusion, with function returned to the OFC, the group differences observed under drug infusion were no longer apparent, and both groups continued to acquire responding at similar levels (Figure 2A, Days 16-17; significant main effect of Day , , but no main effect of Group , , or Group x Day interaction , ). Therefore, the effect of OFC inactivation did not persist, which suggests that the OFC plays a role in the behavioural expression (i.e. performance) of learned value and not in learning *per se*.

**Post-Training OFC lesions**

Next, we used post-training lesions to rule out the possibility that the differences between pre- and post-training OFC manipulations were simply due to differences in the method of manipulation i.e. excitotoxic lesions vs inactivation using a GABA-A agonist. We trained another set of animals on this simple Pavlovian cue-outcome task for 9 days, and then performed post-training excitotoxic or sham OFC lesions before continuing with acquisition acquisition (lesion extent depicted in Figure 2-figure supplement 3). Prior to surgery, animals acquired responding to the cue (Figure 2B, Pre-Surgery; significant main effect of Block , , but no main effect of Group , , or Group x Block interaction , ). After surgery, the sham group continued to acquire responding, but the lesion group did not (Figure 2B, Post-Surgery; significant Group x Block interaction , , but no main effect of Group , , or Day , ). Responding in the sham control group was significantly higher than the lesion group in the final block of 3 days (Block 4 , , Block 5 , , Block 6 , ). Furthermore, further acquisition post-surgery was completely abolished in the lesion group (Lesion: no linear trend over Blocks 4-6 , ), but continued in the sham control group (Sham: significant positive linear trend over Blocks 4-6 , . Therefore, both post-training lesions and inactivation of OFC function effectively block the expression of Pavlovian acquisition.

To facilitate comparisons between experiments, CS-PreCS response rates on Block 6 were Sham: M = 9.61, SD = 3.88, Lesion: M = 7.18, SD = 1.74 (Figure 2B). The terminal levels of responding in the sham group are similar to those of the saline group in Figure 2A, and the sham group in Figure 1A which used identical session parameters. This suggests that the present findings are unlikely to be due to abnormally elevated levels of responding in the control groups in any one of these experiments.

**OFC inactivation early in acquisition**

The results presented so far suggest that OFC inactivation temporarily suppressed performance, but not learning. However, it is also possible that the protocol is not sensitive enough to observe a learning deficit. For example, rates of responding were still quite high during muscimol inactivation (Figure 2A, days 12-15) and the subsequent recovery of responding (Figure 2A, days 16-17) could reflect rapid within-session learning in the muscimol group. Therefore, we tested the effect of OFC inactivation much earlier in the learning process, after only 4 days of acquisition (Figure 2C) when differences in learning should have greater impact. A new set of animals was implanted with bilateral cannulae (Figure 2-figure supplement 4) and then trained on a simple Pavlovian cue-outcome task (CS was a 10s house light).

Prior to drug infusions, all animals acquired responding to the cue (Figure 2C, Days 1-4; Significant main effect of Day , , but no main effect of Group , or Group x Day interaction , ). However, inactivation of OFC during the next 5 days of conditioning significantly impaired acquisition in the muscimol group (Figure 2C, Days 5-9; Significant main effect of Group , , Day , , and Group x Day interaction , ). Responding in the muscimol group was significantly lower than the saline group on days 7-9 (Muscimol vs Saline: Day 5 , , Day 6 , , Day 7 , , Day 8 , , Day 9 , ). Again, this deficit was characterised by significant acquisition over days in the saline group that was abolished in the muscimol group (positive linear trend over days 5-9; Saline , , Muscimol , ). Finally, this reduction in responding persisted on day 10 when all rats were tested without infusion (Figure 2C, Day 10; , ). In contrast to OFC inactivation later in acquisition (Figure 2A), disrupting OFC activity early in learning suppressed performance which persisted when the OFC was active again. This suggests that OFC inactivation early in training disrupted acquisition learning rather than just behavioural performance.

**OFC inactivation prior to associative blocking**

OFC inactivation during acquisition suppresses cue responding, but it is unclear if this reduction in behaviour is due to suppression of learning (Figure 2A) or behavioural performance (Figure 2C). This ambiguity is predominantly driven by the assumption that an animal’s response levels are some monotonic function of acquired learning [Refs – R-W, Wagner, Sutton and Barto etc…]. To disambiguate learning from performance effects we employed an associative blocking design (Figure 3A). In a blocking experiment, first an animal is trained such that a cue (cue A) predicts an outcome (pellet). Next, A is presented in compound with a novel cue (cue B) which also leads to the same pellet outcome. If the animal has learned that cue A sufficiently predicts the pellet outcome already, then very little is learned about cue B i.e. learning about cue A blocks subsequent learning about cue B [Refs - Kamin]. However, if learning about cue A is insufficient, then learning about cue B should not be blocked. We predicted that if OFC inactivation is disrupting learning, then OFC inactivation during initial learning about cue A should disrupt the blocking effect.

To test this prediction, a new set of animals was implanted with bilateral cannulae targeting the OFC and tested in a blocking procedure. During stage 1 of blocking (Figure 3B), all animals were given 10 days of acquisition training to cue A. OFC function was intact during the first 4 days of acquisition, and all animals began to acquire the cue A-outcome relationship (Days 1-4: significant main effect of Day , , but no effect of Group , or Group x Day interaction , ). All animals then received an additional 6 days of acquisition to cue A (Figure 3B, Days 5-10) following either intra-OFC infusions of muscimol or saline. Infusions of muscimol depressed overall responding relative to saline infusions (significant main effect of Group , , and Day , , but no Group x Day interaction , ). Importantly, on the final day (day 10), responding in the muscimol group was significantly lower than the saline group (, ).

Next, animals were trained such that compounds AB and CD also predicted reward (Figure 3C, Stage 2), importantly OFC function was intact in all animals i.e. no infusions. Responding in both the saline and muscimol groups was initially lower to the novel compound CD than to AB (Significant Cue x Day interaction , , and main effect of Day , , but no other main effects or interactions with Group were significant, all remaining effects *F* < 1.91, *p* > .160; Cue AB vs CD: Day 12 , , Day 13 , , Day 14 , ). However, the pattern of means suggests that responding to compound AB in the muscimol group (Figure 3C, Right) was similar to the novel compound CD on Day 12 (Day 12, Muscimol: AB vs CD , ), and lower than compound AB in the saline group (Figure 3C, Left - Day 12; Day 12, Saline: AB vs CD , ). Within-session changes over trials on Day 12 revealed rapid within-session acquisition to both compounds in both groups, but responding was significantly lower in the muscimol group at the start of the session (Figure 3 - Supplemental figure 2; First 2 trials, significant main effect of Group , , and Cue , , but no Group x Cue interaction , ).

The lower responding to cue AB in the muscimol group suggests that acquisition to cue A was impaired following infusions in Stage 1 and this impairment persisted (albeit transiently) when test drug free in stage 2. Indeed, the levels of responding to compound AB in the muscimol group at the start of Day 12 (Figure 3 - Supplemental figure 2) are similar to levels of reposnding to the novel compound CD in the saline group. This would suggest that learning about cue A in the muscimol group was impaired in stage 1, and therefore cue A will not effectively block learning to cue B in stage 2. However, at test it appears that both groups showed significant blocking of learning to cue B relative to the control cue D (Figure 3D; Significant main effect of Cue , , but no main effect of Group , , or Group x Cue interaction , ). This suggests that inactivation of the OFC significantly reduced behavioural performance but not learning to cue A in Stage 1, and this impairment transiently affected compound AB on Day 12 in the absence of OFC inactivation. Therefore, the impairments observed in our earlier findings (Figure 2A & C, post infusion) are unlikely to be due to impairments in learning. In addition to this, we rule out the possibility that the two groups used different attentional solutions to achieve a similar blocking result (Figure 3-Figure supplement 1).

**Figure 3-Figure supplement 1: Caption**

Reacquisition to cues A and B provide a test of whether the successful blocking effect observed in the saline and muscimol groups was the result of different underlying attentional strategies. Down-regulation of attention to a cue can result in retardation of subsequent acquisition (REFS - Papini & Bitterman). There were no differences in the rates of learning to the blocked cue B or to the blocking cue A. (A) The rate of re-acquisition to cue B (day 17-19) and (B) cue A (day 20-21) did not differ between groups (Cue B: Significant main effect of Day , , but no main effect of Group , , or Group x Day interaction , . Cue A:Significant main effect of Day , , but no main effect of Group , , or Group x Day interaction , ). Note: Due to experimenter error one animal in each group was tested with the wrong counterbalancing and excluded from the analysis of re-acquisition data (remaining N = 24, saline n = 12, muscimol n = 12). Error bars depict ± SEM.

**Competing response values**

One possible account of the impaired performance following OFC inactivation in the present study is an inability to potentiate behaviour based on the current value of the outcome. Specifically, the ability to potentiate performance based on the current motivational value of the outcome may be disrupted during OFC inactivation, leaving intact the predictive cue-outcome relationship. The current value of an outcome can be modulated by a number of factors such as current motivation (e.g. hunger), the magnitude of the outcome (e.g. volume, concentration, or number or rewards), and the relative value of competing alternative outcomes. To assess this possibility a novel task was created in which the strength of responding to a Pavlovian cue is modulated by the relative value of a competing unsignalled reward.

The task involved a Pavlovian cue-outcome procedure similar to those described above i.e. a 15s white-noise auditory stimulus predicted the delivery of a food pellet into a reward magazine (Figure 4A). In parallel to this, a second magazine was located on the opposite side of the chamber which could present and retract a sucrose reward in a dipper cup. Sucrose availability in this alternative magazine was presented randomly throughout the session without explicit cues. The probability of sucrose availability was randomized within each session into blocks of low, medium, or high probability (Figure 4 – Supplementary Figure 1). This background reinforcement rate could only be determined by sampling from the alternative magazine. This task provided a measure of a reward guided exploratory behaviour in the sucrose magazine, and Pavlovian behaviour to the pellet magazine driven by the expected value of the predicted outcome (Figure 4 – Supplementary Figure 2). Normally, animals will engage in a range of unmeasured and uncontrolled alternative behaviours in a testing chamber (e.g. exploration, orienting, grooming, etc…) that may compete with Pavlovian magazine approach. Here we provide a means to guide and control these alternative behaviours towards the sucrose magazine, and explicitly measure the integration of un-cued and cued expected value.

**The effect of OFC inactivation on updating relative expected value**

Next, these animals were implanted with bilateral cannulae targeting the OFC to assess the role of the OFC in updating relative expected value. It was predicted that OFC inactivation would impair flexible updating of relative expected value of the Cue during the cue period. It was unclear whether the OFC would also be necessary for tracking and evaluating the changing probability of sucrose during the Baseline period [REFS]. Following muscimol or saline infusions, animals were tested with sucrose probability changing from low to high. This shorter session minimised the probability that the muscimol was no longer effective, and the fixed order of probabilities reduced the possible confound of general satiety during the second half of the session.

All animals were able to detect changes in the probability of the unsignalled sucrose reward, and appropriately update Sucrose approach behaviour during the PreCS period (Figure 4B & D; PreCS period: Significant Probability x Magazine interaction , , and main effects of Probability , , and Magazine , ; No significant main effect or interaction with Drug). Specifically, anticipatory approach to the sucrose magazine increased with the probability of sucrose (Sucrose magazine: Low vs High probability , ), whereas approach to the pellet magazine dd not change (Pellet magazine: Low vs High probability , ).

However, during the CS period, OFC inactivation disrupted activity at the pellet magazine but left activity at the sucrose magazine intact (Figure 4C & E; CS period: significant Drug x Magazine x Probability 3-way interaction , ). Activity at the sucrose magazine was not affected by muscimol infusions (Figure 4C; Sucrose magazine: no significant main effect of Drug , , or Drug x Probability interaction , ), whereas activity at the pellet magazine was significantly disrupted by muscimol infusions (Figure 4E;Pellet magazine: significant Drug x Probability interaction , ). Simple effects revealed that pellet magazine responding was lower after muscimol than saline infusions for the low probability of dipper reward (, ) but did not differ between infusions for the high probability of dipper reward (, ).

Comparing the analysis of the PreCS and CS periods using a full Drug(Saline, Muscimol) x Period(PreCS, CS) x Magazine(Sucrose, Pellet) x Probability (Low, High) repeated measures ANOVA confirmed that the muscimol deficit was specific to the pellet magazine in the CS period (Figure 4 B-E; Significant Drug x Period x Magazine x Probability 4-way interaction , ). This suggests that muscimol specifically dampened responding to the pellet magazine during the CS period when the probability of sucrose was low. OFC inactivation selectively disrupted the ability to modulate behaviour based on integrating Pavlovian expected values with the current rate of alternative rewards i.e. the current subjective value of the predicted reward.

**Supplementary Figure**

Anticipatory approach to the Sucrose and Pellet magazine during the PreCS (top row) and CS period (middle row) following initial acquisition (Acquisition, left column), 24 hrs of water deprivation (Thirst, middle column), and a 4-fold increase in sucrose reward volume (4x Sucrose). Periods of time in which the sucrose reward was present were removed from this analysis to measure anticipatory approach that is not conflated with consummatory behaviour. (Bottom row) Data are also re-presented as a pellet magazine bias score (activity towards pellet – sucrose magazine) to help visualise the response competition where high scores indicate greater approach to the pellet magazine and low scores indicate greater approach to the sucrose magazine. Complete analysis of these data is presented below. Error bars depict ± SEM.

An analysis of the separate magazine approach data (top and middle row) was performed using a Shift(Acquisition, Thirst, 4xSucrose) x Period (PreCS,CS) x Magazine (Sucrose, Pellet) x Probability(Low,Medium,High) repeated measures ANOVA. Pavlovian anticipatory behaviour selectively modulated activity at the relevant (pellet) magazine, and exploratory sampling of the sucrose magazine was modulated by the background probability of unsignalled sucrose availability. In addition to this, the rate of Pavlovian anticipatory activity during the CS was modulated by the sucrose probability. This suggests that the distribution of responding was appropriately gated by a cost-benefit trade-off that appears to depend on the relative value of each behavioural option i.e. expected value of pellet vs sucrose. In addition to this, increasing the value (4-fold volume increase) of the sucrose reward significantly biased this behavioural trade-off towards the sucrose magazine whereas inducing a motivational state of thirst did not. This suggests that task behaviour is sensitive to shifts in relative value, and that the relative value of the liquid sucrose reward was not simply driven by a motivational state of thirst.

The Pavlovian CS selectively increased activity at the relevant Pavlovian/pellet magazine (Pellet magazine: PreCS vs CS , , Sucrose magazine: PreCS vs CS , ; Significant Period x Magazine interaction , , and main effect of Period , ). Furthermore, the effect of the background probability of unsignalled sucrose availability modulated activity at the sucrose and pellet magazines in opposing directions (significant Magazine x Probability interaction , ). As the probability of sucrose availability increased, activity at the sucrose magazine increased whereas activity at the pellet magazine decreased (Sucrose magazine: Low vs Medium , , Low vs High , , Medium vs High , , Pellet magazine: Low vs Medium , ,Low vs High , ,Medium vs High , ).

Finally, increasing the volume of the sucrose reward significantly increased activity directed at the sucrose but not the pellet magazine, whereas increasing thirst did not significantly change behaviour in this task (significant Magazine x Shift interaction , ; Sucrose magazine: Acquisition vs Thirst , 4xSucrose vs Acquisition , 4xSucrose vs Thirst , ; Pellet magazine: Acquisition vs Thirst , 4xSucrose vs Acquisition , 4xSucrose vs Thirst , ). No other meaningful effects were significant (significant Period x Probability interaction , ; all remaining effects *F* < 3.421, *p* > .06).