**Results**

**Pre-training lesions enhance Pavlovian acquisition**

**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 driven performance above baseline. Acquisition of responding to the CS was significantly greater in the lesion group than the sham group. This was supported by a Group (sham, lesion) x Block (1-7) mixed ANOVA revealing significant main effects of Block (*F*(6, 240) = 34.07, *p* < .001), Group (*F*(1, 40) = 10.83, *p* = .002) and a Block x Group interaction (*F*(6, 240) = 7.33, *p* < .001; significant linear trend interaction *F*(1, 40) = 11.11, *p* = .002). Follow up contrasts on each block revealed that there were no significant differences between groups during the first 3 blocks (all *F* < 3.19, *p* > .08), but the lesion group were significantly higher than the sham group during the last 4 blocks (all *F* > 5.38, *p* < .03). 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 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 1B) did not differ between groups (main effect of TimeBin *F*(1, 33) = 62.93, *p* < .001, but no significant main effect of Group, *F*(1, 33) = 2.87, *p* = .10, or Group x TimeBin interaction, *F*(1, 33) = 0.36, *p* = .55). Furthermore, while not statistically significant, the mean locomotor activity was numerically lower in the lesion group than the sham group.

**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 1C). General satiety, following 24 hours access to home-cage food, did not affect the rate of responding in the sham group (*F*(1, 13) = 1.91, *p* = .19) but significantly suppressed responding in the lesion group (*F*(1, 13) = 17.95, *p* = .001) compared to subsequent testing 24 h hours later when hungry again. These differences were supported by a significant interaction between Group and Satiety conditions (*F*(1, 13) = 4.63, *p* = .05). 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 (*t*(13) = 1.31, *p* = .32, 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 (*F*(1, 11) = 9.34, *p* = .01). In contrast, the devaluation effect was abolished in the lesion group, and responding remained high to both the devalued and non-devalued cue (*F*(1, 11) = 1.18, *p* = .30). A mixed Group x Devaluation ANOVA confirmed the observed pattern of data with a significant Group x Devaluation interaction (*F*(1, 11) = 7.55, *p* = .02, all remaining *F <* 1.09, *p* > .32). This finding successfully replicates the finding that larger non-specific OFC lesions abolish the outcome devaluation effect in rodents [REFS – Schoenbaum Series; Elife Panayi/Killcross].

**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 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). The rate of acquisition to the CS was matched prior to surgery (significant main effect of Day (F(8, 176) = 25.42, p < .001, but no effect of Group F(1, 22) = 1.08, p = .31, or Group x Day interaction F(8, 176) = 0.54, p = .83). Following post-operative recovery (histology depicted in Figure 2-figure supplement 2) and prior to infusion, response levels were similar in both groups (t(22) = 1.02, p = .32).

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). A mixed Group x Day (4 days) ANOVA supported this observation with a significant Group x Day interaction (F(3, 66) = 5.03, p < .01, and a significant Group x Day linear trend interaction contrast F(1, 22) = 9.50, p = .01), but no main effects of Group (F(1, 22) = 1.90, p = .18) or Day (F(3, 66) = 0.32, p = .81). Follow up linear trend contrasts found that the saline group increased responding across days (F(1, 11) = 8.78, p = .01) whereas the muscimol group did not (F(1, 11) = 2.21, p = .17). Simple effect comparisons also suggested that group performances were similar on days 12 (F(1, 12) = .45, p = .51) and 13 (F(1, 12) = 1.06, p = .32), but the saline group performance was significantly higher than the muscimol group on days 14 (F(1, 12) = 7.81, p = .01) and 15 (F(1, 12) = 4.34, p = .05). 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). These observations were supported by a Group x Day (2 days) ANOVA which suggested increased responding across days (main effect of Day F(1, 22) = 16.05, p = .001) but no effect of Group (F(1, 22) = 0.11, p = .74) or Group x Day interaction (F(1, 22) = 0.21, p = .65). 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 ruled out the possibility that the differences between pre- and post-training OFC manipulations was due to differences in the method of manipulation i.e. excitotoxic lesions vs inactivation using a GABAA agonist. Therefore, 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. Prior to surgery, animals acquired responding to the cue (Figure 2B, Pre-Surgery; significant main effect of DayBlock (F(2, 38) = 61.98, p < .001, but no main effect of Group or Group x DayBlock interaction, both F < 1.00, p > .49). After surgery, the sham group continued to acquire responding, but the lesion group did not (Figure 2B, Post-Surgery; lesion extent depicted in Figure 2-figure supplement 3). A mixed Group x DayBlock (3 blocks of 3 days) ANOVA supported this observation with a significant Group x DayBlock interaction (F(2, 38) = 6.25, p < .01, and a significant Group x DayBlock linear trend interaction contrast F(1, 19) = 8.93, p = .01) but no main effect of DayBlock (F(2, 38) = 0.66, p = .53) or Group (F(1, 19) = 2.21, p = .15). Separate linear trend contrasts across DayBlock for each group revealed that whereas the sham group increased responding across days (F(1, 11) = 7.11, p = .02), the lesion group did not (F(1, 8) = 2.80, p = .13). Simple effect comparisons also revealed that sham group responding was greater than the lesion group on the final block of training (DayBlock 6 F(1, 19) = 7.03, p = .02) but not on DayBlock 4 (F(1, 19) = 0.06, p = .81) or DayBlock 5 (F(1, 19) = 0.64, p = .43). To facilitate comparisons between experiments, CS response rates on DayBlock 6 were sham M = 9.61, SD = 3.88, lesion M = 7.18, SD = 1.74. 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 sham group in this experiment.

**OFC inactivation early in acquisition**

While the results so far suggest that OFC inactivation temporarily suppressed performance, but not learning (Figure 2A), it is also possible that the procedure was 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). 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. Prior to drug infusions, all animals acquired responding to the cue (Group F1,13 = 0.03, p = .86; Day F3,39 = 9.43, p < .001; Group x Day F3,39 = 0.30, p = .83). However, inactivation of OFC during the next 5 days of conditioning significantly impaired acquisition in the muscimol group (Group F1,13 = 12.57, p = .004; Day F4,52 = 7.72 p < .001; Group x Day F4,52 = 3.05, p = .02). The muscimol group showed significantly lower responding than the saline group on days 7, 8 and 9 (Day 5 F1,13 = 3.42, p = .09; Day 6 F1,13 = 4.40, p = .06; Day 7 F1,13 = 14.20, p = .002; Day 8 F1,13 = 17.40, p = .001; Day 9 F1,13 = 14.43, p = .002; Sidak adjusted significance threshold p < .01). This reduction in responding persisted on day 10 when all rats were tested without infusion (F1,13 = 14.22, p = .002). In contrast to OFC inactivation later in acquisition (Figure 2A), disrupting OFC activity early in learning suppresses performance and persists when the OFC is active again. This persistent deficit suggests that OFC inactivation 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 5B, Days 1-4), 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 (significant main effect of Day *F*(3, 72) = 5.77, *p* = .001, but no Group *F*(1, 24) = 0.63, *p* = .44, or Group x Day interaction *F*(3, 72) = 0.27, *p* = .85). All animals then received an additional 6 days of acquisition to cue A (Figure 5B, Days 5-10) following either intra-OFC infusions of muscimol or saline. Infusions of muscimol depressed overall responding relative to saline infusions. A mixed Group x Day (day 5-10) ANOVA revealed a significant main effect of Day (*F*(5, 120) = 17.50, *p* < .001, significant positive linear trend suggesting that responding increased over days in both groups *F*(1, 24) = 52.63, *p* < .001) and a significant main effect of Group (*F*(1, 24) = 4.25, *p* = .05, non-significant Group x Day interaction *F*(5, 20) = 1.31, *p* = .26). Importantly, on the final day (day 10), responding in the muscimol group was significantly lower than the saline group (t(14) = 2.69, *p* = .01).

Next, animals were trained such that compounds AB and CD also predicted reward (Figure 5C), importantly OFC function was intact in all animals i.e. no infusions. In the saline control groups (Figure 5C, left), responding to AB was significantly higher than to the novel compound CD on the first day stage 2 (Day 12) [Stats]. In contrast the muscimol group was significantly low to both AB and the novel compound CD (Figure 5C, right) [Stats]. However, a mixed Group x Cue (AB, CD) x Day (12-14) ANOVA did not reveal any significant effect or interactions with Group (Group *F*(1, 24) = 0.52, *p* = .48, Group x Cue *F*(1, 24) = 1.16, *p* = .294, Group x Day *F*(2, 48) = 1.91, *p* = .16, Group x Cue x Day *F*(2, 48) = 0.46, *p* = .63). A significant Cue x Day interaction (*F*(2, 48) = 12.12, *p* < .001, significant effect of Day *F*(2, 48) = 20.09, *p* < .001, but no main effect of Cue *F*(1, 24) = 0.63, *p* = .44) supported the observed differences in response acquisition between cues AB and CD. Specifically, simple main effects revealed that responding to CD was lower than AB on day 12 (*F*(1, 24) = 13.96, *p* = .001) but not on days 13 (*F*(1, 24) = 0.20, *p* = .66) or 14 (*F*(1, 24) = 3.24, *p* = .09).

Finally, at test (Figure 5D) both groups showed a significant effect of blocking of learning to cue B compared to the control cue D (main effect of Cue *F*(1, 24) = 7.29, *p* = .01, but no significant effect of Group *F*(1, 24) = 0.54, *p* = .47, or Group x Cue interaction *F*(1, 24) = 0.04, *p* = .84). Therefore, both groups acquired similar learning about the relationship between cue A and reward in stage 1. 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 2, Figure 3, Figure 4) 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 5-Figure supplement 1).

**Competing response values**

An alternative 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 is likely to be affected 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 the standard Pavlovian cue-outcome procedure used in earlier experiments i.e. a 15s white-noise auditory stimulus predicted the delivery of a food pellet (Figure 6A). In parallel to this, there was a second magazine 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 throughout the session without explicit cues. The probability of sucrose availability was randomized within each session into blocks of low, medium, or high probability. This provided a background reinforcement rate that could only be determined by sampling from the alternative magazine. This 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. Normally, animals will engage in a range of unmeasured and uncontrolled alternative behaviours in a testing chamber (e.g. exploration, 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 thirst and an upshift in sucrose reward size was also tested to confirm whether responding in this task was sensitive to value manipulations.

Data were first analysed as the difference between responding to the pellet vs. the sucrose magazine to clearly see response competition. The pattern of results suggests that at the end of acquisition, responding to the sucrose magazine was greatest during the Baseline period and responding was greatest to the pellet magazine during the Cue period (Figure 6B). Furthermore, as the probability of the un-signalled sucrose reward increased, behaviour towards the sucrose magazine increased during both periods (i.e. difference scores decreased). There was minimal effect of water deprivation on this pattern of responding, however increasing sucrose reward magnitude enhanced the response competition by increasing overall responding towards the sucrose magazine (i.e. difference scores decreased overall more than during acquisition). A Shift (Acquisition, Thirst, 4xSucrose) x Period (Baseline, Cue) x Probability (low, medium, high) repeated measures ANOVA supported the observed pattern of results with significant main effects of Shift (*F*(2, 14) = 5.03, *p* = .02), Period (*F*(1, 7) = 13.44, *p* = .01), and Probability (*F*(2, 14) = 22.49, *p* < .001, all interactions failed to reach significance, all *F* < 1.14, *p* > .36). Bonferroni corrected simple main effects revealed that increasing the size of the sucrose reward decreased difference scores compared to acquisition (*F*(1, 7) = 10.20, *p* < .05, remaining comparisons between acquisition, water deprivation and increasing reward size failed to reach significance, all *F* < 2.68, *p* > .44). Bonferroni corrected simple main effects also revealed that difference scores decreased as the probability of reward increased i.e. increasing the probability of the dipper reward systematically increased responding at the dipper magazine in all periods (all *F*(1, 7) > 12.30, *p* < .01). These results suggest that the distribution of responding in the task is sensitive to the un-signalled rate of dipper reinforcement, and to changes in the magnitude of the dipper reward, but not to manipulations of thirst. This was also confirmed by a more comprehensive analysis separating responses to each magazine (Figure X)

**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.

The pattern of responding at test under infusions of saline was similar to the pattern observed prior to surgery (Figure X). Specifically, during the Baseline period behaviour was significantly biased towards the sucrose magazine and this bias (negative score) increased with the probability of sucrose reward. This pattern of responding was similar under saline and muscimol infusions. Following saline infusions, responding during the Cue period was biased towards the pellet magazine when the probability of sucrose was low and biased towards the sucrose magazine when the probability of sucrose was high. In contrast, muscimol infusions disrupted response distribution during the Cue such that behaviour was biased towards the sucrose magazine during both low and high probability of sucrose reward. That is, the Cue failed to control behaviour as it did following saline infusions.

This description was supported by a Drug(saline, muscimol) x Period(Baseline, Cue) x Probability (low, high) repeated measures ANOVA which found a significant Drug x Period x Probability three-way interaction (*F*(1, 5) = 11.99, *p* = .02). A follow up Drug x Probability ANOVA was conducted on the Baseline and Cue periods separately to explore this three-way interaction. During the Baseline period there was a significant main effect of probability (*F*(1, 5) = 9.01, *p* = .03) suggesting that response bias towards the sucrose magazine increased from low to high probability of sucrose, and this effect did not differ as a function of drug infusion (all remaining effects failed to reach significance, (*F*(1, 5) < 5.14, *p* > .07). During the Cue period there was a significant main effect of Drug (*F*(1, 5) = 7.50, *p* = .04) and a Drug x Probability interaction (*F*(1, 5) = 8.35, *p* = .03; non-significant main effect of Probability *F*(1, 5) = 2.74, *p* = .16). Simple effects revealed that there was greater response bias towards the pellet magazine following saline infusions than muscimol infusions during the low probability of sucrose (*F*(1, 5) = 78.85, *p* < .001) but no drug infusion differences during the high probability of sucrose (*F*(1, 5) = 0.12, *p* = .74). Therefore, muscimol specifically disrupted the increase in responding to the pellet magazine normally observed in the Cue period during the low probability of sucrose. Given that the current measure is a difference score, it was important to analyse responding to each magazine separately to determine whether the significant effect of muscimol was a result of disrupting pellet or sucrose magazine responding or both.

Responding to each magazine at test under saline and muscimol is presented in (Figure X and Figure X). Under saline and muscimol infusions, responding to the dipper magazine increased with the probability of dipper reward in both the Baseline and the Cue period. Responding to the pellet magazine was minimal during the Baseline period and this was not differentially affected by saline or muscimol infusions. However, under saline infusions, Cue period responding decreased to the pellet magazine as the rate of dipper reward increased but did not change under muscimol infusions. A Drug (Saline, Muscimol) x Period (Baseline, Cue) x Probability (low, high) x Magazine (Dipper, Pellet) repeated measures ANOVA supported these observations with a significant 4-way Drug x Period x Probability x Magazine interaction (*F*(1, 5) = 12.08, *p* = .02). This 4-way interaction term was explored by conducting separate Drug x Probability x Magazine ANOVAs for the Baseline and Cue periods.

During the Baseline period (Figure X) responding was greater to the dipper than the pellet magazine an effect which increased in magnitude as the probability of dipper reward increased (significant Probability x Magazine interaction *F*(1, 5) = 9.06, *p* = .03, main effect of Magazine *F*(1, 5) = 20.50, *p* = .01 and main effect of Probability *F*(1, 5) = 12.23, *p* = .02, all remaining *F*(1, 5) < 6.183, *p* > .05). During the Cue period there was a significant 3-way Drug x Probability x Magazine interaction (*F*(1, 5) = 8.37, *p* = .03), and a significant Drug x Magazine and Drug x Probability interactions (both *F*(1, 5) > 7.35, *p* = .04, all remaining effects failed to reach significance, all *F*(1, 5) < 4.19, *p* > .10).

Separate Drug x Probability ANOVAs were conducted on each magazine response to understand the nature of the 3-way interaction during the Cue period (Figure X). While there were no significant effects for the dipper magazine (all *F*(1, 5) < 4.67, *p* > .08), there was a significant Drug x Probability interaction for the pellet magazine (*F*(1, 5) = 9.93, *p* = .03, remaining *F*(1, 5) < 2.55, *p* > .17). Simple effects revealed that pellet magazine responding was lower after muscimol than saline infusions for the low probability of dipper reward (*F*(1, 5) = 25.67, *p* < .01) but did not differ between infusions for the high probability of dipper reward (*F*(1, 5) = 0.94, *p* = .38). This suggests that muscimol specifically dampened responding to the pellet magazine during the Cue period when the probability of sucrose was low.

Methods and materials

*Animals.* Subjects were male Long Evans rats (Monash Animal Services, Gippsland, Victoria, Australia) approximately 4 months old. Rats were housed four per cage in ventilated Plexiglass cages in a temperature regulated (22 ± 1­°C) and light regulated (12h light/dark cycle, lights on at 7:00 AM) colony room. At least one week prior to behavioural testing, feeding was restricted to ensure that weight was approximately 95% of ad libitum feeding weight, and never dropped below 85%. All animal research was carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratories Animals (NIH publications No. 80-23, revised 1996) and approved by the University of New South Wales Animal Care and Ethics Committee.

*Apparatus.* Behavioural testing was conducted in eight identical operant chambers (30.5 x 32.5 x 29.5 cm; Med Associates) individually housed within ventilated sound attenuating cabinets. Each chamber was fitted with a 3-W house light that was centrally located at the top of the left-hand wall. Food pellets could be delivered into a recessed magazine, centrally located at the bottom of the right-hand wall. Delivery of up to two separate liquid rewards via rubber tubing into the magazine was achieved using peristaltic pumps located above the testing chamber. The top of the magazine contained a white LED light that could serve as a visual stimulus. Access to the magazine was measured by infrared detectors at the mouth of the recess. Two retractable levers were located on either side of the magazine on the right-hand wall. A speaker located to the right of the house light could provide auditory stimuli to the chamber. In addition, a 5-Hz train of clicks produced by a heavy-duty relay placed outside the chamber at the back-right corner of the cabinet was used as an auditory stimulus. The chambers were wiped down with ethanol (80% v/v) between each session. A computer equipped with Med-PC software (Med Associates Inc., St. Albans, VT, USA) was used to control the experimental procedures and record data.

*Consumption chambers.* To provide individual access to reinforcers during the satiety and devaluation procedures, rats were individually placed into an individual cage (33 x 18 x 14 cm clear Perspex cage with a wireframe top). Pellet reinforcers were presented in small glass ramekins inside the box and liquid reinforcers were presented in water bottles with a sipper tube. 1 day prior to the target procedure, all rats were exposed to the individual cages and given 30 mins of free access to home cage food and water to reduce novelty to the context and consuming from the ramekin and water bottles.

*Locomotor activity.* Locomotor activity was assessed in eight identical boxes measuring 50 x 36x 18 cm (length x width x height), housed in a sound attenuated room. Each box consisted of 4 opaque white polyurethane walls and floor and a removable roof. In the center of the roof was an 18x40 cm grid of 3x3 mm ventilation holes. Two custom pairs of infrared beam detectors spanned the width of the box to detect locomotor activity and were located 15 cm from each end of the box. Beam breaks, corresponding to activity within the box, were recorded on a computer equipped with Med-PC software (Med Associates Inc.).

*Surgery.* Excitotoxic lesions targeting the lateral OFC were performed in experiments [XYZ]. Rats were anesthetized with isoflurane, their heads shaved, and placed in a stereotaxic frame (World Precision Instruments, Inc., Sarasota, FL, USA). The scalp was incised, and the skull exposed and adjusted to flat skull position. Two small holes were drilled into the skull and the dura mater was severed to reveal the underlying cortical parenchyma. A 1-µL Hamilton needle (Hamilton Company, Reno, NV, USA) was lowered through the two holes targeting the lateral OFC (co-ordinates specified below). Stereotaxic co-ordinates were AP: +3.5 mm; ML: ±2.2 mm; D-V: -5.0 mm from bregma. At each site the needle was first left to rest for 1 min. Then an infusion of N-methyl-D-aspartic acid (NMDA; Sigma-Aldrich, Switzerland), dissolved in phosphate buffered saline (pH 7.4) to achieve a concentration of 10μg/μL, was infused for 3 mins at a rate of 0.1 µ/min. Finally, the needle was left in situ for a further 4 mins to allow the solution to diffuse into the tissue. Following the diffusion period, the syringe was retracted, and the scalp cleaned and sutured. Sham lesions proceeded identically to excitotoxic lesions except that no drugs were infused during the infusion period. After a minimum of 1 week of postoperative recovery, rats were returned to food restriction for 2 days prior to further training.

In experiments [XYZ] bilateral guide cannulae were surgically implanted targeting the lateral OFC. Rats were anesthetized with isoflurane, their heads shaved, and placed in a stereotaxic frame (World Precision Instruments, Inc., Sarasota, FL, USA). The scalp was incised, and the skull exposed and adjusted to flat skull position. Two small holes were drilled for the cannulae using a high-speed drill, and four holes were hand drilled on different bone plates to hold fixing screws. Bilateral stainless steel guide cannulae (26 gauge, length 5mm below pedestal; Plastics One, Roanoke, VA, USA) were lowered into the lateral OFC (AP: +3.5 mm; ML: ±2.2 mm; D-V: -4.0 mm from bregma). Cannulae were held in place by dental cement and anchored to the skull with 4 fixing screws. Removable dummy cannulae were inserted into the guide cannulae to prevent them from blocking. After one week of postoperative recovery, rats were returned to food restriction for 2 days prior to further testing.

*Drugs and infusions.* The GABAA agonist muscimol (Sigma-Aldrich, Switzerland) was dissolved in 0.9% (w/v) non-pyrogenic saline to obtain a final concentration of 0.5 *μ*g/0.5 *μ*l. Non-pyrogenic saline 0.9% (w/v) was used as the saline control.During infusions, muscimol or saline was infused bilaterally into the lateral OFC by inserting a 33 gauge internal cannula into the guide cannula which extended 1 mm ventral to the guide tip. The internal cannula was connected to a 25 *μ*l glass syringe (Hamilton Company, Reno, NV, USA) attached to a microinfusion pump (World Precision Instruments, Inc., Sarasota, FL, USA). A total volume of 0.5 *μ*l was delivered to each side at a rate of 0.25 *μ*l/min. The internal cannula remained in place for an additional 1 min after the infusion and then removed. During the infusion procedure animals were allowed to move freely in a bucket to minimize stress. Dummy cannulae were removed prior to, and replaced immediately after, infusions. For the two training sessions prior to infusions, all animals received dummy infusions which were identical to the infusion procedure, except that no liquids were infused. These dummy infusions were performed to familiarize the rats with the microinfusion procedure and thereby minimize stress. Dummy infusions were also conducted on test sessions after the infusions to minimise differences in handling between experimental stages.

*Reinforcers***.** The reinforcers used were a single grain pellet (45 mg dustless precision grain-based pellets; Bio-serv, Frenchtown, NJ, USA), 20% w/v sucrose solution and 20% w/v maltodextrin solution (Myopure, Petersham, NSW, Australia). Liquid reinforcers were flavoured with either 0.4% v/v concentrated lemon juice (Berri, Melbourne, Victoria, Australia) or 0.2% v/v peppermint extract (Queen Fine Foods, Alderley, QLD, Australia) to provide unique sensory properties to each reinforcer. Liquids were delivered over a period of 0.33 s via a peristaltic pump which corresponded to a volume of 0.2 mL. The volume and concentration of liquid reinforcers was chosen to match the calorific value of the corresponding grain pellet reward and have been found to elicit similar rates of Pavlovian and instrumental responding as a pellet reward in other experiments conducted in this lab. In all experiments involving liquids, the magazine was scrubbed with warm water and thoroughly dried between sessions to remove residual traces of the liquid reinforcer. To reduce neophobia to the reinforcers, one day prior to magazine training sessions all animals were pre-exposed to the reinforcers (10 g of pellets per animal and 25 ml of liquid reinforcer per animal) in their home cage.

*Magazine training.* All animals received one session of magazine training for each experimental reinforcer with the following parameters: reward delivery was on an RT60 s schedule for 16 rewards. When necessary, sessions were separated by at least 2 hours and the order of reinforcer identity was counterbalanced between groups.

*Behaviour.* CS responding was operationalized as the number of magazine entries during the CS period. PreCS responding was operationalized as the frequency of responding during the immediately preceding the CS period, and was used as a measure of baseline responding to the testing context. PreCS responding was analysed separately, and any group differences identified and reported. Data were presented as CS – PreCS difference scores, which reflect discriminative responding to the CS. All data were analysed with mixed ANOVAs using SPSS statistical software (REFERENCE), and significant interactions of interest were followed up with ANOVAs on the relevant subset of data. Following significant omnibus ANOVA tests, in addition to simple effects, planned linear and quadratic orthogonal trend contrasts and their interactions between groups were analysed to assess differences in rates of responding.

**Experiment 1: Acquisition with Pre-training lesions**

**Subjects.**

Subjects were forty-eight (N = 48) rats, tested in two cohorts. Cohort 1, n = 16 rats weighing between 280-361 g (M = 312.2 g) and cohort 2, n = 32 rats weighing between 271-328 g (M = 296.3 g).

Training

Pavlovian Acquisition

Following magazine training, all rats received 21 sessions of Pavlovian acquisition training. Each session consisted of 16 presentations of a single auditory CS (a 15 s train of clicks) presented on a VT90s schedule (ranging from 60 to 120 s). A single pellet (US) was delivered at the termination of each CS. The session duration was 28 mins and animals were left in the chamber for an additional 2 mins before being removed. Animals received either one session per day, or two sessions per day separated by at least 2 hours.

Subgroup 1: General Satiety Pre-Feeding

At the end of acquisition training on day 21, a subgroup of animals (sham n = 8, lesion n = 8) were taken off food restriction and given 24 hours free access to their home cage food before further acquisition training on day 22. This session was rewarded as per acquisition training. At the end of day 22 animals were put back on food restriction and continued acquisition training.

***Subgroup 2: Devaluation***

Following initial Pavlovian acquisition of a single CS-US association, a subgroup of animals (sham n = 8, lesion n = 8) were re-trained with two novel unique CS-US associations intended to test devaluation in a taste aversion procedure.

Novel Acquisition

Novel acquisition of two unique CS-US associations was conducted with identical parameters to initial acquisition training, 2 session per day for 14 days, each session consisting of 16 trials consisting of a 15s CS co-terminating with reward with a vITI90s. Unlike initial acquisition the two CSs were an 80dB white noise and a 2800 Hz, 80 dB tone followed by either a single pellet or 20% w/v maltodextrin liquid (CS-US identities counterbalanced between animals).

Taste Aversion

Taste aversion took place in the devaluation chambers and involved 30 mins exposure to one US every day, alternating each day for 4 days. Following fee access to a US animals were immediately injected i.p. with either 0.15M LiCl or 0.9% saline (15 mL/Kg). The outcome paired with nausea induced by injection of LiCl was designated the devalued outcome and the outcome paired with neutral saline injections was designated the non-devalued outcome (counterbalanced between animals). Following the final day of injections all animals were given a day of rest in their home cage to allow hunger levels to return to normal after taste aversion training.

Devaluation Test

Animals were tested with a single session of CS training except that no rewards were delivered i.e. in extinction. The magazine frequency measure that was available was not as sensitive to devaluation as a measure of duration, so only data from the first trial was analysed at test.

Locomotor Activity

At the end of the experimental procedures, all animals were assessed for locomotor activity over a 1-hour period.

**Histology and Group Allocation**

Lesion damage is depicted in Figure XXX. Lesion extent was judged by a trained observer blind to group allocation. A lesion was retained if there was evidence of significant bilateral damage constrained to LO or DLO. Animals were excluded if there was only unilateral LO/DLO damage, evidence of damage to the dorsal part of the anterior olfactory nucleus ventral to LO/DLO or if there was extensive damage to the white matter of the forceps minor of the corpus callosum. One lesioned animal did not recover from surgery, four lesion animals had only unilateral OFC damage, and one lesioned animal had extensive white matter damage. Forty-two animals were retained (N = 42, sham n = 24, lesion n = 18), of which subgroup 1 contained fifteen (*N* = 15; sham *n* = 8, lesion *n* = 7) and subgroup 2 contained thirteen (*N* = 13; sham *n* = 8, lesion *n* = 5).

**PreCS Analysis**

Analysis of the PreCS period using a Group (sham, lesion) x Block (1-7) mixed ANOVA revealed that responding was significantly higher in the lesion group than the sham group (main effect of Group *F*(1, 40) = 7.24, *p* = .01). Furthermore, while responding increased over blocks (main effect of Block *F*(6, 240) = 20.37, *p* < .001; positive linear trend *F*(1, 40) = 33.18, *p* < .001), this increase was greater in the lesion than the sham group (Block x Group interaction *F*(6, 240) = 2.52, *p* = .02; linear trend interaction *F*(1, 40) = 5.34, *p* = .03). During the first block PreCS responding was similar between groups (Sham M = 2.07, SD = 0.60; Lesion M = 2.13, SD = 0.90), by the final block PreCS responding was higher in the Lesion group (M = 4.30, SD = 1.95) than the sham group (M = 2.76, SD = 2.30).

**Experiment 2: Acquisition with Muscimol Inactivation**

**Subjects**

Subjects were thirty-two (total N = 32) male Long Evans rats (Monash Animal Services, Gippsland, Victoria, Australia) approximately 4 months old, weighing between 285-350 g (M = 319.7 g).

***Pavlovian Acquisition***

Animals were given 9sessions, 1 session per day, of Pavlovian acquisition training with session parameters identical to those described in Experiment 3a. This number of session was chosen because the effect of pre-training lesions appeared after around 9 session in Experiments 3a and 3b. Briefly, each session consisted of a VT90s ITI with 16 trials consisting of a 15s click CS co-terminating with a single pellet US. Following the final day of training all animals were taken off food restriction and received surgical implantation of guide cannulae.

**Post-Training**

***Pre-Infusion***

Following post-operative recovery animals were returned to food restriction for a day before receiving a further 2 days of acquisition training as per pre-training. However, immediately prior to entering the chamber all animals received a dummy infusion.

***Infusion***

Animals were assigned to one of two infusion groups such that performance there were no differences between groups on the final day of pre-infusion acquisition. For the next 4 days, all animals received an infusion of saline or Muscimol immediately prior to entering the testing chamber for a Pavlovian acquisition session.

***Post-Infusion***

On the final 2 days of training all animals received a further 2 days of acquisition training immediately preceded by a dummy infusion.

**Histology and Group Allocation**

Cannulae placements are illustrated in (Figure X). One animal did not recover from surgery and was excluded. Three animals were excluded as a result of the cannulae assembly detaching from the skull. A further 3 animals were excluded as a result of failing to consume the pellets after recovery from surgery. One animal from the muscimol group was excluded from analysis as a result of a cannula tip embedded within the white matter of the forceps minor of the corpus callosum. Therefore, a total of 8 animals were excluded leaving *N* = 24 (saline *n* = 12, muscimol *n* = 12).

**PreCS Rates**

PreCS baseline responding did not differ between infusion groups across training and justified the use of CS-preCS difference scores for analyses of discriminative responding. In particular, during the infusion period a Group x Day (4 days) mixed ANOVA on preCS responses revealed a significant effect of Day (*F*(3, 66) = 5.95, *p* = .001) but no significant effect of Group (*F*(1, 22) = 0.01, *p* = .93) or Group x Day interaction (*F*(3, 66) = 0.41, *p* = .741). PreCS response rates on these days were, saline *M* = 0.70, *SD* = .48, muscimol *M* = 0.72, *SD* = .48.

**Experiment 3: Post-training LO Lesions**

**Methods**

**Subjects**

Subjects were twenty-four (total N = 24) male Long Evans rats (Monash Animal Services, Gippsland, Victoria, Australia) approximately 4 months old, weighing between 317-369 g (M = 338.9 g).

**Pre-lesion Training**

***Pavlovian Acquisition***

All animals received 9 days of Pavlovian acquisition training, 1 session per day. On the final day of training all animals were removed from food restriction for at least 24 hours before receiving sham or excitotoxic lesions of the OFC. Lesion conditions were pseudo-randomly assigned to animals such that group performance was matched on the final day of acquisition and an equal number of animals were assigned to each lesion condition in each homecage.

**Post-lesion Training**

***Pavlovian Acquisition***

Following post-operative recovery all animals were returned to food restriction for 24 hrs before receiving an additional 9 days of acquisition training.

**Histology and Group Allocation**

Lesion damage is depicted in Figure X. Lesion extent was judged by a trained observer blind to group allocation. A lesion was retained if there was evidence of significant bilateral damage constrained to LO or DLO. Animals were excluded if there was only unilateral LO/DLO damage, evidence of damage to the dorsal part of the anterior olfactory nucleus ventral to LO/DLO or if there was extensive damage to the white matter of the forceps minor of the corpus callosum. Three lesion animals had only unilateral OFC damage and were excluded from analysis (final *N* = 21; sham *n* = 12, lesion *n* = 9).

**PreCS Responding**

PreCS levels of responding did not differ between groups across days of training, and on the final block of 3 days (post-operative) response rates (15s) were sham *M* = 2.55, *SD* = 2.03, lesion *M* = 2.74, *SD* = 0.94. A mixed Group x DayBlock (6 blocks of 3 days) ANOVA on preCS responding supported this observation with only a significant main effect of DayBlock (*F*(5, 95) = 11.52, *p* < .001, effect of Group and Group x DayBlock interaction *F* < 1.00, *p* > .81).

**Experiment 5: Early training Acquisition with Muscimol Inactivation**

**Subjects**

Subjects were thirty-two (total N = 16) male Long Evans rats (Monash Animal Services, Gippsland, Victoria, Australia) approximately 4 months old, weighing between 321-399 g (M = 357.4 g).

**Surgery**

Surgical implantation of cannulae occurred prior to any behavioural training.

***Pavlovian Acquisition***

Animals were given 10sessions, 1 session per day. Briefly, each session consisted of a VI 200s ITI with 16 trials consisting of a 10s light CS (illumination of the house light at the back of the chmber) co-terminating with a single pellet US. Subjects received mock infusions on days 3 and 4, and either Saline or Muscimol was infused prior to entering the chamber on days 5-9. On day 10 all animals received a mock infusion.

**Histology and exclusions**

One rat in the Muscimol condition had a blocked guide cannulae and was excluded from experimental analysis. Final numbers N = 15 (Muscimol n = 7, Saline n = 8).

**PreCS Rates**

PreCS responding did not differ between infusion groups across the 10 days of Pavlovian conditioning (Group *F*1,13 = 2.72, *p* = .12; Day *F*9,117 = 1.49, *p* = .16; Group x Day *F*9,117 = 2.72, *p* = .25).

**Experiment 4. Pavlovian blocking following LO inactivation during acquisition**

**Subjects**

Subjects were thirty-two (total N = 32) male Long Evans rats (Monash Animal Services, Gippsland, Victoria, Australia) approximately 4 months old, weighing between 299-395 g (M = 331.5 g).

**Surgery**

Surgical implantation of cannulae occurred prior to any behavioural training.

**Training**

The design of the experiment was such that 4 CSs were designated as cues A, B, C and D. Cues A and C were always visual cues, either darkness caused by extinguishing the houselight or flashing panel lights (5Hz; Figure 3A). Cues B and D were always auditory cues, either an 80dB white noise or a 5Hz train of clicks. Throughout all training sessions the house light was always illuminated unless it was extinguished to act as a visual cue. All cues lasted 10s and co-terminated with the delivery of the US, 2 pellets delivered consecutively 0.25s apart. The identity of the cues was counterbalanced between subjects except that A and C were always visual cues and B and D were always auditory cues. Simultaneous audio-visual compounds were designated as AB and CD. Pavlovian training sessions were always 56 mins long such that there were 16 trials with a vITI 200s (range 100 to 300s); animals were left in the chambers for an additional 2 mins before being removed.

***Food Restriction and Magazine Training***

Magazine training sessions consisted of an RT120s reward delivery schedule for 16 rewards. Each reward consisted of 2 pellets delivered to the magazine 0.25s apart.

***Stage 1***

Stage 1 acquisition involved 10 days of acquisition to cue A, 16 trials per session. On days 1-4 of training all animals received dummy infusions to familiarise them to the infusion procedure. Animals were then split into two groups with matched performance on day 4. On days 5-10 all animals received an infusion of saline or muscimol immediately prior to entering the test chambers.

***Pre-exposure***

On day 11 all rats received pre-exposure to auditory cues B and D, 4 non-rewarded presentations of each cue vITI 200s. This was done to minimise novelty to the auditory cues during compound training in stage 2. All animals received dummy infusions prior to the session.

***Stage 2***

On days 12-14 all animals received stage 2 audio-visual compound training. Sessions involved 8 presentations of compound AB and 8 presentations of CD (pseudo randomly presented such that a compound was never repeated more than 2 times in a row). The compounds were rewarded with 2 pellets, the same US that was used in stage 1. All animals received dummy infusions prior to each session.

***Test***

On day 15 and 16 all animals were tested in extinction for responding to the target auditory cue B and the overshadowing control cue D (8 presentations of each cue, pseudorandom trial order, vITI 200s). All animals received dummy infusions prior to each session.

***Re-acquisition***

On days 17-19, all animals received re-acquisition training to cue B (16 trials per session) to test for differences in rates of re-acquisition to the blocked cue. On days 20-21 animals were tested for re-acquisition to cue A (16 trials per session) to test for differences in the rate of re-acquisition to the blocking cue.

**Results**

**Histology and Group Allocation**

Cannulae placements are illustrated in Figure X. 1 animal failed to consume pellets throughout the experiment and was excluded from testing. One animal from the muscimol group lost its cannula assembly during the infusion period and was excluded from testing. One animal in the muscimol group was euthanized due to severe illness. A further 2 animals were excluded after histological analysis revealed that the cannulae were only unilaterally targeting DLO and LO. Therefore, a total of 6 animals were excluded leaving *N* = 26 (saline *n* = 13, muscimol *n* = 13).

**PreCS Responding**

Baseline levels of responding did not differ between groups during training, and on the final day of infusions (day 10 of stage 1) preCS response rates (10s) were saline *M* = 0.122, *SD* = 0.24, muscimol *M* = 0.67, *SD* = 0.87. These observations were supported by mixed Group x Day ANOVAs on preCS responding in stage1 suggesting that there were no group differences on days 1-4 prior to infusion (all *F* < 1.69, *p* > .21) or on days 5-10 during infusions (significant main effect of Day *F*(5, 120) = 15.21, *p* < .001, all remaining *F* < 1.00, *p* > .50).

**Experiment 6. Effect of LO Inactivation on reward competition**

**Subjects**

Subjects were eight (total N = 8) male Long Evans rats (Monash Animal Services, Gippsland, Victoria, Australia) approximately 4 months old, weighing between 285-331 g (M = 314.9 g).

**Apparatus**

The apparatus comprised of 8 operant chambers (Med Associates Inc.) individually housed in light and sound attenuating cabinets. Each chamber comprised of a transparent Perspex back wall, roof and front door, with aluminium left and right-hand walls. The floor consisted of 19 steel bars (3.8mm diameter, spaced 1.6 cm apart), aligned perpendicular to the back of the chamber. Rewards could be independently delivered into one of two recessed magazines located centrally at the bottom of the left and right-hand walls. The magazine on the right-hand wall could be rewarded with food pellets (45 mg; Bio-Serv) whereas the magazine on the left hand wall could be rewarded with liquid rewards delivered by a dipper cup mechanism that could be retracted from the magazine. Access to the magazines was measured by infrared detectors at the mouth of the recess. Two panel lights (2 cm diameter) were located on either side of the right-hand magazine at the top of the right-hand wall. A 3-W house light was located at the top left of the left-hand wall. A speaker located to the right of the house light (on the top far right of the left-hand wall) could provide auditory stimuli to the chamber. In addition, a 5-Hz train of clicks produced by a heavy-duty relay placed outside the chamber at the back-right corner of the cabinet was used as an auditory stimulus. A computer equipped with Med-PC software (Med Associates Inc.) was used to control the experimental procedures and record data.

**Food Restriction and Magazine Training**

All animals were food restricted for at least 2 days prior to any training, and pre-exposed to sucrose (10 mL) and pellets (5g per rat) in their homecage.

Magazine training involved the unsignalled delivery of the reinforcer in the experimental chamber to familiarise the subjects with retrieving rewards from each of the two magazines. All rats received two separate magazine training sessions in one day (separated by at least 2 hours), one for each magazine, order counterbalanced. The dipper magazine was always paired with 20% w/v sucrose solution, the other magazine was always rewarded with pellets. Magazine training involved un-signalled delivery of reward on an RT60s schedule for 16 rewards.

**Acquisition**

Acquisition training lasted for 16 days. In each session rats received 3 consecutive blocks of 8 trials. Each trial consisted of a vITI 105s, a 15s CS (80 dB white noise) co-terminating in a pellet delivered into the pellet magazine. Simultaneously, there was always a probability of un-signalled 5s access to sucrose in the dipper magazine (dipper cup held 0.01cm3 fluid). The probability of sucrose availability changed randomly between each block from low (p = 2/24), medium (p = 4/24) to high (p=8/24). Each trial was defined by 24 bins of 5s (trial length = 120s). During this period the CS would occur across 3 5s bins (15s CS) and there was the possibility of un-signalled reward at the dipper magazine at the start of each 5s time bin. Notably, un-signalled reward could occur during any 5s bin, including the CS period. Unsignalled rewards occurred 2, 4, or 8 times in each trial. The pellets were therefore signalled rewards (as they were reliably preceded by the CS). The sucrose dipper was un-signalled, and if the sucrose was not collected during the 5s period the dipper would be retracted and lost.

**Water deprivation**

On days 17 and 18, all animals were water restricted for 22h prior to testing. Test sessions were identical to acquisition sessions. Animals were given 2 hours of free access to water 2 hours after test sessions. Animals were given 24 hours of ad libitum access to water after day 18 before any further testing to ensure animals were no longer thirsty in subsequent tests.

**Un-Signalled Reward Shift**

On testing days 19 and 20 received 2 sessions of acquisition with an increased magnitude of un-signalled reward delivery. Specifically, the size of the dipper cup was increased from 0.01 to 0.04 cm3 of fluid so that each un-signalled reward was increased in volume.

**Surgery and drug infusions**

Following testing day 20 all animals were taken off food restriction and underwent surgical implantation of guide cannulae targeting the lateral OFC.

**Test**

Following post-operative recovery all animals received 3 days of training immediately preceded by dummy infusions. All post-operative sessions used the larger sucrose dipper cup volume (0.04 cm3). Following this, all animals received 2 days of training in which they received 1 test day under saline infusion and 1 test day under muscimol infusion. The order of infusion days was counterbalanced. Test sessions were shortened to only 2 blocks with a fixed progression from low probability (p = 2/24) to high probability (p = 8/24) of un-signalled reward. This was done to ensure that muscimol was still active during the test session by keeping the session duration to 30 mins.

**Response Analysis**

It is important to note that all magazine responding in this procedure was analysed from periods in which the dipper reward was not physically present to eliminate the possibility that magazine responses simply reflect sucrose consumption.

**Histology**

Cannulae placements are illustrated in (FIGXXX). Two animals were due to the cannulae assembly losing patency during post-operatively. Therefore, a total *N*= 6 animals were tested post-operatively.