Environmental cues are often reliable signals of motivationally significant outcomes allowing one to predict outcomes and to perform appropriate behaviours based on this information. Neural activity in the orbitofrontal cortex (OFC) may form part of the representation of expected outcomes following predictive actions or cues (Lucantonio et al., 2015; Rudebeck & Murray, 2014; Schoenbaum & Esber, 2010; Mark E Walton, Chau, & Kennerley, 2015) and has been found to increase in the presence of cues that reliably predict rewards (Schoenbaum, Roesch, Stalnaker, & Takahashi, 2009; van Wingerden, Vinck, Lankelma, & Pennartz, 2010).

The OFC has been hypothesised as a site that integrates sensory and motivational information to adaptively increase or inhibit behaviour based on the up-to-the-moment expected value of predicted rewards (Rudebeck & Murray, 2014; Mark E Walton et al., 2015). For example, in outcome devaluation procedures an intact OFC is required to appropriately reduce responding for predicted outcomes that are no longer rewarding (Gallagher, McMahan, & Schoenbaum, 1999; Pickens et al., 2003; Pickens, Saddoris, Gallagher, & Holland, 2005; Rudebeck & Murray, 2011; West, DesJardin, Gale, & Malkova, 2011). Conversely, in Pavlovian over-expectation procedures a functional OFC is required to selectively increase responding when two predictive cues are compounded to predict an increase in expected reward value (Takahashi et al., 2009).

Surprisingly, disruption of OFC function does not disturb initial learning about cues that predict outcomes (Gallagher et al., 1999; McDannald, Lucantonio, Burke, Niv, & Schoenbaum, 2011) which is thought to depend on prediction-error signals that calculate the discrepancy between expected and actual outcomes (Rescorla & Wagner, 1972). Instead, current hypotheses attribute OFC involvement to situations in which initial learning must be modified by changes in the value or likelihood of the reward change. For example, in extinction procedures when an expected reward is no longer delivered the expected value of the cue should be updated to reflect this new state of affairs, a process that is predicted to involve OFC function (Panayi & Killcross, 2014; Wilson, Takahashi, Schoenbaum, & Niv, 2014). In line with this prediction, Panayi & Killcross (2014) found that selectively inactivating OFC function during extinction results in abnormally persistent responding to a cue that no longer predicts reward.

While the findings of Panayi & Killcross (2014) are consistent with the hypothesis that the OFC is required to update behaviour based on the current value of predicted rewards, there are two alternative explanations of these results. One possibility is that the role of the OFC is to form inhibitory associations between events and expected outcomes, and therefore the rats never learn to inhibit their established behaviour. In the past, simple inhibitory explanations of OFC function have been ruled out by evidence that subjects with OFC damage are ultimately able to inhibit inappropriate responding (Murray, O’Doherty, & Schoenbaum, 2007). However, suppression of behaviour can occur via a number of alternative mechanisms that do not involve inhibition such as behavioural competition, attention and habituation (Panayi & Killcross, 2014). The objective of this work is to provide the first direct test of the role of the OFC in the acquisition of inhibitory associations.

Extinction learning has been argued to predominantly involve new context-dependent inhibitory learning rather than unlearning of the original association (Bouton, 1993; Delamater, 2004). Therefore, a more recent explanation of the role of the OFC in extinction learning is that the OFC represents a new state to support this new inhibitory learning (Wilson et al., 2014). In the absence of this new state representation it is predicted that extinction will result in the unlearning of the original association which would retard the rate of extinction. Importantly, the OFC is proposed to only be involved in the representation of task states when the states are not explicitly signalled but instead require the use of memory to infer a new state.

Considered together, these hypotheses of OFC function provide three distinct predictions about the role of the OFC in the acquisition and expression of inhibitory associations in a Pavlovian conditioned inhibition task. If the OFC is simply involved in the acquisition of inhibitory associations, then OFC inactivation should disrupt the expression and subsequent learning of a conditioned inhibitor. If the OFC is involved in modulating behaviour based on the current expected value of predicted outcomes, then OFC inactivation should disrupt the expression but not the acquisition of a conditioned inhibitor. If the OFC is involved in representing the states of a task when changes in task contingencies are not explicitly signalled, then OFC inactivation should not disrupt the expression or acquisition of a conditioned inhibitor. Our findings indicate that OFC inactivation impairs the selective expression of behavioural inhibition during task acquisition. However, conditioned inhibition is acquired in rats when subsequently tested with functional OFC. These findings were also replicated when the conditions of training were matched to extinction learning tasks. These findings suggest that the OFC is not simply involved in the acquisition of inhibitory associations, but instead modulates behaviour when expected outcomes change.

**Materials and Methods**

*Animals.* Fifty-six adult male Long-Evans rats (302-406 g prior to surgery; Monash Animal Services, Gippsland, Victoria, Australia) were used (experiment 1, N = 32; experiment 2, N = 24). 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 ensuring that weight was approximately 95%, and never dropped below 85%, of ad libitum feeding weight. 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 a 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 (45 mg dustless precision grain-based pellets; Bio-serv, Frenchtown, NJ, USA) could be delivered into a recessed magazine located at the bottom centre of the right hand wall. The top of the magazine contained a white LED light that could be used 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 80% v/v ethanol 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.

*Surgery.* Surgery was performed prior to testing in experiment 1 and after initial training in experiment 2.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 1mm 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, but no liquids were infused. These dummy infusions were performed to familiarize the rats with the microinfusion procedure and thereby minimize stress.

*Histology.* Following completion of behavioural testing, rats received a lethal dose of sodium pentobarbital. The brains were removed, frozen, and sectioned coronally at 40 *μ*m through the lateral OFC with a cryostat. Every third section was collected on a slide and stained with cresyl violet. The location of cannula tips was determined under a microscope by a trained observer, unaware of subject’s group designations, using the boundaries defined by the atlas of George Paxinos and Watson (1998). Rats with bilateral cannulae placements outside the lateral or dorsolateral OFC were excluded from statistical analyses.

*Magazine training.* Prior to each experiment, all rats were familiarised with retrieving rewards from the magazine in a session of magazine training that lasted approximately 32 mins. Rewards, consisting of two pellets delivered 0.25s apart, were delivered randomly throughout the session every 120s until 32 pellets were delivered. The house light was kept illuminated throughout the session.

*General session parameters.* All sessions consisted of a number of trials in which 10s auditory and/or visual cues were presented. Visual cues designated as X and Y were flashing panel lights (0.1 s illuminated, 0.1s off) or extinguishing the house light (identity counter balanced). Visual cue Z was always a flashing magazine light (0.1 s illuminated, 0.1s off) for all animals. Auditory cues A and B were a 5 Hz train of clicks or a 78 dB white noise (identity counter balanced). Auditory cue C was always an 84 dB, 2.6 kHz tone. On rewarded trials (denoted by the symbol ‘+’) a single reward pellet was delivered upon CS termination. On non-reinforced trials (denoted by the symbol ‘-’), no reward was delivered. The variable inter-trial-interval was 90s (± 45s). Only a single training session occurred per day. All animals were handled in the infusion bucket for 5 minutes prior to each session and handled similarly regardless of whether drug infusions were administered. This was done to equate handling cues and stress on all training days.

*Experiment 1 acquisition days 1-4.* During each acquisition session a total of 36 trials were presented consisting of 12 A+, 12 B+ and 12 Z+ trials. Each session lasted 60 mins. On days 3 and 4, all animals received dummy infusions immediately prior to the session.

Experiment 1 feature negative training days 5-10. During each feature negative training session all animals received a total of 36 trials consisting of 10 A+, 20 AX- and 6 Z+ trials. The non-rewarded AX- trials consisted of the simultaneous presentation of the audio-visual cues A and X. The A+/AX- feature negative discrimination was used to establish cue X as a conditioned inhibitor. Prior to each feature negative training session all animals received an infusion of either muscimol or saline targeting the OFC.

*Experiment 1 cue retraining days 11-12.* During cue retraining sessions a total of 36 trials were presented, consisting of 18 B+ and 18 Z+ trials. This retraining was done to ensure that responding to cue B was high prior to the summation test.

*Experiment 1 summation probe test day 13.* The summation probe test consisted of 27 trials (45 mins session length) in the following order: first 3 Z+ and 3 B+ trials (order: Z+, B+, B+, Z+, B+, Z+). This rewarded start ensured high responding to the critical cue B. Then 2 B- and 2 BX- trials (Order randomised) were presented, followed by a Z+ trial. This cycle of 7 trials (B-/BX-/Z+) was repeated 2 more times. The B-/BX- cues were probe trials to test whether cue X had acquired inhibitory properties that transferred to cue B. The single rewarded Z+ trials were used to maintain responding throughout the probe trials. Finally, all animals received 6 presentations of Y- at the end of the session. This pre-exposure to cue Y was done to minimise any external inhibition that may occur during the retardation test that followed.

*Experiment 1 retardation test days 14-16.* The retardation test sessions contained 36 trials consisting of 12 X+, 12 Y+ and 12 Z- trials. This test shows whether the prior inhibitory training with cue X impairs subsequent excitatory acquisition relative to the novel cue Y. The non-rewarded cue Z was designed to prevent animals from responding non-discriminatively to all cues during this acquisition session.

*Experiment 1 consumption test days 17-18.* Following the retardation test, all animals were given a consumption test to assess whether muscimol infusions into the OFC impaired the motivation or timing of the consumption of pellets, which may have interfered with performance during the Stage 2 feature negative training under infusions. On day 17, all animals were given a dummy infusion immediately prior to entering the test chamber. Prior to the sessions, 40 pellets were placed in the magazine. All animals were given 30 minutes in the chamber. Magazine behaviour was recorded during this session for analysis, but there were no programmed events throughout the session. On day 18 all animals were infused with muscimol or saline before being entered for a consumption test identical to that on day 17.

*Experiment 2 acquisition days 1-6.* During acquisition sessions there were a total of 36 trials, consisting of 9 A+, 9 B+, 9 C+ and 9 Z+ trials. Animals were entered for 2 sessions per day for stage 1 training for a total of 12 sessions across 6 days. On the days following the final stage 1 acquisition session, animals were returned to free feeding and surgery was performed. Immediately following post-operative recovery all animals were returned to food restriction 2 days prior to re-acquisition.

*Experiment 2 re-acquisition days 7-9.* Sessions were identical to pre-surgical Stage 1 acquisition, except that only a single session was administered per day. On the final two days all animals received dummy infusions immediately prior each session.

*Experiment 2 feature negative training days 10-13.* During the feature negative training, each session consisted of 36 trials such that there were 18 AX- and 18 C- trials. Infusions of saline or Muscimol were administered immediately to separate groups (matched on performance to all cues) prior to each of these sessions.

*Experiment 2 extinction test day 14.* During the extinction test there were a total of 24 trials consisting of 12 A- and 12 C- trials.

*Experiment 2 summation and retardation tests*. The summation and retardation tests were identical to those described in Experiment 1.

Data analysis. CS responding was operationalized as the number of magazine entries during the 10s CS. PreCS responding was operationalized as the frequency of responding during the 10s immediately preceding the 10s CS and was used as a measure of baseline responding to the testing context. All data were analysed with mixed ANOVAs, and significant interactions of interest were followed up with ANOVAs on the relevant subset of data. Following significant omnibus ANOVA tests, planned linear and quadratic orthogonal trend contrasts and their interactions between groups were analysed to assess differences in rates of responding.

**Results**

**Experiment 1: LO inactivation disrupts the expression but not the acquisition of conditioned inhibition**

*Histology*

Cannulae placements are depicted in Figure 9. A total of two animals were excluded from further analysis due to misplaced cannulae. During training two further animals from the saline group were excluded and were not trained further as they failed to acquire magazine training after several days. Final numbers for infusion groups in Experiment 1 were saline (n = 13) and muscimol (n = 15).

Baseline responding

Rates of baseline responding did not significantly differ between groups during any of the testing phases, and justified the analysis of CS-PreCS difference scores as measures of discriminative responding to the cues in consequent analyses. Briefly, Group x Day mixed ANOVAs were run separately for each stage of testing. During stage 1 acquisition (main effect of Group F(1, 26) = 3.20, *p* = .09; Group x Day interaction F(3, 78) = 1.47, *p* = .23), stage 2 feature negative training, stage 3 cue re-training, summation and retardation tests all Group and Group x Day interactions did not reach significance (all F < 2.01, *p* > .16).

*Stage 1: Acquisition (Days 1-4)*

Acquisition of discriminative responding to each cue did not differ between groups (Figure 10A). This impression was confirmed by a mixed ANOVA with main factors of Group (saline, muscimol), Cue (A, B) and Day (1-4). Acquisition of responding to cues A and B increased significantly (main effect of day F(3, 78) = 20.41, *p* < .001; significant linear trend across Day F(1, 26) = 37.33, *p* < .001) and did not differ between groups (all other effects F < 1.5, *p* >.23).

Similarly, responding to control cue Z did not differ between groups (*F*igure 12), nor did it increase across Day (main effect of Group, Day and Group x Day interaction, all (all *F* < 1.6, *p* > .21). However, discriminative responding to cue Z was significantly above PreCS levels (test of model intercept i.e. test of grand mean responding above 0, *F*(1, 26) = 24.17, *p* < .001). Therefore, at the end of stage 1, all animals had acquired discriminative responding to all cues, but responding was significantly lower to cue Z.

*Stage 2: LO inactivation abolishes selective behavioural inhibition (days 5-10)*

Selectively inhibiting responding to the non-rewarded compound, AX-, relative to the rewarded cue, A+, indicates that the feature negative discrimination has been acquired successfully. Muscimol infusions into LO completely abolished the acquisition of this discrimination (Figure 10B). This impression was confirmed by a Group x Cue (A+, AX-) x Day (6 days) mixed ANOVA. The analysis revealed a significant 3-way Group x Cue x Day interaction (*F*(5, 130) = 2.89, *p* = .02; and a significant Group x Cue interaction *F*(1, 26) = 8.12, *p* = .008) suggesting that there were group differences in acquisition of the feature negative discrimination across days. Follow up Cue x Day ANOVAs were conducted for each group separately to explore this interaction. The muscimol group increased responding to the cues across days (main effect of Day, *F*(5, 70) = 4.88, *p* = .001; linear trend *F*(1, 14) = 11.66, *p* = .004) but did not discriminate between cues (non-significant effect of Cue and Cue x Day interaction, all *F*<1, *p* > .86). In contrast, the saline group acquired greater responding to A+ than AX- as suggested by significant effects of Cue (*F*(1, 12) = 11.13, *p* = .006), Day (*F*(5, 60) = 7.84, *p* < .001), and a Cue x Day interaction (*F*(5, 60) = 5.95, *p* < .001). Specifically, in the saline group responding to A+ increased (linear trend *F*(1, 12) = 28.04, *p* < .001), whereas responding to AX- did not significantly increase across days (linear trend *F*(1, 12) = 2.68, *p* = .13). Therefore, the saline group showed behavioural evidence of selective inhibition during the feature negative discrimination which was abolished by intra-LO infusions of muscimol.

Overall, responding was suppressed following intra-LO infusions of muscimol. This overall suppression of responding persisted temporarily during re-acquisition to cue B in the absence of further infusions (stage 3; Figure 10C). This was confirmed by a Group x Day (11, 12) mixed ANOVA which revealed that responding to cues increased across days (main effect of Day, *F*(1, 26) = 22.37, *p* = .001). Furthermore, a main effect of Group *F*(1, 26) = 4.59, *p* = .04) and a Group x Day interaction (*F*(1, 26) = 4.23, *p* = .05) revealed group differences in responding to cue B. Simple effects revealed that the muscimol group responded significantly lower than the saline group on day 11 (*F*(1, 26) = 7.52, *p* = .01) but not day 12 (*F*(1, 26) = 1.86, *p* = .19). This suggests that the effect of muscimol infusion in stage 2 temporarily and non-selectively lowered overall performance when trained drug free in stage 3.

*LO inactivation during training does not prevent the acquisition of conditioned inhibition*

While LO inactivation successfully abolished the expression of selective conditioned inhibition in the feature negative discrimination, it is not clear whether this indicates a failure of acquisition of conditioned inhibition or just impaired behavioural expression. To address this question, summation and retardation tests of conditioned inhibition (Papini & Bitterman, 1993; Rescorla, 1969) were administered in the absence of pre-session drug infusions.

The results of the summation test (day 13; *F*igure 11A) suggested that both groups responded less to the compound BX- than B- which suggests that cue X successfully acquired inhibitory properties during the feature negative training. This observation was confirmed by a Group x Cue (B-, BX-) mixed ANOVA. Specifically, there was a significant main effect of Cue (*F*(1, 26) = 7.60, *p* = .01). While the magnitude of the Cue effect may appear weaker in the muscimol group than the saline group, this observation was not supported statistically (no main effect of Group *F*(1, 26) = 0.72, *p* = .40, or Group x Cue interaction *F*(1, 26) = 2.12, *p* = .16). These findings suggest that intra-LO infusions of muscimol did not disrupt the acquisition of conditioned inhibition to cue X as assessed by a summation test. However, enhanced attention to cue X, generalisation decrement or external inhibition may also account for a reduction in responding to the BX compound. To rule out these alternative explanations a retardation test was conducted in which the rate of acquisition to X+ was compared to the relatively novel cue Y+. If cue X has acquired inhibitory properties, then acquisition should be slower to X+ than Y+. Importantly, this result would be incompatible with an account of the summation test appealing to enhanced attention to X.

During the retardation test (days 14-16) acquisition to target cue X+ appeared significantly lower than control cue Y+ in both groups (Figure 11B). A Group x Cue (X+, Y+) x Day (14, 15, 16) mixed ANOVA revealed a significant main effect of Cue (*F*(1, 26) = 8.82, *p* = .006) and Day (*F*(2, 52) = 5.53, *p* = .008) but no other significant effects (Cue x Day interaction *F*(2, 52) = 2.22, *p* = .12, all other *F*< 1.42, *p* > .24). This retarded acquisition to cue X+ relative to Y+ suggests a significant retardation effect of similar magnitude in both the saline and muscimol groups. Together, the results of the summation and retardation tests suggest that cue X has acquired conditioned inhibition, even though LO inactivation abolished discriminative performance during the feature negative training in stage 2.

*LO inactivation disrupts Pavlovian acquisition*

The adverse consequence of disrupting OFC function is usually only detected when task contingencies change (Rudebeck & Murray, 2014; Wilson et al., 2014), but rarely during the initial acquisition of a task when contingencies and response requirements are constant (M E Walton, Behrens, Noonan, & Rushworth, 2011). Therefore, it is surprising that LO inactivation during the feature negative discrimination also disrupted the acquisition of responding to control cue Z (Figure 12). This impression was confirmed by a significant main effect of Group (*F*(1, 26) = 16.46, *p* < .001) and a Group x Day interaction (*F*(5, 130) = 3.47, *p* = .006; Group x Day linear trend contrast *F*(1, 26) = 6.27, *p* = .02). Follow up linear trend contrasts across Day revealed significant increases in responding in the saline group (*F*(1, 12) = 18.97, *p* = .001) but not the muscimol group (*F*(1, 14) = 3.26, *p* = .09; overall muscimol group responding remained significantly above baseline *F*(1, 14) = 22.37, *p* < .001). Therefore, muscimol infusions significantly suppressed acquisition to cue Z, which suggests a role for LO in Pavlovian acquisition.

Suppressed responding to cue Z persisted in the muscimol group when trained drug-free in subsequent sessions. Drug-free acquisition to cue Z (stage 3; Figure 12) was assessed with a Group x Day (11, 12) mixed ANOVA which revealed that responding to cues increased across days (main effect of Day, *F*(1, 26) = 4.24, *p* = .05). Furthermore, a significant main effect of Group *F*(1, 26) = 7.17, *p* = .01) but no significant Group x Day interaction (*F*(1, 26) = 0.31, *p* = .58) revealed that responding to cue Z was significantly lower in the muscimol than the saline group. This difference between groups in responding to cue Z persisted across three days of extinction to cue Z during the retardation test (Figure 12). A Group x Day mixed ANOVA supported this interpretation with a significant main effect of Group (*F*(1, 26) = 4.50, *p* = .04) and Day (*F*(2, 52) = 27.44, *p* < .001) but no Group x Day interaction (*F*(2, 52) = 1.80, *p* = .18). Overall, the pattern of data suggests that muscimol inactivation of LO disrupted responding to all cues which persisted during further drug free training.

*LO inactivation does not disrupt the motivation to consume food reward*

The significant suppression of responding following LO inactivation observed in stage 2 may have been a consequence of reduced motivation to consume the food reward. This explanation is unlikely given the absence of uneaten rewards following sessions in stage 2, however a more direct test of this explanation was necessary to rule out the possibility that the rewards were not eaten towards the end of the session when muscimol was no longer effective. Therefore, a consumption test was conducted within the test chambers with all animals being tested 10 mins after an infusion to ensure that the muscimol was maximally effective. Prior to the consumption test 2 saline and 1 muscimol infused animal lost their cannula assembly and were not eligible for testing (saline n = 11, muscimol n = 14). All animals consumed all pellets delivered by the end of the session on both days, regardless of infusion group. Similarly, there was no evidence that muscimol infusion differentially affected magazine approach for reward (Figure 13). A Group x Infusion (No Infusion, Infusion) x Block (6 blocks of 5 mins) mixed ANOVA found no significant main effect or interactions with Group (all *F* < 1.00, *p* > .34). A main effect of Block (*F*(5, 115) = 246.18, *p* < .001), Infusion (*F*(1, 23) = 6.53, *p* = .02), and Block x Infusion interaction (*F*(5, 115) = 2.69, *p* = .02), revealed that overall responding was lower on infusion day 18. These findings suggest that the low responding to all cues in stage 2 following muscimol infusions is unlikely to be due to suppressed appetite or motivation for the US, or a general suppression of response vigour.

**Experiment 2: LO inactivation disrupts between- but not within- session Pavlovian extinction**

Histology

Cannulae placements are depicted in Figure 14. All cannulae tips were located within LO or DLO. Final group numbers were saline (n = 12) and muscimol (n = 12).

Baseline responding

Rates of baseline responding did not significantly differ between groups during any of the testing phases and justified the analysis of CS-PreCS difference scores as measures of discriminative responding to the cues in consequent analyses. Briefly, one-way Group or Group x Day mixed ANOVAs were run separately for each stage of testing to assess the effects of Group. During stage 2 feature negative training (main effect of Group *F*(1, 22) = 2.55, *p* = .12; Group x Day interaction *F*(3, 66) = 1.72, *p* = .17), stage 3 testing (Group *F*(1, 22) = 3.32, *p* = .08), during stage 1 acquisition, summation and retardation tests all Group and Group x Day interactions failed to reach significance (all *F* < 1.72, *p* > .17).

Stage 1 Acquisition (Days 1-9)

Acquisition of discriminative responding to cues A, B and C did not differ between (infusion) groups across stage 1 of acquisition (Figure 15A). A Group x Cue (A, B, C) x Day mixed ANOVA revealed a significant main effect of Day (*F*(8, 176) = 26.07, *p* < .001) but no significant effects of Cue, Group or their interactions (all *F* < 1, *p* > .65). Therefore, acquisition was successful to all cues and did not differ between groups.

*Stage 2: LO inactivation enhances within- but disrupts between session Pavlovian extinction (days 10-13)*

Extinction of cue C following infusions in stage 2 allowed for a replication of the findings of Panayi & Killcross (Panayi & Killcross, 2014) that LO inactivation disrupts between- but not within- session extinction. Extinction to cue A in compound with cue X was designed to test whether LO inactivation impairs Pavlovian extinction by disrupting the formation of conditioned inhibition that may form during extinction (Delamater, 2004; Rescorla, 1969). Overall, the rate of extinction differed between drug infusion groups (*F*igure 15B) such that behaviour in the muscimol group appeared to extinguish more rapidly within-session but not between-sessions compared to the saline group. A mixed Group x Cue (AX-, C-) x Day (4) x Block (3 blocks of 6 trials) ANOVA supported the observed pattern of results.

In all animals, evidence of extinction between and within-sessions was supported by significant main effects of Day (*F*(3, 66) = 17.65, *p* < .001) and Block (*F*(2, 44) = 24.40, *p* < .001) and a Day x Block interaction (*F*(6, 132) = 2.52, *p* = .02). Overall responding to both cues did not differ (non-significant main effect of Cue *F*(1, 22) = 1.69, *p* = .21) but a significant Cue x Day interaction suggested that extinction between days was more rapid for AX- than C- (an effect that did not differ between groups, non-significant Group x Cue x Day interaction *F*(3, 66) = 0.83, *p* = .48). Follow up analysis of cue differences revealed a significant Cue x Day linear trend interaction *F*(1, 22) = 8.41, *p* = .01, such that the magnitude of significant negative trend across days was greater for C- (*F*(1, 22) = 33.17, *p* < .001) than AX- (*F*(1, 22) = 7.77, *p* = .01). Reduced responding to the compound AX- compared to C- is consistent with external inhibition or generalisation decrement accounts of the novel presence of cue X suppressing responding.

While there was no overall effect of Group (*F*(1, 22) = 0.63, *p* = .44) there was a significant Group x Day (*F*(3, 66) = 2.93, *p* = .04) and a Group x Block interaction (*F*(2, 44) = 16.35, *p* < .001; all other interactions with Group failed to reach significance, all *F* < 2.30, *p* > .11). Follow up analysis of linear and quadratic Group x Day trend interactions failed to reach significance (linear *F*(1, 22) = 3.44, *p* = .08, quadratic *F*(1, 22) = 3.05, *p* = .10). This suggests that the impaired between-session extinction observed in the muscimol group only approached significance. Follow up analysis of linear and quadratic Group x Block trend interactions were significant (linear *F*(1, 22) = 34.64, *p* = .001; quadratic *F*(1, 22) = 20.94, *p* < .001). Simple trend contrasts across Block revealed significant linear and quadratic trend for the muscimol group (linear *F*(1, 11) = 14.69, *p* = .003, quadratic *F*(1, 11) = 5.08, *p* = .046) but only significant linear trend in the saline group (linear *F*(1, 11) = 21.73, *p* = .001, quadratic *F*(1, 11) = 01, *p* = .93). This pattern of results suggests that the linear decrease in within-session extinction was greater in the muscimol group compared to the saline group. This greater linear increase in the muscimol group is likely to be due to higher responding at the start of each session in the muscimol group, whereas the lower responding in the saline group at the start of each session provided less opportunity for any further reduction in responding.

Given the failure to find the source of the significant Group x Day interaction described above a Group x Cue x Day analysis was run on the first block of trials only. This analysis will allow for a more direct assessment of impairments in the retention of between-session extinction. This analysis revealed a significant main effect of Day (*F*(3, 66) = 10.19, *p* < .001), a Cue x Day interaction Day (*F*(3, 66) = 2.88, *p* = .04) and a significant main effect of Group (*F*(1, 22) = 4.46, *p* < .05). This suggests that there was evidence of between-session extinction in both the saline and the muscimol groups, however overall responding was higher in the muscimol group. Therefore, there is some evidence of poorer between-session extinction retention in the muscimol group compared to the saline group.

Drug free tests of A- and C- revealed that the muscimol group did not acquire extinction to both cues to the same extent as the saline group (Figure 15C). Surprisingly, there was no evidence that compound extinction of cue A with cue X had “protected” cue A from extinction relative to cue C, in fact the mean responding to both cues were identical in both groups. A mixed Group x Cue (A-, C-) x Block (4 blocks of 3 trials) ANOVA supported this observation with no significant effect of Cue or Group x Cue interaction (both *F*(1, 22) = 0.00, *p* = 1.00). However, there was a significant effect of Block (*F*(3, 66) = 3.45, *p* = .02) suggesting within-session extinction behaviour at test and a significant main effect of Group (*F*(1, 22) = 16.02, *p* = .001) showing higher responding in the muscimol than the saline group (all other effects did not reach significance, all *F* < 1.38, *p* > .26).

*Stage 4 Summation test (Day 15)*

Responding to compound BX was lower than to cue B alone in both groups at test (Figure 16A). A Group x Cue (B-, BX-) mixed ANOVA supported this with a significant main effect of Cue (*F*(1, 22) = 4.67, *p* = .04) but no significant effect of Group (*F*(1, 22) <.01, *p* = .96) or Group x Cue interaction (*F*(1, 22) = 0.10, *p* = .75). Therefore, the summation test provided evidence of conditioned inhibition to cue X in both groups.

Stage 5 Retardation test (Days 16-18)

Responding during the retardation test suggested that the rate of acquisition to cue Y was greater than cue X in the muscimol group but not the saline group (Figure 16B). However, this observation was not fully supported statistically by a Group x Cue (X, Y) x Day mixed ANOVA which failed to reveal a significant Group x Cue x Day 3-way interaction (*F*(2, 44) = 2.23, *p* = .12; there was a significant main effect of Day *F*(2, 44) = 10.87, *p* < .001, but all other effects failed to reach significance, all *F* < 2.68, *p* > .12). Given the weak evidence for conditioned inhibition in this experimental design in the literature (REF), planned orthogonal linear and quadratic Group x Cue x Day trend contrasts were tested. This planned analysis revealed a significant quadratic (*F*(1, 22) = 5.42, *p* = .03) but not linear (*F*(1, 22) = 0.68, *p* = .42) 3-way interaction. Follow up Cue x Day quadratic trend was found to be significant in the muscimol group (*F*(1, 11) = 7.53, *p* = .02) but not the saline group (*F*(1, 11) = 0.14, *p* = .71). This suggested that the rate of increase during acquisition was greater for cue Y than cue X in the muscimol but not the saline group.

Cue Z

Responding to control cue Z did not differ throughout training (Figure 17). For completeness, responding during acquisition to cue Z did not differ between groups in stage 1. A mixed Group x Day(1-9) ANOVA supported this observation revealing only a main effect of day (*F*(8, 176) = 8.80, *p* < .001) but no main effect of Group or Group x Day interaction (all *F* < 0.18, *p* > .99). The rate of extinction to control cue Z during the retardation test did not differ between groups as confirmed by a mixed Group x Day(16, 17, 18) ANOVA with no significant effect of Group (*F*(1, 22) = 2.07, *p* = .16) or Group x Day interaction (*F*(2, 44) = 0.08, *p* > .93; significant main effect of Day *F*(2, 44) = 20.78, *p* < .001).

Locomotor activity

There was no differential effect of drug infusion on general locomotor activity (Figure 14D). This was supported by a mixed Group x Block (6 blocks of 10 mins) on total ambulatory distance which revealed a significant effect of Block (*F*(5, 110) = 93.52, *p* < .001) but no Group or Group x Block interaction effects (all *F* < 1, *p* > .96).

Discussion

To test whether deficits in extinction learning following OFC inactivation are due to disrupting the acquisition of conditioned inhibition, we used two procedures to establish inhibition to a novel cue. We found that, compared to control infusions of saline, OFC inactivation via infusions of muscimol reliably disrupted the retention of extinction learning between sessions while increasing the rate of extinction within sessions. Furthermore, when extinction of a different predictive cue occurred in compound with a novel cue, regardless of OFC function during the extinction phase, rats did not readily acquire inhibitory properties to the novel cue as assessed by a retardation test. This suggests that the parameters used in this procedure did not reliably promote the formation of conditioned inhibition between incidental cues and the outcome. OFC inactivation during a feature negative discrimination procedure designed to reliably produce conditioned inhibition did not affect the formation and subsequent expression of conditioned inhibition as assessed by both a summation and retardation test of inhibition. However, discriminative behavioural control during the feature negative discrimination was abolished following OFC inactivation which resulted in similarly low levels of responding to all of the cues presented. Surprisingly, the suppression of responding to the control cue during OFC inactivation persisted on subsequent training sessions in the absence of infusions. These findings extend our understanding of the role of the OFC in reward guided behavioural control and have implications for the current dominant theories of OFC function.

**LO is not necessary for the learning of conditioned inhibition**

* Consistent with findings that the OFC is not required for novel learning, especially when changes in task demands are explicitly signalled by cues (c.f. Wilson et al)

**LO inactivation disrupts the expression of conditioned inhibition**

Impairments to appropriate behaviour following inactivation or lesions of the OFC normally results in a failure to suppress or reduce inappropriate behaviour {Reversal, Devaluation, Extinction}. In the present study, OFC inactivation disrupted the expression of selective inhibition during a feature negative discrimination

* OFC inactivation abolishes discriminative responding (i.e. selective behavioural inhibition)
* Papers looking at discrimination learning (5-CSRRT?) -? Similar?

LO inactivation disrupts between- but not within-session extinction

LO inactivation suppresses acquisition behaviour

*LO modulates behaviour based on the current value of expected outcomes*

**Over-expectation papers**? The failure to disrupt new inhibitory learning in the present experiments is not consistent with these findings. However, the persistent suppression to cue Z is similar to the findings of over-expectation experiments. However, it is not clear if the suppression observed here is necessarily due to impairments in learning or merely behavioural suppression… something that applies equally to the overexpectation results.

* In terms of explanations using the Wilson model, it is not clear how the model would account for the suppression in behaviour seen during normal acquisition… Doing so would have to concede that continued acquisition (sub-asymptotic) requires the mental simulation of hidden states… This throws out all the other unique predictions made by this model
* Furthermore, the failure to account for the extinction results replicated here is in direct opposition the explicitly predicted results!

Solution, the function of the LO is not described by this model, it may describe other parts of the OFC (Cite Mel’s Neuron commentary …. ?).

**Credit assignment?** It would seem that poor credit assignment could account for the overall suppression in performance found in the muscimol group, this could be a misattribution of the non-rewarded trials to cue A… This account fits nicely with the infusion data, but not with the intact acquisition of conditioned inhibition when tested drug-free which suggests unimpaired credit assignment during this learning period.

Interaction between LO and other neural circuits

* (Keiflin, Reese, Woods, & Janak, 2013) – Hierarchical view
  + Placements were accurately within LO, a bit more posterior i.e. focussed around +3.70
  + Expt 1b. extinction test – inactivation resulted in rapid extinction as measured by # f trials initiated
  + Impaired responding to cue Z persists when tested drug free on the following day unlike the hierarchical results found in kieflin et al
    - Similarly, extinction to cue C (replicating Panayi & Killcross…) retained deficits from stage 2 when tested drug free
    - Unfortunately, it is not clear how long term the deficits in Keiflin et al’s experiments are i.e. Figure 6C, there was no test drug free following the second infusion day to see if the MB/MB group retained their preference. Furthermore, both MB groups started at around 0 preference suggesting conflicting response control.
* (Wilson et al., 2014) – Hidden state space view
  + Impoverished extinction between days (cue C) is consistent with the model predictions, except that the model data are based on extinction within session (Butter, 1969; 10 min blocks Fig 3).
  + Another troubling result is the explicitly cued absence of the outcome in the feature negative design i.e. cue X is clearly an explicit rather than a hidden state, yet performance was impoverished throughout LO inactivation despite the clear cues to help guide behaviour.
* Current outcome value view - (Rudebeck & Murray, 2014; Mark E Walton et al., 2015)
  + Current value of outcome is not available under drug infusions, thus affecting responding but not necessarily affecting learning – other systems? [tie in with hierarchical system view of Keiflin?]
  + A method by which CS representation hangs around in A2 and never returns to I for re-activation to A1 in the presence of the cue
  + Walton experiment with credit assignment -> shows that learning was driven by specific Pavlovian contingencies, however since this was a lesion study it is not clear if this is a disruption of learning or behavioural performance per se.

??? Change entire article to OFC and then discuss functional heterogeneity in the discussion???