**Figure 4 – Supplementary Figure 2**

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

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

The Pavlovian CS selectively increased activity at the relevant Pavlovian magazine (Pellet: PreCS vs CS *F*(1, 7) = 29.62, *p* = .001; Sucrose: PreCS vs CS *F*(1, 7) = 1.14, *p* = .33; Period\*Magazine interaction *F*(1, 7) = 13.44, *p* = .008; main effect of Period *F*(1, 7) = 71.86, *p* < .001). Furthermore, the effect of the background rate of unsignalled sucrose availability modulated activity at the sucrose and pellet magazines in opposing directions. As the probability of sucrose availability increased, activity at the sucrose magazine increased whereas activity at the pellet magazine decreased (Sucrose: Low vs Medium *F*(1, 7) = 81.66, *p* < .001, Low vs High *F*(1, 7) = 46.47, *p* < .001, Medium vs High *F*(1, 7) = 23.66, *p* = .002; Pellet: Low vs Medium *F*(1, 7) = 2.84, *p* = .14, Low vs High *F*(1, 7) = 7.71, *p* = .027, Medium vs High *F*(1, 7) = 15.73, *p* = .005; Sidak corrected significance threshold *p <* .017; significant Magazine\*Probability interaction *F*(2, 14) = 22.49 *p* < .001).

Finally, increasing the volume of the sucrose reward significantly increased activity directed at the sucrose but not the pellet magazine, whereas increasing thirst did not significantly change behaviour in this task (Sucrose: Acquisition vs Thirst *F*(1, 7) = 6.26, *p* = .041, Acquisition vs 4xDipper *F*(1, 7) = 14.89, *p* = .006, Thirst vs 4xDipper *F*(1, 7) = 17.53, *p* = .004; Pellet: Acquisition vs Thirst *F*(1, 7) = 0.42, *p* = .54, Acquisition vs 4xDipper *F*(1, 7) = 3.45, *p* = .11,Thirst vs 4xDipper *F*(1, 7) = 1.01, *p* = .35; Sidak corrected significance threshold *p <* .017; significant Magazine\*Shift interaction *F*(2, 14) = 5.03, *p* = .02). No other meaningful effects were significant (Period\*Probability *F*(2, 14) = 9.59, *p* = .002; All remaining *F* <3.42, *p* > .06).

Data were also analysed as the difference between responding to the pellet vs. the sucrose magazine to clearly see response competition (bottom row). The pattern of results suggests that at the end of acquisition, responding to the sucrose magazine was greatest during the PreCS baseline period and responding was greatest to the pellet magazine during the CS period. 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). Sidak 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). Sidak 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.

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**Competing response values**

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

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

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

The pattern of responding at test under infusions of saline (Figure 4B) was similar to the pattern observed prior to surgery (Figure 4 – Figure Supplement 2). Specifically, during the baseline PreCS 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.

**Competing response values**

One possible account of the impaired performance following OFC inactivation in the present study is an inability to potentiate behaviour based on the current value of the expected 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.

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