

## BEHAVIORAL NEUROSCIENCE

# Orbitofrontal inactivation impairs reversal of Pavlovian learning by interfering with ‘disinhibition’ of responding for previously unrewarded cues

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**Keywords:** associative learning, expectancies, orbitofrontal, Pavlovian, rat, reversal

## Abstract

Orbitofrontal cortex (OFC) is critical for reversal learning. Reversal deficits are typically demonstrated in complex settings that combine Pavlovian and instrumental learning. Yet recent work has implicated the OFC specifically in behaviors guided by cues and the features of the specific outcomes they predict. To test whether the OFC is important for reversing such Pavlovian associations in the absence of confounding instrumental requirements, we trained rats on a simple Pavlovian task in which two auditory cues were presented, one paired with a food pellet reward and the other presented without reward. After learning, we reversed the cue–outcome associations. For half the rats, OFC was inactivated prior to each reversal session. Inactivation of OFC impaired the ability of the rats to reverse conditioned responding. This deficit reflected the inability of inactivated rats to develop normal responding for the previously unrewarded cue; inactivation of OFC had no impact on the ability of the rats to inhibit responding to the previously rewarded cue. These data show that OFC is critical to reversal of Pavlovian responding, and that the role of OFC in this behavior cannot be explained as a simple deficit in response inhibition. Furthermore, the contrast between the normal inhibition of responding, reported here, and impaired inhibition of responding during Pavlovian over-expectation, reported previously, suggests the novel hypothesis that OFC may be particularly critical for learning (or behavior) when it requires the subject to generate predictions about outcomes by bringing together or integrating disparate pieces of associative information.

## Introduction

Orbitofrontal cortex (OFC) is critical for reversal learning. This has been demonstrated in rats, cats, mice, monkeys and humans (Teitelbaum, 1964; Butter, 1969; Dias *et al.*, 1996; Bechara *et al.*, 1997; Ferry *et al.*, 2000; Chudasama & Robbins, 2003; Fellows & Farah, 2003; Schoenbaum *et al.*, 2003; Hornak *et al.*, 2004; Izquierdo *et al.*, 2004; Bissonette *et al.*, 2008). Indeed, reversal deficits have come to epitomize the effects of damage to OFC.

More recently, work using very different behavioral paradigms has highlighted a role for OFC in the performance of behaviors that depend on Pavlovian associations, specifically associations between cues and outcomes. For example, OFC is critical for spontaneous changes in conditioned responding after reinforcer devaluation (Gallagher *et al.*, 1999; Izquierdo *et al.*, 2004; Pickens *et al.*, 2005), and also for changes in the expression of outcome-specific Pavlovian-

to-instrumental transfer (Ostlund & Balleine, 2007a) and in outcome-guided conditioned reinforcement (Burke *et al.*, 2008). All of these settings illustrate a specific role for OFC in mediating Pavlovian representations, linking cues to the outcomes they predict. Consistent with this, recording studies in both rats and monkeys and imaging work in humans have shown that cue-evoked neural signals in OFC are particularly attuned to these associations (Thorpe *et al.*, 1983; Rolls *et al.*, 1996; Schoenbaum *et al.*, 1999; Ramus & Eichenbaum, 2000; Gottfried *et al.*, 2003; O’Doherty *et al.*, 2003; Wallis & Miller, 2003; Roesch & Olson, 2004; Hampton *et al.*, 2006; Morrison & Salzman, 2006; Padoa-Schioppa & Assad, 2006; Roesch *et al.*, 2006). Indeed, at least one recent report has suggested that when behaviors do not depend on cue-evoked information about outcomes, OFC may not even be involved (Ostlund & Balleine, 2007a,b).

Nearly all of the evidence linking OFC to reversal learning is derived from complex settings that combine Pavlovian and instrumental learning. This is because reversal deficits are typically demonstrated in discrimination settings in which instrumental responses are rewarded after presentation of one cue but not the other. Thus, the role of OFC in reversal learning might be related to its

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Received 14 September 2009, accepted 15 September 2009

role in guiding other Pavlovian behaviors or it might reflect another function, such as rule learning, required in these complex settings (Murray & Izquierdo, 2007).

Here we tested this simple question by inactivating OFC during reversal of a Pavlovian discrimination. This setting differs from the reversal tasks cited above in that there was no contingency between the animal's response and reward delivery. As expected, OFC inactivation impaired the ability of the rats to reverse conditioned responding. However, somewhat surprisingly, the deficit reflected an inability of these rats to develop normal conditioned responding for the previously unrewarded cue. Inactivation of OFC had no impact on the ability of the rats to inhibit responding to the previously rewarded cue. These data show that although OFC is critical to reversal of Pavlovian responding, the role of OFC in this function cannot be explained as a simple deficit in response inhibition. Notably, OFC inactivation in these same rats impaired the normal inhibition of responding in a Pavlovian over-expectation task (Takahashi *et al.*, 2009). As we will discuss, the contrast between the impairment reported in that study and the lack of effect of OFC inactivation on response inhibition here suggests that orbitofrontal signaling may be particularly critical when normal learning (or behavior) requires the subject to generate predictions about outcomes by bringing together or integrating disparate pieces of associative information.

## Materials and methods

### Subjects

A total of 16 Long-Evans rats (Charles River Laboratories, Wilmington, MA, USA), weighing between 320 and 450 g, were housed individually and were placed on a 12-h light : dark schedule. During non-testing periods, all rats had *ad libitum* access to food and water but, during behavioral testing, all rats were food restricted to 85% of their baseline weights. Testing was conducted during the light period of the rats' cycles. All testing was conducted in accordance with NIH guidelines, and was approved by the Animal Care and Use Committee at the University of Maryland, Baltimore, USA.

### Surgical procedures

Twenty-three gage cannulae (Plastics One, Roanoke, VA, USA) were implanted in all rats bilaterally into OFC (coordinates: 3.0 mm anterior to bregma; 3.2 mm lateral and 5.0 mm ventral; Paxinos & Watson, 1998) during stereotaxic surgery to allow for infusions of a  $\gamma$ -aminobutyric acid (GABA)-A/B agonist cocktail (muscimol/baclofen;  $n = 8$ ) or saline for controls ( $n = 8$ ). Surgical and infusion protocols were identical to those used previously (Schoenbaum *et al.*, 2007; Takahashi *et al.*, 2009). Rats were anesthetized with isoflurane and then placed into a stereotax. Here the head placement was adjusted to ensure that bregma and lambda were placed at the same level. A midline incision was made to expose the skull, burr holes were drilled over the OFC, and guide + dummy cannulae (Plastics One) were lowered and cemented to the skull. During non-infusion periods, dummy cannulae were left in place. Two rats in the experimental group lost one of their cannulae during the course of the experiment and were excluded from the study.

### Apparatus

Testing was conducted in eight standard-sized rat behavioral boxes from Coulbourn Instruments (12"W  $\times$  10"D  $\times$  12"H; Allentown, PA,

USA), enclosed in a sound-resistant shell. One recessed food cup was placed in the center panel, 2 cm above the floor. Infrared photocells placed inside the food troughs were used to measure conditioning behavior. Each food trough was connected to a feeder placed outside the behavior chamber. Each feeder was set up to deliver 45 mg sucrose pellets (banana- or grape-flavored pellets; Bio-Serv, Frenchtown, NJ, USA). Infrared illuminating lights placed above the food trough were used for the experimenter to detect cue periods recorded on DVDs. Auditory cues were used in the conditioning training. Speakers mounted inside the behavior chamber were used to deliver noise cues, such as a white noise and a tone cue (4 kHz,  $\sim$ 76 dB). Additionally, a clicker auditory cue was used (2 Hz).

### Pavlovian conditioning

Prior to training, all rats in the current study received 2 weeks of training in a Pavlovian over-expectation task (Takahashi *et al.*, 2009). In this task, four cues were used: three auditory cues (white noise, tone and clicker) and one visual cue (cue light). Rats received all four cues (30 s each) in a blocked design. In this design, each cue was presented eight times in their own respective block (four blocks, counterbalanced in terms of block) over 10 days. The average inter-trial interval (ITI) between cues in a particular block was 2.5 min, and between blocks was 5–10 min. Two cues (one visual and one auditory) terminated with the delivery of three pellets, noted as O1 (grape or banana flavored; Bio-Serv). Additionally, a second auditory cue terminated with three pellets of the alternative flavored sucrose pellet, O2. The fourth cue served as a control cue and was paired with no food. Next, in compound conditioning, the visual cue and the auditory cue that led to the same outcome were presented together as a compound and led to the same three O1 pellets. The other two auditory cues were presented separately during this time and remained unchanged. These two cues subsequently served as the CS+ and CS- in this current study, and were trained in an identical fashion as used previously. Note, during compound conditioning, some rats in the experimental groups received infusions of baclofen/muscimol (see methods below). Additionally, rats received a probe test in the earlier study at the end of training where all cues were presented eight times each under extinction settings. After approximately 3 weeks between the completion of this testing, training for this current experiment began. During this time, the rats remained in their home cages, and received periodic handling and *ad libitum* food and water.

Reminder training was conducted over 3 days. During each daily session, the rats were presented with two of the auditory cues used in the previous training: a 30-s CS+, designated A1, which terminated with the delivery of three flavored sucrose pellets (same pellets that were paired with this cue previously) and a 30-s CS-, designated A2, which terminated with no reward. Each cue was presented eight times in a blocked design (counterbalanced in terms of order, using the same ITIs as listed above both within and between blocks). The specific cues (tone, white noise and clicker) and food pellet rewards (banana- and grape-flavored sucrose pellets) were counterbalanced. By the third day, responding in both groups had returned to a ceiling similar to that observed in the initial training. There were no effects of prior experience on responding during reminder training in the current study. After 3 days of reminder training, we began reversal sessions. These sessions were identical to the reminder sessions except that the cue-outcome associations were switched such that A2 predicted reward and A1 predicted no reward. Training on this one reversal continued for five sessions.

### OFC inactivation

Prior to each of the five Pavlovian reversal sessions, rats in each group were bilaterally infused with either a GABA agonist cocktail (experimental group,  $n = 6$ ) or saline (control group,  $n = 8$ ). Both muscimol, a GABA-A agonist (Sigma, St Louis, MO, USA), and baclofen, a GABA-B agonist (Sigma), were used as the inactivating agents. For each infusion, a 30-G injector cannula was inserted into each guide cannula. The injector extended 1 mm below the end of the guide. Injectors were connected to a Hamilton Syringe (Hamilton, Reno, NV, USA) with PE20 tubing (Thermo Fisher Scientific, Waltham, MA, USA). The syringe was placed in an infusion pump (Thermo Fisher Scientific, Waltham, MA, USA). Infusions into each hemisphere consisted of 103 ng muscimol and 32 ng baclofen in 150 nL saline, infused at a rate of 250 nL/min. We have previously found that this concentration and procedure is sufficient to induce discrimination reversal deficits equivalent to those caused by neurotoxic lesions of OFC (Takahashi *et al.*, 2009). Furthermore, larger amounts of fluorescently-conjugated muscimol injections into other prefrontal regions have produced significant spreads (approx. 0.5–0.7 mm from cannula tip in the medial–lateral and dorsal–ventral axis, respectively; Allen *et al.*, 2008). After the infusion, the cannulae were left in place for 2–3 min to allow for proper diffusion of the drugs. Approximately 5–10 min following the bilateral injections, rats were placed in the behavioral chambers for reversal sessions.

### Histology

After the completion of the study, all rats were anesthetized with isoflurane and then infused with 150 nL thionin (0.25%), using the same procedure used to infuse the inactivating GABA agents into the OFC. Rats were then killed with an overdose of isoflurane and perfused with saline followed by 4% paraformaldehyde. Brains were extracted and later cut on a microtome at 40  $\mu$ m per section. Every other section was kept to determine cannulae placement. Both cannulae tracks and thionin deposits helped to identify the exact location of the injection site in the OFC. Sections were later Nissl stained.

### Behavioral measurements and statistical analyses

The percentage of time spent in the food cup was analysed during both cue and non-cue periods, pre-CS periods (30 s before cue onset)

before and after reversal learning. Data are shown as percentage of time spent in the food cup during the 30-s CS cue period. These data, measured by photobeam breaks, were collected from Graphic State software (Coulbourn, PA, USA) and analysed in Matlab (Mathworks) and Statistica (Statsoft).

### Results

Cannulae placement is illustrated in Fig. 1A. Cannulae were located within OFC for all rats, and there were no obvious differences in placement between groups. Figure 1B shows a photomicrograph of a guide cannula track in one of our experimental rats. No additional damage was found outside cannula track damage in our experimental group.

Average responding during reminder training is shown in Fig. 2A (to be saline) and B (to be musc/bac). No infusions were given during this time. Rats in both groups responded significantly more for the rewarded A1 cue than for the non-rewarded A2 cue, and there were no differences between groups. A three-way ANOVA (cue  $\times$  treatment  $\times$  day) revealed a main effect of cue ( $F_{1,12} = 21.429$ ,  $P < 0.001$ ), but no main effects or interactions with treatment ( $F < 1.663$ ,  $P > 0.2107$ ). A comparison of responding to A1 and A2 during the three reminder days vs. the last 3 days of initial conditioning showed a main effect of cue ( $F_{1,12} = 27.080$ ,  $P < 0.001$ ), but no main effects or any interactions with any other factors ( $F < 3.273$ ,  $P > 0.05$ ).

Average responding during subsequent reversal sessions is shown in Fig. 2A (saline) and B (muscimol/baclofen). Saline controls learned to inhibit responding to A1 while at the same time increasing responding for A2 over the 5 days of reversal learning. By contrast, OFC-inactivated rats showed a normal decline in their conditioned responding to A1 but failed to increase their responding to A2 normally. This failure was particularly evident in the final several sessions. In accord with this interpretation, two-way ANOVA (cue  $\times$  day) revealed a main effect of cue ( $F_{1,7} = 7.348$ ,  $P < 0.0302$ ) and a cue  $\times$  day interaction ( $F_{4,28} = 9.165$ ,  $P < 0.0001$ ) in saline controls, and further planned comparisons on each day indicated that there were significant differences in responding for the two cues on each of the last three days of reversal training. A similar analysis of responding in OFC-inactivated rats revealed a main effect of cue ( $F_{1,5} = 9.334$ ,  $P < 0.028$ ) but no cue  $\times$  day interaction ( $F_{4,20} = 0.953$ ,  $P < 0.4545$ ),

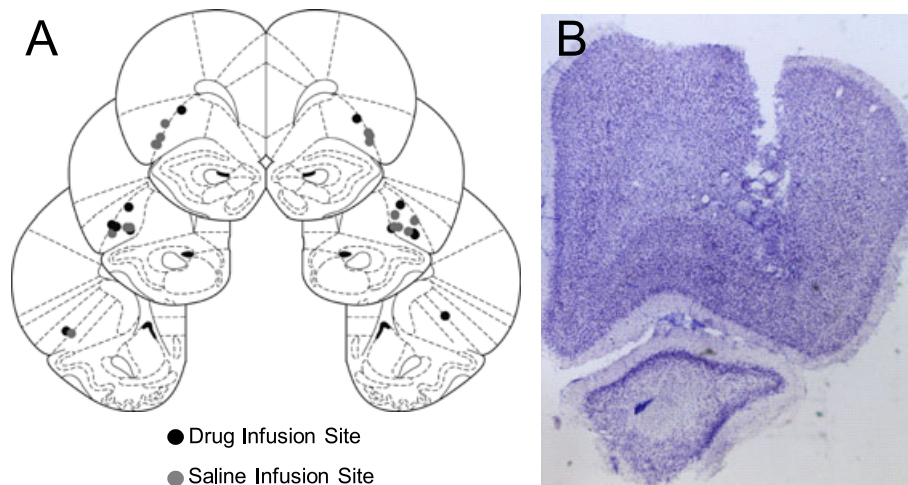


FIG. 1. (A) Location of cannulae in OFC. (B) Photomicrograph of one experimental rat that received infusions of baclofen/muscimol. Note the damage made by the guide cannula ends above OFC; there was little or no apparent permanent damage from the repeated infusions.

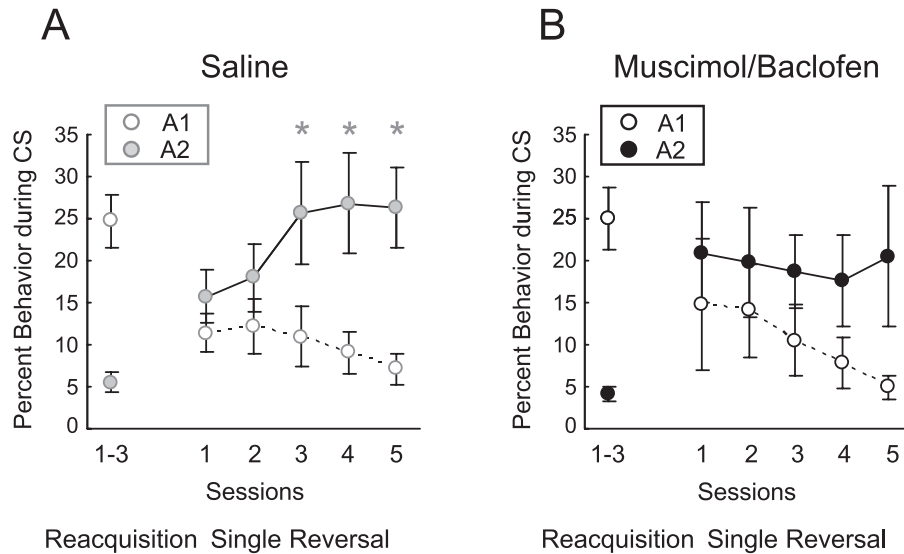


FIG. 2. Effects of OFC inactivation on reversal of Pavlovian responding. (A) Conditioned responding (as measured by percent behavior spent in the food cup only during the CS) during reminder conditioning days (as an average over 3 days) and reversal training. Rats only received infusions of saline in OFC during reversal sessions (5 days). (B) Conditioned responding during reminder conditioning days (as an average) and reversal training in rats that received infusions of muscimol and baclofen in OFC (only during the five reversal sessions;  $*P < 0.05$ ).

and planned comparisons demonstrated no significant differences in responding for the two cues at any point during reversal learning. Additionally, ANOVA (group  $\times$  day) comparing responding for the rewarded A2 cue and the non-rewarded A1 cue in the two groups during reversal training demonstrated no main effect nor any interactions for cue A1 ( $F_{4,48} < 0.310$ ,  $P > 0.870$ ), but a significant group  $\times$  day interaction in responding for cue A2 ( $F_{4,48} = 3.921$ ,  $P < 0.008$ ). Thus, OFC inactivation did not disrupt the rats' ability to inhibit responding for the previously rewarded cue (A1), but did prevent them from learning to respond normally for the previously non-rewarded cue (A2).

## Discussion

Here we tested the role of OFC in reversal learning using a Pavlovian discrimination task. This setting differs from the reversal tasks typically used to assess OFC function in that there was no contingency between the animal's response and reward delivery. As expected, OFC inactivation impaired the ability of the rats to reverse conditioned responding. However, somewhat surprisingly, the deficit reflected an inability of these rats to develop normal conditioned responding for the previously unrewarded cue; inactivation of OFC had no impact on the ability of the rats to inhibit responding to the previously rewarded cue. This result is consistent with a recent report from our lab, in which OFC-lesioned rats exhibited normal extinction of responding to previously rewarded Pavlovian cues (Burke *et al.*, 2008). Further it replicates findings from Roberts and colleagues in marmosets, showing that OFC damage also prevented normal changes in conditioned motor and autonomic responses for the previously rewarded cue (Reekie *et al.*, 2008). In this study, marmosets were trained on a similar task with two cues: one paired with reward (CS+) and one paired with nothing (CS-). After initial training, half of the animals were lesioned, retrained to reach similar levels of responding and then reversed on cue–outcome contingencies. As in the current study, they found that lesioned animals had significant behavioral impairments in reversing their behavior, primarily due to an inability

to learn to respond appropriately to the new CS+. The lesioned animals had no significant difficulty inhibiting their responding to the previously rewarded CS.

There are three aspects of these results that are worth comment. The first concerns the general role of the OFC in reversal learning. The prior report in marmosets and the results presented here show that OFC is critical to reversal of Pavlovian responding. As noted in the Introduction, this is important because the discrimination tasks typically used to study the contribution of OFC to reversal learning include both Pavlovian and instrumental elements. That OFC is specifically necessary for Pavlovian reversals is consistent with the view that OFC is critical to reversal learning (and in a variety of other tasks) due to its role in signaling the Pavlovian associations between the cues and outcomes rather than due to any specific role in instrumental learning. OFC neurons encode associations between cues and outcomes in reversal settings (Thorpe *et al.*, 1983; Rolls *et al.*, 1996; Schoenbaum *et al.*, 1999), and such encoding seems to be similar even without instrumental contingencies (Morrison & Salzman, 2006). Indeed it has recently been suggested that OFC plays no role in learning about actions and is involved exclusively in Pavlovian learning (Ostlund & Balleine, 2007a,b). Our data are consistent with this idea.

However, while OFC may not be critical for action–outcome learning, clearly signaling from OFC can influence the selection of actions. This is true in discrimination reversal, and we have also recently shown a critical role for the OFC in instrumental learning for conditioned reinforcement (Burke *et al.*, 2008). Additionally neural activity appears to signal the value of different actions, both in rat OFC (Feierstein *et al.*, 2006; Roesch *et al.*, 2006) and also in ventromedial OFC in humans (Valentin *et al.*, 2007). While these correlates may reflect the unique sensory aspects of the different responses, it is also possible that they reflect a complex role for OFC in influencing action selection, which is not revealed by lesion studies.

The second aspect of these results worth commenting on is their significance for the notion that OFC plays a general role in response inhibition. While it is true that damage to OFC often affects behavior in situations that require animals to inhibit a response, these data, as

well as evidence of normal inhibition of prepotent responding in the reversed-reward-contingency task (Chudasama *et al.*, 2007) and even in go, no-go tasks (Schoenbaum *et al.*, 2002b), indicate that 'response inhibition' is unlikely to be a root function of the OFC. Instead it may be a symptom of an underlying failure in signaling of outcome expectancies (Schoenbaum & Roesch, 2005). This failure is clearly evident in reinforcer devaluation settings, in which there is no requirement for learning (Gallagher *et al.*, 1999; Izquierdo *et al.*, 2004). We have argued that this same function – signaling of expected outcomes by OFC – may also facilitate learning in the face of unexpected outcomes via a contribution to the generation of prediction errors. Consistent with this idea, OFC damage is associated with miscoding of old associations in basolateral amygdala during reversal learning (Saddoris *et al.*, 2005; Stalnaker *et al.*, 2007).

Of course OFC is a large area, encompassing both lateral and medial regions and extending back into caudal agranular insular regions in some reports; our conclusions may not be relevant to orbital areas outside our target region. In the current study, we targeted a region of lateral OFC in rats that has reciprocal connections with subregions of amygdala, ventral striatum and mediodorsal thalamus that parallel the connectivity of areas 11, 12 and 13 in primates (Schoenbaum *et al.*, 2002a). This region, located in the dorsal bank of the rhinal sulcus, would include the lateral and ventral orbital areas and agranular insular cortex back to the genu of the corpus callosum. We specifically avoided posterior agranular insular regions, presumed to be primary gustatory regions (Saper, 1982; Kosar *et al.*, 1986; Krushel & Van Der Kooy, 1988). Additionally we did not target medial orbital areas, and it is unlikely that our infusions reached these regions. It is possible that these medial regions mediate inhibitory functions reported in some studies of OFC function that utilize very large or more medial lesions.

However, the area targeted in the current report does mediate reversal learning; indeed, inactivation of the lateral part of OFC targeted here, using the same agents, dose and cannulae location caused reversal deficits in an olfactory discrimination task identical to those caused by neurotoxic lesions (Takahashi *et al.*, 2009). Furthermore, lesions centered on this same area (and not extending into medial regions) also cause devaluation deficits in rats (Pickens *et al.*, 2003, 2005). Thus, at least in rats, these functions appear to be mediated by this lateral area (but see also Kazama & Bachevalier, 2009).

Finally, the third and perhaps most interesting aspect of these results that deserves comment is the contrast between the negative effect of OFC inactivation on the inhibition of responding to a previously rewarded cue, reported here, and the positive effect of OFC inactivation on inhibition of responding to a previously compounded cue in a Pavlovian over-expectation task, reported previously (Takahashi *et al.*, 2009). Indeed, as noted in the Materials and methods, the rats used here were a subset of those animals. The striking contrast between the ability of these rats to inhibit responding in extinction here but not as a result of over-expectation may point to a specific role for OFC when it is necessary to integrate elemental Pavlovian associations in order to generate accurate predictions about expected outcomes. OFC is critical in the final stage of performance in a number of tasks, such as devaluation and discounting tasks and outcome-specific transfer (Mobini *et al.*, 2002; Pickens *et al.*, 2003, 2005; Izquierdo *et al.*, 2004; Winstanley *et al.*, 2004; Rudebeck *et al.*, 2006; Ostlund & Balleine, 2007a). Each of these settings requires the subject to generate predictions about outcomes and to do so by bringing together or integrating disparate pieces of associative information. Summation – which is required for normal learning during over-expectation – is just a special case of such integration. OFC

inactivation prevents learning from over-expectation and, critically, also disrupts the increased responding observed in controls to the compound cue (Takahashi *et al.*, 2009). By contrast, extinction does not necessarily require summation, though it can be an incidental feature of some procedures (e.g. if the context has also been disproportionately rewarded or the animal has a prior training history of some sort). This could explain why OFC is sometimes necessary for extinction learning (Butter, 1969; Izquierdo & Murray, 2005).

Of course, if OFC is only critical when normal learning (or behavior) requires summation, then it is perhaps surprising that the acquisition of a new response was impaired by OFC inactivation in the current experiment, as this would seem to only require simple elemental expectancies. While this seems at odds with the proposal that OFC is important for integrating information about expected outcomes, it is worth considering that reversal learning is quite complex, thus new learning in this setting may not be as straightforward as it appears. Processes involving contextual conditioning, occasion setting, temporal features of the environment and even uncertainty may all play a larger role in reversal learning than they do in initial conditioning. Indeed, initial appetitive Pavlovian conditioning in naïve rats is not normally affected by OFC lesions (Gallagher *et al.*, 1999; Burke *et al.*, 2008; Takahashi *et al.*, 2009). We would speculate some feature or requirement of the task or training history of the rats used here may be allowing summation to facilitate new learning after reversal but not extinction. Further work using behavioral procedures designed specifically to manipulate the need for summation is necessary to directly test this hypothesis.

## Acknowledgements

This research was supported by grants from the NIA (GS, R01 AG027097) and NIDA (KB, F31 DA021989).

## Abbreviations

GABA,  $\gamma$ -aminobutyric acid; ITI, inter-trial interval; OFC, orbitofrontal cortex.

## References

- Allen, T.A., Narayanan, N.S., Kholodar-Smith, D.B., Zhao, Y., Laubach, M. & Brown, T.H. (2008) Imaging the spread of reversible brain inactivations using fluorescent muscimol. *J. Neurosci. Methods*, **171**, 30–38.
- Bechara, A., Damasio, H., Tranel, D. & Damasio, A.R. (1997) Deciding advantageously before knowing the advantageous strategy. *Science*, **275**, 1293–1294.
- Bissonette, G.B., Martins, G.J., Franz, T.M., Harper, E.S., Schoenbaum, G. & Powell, E.M. (2008) Double dissociation of the effects of medial and orbital prefrontal cortical lesions on attentional and affective shifts in mice. *J. Neurosci.*, **28**, 11124–11130.
- Burke, K.A., Franz, T.M., Miller, D.N. & Schoenbaum, G. (2008) The role of orbitofrontal cortex in the pursuit of happiness and more specific rewards. *Nature*, **454**, 340–344.
- Butter, C.M. (1969) Perseveration and extinction in discrimination reversal tasks following selective frontal ablations in *Macaca mulatta*. *Physiol. Behav.*, **4**, 163–171.
- Chudasama, Y. & Robbins, T.W. (2003) Dissociable contributions of the orbitofrontal and infralimbic cortex to pavlovian autoshaping and discrimination reversal learning: further evidence for the functional heterogeneity of the rodent frontal cortex. *J. Neurosci.*, **23**, 8771–8780.
- Chudasama, Y., Kralik, J.D. & Murray, E.A. (2007) Rhesus monkeys with orbital prefrontal cortex lesions can learn to inhibit prepotent responses in the reversed reward contingency task. *Cereb. Cortex*, **17**, 1154–1159.
- Dias, R., Robbins, T.W. & Roberts, A.C. (1996) Dissociation in prefrontal cortex of affective and attentional shifts. *Nature*, **380**, 69–72.
- Feierstein, C.E., Quirk, M.C., Uchida, N., Sosulski, D.L. & Mainen, Z.F. (2006) Representation of spatial goals in rat orbitofrontal cortex. *Neuron*, **51**, 495–507.

- Fellows, L.K. & Farah, M.J. (2003) Ventromedial frontal cortex mediates affective shifting in humans: evidence from a reversal learning paradigm. *Brain*, **126**, 1830–1837.
- Ferry, A.T., Lu, X.C. & Price, J.L. (2000) Effects of excitotoxic lesions in the ventral striatopallidal-thalamocortical pathway on odor reversal learning: inability to extinguish an incorrect response. *Exp. Brain Res.*, **131**, 320–335.
- Gallagher, M., McMahan, R.W. & Schoenbaum, G. (1999) Orbitofrontal cortex and representation of incentive value in associative learning. *J. Neurosci.*, **19**, 6610–6614.
- Gottfried, J.A., O'Doherty, J. & Dolan, R.J. (2003) Encoding predictive reward value in human amygdala and orbitofrontal cortex. *Science*, **301**, 1104–1107.
- Hampton, A.N., Bossaerts, P. & O'Doherty, J.P. (2006) The role of the ventromedial prefrontal cortex in abstract state-based inference during decision-making in humans. *J. Neurosci.*, **26**, 8360–8367.
- Hornak, J., O'Doherty, J., Bramham, J., Rolls, E.T., Morris, R.G., Bullock, P.R. & Polkey, C.E. (2004) Reward-related reversal learning after surgical excisions in orbito-frontal or dorsolateral prefrontal cortex in humans. *J. Cogn. Neurosci.*, **16**, 463–478.
- Izquierdo, A.D. & Murray, E.A. (2005) Opposing effects of amygdala and orbital prefrontal cortex lesions on the extinction of instrumental responding in macaque monkeys. *Eur. J. Neurosci.*, **22**, 2341–2346.
- Izquierdo, A.D., Suda, R.K. & Murray, E.A. (2004) Bilateral orbital prefrontal cortex lesions in rhesus monkeys disrupt choices guided by both reward value and reward contingency. *J. Neurosci.*, **24**, 7540–7548.
- Kazama, A. & Bachevalier, J. (2009) Selective aspiration or neurotoxic lesions of orbital frontal areas 11 and 13 spared monkeys' performance on the object discrimination reversal task. *J. Neurosci.*, **29**, 2794–2804.
- Kosar, E., Grill, H.J. & Norgren, R. (1986) Gustatory cortex in the rat. I. Physiological properties and cytoarchitecture. *Brain Res.*, **379**, 329–341.
- Kruschel, L.A. & Van Der Kooy, D. (1988) Visceral cortex: integration of the mucosal senses with limbic information in the rat agranular insular cortex. *J. Comp. Neurol.*, **270**, 39–54.
- Mobini, S., Body, S., Ho, M.-Y., Bradshaw, C.M., Szabadi, E., Deakin, J.F.W. & Anderson, I.M. (2002) Effects of lesions of the orbitofrontal cortex on sensitivity to delayed and probabilistic reinforcement. *Psychopharmacology*, **160**, 290–298.
- Morrison, S.E. & Salzman, C.D. (2006) Representation of stimulus value in primate orbitofrontal cortex during reinforcement learning. *Soc. Neurosci. Abstr.*, **164**, 165.
- Murray, E.A. & Izquierdo, A.D. (2007) Orbitofrontal cortex and amygdala contributions to affect and action in primates. *Ann. N. Y. Acad. Sci.*, **1121**, 273–296.
- O'Doherty, J., Critchley, H.D., Deichmann, R. & Dolan, R.J. (2003) Dissociating valence of outcome from behavioral control in human orbital and ventral prefrontal cortices. *J. Neurosci.*, **23**, 7931–7939.
- Ostlund, S.B. & Balleine, B.W. (2007a) Orbitofrontal cortex mediates outcome encoding in Pavlovian but not instrumental learning. *J. Neurosci.*, **27**, 4819–4825.
- Ostlund, S.B. & Balleine, B.W. (2007b) The contribution of orbitofrontal cortex to action selection. *Ann. N. Y. Acad. Sci.*, **1121**, 174–192.
- Padoa-Schioppa, C. & Assad, J.A. (2006) Neurons in orbitofrontal cortex encode economic value. *Nature*, **441**, 223–226.
- Paxinos, G. & Watson, C. (1998) *The rat brain in stereotaxic coordinates*. Academic Press, San Diego, California, USA.
- Pickens, C.L., Setlow, B., Saddoris, M.P., Gallagher, M., Holland, P.C. & Schoenbaum, G. (2003) Different roles for orbitofrontal cortex and basolateral amygdala in a reinforcer devaluation task. *J. Neurosci.*, **23**, 11078–11084.
- Pickens, C.L., Saddoris, M.P., Gallagher, M. & Holland, P.C. (2005) Orbitofrontal lesions impair use of cue-outcome associations in a devaluation task. *Behav. Neurosci.*, **119**, 317–322.
- Ramus, S.J. & Eichenbaum, H. (2000) Neural correlates of olfactory recognition memory in the rat orbitofrontal cortex. *J. Neurosci.*, **20**, 8199–8208.
- Reekie, Y.L., Braesicke, K., Man, M.S. & Roberts, A.C. (2008) Uncoupling of behavioral and autonomic responses after lesions of the primate orbitofrontal cortex. *Proc. Natl Acad. Sci. USA*, **105**, 9787–9792.
- Roesch, M.R. & Olson, C.R. (2004) Neuronal activity related to reward value and motivation in primate frontal cortex. *Science*, **304**, 307–310.
- Roesch, M.R., Taylor, A.R. & Schoenbaum, G. (2006) Encoding of time-discounted rewards in orbitofrontal cortex is independent of value representation. *Neuron*, **51**, 509–520.
- Rolls, E.T., Critchley, H.D., Mason, R. & Wakeman, E.A. (1996) Orbitofrontal cortex neurons: role in olfactory and visual association learning. *J. Neurophysiol.*, **75**, 1970–1981.
- Rudebeck, P.H., Walton, M.E., Smyth, A.N., Bannerman, D.M. & Rushworth, M.F. (2006) Separate neural pathways process different decision costs. *Nat. Neurosci.*, **9**, 1161–1168.
- Saddoris, M.P., Gallagher, M. & Schoenbaum, G. (2005) Rapid associative encoding in basolateral amygdala depends on connections with orbitofrontal cortex. *Neuron*, **46**, 321–331.
- Saper, C.B. (1982) Convergence of autonomic and limbic connections in the insular cortex of the rat. *J. Comp. Neurol.*, **210**, 163–173.
- Schoenbaum, G. & Roesch, M.R. (2005) Orbitofrontal cortex, associative learning, and expectancies. *Neuron*, **47**, 633–636.
- Schoenbaum, G., Chiba, A.A. & Gallagher, M. (1999) Neural encoding in orbitofrontal cortex and basolateral amygdala during olfactory discrimination learning. *J. Neurosci.*, **19**, 1876–1884.
- Schoenbaum, G., Setlow, B. & Gallagher, M. (2002a) Orbitofrontal cortex: modeling prefrontal function in rats. In Squire, L. & Schacter, D. (Eds), *The Neuropsychology of Memory*, 3rd edn. Guilford Press, New York, pp. 463–477.
- Schoenbaum, G., Nugent, S., Saddoris, M.P. & Setlow, B. (2002b) Orbitofrontal lesions in rats impair reversal but not acquisition of go, no-go odor discriminations. *Neuroreport*, **13**, 885–890.
- Schoenbaum, G., Setlow, B., Nugent, S.L., Saddoris, M.P. & Gallagher, M. (2003) Lesions of orbitofrontal cortex and basolateral amygdala complex disrupt acquisition of odor-guided discriminations and reversals. *Learn. Mem.*, **10**, 129–140.
- Schoenbaum, G., Saddoris, M.P. & Stalnaker, T.A. (2007) Reconciling the roles of orbitofrontal cortex in reversal learning and the encoding of outcome expectancies. *Ann. N. Y. Acad. Sci.*, **1121**, 320–325.
- Stalnaker, T.A., Franz, T.M., Singh, T. & Schoenbaum, G. (2007) Basolateral amygdala lesions abolish orbitofrontal-dependent reversal impairments. *Neuron*, **54**, 51–58.
- Takahashi, Y., Roesch, M.R., Stalnaker, T.A., Haney, R.Z., Calu, D.J., Taylor, A.R., Burke, K.A. & Schoenbaum, G. (2009) The orbitofrontal cortex and ventral tegmental area are necessary for learning from unexpected outcomes. *Neuron*, **62**, 269–280.
- Teitelbaum, H. (1964) A comparison of effects of orbitofrontal and hippocampal lesions upon discrimination learning and reversal in the cat. *Exp. Neurol.*, **9**, 452–462.
- Thorpe, S.J., Rolls, E.T. & Maddison, S. (1983) The orbitofrontal cortex: neuronal activity in the behaving monkey. *Exp. Brain Res.*, **49**, 93–115.
- Valentin, V.V., Dickinson, A. & O'Doherty, J.P. (2007) Determining the neural substrates of goal-directed learning in the human brain. *J. Neurosci.*, **27**, 4019–4026.
- Wallis, J.D. & Miller, E.K. (2003) Neuronal activity in primate dorsolateral and orbital prefrontal cortex during performance of a reward preference task. *Eur. J. Neurosci.*, **18**, 2069–2081.
- Winstanley, C.A., Theobald, D.E.H., Cardinal, R.N. & Robbins, T.W. (2004) Contrasting roles of basolateral amygdala and orbitofrontal cortex in impulsive choice. *J. Neurosci.*, **24**, 4718–4722.