When submitting a revised manuscript, please list your responses to the Reviewers, explaining how each point raised has been addressed in the area provided in Step #1 of the submission. You may type directly into the assigned space or cut and paste from a word document. Alternatively, you may upload a response file and designate as "Response to decision". Please highlight in the manuscript the new text, or if major changes have been made, instead of highlighting each word highlight what sections are new. This way we can make decision in the Editorial Office without asking again reviewer’s opinion.

Please take a moment to check the format of your references. Here is an example of the correct format:

Author SP, Author DJ, Author RT. 2007. Title of paper. Journal Name. 8:123-154.

ONLY ONE FILE NEEDED FOR REVIEW, please upload a single file, double-spaced, inclusive of all main text, tables, and figures with legends. The tables and figures with legends should be embedded within the text at the relevant location.

Reviewer: 1  
  
Comments to Author  
The work by Panayi and Killcross examines the role of the lateral orbitofrontal cortex (OFC) in a simple CS-US Pavlovian conditioning task in laboratory rats (male, adult, Long-Evans, socially housed, food restricted). Specifically, rats were trained to associate the occurrence of a 10-s auditory (or visual) cue with the delivery of food at a specific location in the testing cage. Rats’ visits of this location during the cue increased during the first 10 training sessions (called thereafter “behavioral ascent”) before eventually leveling off after extended training (“behavioral plateau”). Inactivation of the lateral OFC had a differential effect on conditioned visits depending on the level of prior CS-US training. Briefly, if it occurred before the very first training session, it did not affect behavioral ascent but elevated behavioral plateau. In contrast, if it occurred during behavioral ascent, it stopped further behavioral ascent and, as a result, led to a lower behavioral plateau. Importantly, a Pavlovian conditioning blocking experiment revealed that the latter effect was not due to a failure of CS-US learning per se. None of these effects were predicted by current theories of OFC functions, at least as they were initially formulated. In their Discussion, the authors have tried to see how some of these theories could be amended to explain these unexpected findings. Overall, this is a well-designed and well-controlled series of behavioral neuroscience experiments that provide important novel findings for better understanding the functions of the still elusive OFC. I found the Discussion particularly thoughtful and insightful.  
  
My only major criticism concerns the Methods section which I found very hard to follow. It also contains many analysis and results. I urge the authors to make an effort to simplify the description of this section and displace all results to where they belong, that is, to the Results section. The text must also be edited more carefully as it contains several typos and few incomplete or awkward sentences. Finally, in the Discussion, the authors suggest that a habit system left alone (e.g., in animals with pre-training OFC lesions) could lead to an elevated behavioral plateau after extended training, but they did not exactly explain how and why this should happen. Please provide more explanation here.

Response to Reviewer 1:

We thank the reviewer for this extremely positive feedback.

We have also considered the following points that were raised:

1) The methods section was hard to follow.

We appreciate this feedback and have modified the methods section to make this section clearer and easier to follow.

2) There are a number of analyses in the methods section that should be placed in the results section.

These were the histology and analyses of baseline levels of responding and were preliminary analyses that informed the interpretation of the main measure, the CS-PreCS difference scores. They were placed in the methods section to allow the results section to focus on the more relevant/meaningful analyses given the aim of the experiment. However, we agree that the results section is an appropriate place for these analyses and have now moved these sections to the relevant section of the main body of the results. For supplementary experiments, these analysis sections have been moved to the relevant figure captions.

3) The text contains several typos and awkward/incomplete sentences.

We thank the reviewer for raising this issue, and apologise that these had not been noticed by the author’s earlier. The manuscript has been reviewed thoroughly to identify and correct these issues.

4) In the Discussion, the authors suggest that a habit system left alone (e.g., in animals with pre-training OFC lesions) could lead to an elevated behavioral plateau after extended training, but they did not exactly explain how and why this should happen. Please provide more explanation here.

This point has been expanded upon in the Discussion with the addition of the following section (original text in blue):

Following pre-training lesions, this may lead to an unconstrained habit learning system (Coutureau & Killcross, 2003; Dickinson, 1985; Dolan & Dayan, 2013; Killcross & Coutureau, 2003) that is not necessarily bounded by the current value of the outcome, and overly sensitive to current general motivational states (e.g. overall hunger levels; Figure 1B) of the organism. This is consistent with evidence that a stimulus-response habit like system develops in Pavlovian conditioning paradigms (Hall, 2002; Killcross & Blundell, 2002; Parkinson et al., 2005), and is likely to interact and compete with stimulus-outcome learning systems for behavioural control, similar to the interaction found between instrumental habit and goal-directed systems (Balleine & Killcross, 2006; Coutureau & Killcross, 2003; Killcross & Coutureau, 2003; Kim et al., 2019; Lee et al., 2014; Yin et al., 2005, 2006).

Reviewer: 2  
  
Comments to Author  
In this manuscript, the authors explore the potential involvement of the lateral OFC in stimulus-outcome learning. In Experiment 1, they report that pre-acquisition lOFC lesions enhance cue responding but inactivation of this region later in acquisition impairs responding (Experiment 2). Inactivation of lOFC was also found to leave blocking intact (Experiment 3), suggesting that the effects observed on cue responding in Experiments 1 and 2 relate to behavioural performance and not learning. It is difficult to question the robustness of these results given that several findings are replicated throughout the manuscript (including in the supplementary materials).  
  
Overall, this study is particularly interesting and important as it explicitly tests a null effect that is largely accepted in the literature – that lOFC inhibition leaves intact “simple” S-O responding. Here, the authors demonstrate quite convincingly that the lOFC is indeed involved in simple Pavlovian acquisition. The contribution of this work to the field is summarized by the authors in the discussion: “Even within a putatively simply behavioural task, there are many potential underlying psychological processes that can contribute to performance and change over time”. Indeed, while this work clearly contributes to our understanding of OFC function in value-based behaviours, it also serves as a reminder of the complexity of simple psychological processes and highlights the importance of understanding these processes when interpreting neural data. I have some suggestions that the authors may wish to consider to improve the clarity of the manuscript.

Response to Reviewer 3:

We thank the reviewer for this extremely positive feedback.

We have addressed the reviewer’s suggestions below:

1.      These authors have previously highlighted the functional hetereogeneity of the anterior versus posterior divide in lOFC (e.g., in Pavlovian reversal learning). Could the authors comment on this divide for the current results? Particularly as, despite the AP coordinates, the majority of the damage/placements seem quite anterior.

Yes, we completely agree that the distinction between anterior and posterior lOFC that we have raised before, might be of relevance when considering the present results. The reviewer is also correct in their assessment that the majority of lesion damage and cannulae placement in the present experiments are quite anterior, and would likely fit the criteria we have previously used to define an anterior lOFC (i.e. bilateral damage anterior to +3.7 mm from bregma/anterior to the corpus callosum in the coronal section). We chose not to emphasize this point as the present experiments did not systematically compare anterior and posterior regions, and selective targeting of the anterior lOFC was not intentional when the study was planned. However, we agree with the reviewer that this distinction should be raised in the discussion to highlight the possibility that the present results might also be dissociable between anterior and posteror lOFC. Particularly given that the majority of studies selectively targeting lOFC have targeted posterior regions, or the whole structure (i.e. both anterior and posterior lOFC).

Accordingly, the following paragraph has been added to the Conclusion of the Discussion section:

Recently, we demonstrated functional heterogeneity within the lateral OFC between anterior and posterior subregions (Panayi & Killcross, 2018). While the present experiments did not explicitly target and compare anterior and posterior subregions, it is notable that present lesion and cannula placements targeted predominantly anterior lateral OFC. Therefore, one possible account of the surprising role of lateral OFC in simple Pavlovian acquisition is that prior research has often focused on the posterior lateral OFC or the structure as a whole (Gallagher et al., 1999; Izquierdo, 2017; Ostlund & Balleine, 2007). However, further studies systematically comparing anterior and posterior subregions within lateral OFC are still needed.

2.      The authors use the term « acquisition behaviour » throughout the manuscript (e.g., OFC lesions enhance acquisition behaviour). To what extent does « acquisition behaviour » simply equate to “performance” or “expression”? And why favour this term over “performance” or “expression”?

The term “acquisition behaviour” was chosen to describe the observed deficits without presupposing an interpretation of the effect as a deficit in performance rather than learning (until this is directly tested in Figure 3 with the blocking procedure). The term is also consistent with the OFC literature often describing the null effect as a null effect on “acquisition”. However, the reviewer is correct that the term could be exchanged with “performance” or “expression”.

3.      Page 8, line 17: “no drugs were infused”, suggest changing to “PBS was infused”.

Sham lesions were performed by leaving the needle (filled with PBS) in situ, but no infusion occurred. The manuscript has been updated to clarify this point.

4.      Page 12, line 14: the authors state that a pellet was used for novel acquisition but it seems that the pellet was used for initial Pavlovian acquisition (page 11, line 22) – I assume this is a mistake and one should be changed to the sucrose reinforcer?

The reviewer is correct that there is a mistake in this description. In fact, the reinforcers in this experiment were a sucrose pellet and lemon flavoured maltodextrin liquid. The description in the general methods describing two separate liquid reinforcers was the actual source of the error. We are grateful for the reviewer identifying this error. The manuscript has been updated accordingly.

5.      Page 12, lines 47-48: “the magazine frequency that was available was not as sensitive to devaluation as a measure of duration, so only data from the first trial was analysed at test.” This is a bit unclear for me, could the authors please elaborate a little? Why does an “insensitive” frequency measure mean that only the first trial can be analysed?

We have found that magazine duration measures often exhibit more robust outcome devaluation effects which persist over multiple trials. In contrast, magazine frequency in this protocol extinguished quite rapidly, which reduced power to detect significant differences between devalued and non-devalued cues (i.e., a floor effect averaging over lots of non-responding after the first non-reinforced presentation of each CS). Unfortunately, when this experiment was conducted the chambers were not capable of recording magazine duration.

This difference in the sensitivity of these measures is also reflected in the fact that the majority of published examples of Pavlovian devaluation effects use magazine duration as the outcome measure (e.g. for lateral OFC devaluation - Panayi & Killcross, 2018; Pickens et al., 2003, 2005; Schoenbaum et al., 1999). This is why we described the magazine frequency measure as “insensitive” in the manuscript. However, we appreciate that this description is confusing without the additional context we have provided in this response. Therefore, the description of magazine frequency measures being insensitive has been removed from the manuscript.

6.      Page 15, lines 45-46: “PreCS baseline responding did not differ between infusion groups across training and justified the use of CS-preCS difference scores for analyses of discriminative responding.” Do the authors mean to say that if preCS baselines differ then CS-preCS scores should not be used? (i.e., like they did in Experiment 1?)

The reviewer is correct to point out this issue. If the PreCS levels differed, then it would suggest that analysis of uncorrected responding during the CS period alone would be inappropriate. This sentence has been removed accordingly.

7.      Page 31, line 16: days 5-10 should be changed to 5-9, as I understood that there was no infusion on day 10.

This has been corrected.

8.      Page 42, lines 13-15: “In the stable single cue-outcome learning situation employed in the present studies, this would result in disruption of further acquisition” - but this doesn’t appear to be the case as responding decreased in Figure 2, i.e., these rats didn’t simply fail to increase responding.

Statistically, this was not the case. The data were analyzed to confirm whether this was indeed the case for Figure 2:

“Contrary to our prediction, intra-OFC muscimol infusions disrupted rather than enhanced further acquisition of responding relative to the saline group (Figure 2, Infusion - Days 12-15; Significant Group x Day interaction F(3,66)=5.03, p=.003, but no main effect of Group F(1,22)=1.90, p=.182, or Day F(3,66)=0.32, p=.809). Simple effects revealed significantly greater responding in the saline group on the last 2 days of infusions (Muscimol vs Saline: Day 12 t(22)=0.67, p=.508, Day 13 t(22)=-1.03, p=.315, Day 14 t(22)=-2.79, p=.011, Day 15 t(22)=-2.08, p=.049). Furthermore, the saline group increased responding across infusion days 12-15 (Saline: significant positive linear trend t(22)=2.79, p=.011), whereas the muscimol group did not (Muscimol: no significant linear trend t(22)=-1.57, p=.131). Therefore, post-training inactivation of the OFC impaired acquisition.”

Reviewer: 3  
  
Comments to Author  
 The orbitofrontal cortex (OFC) is of interest to behavioral neuroscientists for a multitude of reasons. Some see the OFC as the seat of economic choice - the brain region in which  apples and oranges must be compared. Other argue the OFC acts as a cognitive map - representing the causal texture of the environment. Many more roles for the OFC have been proposed. Regardless of the exact view, all of these theories assume that the OFC is not necessary for the acquisition of simple cue-outcome associations. In a clever and rigorous set of studies, Panayi and Killcross cast doubt on this assumption. The authors first demonstrate that OFC lesions enhance the acquisition of cue-outcome responding, then show these same OFC-lesioned rats were profoundly impaired in reinforcer devaluation - long known to depend on OFC function. In the second experiment, rats are permitted to acquire cue-outcome responding, then the OFC is inhibited with baclofen muscimol. The effect is clear, suppressing OFC activity suppresses cue responding. Extending this, the authors then replicate the effect of OFC suppression of acquired cue-outcome responding, but then show that this inactivation did not alter associative blocking.  
  
All experiments are expertly designed and rigorously analyzed. The discussion is thorough, providing a complete account of their effects in the context of the larger literature. This is an excellent manuscript and I have no suggestions for edits or revisions. Very well done, this was a delight to read and really made me think more deeply about OFC function in appetitive conditioning.

Response to Reviewer 3:

We thank the reviewer for this extremely positive feedback.