Brief Communication

A novel multichoice touchscreen paradigm for assessing cognitive flexibility in mice

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Cognitive flexibility refers to various processes which enable behaviors to be modified on the basis of a change in the contingencies between stimuli or responses and their associated outcomes. Reversal learning is a form of cognitive flexibility which measures the ability to adjust responding based on a switch in the stimulus–outcome contingencies of, typically two, perceptually distinct stimuli. Reversal tasks have provided valuable insight into the neural basis of cognitive flexibility, implicating brain regions including the lateral orbitofrontal cortex (lOFC) and dorsomedial prefrontal cortex (dmPFC). However, with two-stimulus reversal, it is difficult to determine whether response errors are due excessive perseveration, deficient learning, or other problems with updating. To address this limitation, we developed a mouse three-choice touchscreen-based visual reversal task, in which the contingencies of two stimuli were switched on reversal but a third, simultaneously presented, stimulus was never reinforced. We found that, in male C57BL/6J mice, responding at the previously rewarded stimulus predominated over the newly and never-reinforced stimuli during early reversal. Next, we showed that acute pharmacological inhibition of lOFC, but not dmPFC, impaired early reversal performance, relative to noninactivated controls. Interestingly, however, lOFC inactivation deficits were characterized by increased choice of the never-reinforced stimulus and a decrease in (perseverative-like) responding at the previously rewarded stimulus. These effects are inconsistent with the historical notion of lOFC mediating response inhibition and closer to recent views of the lOFC's role in response/outcome tracking. Overall, these findings provide initial support the utility of this novel paradigm for studying cognitive flexibility and its underlying neural substrates.

[Supplemental material is available for this article.]

Cognitive inflexibility in addictions including alcohol use disorders (AUDs) can prevent effective disengagement from destructive patterns of drug-seeking (Jentsch and Taylor 1999; Belin et al. 2016). In laboratory settings, measures of cognitive flexibility such as reversal learning are slowed in AUD patients (Vanes et al. 2014; Le Berre et al. 2017), while rodents chronically exposed to alcohol or other drugs of abuse (e.g., cocaine), exhibit abnormalities in reversal performance and other forms of cognitive flexibility, including attentional set-shifting (Schoenbaum et al. 2004; Coleman et al. 2012; DePoy et al. 2013; Trantham-Davidson et al. 2014; Hu et al. 2015; Varodayan et al. 2018). In turn, these behavioral disturbances have been attributed to drug-induced adaptations in certain cortical and striatal regions that are known substrates for these cognitive processes (Schoenbaum and Shaham 2008; Moorman 2018).

Prior studies in the rodent and nonhuman primate have shown that neurons in the lateral orbitofrontal cortex (lOFC) exhibit correlates of successful reversal learning, while experimentally induced disruptions of lOFC function impair reversal (Iversen and Mishkin 1970; Jones and Mishkin 1972; Dias et al. 1996, 1997; Rolls 1996; Tremblay et al. 1998; Schoenbaum et al. 2002, 2003; Chudasama and Robbins 2003; Izquierdo et al. 2004; Walton et al. 2004, 2010; Kim and Ragozzino 2005; Stalnaker et al. 2006; Boulougouris et al. 2007; Clarke et al. 2008; Ghods-Sharifi et al. 2008; Brigman et al. 2013; Bissonette et al. 2014; Dalton et al. 2016; Marquardt et al. 2017). However, although lOFC disruptions reliably disrupt reversal, differing views regarding what this reflects about the function of the region exist. Early hypotheses suggested the lOFC was important for response inhibition (Jones and Mishkin 1972), while more recent accounts posit a role in tracking response and outcome histories to guide decisions, and in using stimuli-associated specific outcomes to make choices among the available options (Balleine et al. 2011; Noonan et al. 2012; Rudebeck and Murray 2014; Costa et al. 2015; Stalnaker et al. 2015; Izquierdo et al. 2016).

In addition to the prominent role ascribed to the lOFC, the medial prefrontal cortex (mPFC), as well as a number of other brain regions such as the dorsal striatum, have been shown to subserve reversal (Palencia and Ragozzino 2004, 2006; Ragozzino and Choi 2004; Tzavos et al. 2004; Brown et al. 2010; Graybeal et al. 2011; Amodeo et al. 2017; Grospe et al. 2018). For example, lOFC lesions in rats produced a perseverative-like reversal deficit (increased early reversal responding at the previously rewarded stimulus [S^{prior}]), whereas mPFC lesions appeared to impair learning of the new stimulus–reward contingency (more late-reversal responding at the S^{prior}) (Chudasama and Robbins 2003). However, other studies found that while mPFC lesions or inactivations disrupted attentional shifts, there were null, and even facilitatory, effects on reversal (Dias et al. 1996; Birrell and Brown 2000; McAlonan and Brown 2003; Ragozzino et al. 2003; Brigman and Rothblat 2007; Bissonette et al. 2008, 2013; Floresco et al. 2008; Dalton et al. 2016).

Some of the inconsistencies and interpretative issues evident in the literature might stem in part from limitations inherent to the most typically used reversal paradigms. In such preparations, subjects learn the outcome contingencies of two stimuli, which are

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then switched on reversal. Because both stimuli have well-established antecedent reward versus nonreward associations at reversal, it is difficult to disambiguate perseverative responding from impaired learning about the new contingencies or other problems, such as deficient on-task response/outcome tracking. This method also lacks the possibility to evaluate the presence of irrelevant/distracting stimuli that are known to potently influence human decision-making in a manner related to OFC and mPFC recruitment (Chau et al. 2014).

One approach to circumventing some of these problems is to expand the range of choices available on reversal by adding a third (or more) stimulus that is never rewarded (Bussey et al. 1997; Ragozzino et al. 2003; Kim and Ragozzino 2005; Lee et al. 2007; Ragozzino and Rozman 2007; Rudebeck et al. 2008; Seu et al. 2009; Walton et al. 2010; D'Cruz et al. 2011; Kosaki and Watanabe 2012; Noonan et al. 2012, 2017; Riceberg and Shapiro 2012). Yet despite rapid advances in the availability of powerful genetic tools to monitor and manipulate neural substrates of higher-order behaviors in mice, there is currently a lack of multistimulus reversal paradigms to test cognitive flexibility in this species.

The goal of the present study was to first develop a novel, three-choice, version of a previously described mouse visual discrimination and reversal touchscreen paradigm (Izquierdo et al. 2006; Mar et al. 2013), and then to evaluate the effects on early reversal performance after inactivating the lOFC and dorsomedial prefrontal cortex (dmPFC). We began by assessing a cohort of male C57BL/6J mice (a commonly used inbred strain) using a modified version of the Bussey–Saksida Touch Screen System (Graybeal et al. 2014) with three response windows on a touchscreen panel, within each of which was presented a single two-dimensional visual stimulus (Fig. 1A; see Supplemental Material for full experimental procedures).

To characterize the task, mice were first pretrained to reliably make a single touch at one response window to obtain a food-pellet reward (~10 sessions). During discrimination sessions, three novel stimuli were simultaneously presented on each trial, with responses at one stimulus (S^+, "bars" or "dots" counterbalanced across mice) producing reward at a continuous rate of reinforcement, followed by another trial in which the stimuli were presented in a randomly selected spatial configuration (Fig. 1B). Responses at either of the other two stimuli ($S1^-$, $S2^-$) produced no reward and a 15-sec timeout period (signaled by extinguishing the house lights), followed by a correction trial in which the stimuli were presented in the same spatial configuration. Correction trials were repeated until a correct response was made. Testing proceeded on daily sessions comprising either 36 (for task characterization) or 30 (for inactivation) trials (excluding correction trials) until a criterion of >75% correct performance on two consecutive sessions was attained (=11.9 ± 1.4 sessions).

Reversal began on the next session after discrimination criterion was achieved. Here, the same three stimuli were presented, but the previously rewarded stimulus was now unrewarded (S^+ now S^{prior}), while one of the previously unrewarded stimuli ("moon" for all mice) was now rewarded ($S1^-$ now S^{new}) and the other stimulus remained unrewarded ($S2^-$ now S^{never}) (Fig. 1B). The procedure was otherwise the same as for discrimination (including use of correction trials). Testing proceeded until a >75% correct performance on two consecutive sessions criterion was met (=11.2 ± 1.2 sessions). For each mouse, sessions to discrimination criterion and, separately, reversal criterion were tallied and subdivided into early, mid, and late phases (Bergstrom et al. 2018). Of the total responses made during each phase, the percentage of responses at each of the three stimuli was calculated. In addition, to evaluate the microstructure of responding at each phase, the average length of unbroken strings of responding at the same stimulus was also analyzed for each phase (Brigman et al. 2013).

Results showed that choice of the S^+/S^{prior} stimulus increased significantly from early to late discrimination and remained high at early reversal before decreasing across subsequent reversal training (Fig. 1D; e.g., behavioral raster plots, see Fig. 1C). Conversely, selection of the $S1^-/S^{new}$ decreased from early to late discrimination and progressively increased over reversal, while $S2^-/S^{never}$ choice was low by late discrimination and spiked during mid-reversal—apparently reflecting sampling of both previously unrewarded options at this mid-stage (Fig. 1E,F). The patterns evident in overall percent responding were substantiated by the trial-by-trial analysis showing that strings of consecutive S^+/S^{prior} responses increased over discrimination and decreased over reversal. Strings of $S1^-/S^{new}$ responses, in contrast, were low by late discrimination before increasing across reversal, whereas $S2^-/S^{never}$ strings were also reduced by late discrimination and remained flat across reversal (Fig. 1G–I). The number of S^{prior} ($r = +0.72$, $P < 0.003$) and S^{never} ($r = +0.55$, $P < 0.044$), but not S^{new} ($r = +0.39$), responses made at early reversal positively correlated with S^{new} choice at late reversal—suggesting that greater negative feedback early in the task led to superior performance later (Supplemental Fig. S1A–C). Lastly, choice latencies for all three stimuli quickened across the phases of discrimination and again at reversal (Supplemental Fig. S2).

These initial data establish the feasibility of assessing multichoice reversal in mice using a touchscreen-based procedure. They also demonstrate this paradigm's value in dissociating patterns of errors that are perseverative in nature ($S^{prior} > S^{new}$) from those reflecting random responding or exploration of the alternative stimulus options ($S^{prior} = S^{new}$). On the basis of these findings, we next evaluated the consequences of pharmacologically inactivating either the lOFC or dmPFC during early reversal, given the known contributions of these regions to cognitive flexibility (Fig. 2A). Following pretraining, male C57BL/6J mice were implanted with indwelling guide cannula into the lOFC (Fig. 2B) or dmPFC (Fig. 2E) and trained to the discrimination criterion in 12–15 sessions. To inhibit neuronal activity during early reversal, the GABA receptor agonist, muscimol (MUS), or saline (SAL) vehicle was bilaterally infused into the lOFC or dmPFC prior to each of the first two reversal sessions (capturing early reversal).

lOFC inactivation produced significant alterations in the pattern of responding during early reversal, as well as a reduction in the number of trials performed overall, relative to saline-infused controls (SAL = 72.2 ± 4.9, MUS = 33.4 ± 8.2; $t_{(16)} = 4.03$, $P < 0.0010$). Specifically, mice with the lOFC inactivated made significantly fewer responses at the S^{prior} than controls but showed a significant increase in choices at the S^{never}, as a percentage of total trials completed. Selection of the S^{new} option was equivalently low across the inactivated and saline groups (Fig. 2C). Microstructural analysis of trial-by-trial responding revealed that lOFC-inactivated mice made significantly shorter strings of consecutive of S^{prior} responses, with a corresponding increase in S^{never} strings (and no change in S^{new} strings) (Fig. 2D). Lastly, the latency to choose the S^{prior}, but not S^{new} or S^{never} stimulus, was longer after lOFC inactivation (Supplemental Fig. S2). The fact that choice latency was selectively higher for the S^{prior} stimulus after lOFC inactivation argues against the kind of general loss of response vigor that has been reported in rats studies (St Onge and Floresco 2010; Dalton et al. 2016). It could instead reflect deliberation or hesitancy at the S^{prior} option. Why this would be greater after lOFC inactivation is unclear; one intriguing possibility is that this renders animals more sensitive to the S^{prior}'s strong violation of outcome expectancies.

These data demonstrate that early reversal performance in this three-choice task was disrupted by lOFC inactivation. However, the pattern of effects does not fit with the historically favored notion that the lOFC mediates response inhibition—which would be expected to produce an increase, not a decrease, in

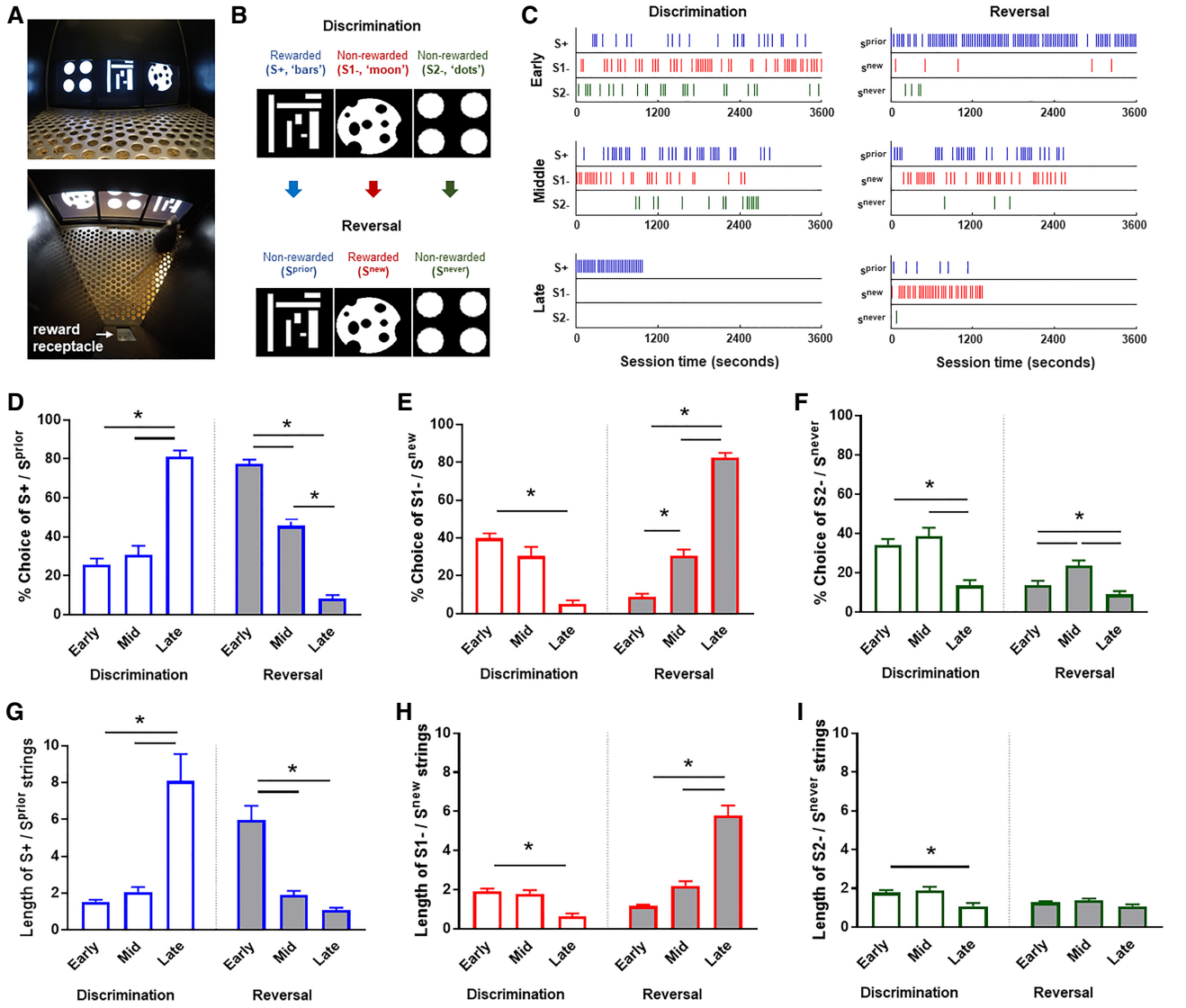

Figure 1. Performance in a novel three-choice visual discrimination and reversal touchscreen paradigm. (*A*) A mouse-eye (*upper*) and overhead (*lower*) view of the apparatus. (*B*) Visual stimuli used for discrimination and reversal (note, the designation of bars and dots as the S^+ on discrimination, and the corresponding S^{prior} designation on reversal, was counterbalanced across mice and did not affect performance). (*C*) Behavioral raster from single training sessions of a representative mouse at each relevant training phase. Blue lines represent choice of the S^+ or S^{prior} during discrimination (*left*) or reversal (*right*) sessions. Red lines represent choice of the $S1^-$ (discrimination sessions, *left*) or S^{new} (reversal sessions, *right*), while green lines represent the $S2^-$ (discrimination sessions, *left*) or S^{never} (reversal sessions, *right*). The maximum session length was 3600 sec (1 h). Note the shift from random stimulus selection, to efficient discrimination displayed across discrimination, and then the marked degree of perseveration in early reversal, followed by characteristic sampling and then reversal during the *middle* and late reversal phases, respectively. (*D*) The percentage of S^+/S^{prior} responses increased across discrimination and remained high at early reversal before decreasing across subsequent reversal training (task × phase interaction: $F_{(2,26)} = 217.9$, $P < 0.0001$; effect of task: $F_{(1,13)} = 0.68$, $P = 0.4245$; effect of phase: $F_{(2,26)} = 9.51$, $P = 0.0008$, followed by individual comparisons via Tukey's post-hoc tests). (*E*) The percentage of $S1^-/S^{new}$ responses decreased from early to late discrimination and progressively increased over reversal (task × phase interaction: $F_{(2,26)} = 239.1$, $P < 0.0001$; effect of task: $F_{(1,13)} = 34.32$, $P < 0.0001$; effect of phase: $F_{(2,26)} = 24.25$, $P < 0.0001$, followed by Tukey's post-hoc tests). (*F*) The percentage of $S2^-/S^{never}$ choice was low by late discrimination and did not change during reversal (task × phase interaction: $F_{(2,26)} = 4.01$, $P = 0.0302$; effect of task: $F_{(1,13)} = 43.44$, $P < 0.0001$; effect of phase: $F_{(2,26)} = 22.52$, $P < 0.0001$, followed by Tukey's post-hoc tests). (*G*) Strings of consecutive S^+/S^{prior} responses increased over discrimination and decreased over reversal (task × phase interaction: $F_{(2,26)} = 5.50$, $P < 0.0102$; effect of task: $F_{(1,13)} = 26.26$, $P = 0.0002$; effect of phase: $F_{(2,26)} = 19.86$, $P < 0.0001$, followed by Tukey's post-hoc tests). (*H*) Strings of $S1^-/S^{new}$ responses were decreased by late discrimination before increasing across reversal (task × phase interaction: $F_{(2,26)} = 91.38$, $P < 0.0001$; effect of task: $F_{(1,13)} = 34.57$, $P < 0.0001$; effect of phase: $F_{(2,26)} = 25.87$, $P < 0.0001$, followed by Tukey's post-hoc tests). (*I*) $S2^-/S^{never}$ strings were reduced at late discrimination and stayed flat across reversal (task × phase interaction: $F_{(2,26)} = 2.39$, $P = 0.1116$; effect of task: $F_{(1,13)} = 14.92$, $P = 0.0020$; effect of phase: $F_{(2,26)} = 10.03$, $P < 0.0006$, followed by Tukey's post-hoc tests). $n = 14$. Data are means ± SEM. (*) $P < 0.05$.

perseverative-like responding at the S^{prior}. Rather, these data are more readily explained by more recent views that the lOFC integrates a record of choices and their respective outcomes to guide the optimal response based on the calculated value of relative options available (Noonan et al. 2012; Rudebeck and Murray 2014; Stalnaker et al. 2015; Izquierdo et al. 2016). For example, multi-choice reversal studies in nonhuman primates find that, without the normal contribution of the lOFC (or mPFC), animals do not perseverate more, but instead more frequently shift between choices (Walton et al. 2010; Noonan et al. 2012, 2017; Chau et al. 2014).

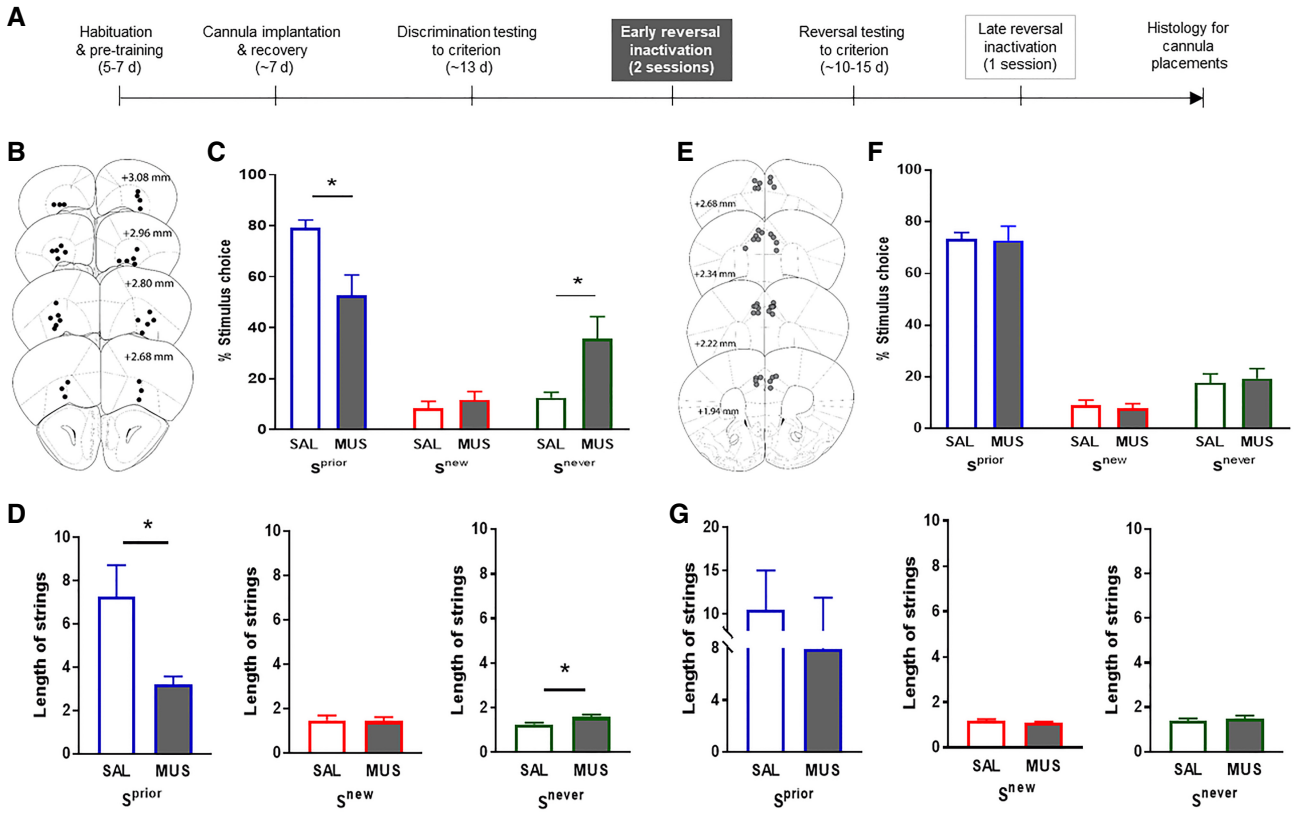

Figure 2. Inactivation of the lOFC, but not dmPFC, disrupts early three-choice reversal performance. (*A*) Experimental timeline for pretraining, discrimination testing, and early reversal inactivation. (*B*) Ventral extent of the infusion located in lOFC. (*C*) Inactivation of lOFC resulted in a decrease in the percentage of trials ending in S^{prior} choices (*left* panel; corrected $t_{(16)} = 3.09$, $P < 0.0210$), without affecting the percentage of S^{new} trials (*middle* panel; corrected $t_{(16)} = 0.80$, $P > 0.05$). In contrast, the percentage of trials ending in S^{never} choices were increased following lOFC inactivation (*right* panel; corrected $t_{(16)} = 2.56$, $P < 0.0412$). (*D*) lOFC inactivation decreased the average length of strings of S^{prior} (*left* panel; $t(16) = 2.72$, $P = 0.0151$) without affecting strings of S^{new} responses (*middle* panel; $t_{(16)} = 0.24$, $P = 0.8157$). The length of strings of S^{never} responses tended to be increased by lOFC inactivation (*right* panel; $t_{(16)} = 2.12$, $P = 0.0502$). (*E*) Ventral extent of the infusion located in dmPFC. (*F*,*G*) Inactivation of the dmPFC did not significantly affect any measure (all *t*-values < 1.5, all *P*-values > 0.15). $n = 9$–10 per group. Data are means ± SEM. (*) $P < 0.05$.

This effect has been explained by an inability to integrate recent and historical choice-outcomes—which are mixed and conflicting during reversal—to support decisions, leading to the misattribution of positive outcomes to the unrewarded stimuli ("deficient credit assignment"). While a deficiency of this kind could conceivably contribute to the reversal abnormalities observed in the current study, it cannot adequately account for the specific pattern we see. In particular, if mice had difficulty with credit assignment per se, then we would expect to see an increase in responding at the S^{new}, as well as the S^{never}, because credit misattributed to the latter would derive from more frequent choice of the former. To decipher the nature of the effects we do see, it could be informative to inactivate the lOFC at the mid-reversal stage, when S^{new} responding is higher than at early reversal, and ask whether S^{never} responding is still excessively high after inactivation.

The current data also bear comparison with prior rodent studies of lOFC inactivation in reversal tasks that vary in stimulus number (two or more), stimulus type (visual, olfactory, or spatial) and/or reinforcement schedule (deterministic or probabilistic). In rats, lOFC inactivation has been found to impair performance of a probabilistic spatial reversal, without affecting performance on a deterministic version (Dalton et al. 2016). Conversely, rat or mouse lOFC inactivation or NMDA receptor antagonism was reported to increase putative perseverative-like errors in two-choice odor (Kim and Ragozzino 2005) and visual touchscreen (Chudasama and Robbins 2003; Brigman et al. 2013) reversals, but cause a general increase in perseverative, regressive and irrelevant errors in a four-choice odor reversal (Kim and Ragozzino 2005; Ragozzino 2007). One preliminary conclusion to draw from these studies is that the manner in which lOFC disruptions behaviorally manifest critically depends on the range of options available and their relative reinforcement histories.

There are also some noteworthy differences between the current visual reversal task and prior studies that use olfactory, tactile and visual stimuli, often in compound. In the touchscreen procedure, mice perform many more trials to reach discrimination and reversal criteria than rats typically do in, for example, digging tasks (i.e., hundreds versus tens). As such, all the stimuli are highly familiar by reversal in our task, whereas rats are sometimes introduced to novel stimuli across serial discriminations and reversals. These differing levels of stimulus-familiarity could have important consequences for how reversals are performed and recruit the lOFC. Indeed, Tait and Brown (2007) found that juxtaposing two reversal stimuli based on their familiarity and relative reinforcement histories markedly affected the nature of lOFC loss-of-function effects in a rat digging task. lOFC-lesioned rats displayed poor learning and increased omissions ("refusals to dig") when required to select the previously unrewarded stimulus over a novel option, but actually had superior learning when rewarded for choosing a novel stimulus over the previously reward option (Tait and Brown 2007). One interesting avenue for future studies will be dissecting the influence of these parameters. As a behavioral platform, the

touchscreen provides a tractable means to do so, given that the number, familiarity, and reinforcement histories of the choice-options can be varied, and their influence examined across single as well as potentially serial reversals.

In contrast to the marked effects of lOFC inactivation, inactivating the dmPFC was without any discernible effect (Fig. 2E–G). Prior reports of dmPFC inactivation effects on reversal tasks (including multichoice) in rats have been mixed, with examples of improvement, impairment and, as in the current case, no change in performance (cf. Becker et al. 1981; Bussey et al. 1997; Ragozzino et al. 2003; Boulougouris et al. 2007; Ragozzino and Rozman 2007; Floresco et al. 2008; Dalton et al. 2016). Again, this might reflect important variants in task parameters. Tasks that require cross-modal cognitive flexibility and resolution of rule-conflict appear to be particularly sensitive to mPFC disruption (Ragozzino et al. 2003; Floresco et al. 2008; Bissonette and Roesch 2017). For example, rats with dmPFC lesions exhibited deficits in a Y-maze reversal after previously learning a strategy shift; an effect attributed to a deficit in maintaining performance under conditions of conflicting strategies (Oualian and Gisquet-Verrier 2010).

Following early reversal inactivation in the current study, mice were trained to reversal criterion (without further infusions). Groups took an equivalent number of sessions to reach reversal criterion (lOFC: SAL = 15.0 ± 2.8, MUS = 13.7 ± 1.1; mPFC: SAL = 10.3 ± 0.9, MUS = 10.3 ± 1.4), indicating that the initial manipulation left no residual effects on performance when the cortical regions were back "online." The lOFC or dmPFC was then inactivated during a single post-criterion session to test for effects on the expression of the now well-learned stimulus–reward contingencies (Fig. 3A), but neither inactivation significantly affected overall percent stimulus-choice (Fig. 3B,D). lOFC, but not dmPFC, inactivation did produce a modest increase in the length of S^{prior} strings, while decreasing consecutive selections of the S^{new} option (Fig. 3C, E) and increasing the number of trials performed overall (SAL = 35.0 ± 1.2, lOFC = 45.1 ± 4.2; $t_{(16)} = 2.27$, $P < 0.0378$). Also, and in contrast to the selective increase in S^{new} choice latency after early reversal lOFC inactivation, inactivation at late reversal increased latencies generally (Supplemental Fig. S3).

The slight shift in favor of the S^{prior} after late reversal lOFC inactivation could reflect partial reversion to the discrimination associations (Delamater 2007) which, as noted above, could be expressed by other brain regions. However, the fact that correct choice was reduced and both error types were increased (not just the S^{prior}) suggests a problem with maintaining the stimulus–reward/nonreward associations formed during reversal. This is somewhat surprising considering that lOFC is typically implicated when behaviors are being adjusted (as in early reversal), rather than after adjustments have been made (as in late reversal) (Boulougouris et al. 2007). However, the lOFC was also found to support established reversal performance on a probabilistically reinforced reversal task conducted in rats (Dalton et al. 2016). It may be that the multichoice and probabilistic reversals are especially taxing on the lOFC's capacity for predicting and tracking stimulus–outcome associations, such that the region retains a hand in maintaining performance even after behavior has successfully adjusted to the new stimulus–reward contingencies. To address this question

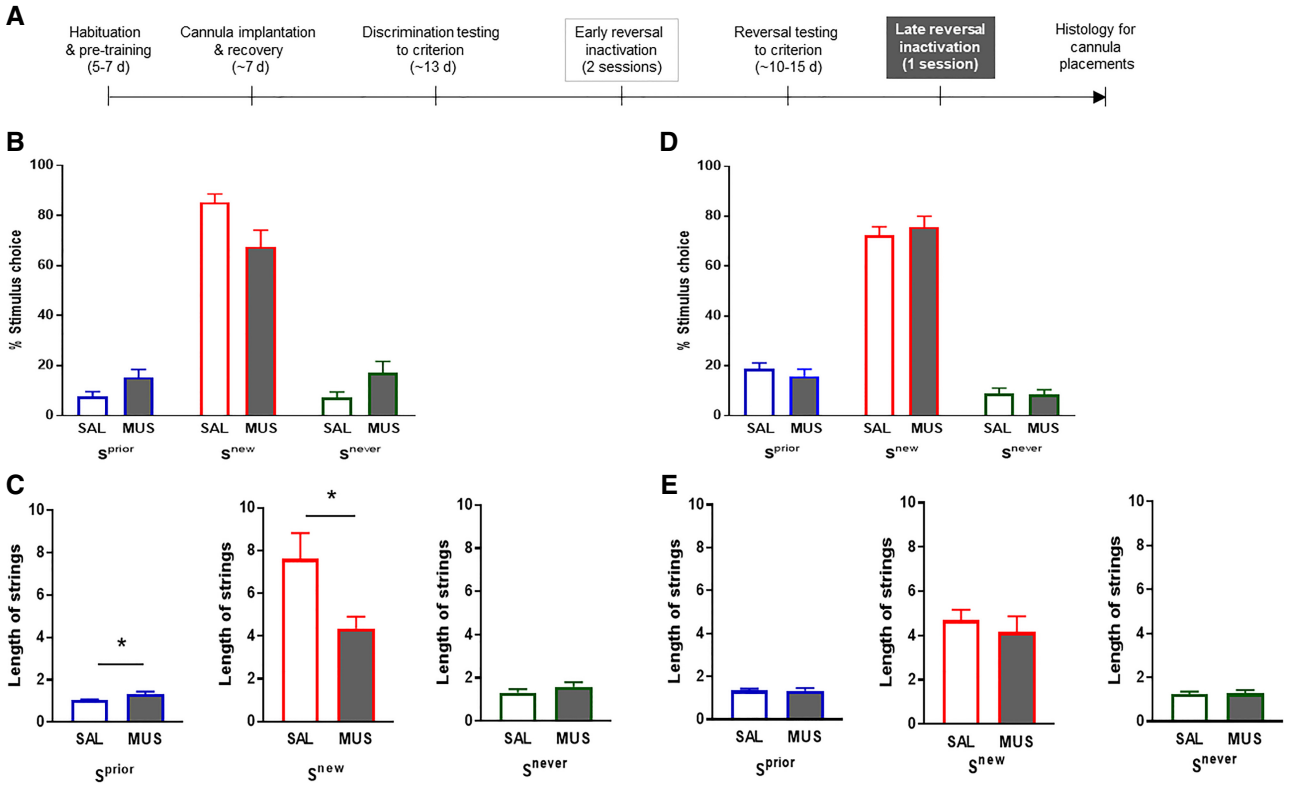

Figure 3. Effects of lOFC and dmPFC inactivation on late three-choice reversal performance. (*A*) Experimental timeline for post-reversal criterion inactivation. (*B*) lOFC inactivation did not significantly affect the percentage of S^{prior} (*left* panel; corrected $t_{(16)} = 2.03$, $P = 0.1160$), S^{new} (*middle* panel; corrected $t_{(16)} = 2.39$, $P = 0.0856$), or S^{never} (*right* panel; corrected $t_{(16)} = 1.99$, $P = 0.1160$) choices. (*C*) The average length of S^{prior} strings was increased by lOFC inactivation (*left* panel; $t_{(16)} = 2.61$, $P = 0.0190$), while the average S^{new} string length was diminished by lOFC inactivation (*middle* panel; $t_{(16)} = 2.45$, $P = 0.0261$). In contrast, S^{never} string length was not altered by lOFC inactivation (*right* panel; $t_{(14)} = 0.98$, $P = 0.3450$). (*D*,*E*) Inactivation of the dmPFC at late reversal did not significantly affect any measure (all *t*-values <1.5, all *P*-values > 0.15). $n = 9$–10 per group. Data are means ± SEM. (*) $P < 0.05$.

going forward, it would be valuable to chronically measure (e.g., via neuronal recordings or imaging) the sustained engagement of the lOFC across reversal learning in the three-choice task.

In conclusion, the current three-choice touchscreen task represents novel paradigm for assessing murine reversal learning and cognitive flexibility that may be useful for translational research given its overt similarity to cognitive testing procedures used in higher species, including humans (Mar et al. 2013; Akaishi et al. 2016; Izquierdo et al. 2016; Noonan et al. 2017). Further speaking to this potential, we show that the reversal task is highly sensitive to disruption of the lOFC, a brain region subserving the modification of choices to accommodate new outcome contingencies, and implicated in narrowed, intransigent patterns of behavior characteristic of AUDs and other addictions.

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References

Akaishi R, Kolling N, Brown JW, Rushworth M. 2016. Neural mechanisms of credit assignment in a multicue environment. *J Neurosci* **36:** 1096–1112. doi:10.1523/JNEUROSCI.3159-15.2016

Amodeo DA, Rivera E, Cook EH Jr, Sweeney JA, Ragozzino ME. 2017. 5HT2A receptor blockade in dorsomedial striatum reduces repetitive behaviors in BTBR mice. *Genes Brain Behav* **16:** 342–351. doi:10.1111/gbb.12343

Balleine BW, Leung BK, Ostlund SB. 2011. The orbitofrontal cortex, predicted value, and choice. *Ann N Y Acad Sci* **1239:** 43–50. doi:10.1111/j.1749-6632.2011.06270.x

Becker JT, Olton DS, Anderson CA, Breitinger ER. 1981. Cognitive mapping in rats: the role of the hippocampal and frontal system in retention and reversal. *Behav Brain Res* **3:** 1–22. doi:10.1016/0166-4328(81)90025-5

Belin D, Belin-Rauscent A, Everitt BJ, Dalley JW. 2016. In search of predictive endophenotypes in addiction: insights from preclinical research. *Genes Brain Behav* **15:** 74–88. doi:10.1111/gbb.12265

Bergstrom HC, Lipkin AM, Lieberman AG, Pinard CR, Gunduz-Cinar O, Brockway ET, Taylor WW, Nonaka M, Bukalo O, Wills TA, et al. 2018. Dorsolateral striatum engagement interferes with early discrimination learning. *Cell Rep* **23:** 2264–2272. doi:10.1016/j.celrep.2018.04.081

Birrell JM, Brown VJ. 2000. Medial frontal cortex mediates perceptual attentional set shifting in the rat. *J Neurosci* **20:** 4320–4324. doi:10.1523/JNEUROSCI.20-11-04320.2000

Bissonette GB, Roesch MR. 2017. Neurophysiology of rule switching in the corticostriatal circuit. *Neuroscience* **345:** 64–76. doi:10.1016/j.neuroscience.2016.01.062

Bissonette GB, Martins GJ, Franz TM, Harper ES, Schoenbaum G, Powell EM. 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. doi:10.1523/JNEUROSCI.2820-08.2008

Bissonette GB, Powell EM, Roesch MR. 2013. Neural structures underlying set-shifting: roles of medial prefrontal cortex and anterior cingulate cortex. *Behav Brain Res* **250:** 91–101. doi:10.1016/j.bbr.2013.04.037

Bissonette GB, Schoenbaum G, Roesch MR, Powell EM. 2014. Interneurons are necessary for coordinated activity during reversal learning in orbitofrontal cortex. *Biol Psychiatry* **77:** 454–464. doi:10.1016/j.biopsych.2014.07.023

Boulougouris V, Dalley JW, Robbins TW. 2007. Effects of orbitofrontal, infralimbic and prelimbic cortical lesions on serial spatial reversal learning in the rat. *Behav Brain Res* **179:** 219–228. doi:10.1016/j.bbr.2007.02.005

Brigman JL, Rothblat LA. 2007. Stimulus specific deficit on visual reversal learning after lesions of medial prefrontal cortex in the mouse. *Behav Brain Res* **87:** 405–410.

Brigman JL, Daut RA, Wright T, Gunduz-Cinar O, Graybeal C, Davis MI, Jiang Z, Saksida LM, Jinde S, Pease M, et al. 2013. GluN2B in corticostriatal circuits governs choice learning and choice shifting. *Nat Neurosci* **16:** 1101–1110. doi:10.1038/nn.3457

Brown HD, Baker PM, Ragozzino ME. 2010. The parafascicular thalamic nucleus concomitantly influences behavioral flexibility and dorsomedial striatal acetylcholine output in rats. *J Neurosci* **30:** 14390–14398. doi:10.1523/JNEUROSCI.2167-10.2010

Bussey TJ, Muir JL, Everitt BJ, Robbins TW. 1997. Triple dissociation of anterior cingulate, posterior cingulate, and medial frontal cortices on visual discrimination tasks using a touchscreen testing procedure for the rat. *Behav Neurosci* **111:** 920–936. doi:10.1037/0735-7044.111.5.920

Chau BK, Kolling N, Hunt LT, Walton ME, Rushworth MF. 2014. A neural mechanism underlying failure of optimal choice with multiple alternatives. *Nat Neurosci* **17:** 463–470. doi:10.1038/nn.3649

Chudasama Y, Robbins TW. 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. doi:10.1523/JNEUROSCI.23-25-08771.2003

Clarke HF, Robbins TW, Roberts AC. 2008. Lesions of the medial striatum in monkeys produce perseverative impairments during reversal learning similar to those produced by lesions of the orbitofrontal cortex. *J Neurosci* **28:** 10972–10982. doi:10.1523/JNEUROSCI.1521-08.2008

Coleman LG Jr, He J, Lee J, Styner M, Crews FT. 2012. Adolescent binge drinking alters adult brain neurotransmitter gene expression, behavior, brain regional volumes, and neurochemistry in mice. *Alcohol Clin Exp Res* **35:** 671–688. doi:10.1111/j.1530-0277.2010.01385.x

Costa VD, Tran VL, Turchi J, Averbeck BB. 2015. Reversal learning and dopamine: a Bayesian perspective. *J Neurosci* **35:** 2407–2416. doi:10.1523/JNEUROSCI.1989-14.2015

Dalton GL, Wang NY, Phillips AG, Floresco SB. 2016. Multifaceted contributions by different regions of the orbitofrontal and medial prefrontal cortex to probabilistic reversal learning. *J Neurosci* **36:** 1996–2006. doi:10.1523/JNEUROSCI.3366-15.2016

D'Cruz AM, Ragozzino ME, Mosconi MW, Pavuluri MN, Sweeney JA. 2011. Human reversal learning under conditions of certain versus uncertain outcomes. *Neuroimage* **56:** 315–322. doi:10.1016/j.neuroimage.2011.01.068

Delamater AR. 2007. The role of the orbitofrontal cortex in sensory-specific encoding of associations in pavlovian and instrumental conditioning. *Ann N Y Acad Sci* **1121:** 152–173. doi:10.1196/annals.1401.030

DePoy L, Daut R, Brigman JL, Macpherson K, Crowley N, Gunduz-Cinar O, Pickens CL, Cinar R, Saksida LM, Kunos G, et al. 2013. Chronic alcohol produces neuroadaptations to prime dorsal striatal learning. *Proc Natl Acad Sci* **110:** 14783–14788. doi:10.1073/pnas.1308198110

Dias R, Robbins TW, Roberts AC. 1996. Dissociation in prefrontal cortex of affective and attentional shifts. *Nature* **380:** 69–72. doi:10.1038/380069a0

Dias R, Robbins TW, Roberts AC. 1997. Dissociable forms of inhibitory control within prefrontal cortex with an analog of the Wisconsin Card Sort Test: restriction to novel situations and independence from "on-line" processing. *J Neurosci* **17:** 9285–9297. doi:10.1523/JNEUROSCI.17-23-09285.1997

Floresco SB, Block AE, Tse MT. 2008. Inactivation of the medial prefrontal cortex of the rat impairs strategy set-shifting, but not reversal learning, using a novel, automated procedure. *Behav Brain Res* **190:** 85–96. doi:10.1016/j.bbr.2008.02.008

Ghods-Sharifi S, Haluk DM, Floresco SB. 2008. Differential effects of inactivation of the orbitofrontal cortex on strategy set-shifting and reversal learning. *Neurobiol Learn Mem* **89:** 567–573. doi:10.1016/j.nlm.2007.10.007

Graybeal C, Feyder M, Schulman E, Saksida LM, Bussey TJ, Brigman JL, Holmes A. 2011. Paradoxical reversal learning enhancement by stress or prefrontal cortical damage: rescue with BDNF. *Nat Neurosci* **14:** 1507–1509. doi:10.1038/nn.2954

Graybeal C, Bachu M, Mozhui K, Saksida LM, Bussey TJ, Sagalyn E, Williams RW, Holmes A. 2014. Strains and stressors: an analysis of touchscreen learning in genetically diverse mouse strains. *PLoS One* **9:** e87745. doi:10.1371/journal.pone.0087745

Grospe GM, Baker PM, Ragozzino ME. 2018. Cognitive flexibility deficits following 6-OHDA lesions of the rat dorsomedial striatum. *Neuroscience* **374:** 80–90. doi:10.1016/j.neuroscience.2018.01.032

Hu W, Morris B, Carrasco A, Kroener S. 2015. Effects of acamprosate on attentional set-shifting and cellular function in the prefrontal cortex of chronic alcohol-exposed mice. *Alcohol Clin Exp Res* **39:** 953–961. doi:10.1111/acer.12722

Iversen SD, Mishkin M. 1970. Perseverative interference in monkeys following selective lesions of the inferior prefrontal convexity. *Exp Brain Res* **11:** 376–386. doi:10.1007/BF00237911

Izquierdo A, Suda RK, Murray EA. 2004. Bilateral orbital prefrontal cortex lesions in rhesus monkeys disrupt choices guided by both reward value and reward contingency. *J Neurosci* **24:** 7540–7548. doi:10.1523/JNEUROSCI.1921-04.2004

Izquierdo A, Wiedholz LM, Millstein RA, Yang RJ, Bussey TJ, Saksida LM, Holmes A. 2006. Genetic and dopaminergic modulation of reversal learning in a touchscreen-based operant procedure for mice. *Behav Brain Res* **171:** 181–188. doi:10.1016/j.bbr.2006.03.029

Izquierdo A, Brigman JL, Radke AK, Rudebeck PH, Holmes A. 2016. The neural basis of reversal learning: an updated perspective. *Neuroscience* **345:** 12–26. doi:10.1016/j.neuroscience.2016.03.021

Jentsch JD, Taylor JR. 1999. Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. *Psychopharmacology* **146:** 373–390. doi:10.1007/PL00005483

Jones B, Mishkin M. 1972. Limbic lesions and the problem of stimulus–reinforcement associations. *Exp Neurol* **36:** 362–377. doi:10.1016/0014-4886(72)90030-1

Kim J, Ragozzino ME. 2005. The involvement of the orbitofrontal cortex in learning under changing task contingencies. *Neurobiol Learn Mem* **83:** 125–133. doi:10.1016/j.nlm.2004.10.003

Kosaki Y, Watanabe S. 2012. Dissociable roles of the medial prefrontal cortex, the anterior cingulate cortex, and the hippocampus in behavioural flexibility revealed by serial reversal of three-choice discrimination in rats. *Behav Brain Res* **227:** 81–90. doi:10.1016/j.bbr.2011.10.039

Le Berre AP, Fama R, Sullivan EV. 2017. Executive functions, memory, and social cognitive deficits and recovery in chronic alcoholism: a critical review to inform future research. *Alcohol Clin Exp Res* **41:** 1432–1443. doi:10.1111/acer.13431

Lee B, Groman S, London ED, Jentsch JD. 2007. Dopamine D2/D3 receptors play a specific role in the reversal of a learned visual discrimination in monkeys. *Neuropsychopharmacology* **32:** 2125–2134. doi:10.1038/sj.npp.1301337

Mar AC, Horner AE, Nilsson SR, Alsiö J, Kent BA, Kim CH, Holmes A, Saksida LM, Bussey TJ. 2013. The touchscreen operant platform for assessing executive function in rats and mice. *Nat Protoc* **8:** 1985–2005. doi:10.1038/nprot.2013.123

Marquardt K, Sigdel R, Brigman JL. 2017. Touch-screen visual reversal learning is mediated by value encoding and signal propagation in the orbitofrontal cortex. *Neurobiol Learn Mem* **139:** 179–188. doi:10.1016/j.nlm.2017.01.006

McAlonan K, Brown VJ. 2003. Orbital prefrontal cortex mediates reversal learning and not attentional set shifting in the rat. *Behav Brain Res* **146:** 97–103. doi:10.1016/j.bbr.2003.09.019

Moorman DE. 2018. The role of the orbitofrontal cortex in alcohol use, abuse, and dependence. *Prog Neuropsychopharmacol Biol Psychiatry* **87:** 85–107. doi:10.1016/j.pnpbp.2018.01.010

Noonan MP, Kolling N, Walton ME, Rushworth MF. 2012. Re-evaluating the role of the orbitofrontal cortex in reward and reinforcement. *Eur J Neurosci* **35:** 997–1010. doi:10.1111/j.1460-9568.2012.08023.x

Noonan MP, Chau BKH, Rushworth MFS, Fellows LK. 2017. Contrasting effects of medial and lateral orbitofrontal cortex lesions on credit assignment and decision-making in humans. *J Neurosci* **37:** 7023–7035. doi:10.1523/JNEUROSCI.0692-17.2017

Oualian C, Gisquet-Verrier P. 2010. The differential involvement of the prelimbic and infralimbic cortices in response conflict affects behavioral flexibility in rats trained in a new automated strategy-switching task. *Learn Mem* **17:** 654–668. doi:10.1101/lm.1858010

Palencia CA, Ragozzino ME. 2004. The influence of NMDA receptors in the dorsomedial striatum on response reversal learning. *Neurobiol Learn Mem* **82:** 81–89. doi:10.1016/j.nlm.2004.04.004

Palencia CA, Ragozzino ME. 2006. The effect of N-methyl-D-aspartate receptor blockade on acetylcholine efflux in the dorsomedial striatum during response reversal learning. *Neuroscience* **143:** 671–678. doi:10.1016/j.neuroscience.2006.08.024

Ragozzino ME. 2007. The contribution of the medial prefrontal cortex, orbitofrontal cortex, and dorsomedial striatum to behavioral flexibility. *Ann N Y Acad Sci* **1121:** 355–375. doi:10.1196/annals.1401.013

Ragozzino ME, Choi D. 2004. Dynamic changes in acetylcholine output in the medial striatum during place reversal learning. *Learn Mem* **11:** 70–77. doi:10.1101/lm.65404

Ragozzino ME, Rozman S. 2007. The effect of rat anterior cingulate inactivation on cognitive flexibility. *Behav Neurosci* **121:** 698–706. doi:10.1037/0735-7044.121.4.698

Ragozzino ME, Kim J, Hassert D, Minniti N, Kiang C. 2003. The contribution of the rat prelimbic-infralimbic areas to different forms of task switching. *Behav Neurosci* **117:** 1054–1065. doi:10.1037/0735-7044.117.5.1054

Riceberg JS, Shapiro ML. 2012. Reward stability determines the contribution of orbitofrontal cortex to adaptive behavior. *J Neurosci* **32:** 16402–16409. doi:10.1523/JNEUROSCI.0776-12.2012

Rolls ET. 1996. The orbitofrontal cortex. *Philos Trans R Soc Lond B Biol Sci* **351:** 1433–1443; discussion 1443–1434. doi:10.1098/rstb.1996.0128

Rudebeck PH, Murray EA. 2014. The orbitofrontal oracle: cortical mechanisms for the prediction and evaluation of specific behavioral outcomes. *Neuron* **84:** 1143–1156. doi:10.1016/j.neuron.2014.10.049

Rudebeck PH, Behrens TE, Kennerley SW, Baxter MG, Buckley MJ, Walton ME, Rushworth MF. 2008. Frontal cortex subregions play distinct roles in choices between actions and stimuli. *J Neurosci* **28:** 13775–13785. doi:10.1523/JNEUROSCI.3541-08.2008

Schoenbaum G, Shaham Y. 2008. The role of orbitofrontal cortex in drug addiction: a review of preclinical studies. *Biol Psychiatry* **63:** 256–262. doi:10.1016/j.biopsych.2007.06.003

Schoenbaum G, Nugent SL, Saddoris MP, Setlow B. 2002. Orbitofrontal lesions in rats impair reversal but not acquisition of go, no-go odor discriminations. *Neuroreport* **13:** 885–890. doi:10.1097/00001756-200205070-00030

Schoenbaum G, Setlow B, Nugent SL, Saddoris MP, Gallagher M. 2003. Lesions of orbitofrontal cortex and basolateral amygdala complex disrupt acquisition of odor-guided discriminations and reversals. *Learn Mem* **10:** 129–140. doi:10.1101/lm.55203

Schoenbaum G, Saddoris MP, Ramus SJ, Shaham Y, Setlow B. 2004. Cocaine-experienced rats exhibit learning deficits in a task sensitive to orbitofrontal cortex lesions. *Eur J Neurosci* **19:** 1997–2002. doi:10.1111/j.1460-9568.2004.03274.x

Seu E, Lang A, Rivera RJ, Jentsch JD. 2009. Inhibition of the norepinephrine transporter improves behavioral flexibility in rats and monkeys. *Psychopharmacology* **202:** 505–519. doi:10.1007/s00213-008-1250-4

Stalnaker TA, Roesch MR, Franz TM, Burke KA, Schoenbaum G. 2006. Abnormal associative encoding in orbitofrontal neurons in cocaine-experienced rats during decision-making. *Eur J Neurosci* **24:** 2643–2653. doi:10.1111/j.1460-9568.2006.05128.x

Stalnaker TA, Cooch NK, Schoenbaum G. 2015. What the orbitofrontal cortex does not do. *Nat Neurosci* **18:** 620–627. doi:10.1038/nn.3982

St Onge JR, Floresco SB. 2010. Prefrontal cortical contribution to risk-based decision making. *Cereb Cortex* **20:** 1816–1828. doi:10.1093/cercor/bhp250

Tait DS, Brown VJ. 2007. Difficulty overcoming learned non-reward during reversal learning in rats with ibotenic acid lesions of orbital prefrontal cortex. *Ann N Y Acad Sci* **1121:** 407–420. doi:10.1196/annals.1401.010

Trantham-Davidson H, Burnett EJ, Gass JT, Lopez MF, Mulholland PJ, Centanni SW, Floresco SB, Chandler LJ. 2014. Chronic alcohol disrupts dopamine receptor activity and the cognitive function of the medial prefrontal cortex. *J Neurosci* **34:** 3706–3718. doi:10.1523/JNEUROSCI.0623-13.2014

Tremblay L, Hollerman JR, Schultz W. 1998. Modifications of reward expectation-related neuronal activity during learning in primate striatum. *J Neurophysiol* **80:** 964–977. doi:10.1152/jn.1998.80.2.964

Tzavos A, Jih J, Ragozzino ME. 2004. Differential effects of M1 muscarinic receptor blockade and nicotinic receptor blockade in the dorsomedial striatum on response reversal learning. *Behav Brain Res* **154:** 245–253. doi:10.1016/j.bbr.2004.02.011

Vanes LD, van Holst RJ, Jansen JM, van den Brink W, Oosterlaan J, Goudriaan AE. 2014. Contingency learning in alcohol dependence and pathological gambling: learning and unlearning reward contingencies. *Alcohol Clin Exp Res* **38:** 1602–1610. doi:10.1111/acer.12393

Varodayan FP, Sidhu H, Kreifeldt M, Roberto M, Contet C. 2018. Morphological and functional evidence of increased excitatory signaling in the prelimbic cortex during ethanol withdrawal. *Neuropharmacology* **133:** 470–480. doi:10.1016/j.neuropharm.2018.02.014

Walton ME, Devlin JT, Rushworth MF. 2004. Interactions between decision making and performance monitoring within prefrontal cortex. *Nat Neurosci* **7:** 1259–1265. doi:10.1038/nn1339

Walton ME, Behrens TE, Buckley MJ, Rudebeck PH, Rushworth MF. 2010. Separable learning systems in the macaque brain and the role of orbitofrontal cortex in contingent learning. *Neuron* **65:** 927–939. doi:10.1016/j.neuron.2010.02.027

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