

Orbitofrontal lesions in rats impair reversal but not acquisition of go, no-go odor discriminations

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Recent evidence suggests that orbitofrontal cortex lesions cause an inability to withhold inappropriate responses particularly when learned behavior must be modified to reflect changes in the likely outcome or consequence of responding. By this account, orbitofrontal cortex should not be necessary for acquisition of simple discrimination problems, but should be critical for acquiring reversals of those problems. However, previous work in rats has shown orbitofrontal cortex to be critical for withholding responses even in a simple go, no-go discrimination task. Here we have reexamined the contribution of rat orbitofrontal cortex to acquisition and

reversal of go, no-go odor discrimination problems. Contrary to prior reports, we found that rats with lesions of the orbitofrontal cortex acquired novel discrimination problems at the same rate as controls. Impairments were evident in lesioned rats when the response contingencies of the odors in the discrimination problem were reversed. These findings suggest that orbitofrontal cortex is not necessary for inhibiting responses unless responses must be altered to reflect changing relationships between cues and outcomes. *NeuroReport* 13:885–890 © 2002 Lippincott Williams & Wilkins.

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INTRODUCTION

Lesions of the orbitofrontal cortex (OFC) result in disinhibition, perseveration and impulsive behavior [1,2]. These deficits are particularly evident in discrimination tasks when existing relationships between cues and outcomes are altered [3–7]. As a result, early accounts suggested that this region of prefrontal cortex was particularly important for response inhibition: that is, withholding of normal, default, or *a priori* responses after those responses become inappropriate [1,2]. It has since become apparent that deficits in response inhibition following OFC lesions may be one consequence of a more fundamental deficit in using information regarding incentive value to guide behavioral responses [3,8–12]. By this account, animals with OFC lesions engage in perseverative or impulsive behavior in certain circumstances because their actions are not goal-directed (i.e. their actions are not mediated by representations of their likely outcome [13]), and not because they cannot inhibit *a priori* responses *per se*. Consistent with this hypothesis, humans [5] and non-human primates [3,4,6,7] with damage to OFC are often unimpaired on discrimination tasks in which they must inhibit normal or default response tendencies. This apparently normal performance may reflect the unambiguous relationships between cues, responses and outcomes that allow other associative representations, such as those linking cues and responses, to support behavior. Deficits in lesioned subjects become apparent in many of these studies only during reversal

learning, when relationships between cues and the appropriate responses become ambiguous [3,4,6,7].

Although experiments in primates suggest that OFC is not necessary for simple discrimination learning, experiments in rats suggest that it is [14,15]. These impairments in OFC-lesioned rats mark a substantial divergence between findings in primate and rat studies, raising the possibility that rat OFC and primate OFC may play different roles in modulating behavioral responses. Here we have reexamined the contribution of rat OFC to discrimination learning requiring response inhibition. Rats with OFC lesions were trained in a series of go, no-go odor discrimination problems, in which one (positive) odor signaled availability of a water reward following a response at a fluid well, and the other (negative) odor signaled no reward. Rats had to learn to inhibit responses at the fluid well following the negative odor to avoid the longer inter-trial interval associated with these incorrect responses. Following acquisition of the discrimination problems, the rats underwent serial reversals of the last problem in order to assess their ability to inhibit responding during manipulation of the relationships between the cues and the outcomes.

MATERIALS AND METHODS

Subjects: Twenty-four male Long-Evans rats (300–350 g), obtained from Charles River Laboratories, Wilmington, MA

served as subjects. Rats were housed individually on a 12:12 h light:dark cycle. During training, access to water was restricted to 30–60 min each day. Food was always available *ad lib*. Prior to odor discrimination training (but after surgery), rats in the current study received training in a food-motivated Pavlovian second-order conditioning task [16] using visual and auditory conditioned stimuli. This training occurred in a separate apparatus in a location different from that used in the present experiment, using different cues and reinforcers than the current task. Approximately 10 days elapsed between the completion of second-order conditioning and the start of odor discrimination training. During this time, the rats remained in their home cages and received periodic handling and *ad lib* food and water. The total time elapsed between surgery and odor discrimination training was ~6 weeks.

Surgery: Surgery was conducted as described previously [9]. Briefly, rats were anesthetized with isoflurane and placed in a stereotaxic frame fitted with an isoflurane gas anesthesia system. The skull was exposed, holes were drilled through the skull over the lesion sites, and intracerebral injections were made using a glass micropipette attached by a length of plastic tubing to a picospritzer (General Valve Corporation, Fairfield, NJ). Twelve rats received infusions of NMDA (20 $\mu\text{g}/\mu\text{l}$, Sigma, St. Louis, MO) in phosphate buffer vehicle to lesion the OFC; 12 rats received infusions of vehicle alone to create sham lesions. Infusions were made at four separate injection sites in each hemisphere (0.1 $\mu\text{l}/\text{site}$). One set of injections was made bilaterally 4.0 mm anterior to bregma. Injection at this level were made 2.2 and 3.7 mm lateral to the midline, and 4.2 mm ventral to the skull surface. A second set of injections was made bilaterally 3.0 mm anterior to bregma, 3.2 and 4.2 mm lateral to the midline and 5.2 mm ventral to the skull surface.

Apparatus and training procedure: Odor discrimination training was conducted using a set of four identical operant chambers similar to those described previously [17]. Odors were selected from a set of compounds from International Flavors and Fragrances (New York, NY) classified subjectively by smell. Each discrimination problem consisted of odors from different categories (fruity, spicy, herbal, etc.), and categories did not repeat in sequentially presented problems. These odor compounds were diluted 1:20 in propylene glycol and isolated on a removable cartridge connected to a system of solenoid valves and flowmeters to allow each odor to be individually delivered to the training chamber.

Odors were presented at an odor delivery port located in a polycarbonate panel bolted into an opening in the right wall of each operant box (Fig. 1). The odor sampling port consisted of a 2.5 cm diameter opening in this panel. Responses at the odor port were detected by a photobeam passing across this opening. Behind this port was a small hemicylinder where odorized air streams could be presented when a rat nose-poked at the odor port. Odors were delivered through tubing connected to the base of the hemicylinder behind the sampling port. A system of

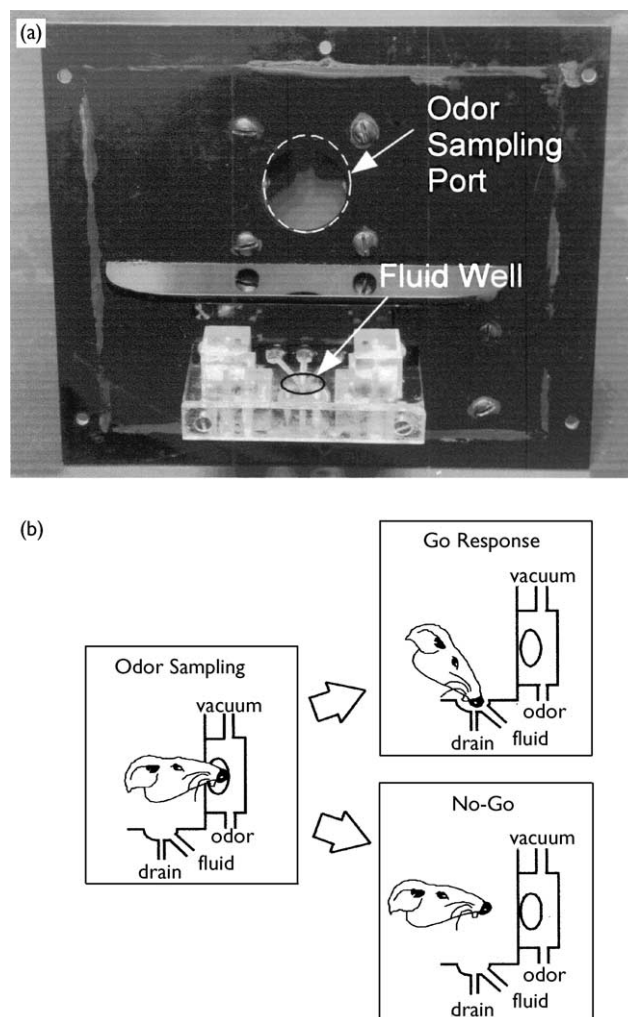


Fig. 1. Illustration of training apparatus and behaviors in the task. (a) Photograph of the polycarbonate panel removed from the operant chamber to show the odor sampling port (white circle) and the fluid delivery well (black circle). (b) Schematic drawings illustrating the sequence of behaviors in the go, no-go olfactory discrimination task employing the apparatus in the photograph.

solenoid valves and air and vacuum lines allowed rapid delivery and termination of odor cues.

A fluid well was located in a ledge 2.5 cm below the odor sampling port. Responses at the fluid well were detected by a photobeam passing parallel to the ledge ~1 mm above the well depression. Water was delivered to the well through activation of a solenoid controlling a water line concealed in the bottom of the fluid well.

Trials were signaled to the rat by illumination of a set of panel lights inside the box. When these lights were on, nose-poke into the odor port resulted in delivery of a preselected odor cue. The rat terminated odor sampling by leaving the odor port. The rat then had 3 s to make a response at the fluid well. If a response was made after sampling a positive odor, then a 0.05 ml bolus of water was delivered to the well. If the same response was made after sampling a negative odor, no fluid was delivered and instead the panel lights were extinguished. On rewarded

trials, the panel lights remained on until the rat left the fluid well, then the lights were extinguished to end the trial. If the rat did not respond within 3 s of exiting the odor port, the trial was counted as a no-go, and the panel lights were extinguished. Inter-trial intervals (which began when the panel lights were extinguished) were 4 s after correct responses and 9 s after incorrect responses.

The rats were trained to nosepoke at the odor port to receive a water reward and then the first phase of odor discrimination training began. In this first phase, the rats were trained on a series of four odor discrimination problems (D1–D4). Each odor problem consisted of two odors. One odor was positive, indicating that a response after sampling would result in delivery of a water reward. The other odor was negative, indicating that the same response after sampling would result in termination of the trial and a prolonged inter-trial interval. Behavior on new discrimination and reversal problems consisted of initially making 'go' responses to both odors, and later inhibiting responding to the negative odor as learning progressed. The rats were trained until they met a criterion of 18 correct responses within the previous 20 trials. When a rat met this behavioral criterion on one of these problems, training was begun on the next problem in the following session. Training sessions were given only once per day and lasted ~60 min.

Once all four discriminations had been acquired, the second phase of training began, in which the rats were trained on five serial reversals of the final discrimination problem (D4). For each reversal, the rats were required to demonstrate retention of the most recently acquired configuration of the discrimination problem (defined as 18/20 correct and 80% performance over a 60 trial block thereafter) and then acquire the reversal of that configuration. Retention and reversal occurred in separate consecutive sessions. Training on a reversal was continued until the rat met criterion performance (18/20 correct).

After the serial reversal phase, the ability of the rats to acquire and reverse a new odor problem (D5) was assessed in a third phase of training. The odor categories in this problem were different from those in the problem employed in the serial reversal phase (D4). Acquisition and reversal were conducted as described above.

Data analysis: Acquisition on each discrimination problem was evaluated by calculating the trials required to reach the behavioral criterion (TTC). Data were analyzed using multifactor ANOVAs with both between- and within-subjects variables, followed by contrasts and step-down ANOVAs where appropriate. Statistical analyses were performed using routines in Statistica (Statsoft, Tulsa, OK) at a significance level of $p < 0.05$.

RESULTS

Histology: Of the 12 rats that underwent surgery to create OFC lesions, eight were judged to have acceptable bilateral lesions of OFC. These rats had a marked loss of neurons in OFC, including the medial ventrolateral, and lateral orbital regions, and both dorsal and ventral agranular insular cortex. On average, lesions encompassed 75% of OFC bilaterally, ranging from 67 to 82%. The minimal and

maximal lesions are shown in Fig. 2. Rats were excluded if damage to OFC was $< 50\%$ in either hemisphere or if there was extensive bilateral damage beyond the borders of OFC.

Phase 1: Acquisition of new odor discrimination problems: The acquisition of each odor problem by the OFC-lesioned rats and controls is shown in Fig. 3 (Phase 1). All rats acquired each of the four discriminations. A two-factor ANOVA (lesion \times discrimination problem) revealed no effect of lesion on acquisition, nor was there any interaction between lesion and problem. Thus, OFC lesions did not impair the rats' ability to acquire odor discriminations at a rate comparable to that of control animals. As expected, there was a significant main effect of discrimination problem ($F(3,54) = 31.3, p < 0.01$).

Phase 2: Acquisition of serial reversals of a single odor discrimination problem: Performance on serial reversals is shown in Fig. 3 (Phase 2). Rats with OFC lesions were impaired initially relative to controls, but improved thereafter such that their performance exceeded that of controls. Performance on intervening retention sessions (not shown) did not differ between the two groups. Accordingly, a

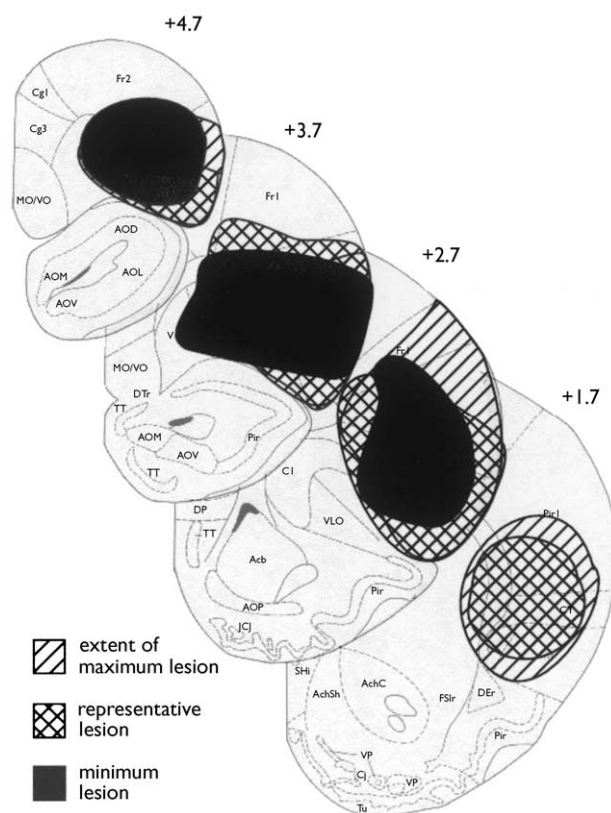


Fig. 2. Drawings showing a reconstruction of the region of damage in OFC in lesioned subjects, indicating the largest (diagonal hatched areas) and smallest (black areas) lesions in each section from animals used in the experiment. A representative lesion is also shown (cross hatched areas). Plates are adapted from the atlas of LW Swanson *Brain maps: structure of the rat brain*, New York: Elsevier (1992).

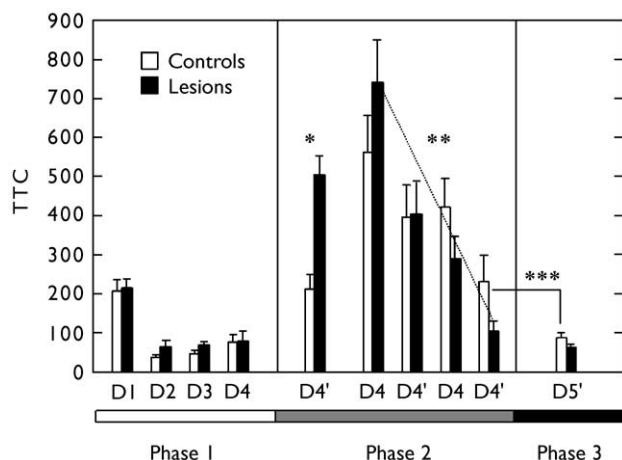


Fig. 3. The effects of OFC lesions on discrimination learning and reversal performance. Phase I: acquisition of successive discrimination problems by OFC-lesioned and control rats. Rate of acquisition of each discrimination problem (D1, D2, D3, D4) is represented as the trials it took to meet a criterion of 18 correct responses in a moving block of 20 trials (TTC). There was no effect of lesion on acquisition of any of the individual discriminations or on the improvement observed across successive problems. Phase 2: acquisition of serial reversals of a single odor discrimination problem (D4) by OFC-lesioned and control rats. D4 notation indicates a reversal session involving the same contingencies that were presented in the original learning in Phase I; D4' notation indicates a reversal session involving contingencies opposite from those presented originally in Phase I. TTC were calculated as in Phase I. OFC-lesioned rats were impaired at acquiring the first reversal (*) but then improved more rapidly than controls on subsequent reversals (**). Phase 3: acquisition of a reversal of a novel odor pair (D5') by OFC-lesioned and control rats. TTC were calculated as in Phase I. OFC-lesioned rats performed similarly on this reversal and the last one in Phase 2, whereas control rats acquired the reversal of the novel odor pair in this phase significantly faster than they acquired the last reversal in Phase 2 (***).

three-factor ANOVA (lesion \times serial problem \times retention/reversal) revealed significant main effects of serial problem ($F(4,72) = 13.4, p < 0.01$) and whether it was a retention or reversal session ($F(1,18) = 37.9, p < 0.01$), and significant interactions between problem and retention/reversal ($F(4,72) = 11.7, p < 0.01$) and between lesion, problem and retention/reversal ($F(4,72) = 5.1, p < 0.01$). Contrast testing showed that rats with OFC lesions were impaired relative to controls at acquiring the first reversal, but not thereafter across serial reversals of this problem (reversals 2–5). This reflected a decline in performance by control rats. A two-factor ANOVA across the serial reversals (excluding data from retention sessions) revealed a significant interaction between lesion and serial problem ($F(3,54) = 2.9, p < 0.05$), indicating that the performance of the lesioned rats improved more rapidly than controls across reversals 2–5.

Phase 3: Acquisition of a reversal of a novel odor discrimination problem: The data of interest from the critical test with a novel odor discrimination problem are shown in Fig. 3 (Phase 3). Acquisition (not shown) and reversal of this odor discrimination problem did not differ between the two groups. A two-factor ANOVA comparing acquisition of the last serial reversal (D4') with acquisition of

this novel reversal (D5') showed a significant interaction between lesion and reversal problem ($F(1,18) = 5.5, p < 0.05$). Contrast testing indicated that performance of OFC-lesioned rats on the reversals was unchanged between D4' and D5', whereas the control rats acquired the novel reversal significantly faster than they acquired the final serial reversal.

Interestingly, four rats with comparable OFC lesions that were excluded due to extensive bilateral damage beyond OFC (primarily to the striatum adjacent to posterior third of OFC), exhibited somewhat different results in this third phase. Like the OFC-lesioned rats included in the analysis, these rats exhibited impaired performance on reversals in Phase 2 that improved with practice. However, this impairment reemerged on the novel reversal (TTC on D4' = 157 ± 52 ; TTC on D5' = 307 ± 46). As a result, these rats performed significantly worse than controls on this reversal ($F(1,14) = 40.9, p < 0.01$).

DISCUSSION

Here we have demonstrated that rat OFC is not necessary for inhibiting a priori responses in a simple go, no-go odor discrimination task. Rats with OFC lesions performed as well as controls in acquiring new discrimination problems, despite the need to inhibit the normal prepotent tendency to respond after odor sampling on negative trials. This tendency was developed over several hundred shaping trials and reappeared with each new discrimination problem, indicating that it represented a strong default response strategy. OFC-lesioned rats were impaired, however, at acquiring reversals of previously learned discriminations, suggesting that this region may play a role in adjusting behavior to reflect changing relationships between cues and outcomes.

These results are substantially similar to those reported in discrimination and reversal tasks following damage to OFC in humans [5] and non-human primates [3,4,6,7]. For example, Diaz *et al.* [3] reported that marmosets given neurotoxic lesions of OFC before training acquired a series of novel discrimination problems normally but were impaired if the response contingencies of a previously acquired discrimination problem were subsequently reversed. Notably, as in the present study, these animals became substantially better at these reversals with additional training and were no longer impaired relative to controls, again indicating that a basic mechanism to inhibit responding remains intact after OFC lesions. They were also unimpaired at reversals in which the feature or dimension of the cue (shape or color for example) associated with reward changed. These so-called extra-dimensional reversals also require the ability to inhibit responding.

Our results differ from those reported in two prior studies in rats that examined the effect of OFC lesions on performance in an odor-guided go, no-go discrimination task [14,15]. In these reports, the rats received training on several discrimination problems before aspiration lesions of OFC were made. The resulting impairment and perseverative responding that was observed may have reflected differences in lesion method (unlike the neurotoxic lesions used in the present experiment, aspiration lesions cause damage to fibers of passage). The discrimination deficits

observed in prior experiments may also have been due to the use of pre-operative training on the task. In particular, pre-operative training may have encouraged the use of a learning or performance strategy dependent on an intact OFC (i.e. goal directed responding) at the expense of other options (e.g. S-R or habit learning). Having learned to solve discrimination problems using an OFC-dependent strategy, impairments may have been evident in previous experiments because rats were no longer able to access this strategy post-lesion. In contrast, rats in the present experiment, which never had the opportunity to learn using an OFC-dependent strategy, were able to perform normally through the use of alternative strategies. Consistent with this hypothesis, Diaz *et al.* [3] directly compared the effects of pre- and post-training lesions of OFC on discrimination learning, and found a trend toward impairment in animals given training before lesions were made that was not apparent in animals given lesions prior to training [3].

Classic effects of OFC damage on response inhibition may reflect a more fundamental deficit: Our results suggest that in rats, as in primates, the classic effects of OFC lesions on response inhibition are consequences of a more basic underlying deficit. This deficit may be in the integration of information regarding the outcome of behavior with other forms of processing to guide goal-directed responses (responses guided by a representation of the goal object or outcome) [9,12,17,18]. Such a function has been demonstrated for OFC [9,12] and for areas of amygdala with which it is connected [12,16]. Within this system, OFC may be particularly important for integrating afferent input regarding incentive value with ongoing processing to guide responding, in effect constructing neural representations that link affective processing to action [9,10,17,18]. The loss of this function after OFC lesions would be evident as perseveration in a go, no-go task if the training paradigm were biased towards a requirement for goal-directed behavior or if the task were sufficiently difficult to reveal the added contributions of these associations to performance. As we and others have noted, this may be the case in reversal learning [3,4,6,7] or when ambiguous or conditional cues are used [6,19].

In accordance with the hypothesis that OFC is important for using incentive information to guide behavior, neurons in OFC acquire responses to cues in both simple and complex discrimination tasks that reflect associations between cue features (such as identity or match/non-match comparisons) and outcomes [10,20], and this firing activity appears to reflect the relative value of the associated outcomes [21]. Although such encoding is apparently not critical for inhibiting responses during initial learning, representations of outcome present in OFC during odor sampling after reversal could allow intact rats in the present study to quickly modify, rather than unlearn, existing responses tendencies. OFC-lesioned rats lacking these representations would be required to unlearn associations (such as those between cues and responses) supporting the original discrimination and replace them with new associative structures. In support of this hypothesis, reversals of previously-learned discrimination problems result in modulation, rather than replacement, of existing patterns of

neural activity in OFC [17,22]. New populations of neurons become selective for cues after reversal [17], while increases in functional connectivity during initial learning that are evident in correlated firing between simultaneously recorded cells are preserved [22].

It is important to note that the spared discrimination performance in OFC-lesioned rats in Phase I does not argue against this interpretation. Animals with damage to OFC show no impairments in other forms of associative (including discrimination) learning [9,12], despite their impairments in goal-directed behavior. Intact learning in these subjects is presumably based on associative structures that do not involve goal representations, such as stimulus-response (habit) learning [13]. Although these associations would depend on the outcome for their formation, in that the outcome might serve to reinforce, or 'stamp in' the associations, the outcome is not represented as part of the associative structure supporting the behavior [23]. As a result, one might say that apparently normal behavior is based on abnormal representations. The abnormal representations are only evident in situations such as devaluation [9,12] or reversal [3–7], which reveal the disconnection between representations of outcome and the associative structures underlying performance in OFC-lesioned subjects.

Recovery of function after OFC lesions indicates parallel processing in other structures: Despite their initial impairment on reversals, performance of OFC-lesioned rats improved with practice. This improvement may have been aided by the between-session design of the reversal paradigm (reversals took place in sessions separate from acquisition), which may have allowed the rats to acquire reversals by simply treating each day as a new discrimination problem. By comparison, controls seemed to have some difficulty with serial reversals, possibly suffering interference from earlier reversals not observed in OFC-lesioned subjects. This interpretation is supported by the improved performance of controls on the novel reversal problem. It is also consistent with neurophysiological data, which indicate that traces of the original learning, evident in correlated firing between simultaneously recorded cells, persist in OFC after reversal [22]. These trace representations may interfere with new encoding across multiple reversals in controls.

Finally, it is notable that the improvement in OFC-lesioned rats generalized to reversal of a novel odor pair. In other words, the lesioned rats did not develop a specific strategy for solving reversals of a single odor pair, but rather developed a more general rule for solving reversals that did not depend on OFC. This finding is consistent with speculation that prefrontal functions are subsumed by other structures with practice [24] and, in fact, suggests that these systems operate in parallel with OFC to some extent, since the practice effect developed in OFC-lesioned rats. Interestingly, collateral damage to striatal areas media to posterior OFC was associated with an improvement across serial reversals that failed to generalize to a new odor discrimination problem. This failure to develop more generalized rules may relate to interactions between the striatum and prefrontal cortex, and suggests that the striatum may be involved in consolidating response strategies in a way that

is only loosely bound to particular cues. In support of this hypothesis, a recent report indicates that depletion of striatal dopamine leads to response patterns that are closely bound to the currently relevant stimulus features [25]. Such results could reflect a role for the striatum in abstracting rules after extended experience.

CONCLUSION

Here we have examined the contribution of rat OFC to discrimination and reversal learning in an attempt to reconcile older evidence that rat OFC is required for response inhibition and new data indicating that rat OFC is important for integrating stimulus outcome associations with behavior. We found that OFC lesions had no effect on the ability of rats to inhibit strong prepotent response tendencies, despite the use of a go, no-go paradigm in which the requirement for response inhibition was maximal. Instead, the inability to inhibit responses was evident in OFC-lesioned rats when they were required to react to changes in the relationships between the cues and the predicted outcomes during reversals. These results are substantially similar to those reported in primates after OFC lesions on comparable tasks, consistent with a more fundamental role for OFC across species in integrating stimulus-outcome associations to guide goal-directed behavior.

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