

The Role of the Orbitofrontal Cortex and Medial Striatum in the Regulation of Prepotent Responses to Food Rewards

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An impairment in learning to inhibit prepotent responses to positive stimuli is associated with damage to the orbitofrontal cortex (OFC) in rats, monkeys, and humans performing discrimination reversal, extinction, and detour reaching tasks. In contrast, a recent study showed that OFC-lesioned rhesus monkeys could learn to select the smaller of 2 quantities of food reward in order to receive the larger reward, at an equivalent rate to controls, despite the requirement to inhibit a prepotent response. Given this result, the aim of the present study was to further specify the contexts under which the OFC regulates responding and to identify additional components of limbic circuitry that contribute to such regulation. Marmosets with lesions of the OFC and medial striatum (MS), but not the amygdala, made more prepotent responses to a clear Perspex box containing high incentive food before learning to choose the box containing low incentive food, to obtain reward. However, having learned the incongruent incentive discrimination OFC- and MS-lesioned monkeys were impaired upon reversal of the reward contingencies, repeatedly selecting the previously rewarded low incentive object. These findings identify the critical contribution of the OFC and MS in the regulation of responding by affective cues.

Keywords: affective inhibition, amygdala

Introduction

Damage to the orbitofrontal cortex (OFC) can lead to the persistent expression of a previously rewarded response despite that response no longer resulting in reward. This has often been described as perseverative responding and has been shown to occur in a variety of behavioral settings in which animals are responding either to stimuli in the environment that are associated with reward (Izquierdo et al. 2005; Jones and Mishkin 1972) or to the rewards themselves. In particular, marmosets and rats with excitotoxic lesions of the OFC display perseverative responding to the previously rewarded visual stimulus following reversal of the reward contingencies in a visual discrimination task (Dias et al. 1997; Chudasama and Robbins 2003). In addition, marmosets display perseverative reaching along their direct line of sight in a task, in which successful retrieval of a food reward from within a Perspex box requires a “detour reach” around the sides of the box (Wallis et al. 2001). The common element in these 2 examples is the existence of a prepotent response tendency to perform a response that is no longer appropriate. The impairment does not extend to a context in which there is a prepotent bias at the level of rules or higher-order attentional selection (Dias et al. 1996) and thus is specific to conditions of response control at the level of concrete stimuli.

A recent study, however, has shown that even at the level of concrete stimuli, in certain contexts the existence of a pre-

potent response tendency can be overcome by monkeys with OFC lesions, as easily as it is in controls (Chudasama et al. 2007). In this case, rhesus monkeys had to select the smaller of 2 food quantities (i.e., one half peanut rather than 4 half peanuts) in order to receive the larger quantity (i.e., 4 half peanuts) in the reversed reward contingency (RRC) task. A variety of explanations were proposed to explain why the behavior of OFC-lesioned monkeys was intact on the RRC task in contrast to discrimination reversal and detour reaching. First, that the OFC was more important in learning the changing associations between nonfood objects and their current biological value (object discrimination reversal) rather than comparable decisions based on food quantities (RRC). Second, that the OFC was more important for substituting a different response to reach the same high incentive food (detour reaching) compared with making an alternative response to a smaller quantity and thus lower incentive food (RRC).

The present study was designed to address these issues and at the same time to reduce some of the complexity inherent in the RRC task that had made the task very difficult, even for controls to learn. First, instead of monkeys choosing between different quantities, and thus different incentive values, of the same food (RRC), marmosets in the present study were required to choose between 2 different foods of varying incentive value (i.e., highly valued marshmallows versus far less valued, lab chow). Second, the reward which marmosets received for selecting the food of lower incentive value (i.e., lab chow) was not, as in the RRC task, the same as the food that they were required to avoid at the selection stage (i.e., marshmallow). Instead, by avoiding high incentive marshmallow and selecting low incentive lab chow, marmosets received high incentive syrup bread. Finally, the reward the marmosets received was in the same spatial location as their response, rather than, as in the RRC task, in the location that the monkeys had just avoided in the selection stage. Thus, 1) as in the RRC task, marmosets were required to inhibit a prepotent tendency to select the preferred of 2 foods, rather than, as in the discrimination reversal task, the preferred of 2 nonfood objects and 2) as in the RRC task, marmosets had to reach for a nonpreferred food rather than, as in the object retrieval task, substitute a new response to the preferred food.

In addition to examining the effects of OFC lesions on performance on this task, the effects of lesioning the medial striatum (MS) and amygdala were also investigated. These subcortical structures are intimately connected to the OFC (Price et al. 1996; Ferry et al. 2000; Ghashghaei and Barbas 2002) and may form part of the neural circuit through which the OFC controls behavioral flexibility. The MS, which includes the medial caudate nucleus and the nucleus accumbens, is the region of

striatum that receives projections from the orbitofrontal cortex in the marmoset (Roberts et al. 2007). It was predicted that lesions to this region would produce similar deficits in inhibiting responding to the preferred of the 2 food objects as was predicted for the OFC lesions. This would be consistent with the finding that these same groups of marmosets, in a separate study, had been shown to display perseverative responding to the previously rewarded pattern on a visual discrimination task following reversal of the reward contingencies (Clarke et al. 2006). In contrast, it was predicted that lesions of the amygdala may actually improve performance, as without the amygdala the tendency to approach high incentive food objects would be reduced, making it easier for the animals to select the less preferred food object in order to receive reward.

Materials and Methods

Subjects

Thirteen common marmosets (*Callithrix jacchus*), were used in the present study (7 males, 6 females). Animals were housed in male/female pairs under temperature controlled and humidity conditions on a 12-h light/dark cycle. Mean age at the start of testing was 24 months. Daily weekday behavioral testing occurred between 0900 and 1230. Monkeys were fed 20 g of MPE1 primate diet food pellets (Special Diet Services, Essex, UK) and 2 pieces of carrot at 1530 h. This diet was supplemented at the weekends with additional fruit, eggs, bread, marmoset jelly (Special Diet Services), and peanuts. Water was available ad libitum.

Prior to entering the present study, all animals had received training on a serial visual discrimination reversal learning task presented on a computer-based touch screen apparatus with liquid reward and had also all received sham control, or excitotoxic lesions of the OFC, amygdala or striatum as part of another study (Clarke et al. 2006). The training and test histories of all animals were identical. All procedures were conducted in accordance with the project and personal licenses held by the authors under the UK Animals (Scientific Procedures) Act of 1986.

Surgery

All marmosets were anaesthetized with a combination of an injection of 0.05 mL of ketamine sulfate (100 mg/mL solution; Pharmacia and Upjohn, Crawley, UK) i.m. followed by 0.4 mL of saffan (alfaxalone 0.9% w/v, alphadolone acetate 0.3% w/v; Schering-Plough, Welwyn Garden City, UK) i.m. and maintained with supplementary doses of 0.4 mL of saffan for the duration of surgery. A prophylactic analgesic (Rimadyl, 0.03 mL 50 mg/mL; Pfizer, Kent, UK) was also administered prior to surgery. Monkeys were held in a stereotaxic frame with specially adapted incisor and zigoma bars. Due to the inherent individual variability in brain size, infusion co-ordinates were tailored-made for each animal, using a standardization technique described in Dias et al. (1997) which involved measuring the depth of the frontal pole of individual marmoset's brains to determine whether adjustments to the standard lesion coordinates were necessary. Excitotoxic lesions were made using 0.09 M solution of Quinolinic acid (Sigma-Aldrich, UK) in 0.01 M phosphate buffer, pH 7.0. Volumes administered varied in accordance to co-ordinates and brain region lesioned (see Table 1). For all placements, infusions were made over 100 s through a stainless steel cannula (30 gauge) attached to a 2- μ L precision Hamilton sampling syringe (Precision Sampling Co., Baton Rouge, LA). The cannula then remained in place for 4 min before being withdrawn slowly.

Following surgery, all animals were administered 5 mL of glucose in saline solution (0.9% saline, 1% sucrose) i.p., followed by Valium (Roche, Hertfordshire, UK) in the range of 0.05-0.25 mL i.m. as necessary over the first 24 h to suppress any epileptic seizure activity. The analgesic Metacam (meloxicam, 0.1 mL of a 1.5 mg/mL oral suspension; Boehringer Ingelheim, Germany) was given 24-h post-operatively for further pain relief. Animals were returned to their home cage and had *ad libitum* access to water and supplementary diet during a recovery period of at least 12 days.

Apparatus

All testing took place in a hand-operated Wisconsin General Test Apparatus (WGTA, Fig. 1A) in a darkened, sound attenuated room. Animals sat in a Perspex carry box (dimensions 160 mm \times 240 mm \times 18 mm) which was aligned with the door of the WGTA, where they could see into the test compartment (dimensions 320 mm \times 520 mm \times 500 mm) lit by 2 8W/35A strip lights. Through the bars of the carry box, spaced 25 mm apart, animals could reach toward a test tray containing 2 food

Table 1

Lesion parameters including the toxin concentration, stereotaxic coordinates of each injection (based on the interaural plane) and the injection volume

| Lesion area | Quinolinic acid concentration | Coordinates (mm) | | | Volume injected (μ L) | | |
|-------------|-------------------------------|------------------|-----------|------------------|----------------------------|------|------|
| | | AP | LM | V | | | |
| OFC | 0.09 M | 16.75 | ± 2.5 | 0.7 ^a | 0.50 | | |
| | | | ± 4.8 | 0.7 ^a | 0.50 | | |
| | | 17.75 | ± 2.0 | 0.7 ^a | 0.50 | | |
| | | | ± 4.8 | 0.7 ^a | 0.50 | | |
| | | 18.5 | ± 2.0 | 0.7 ^a | 0.60 | | |
| | | | ± 4.0 | 0.7 ^a | 0.50 | | |
| MS | 0.1 M | 13.8 | ± 2.3 | 9.3 | 0.25 | | |
| | | | | | 10.6 | 0.20 | |
| | | 12.5 | ± 2.3 | 9.3 | 0.20 | | |
| | | | | | 10.6 | 0.25 | |
| | | 11.5 | ± 2.3 | 11.5 | 0.20 | | |
| | | | | | 12.5 | 0.20 | |
| | | Amygdala | 0.12 M | 9.3 | ± 5.6 | 4 | 0.35 |
| | | | | | | | 5 |

^a0.7 mm above base of brain.

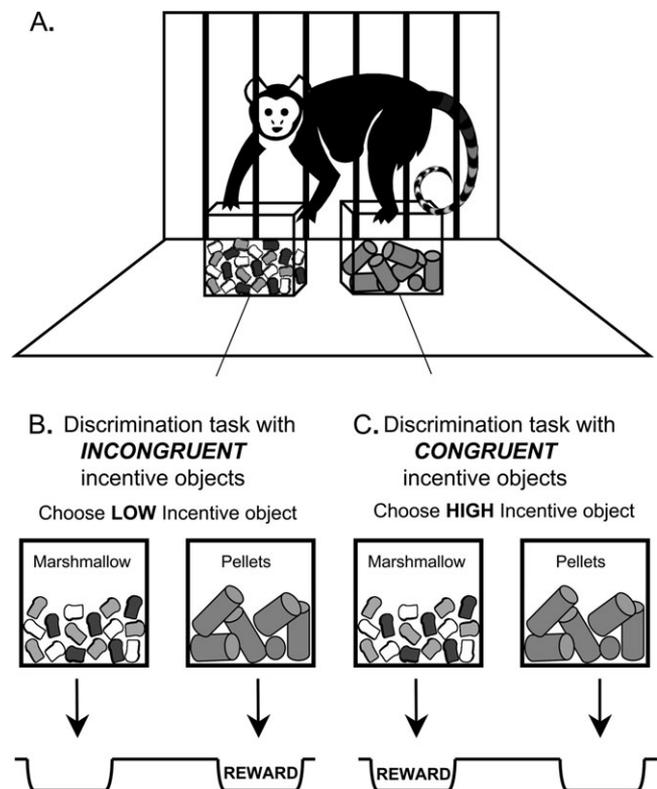


Figure 1. A schematic diagram of the apparatus (A) and the incongruent (B) and congruent (C) versions of the discrimination task. In the incongruent version (B), selecting the high incentive foodbox was not rewarded whilst selecting the low incentive foodbox was rewarded with syrup bread. Once this discrimination had been learnt, the contingent relationship between the high and low incentive foodboxes and the syrup bread reward was reversed (C).

wells (20 mm diameter and 6 mm deep, spaced 115 mm apart, and 10 mm from the edge closest to the animal). The experimenter could view the test compartment through a one-way mirror and control the door and objects using pulleys and string. Therefore, the animals could not see, and their behavior could not be biased by, the experimenter.

Preliminary Training

Animals were first trained to perform simple 2-choice object discriminations. Upon raising an opaque screen, the trial commenced and subjects were presented with 2, 3-dimensional objects of differing color and shape, placed directly over the prebaited food wells. An animal had to touch an object to choose it, whereby the object was retracted by the experimenter pulling on an attached piece of white string. This revealed the underlying food well, which if the choice was correct contained a reward (0.5-cm cube of whole meal bread soaked in glucose B.P. solution; William Ranson & Son Plc, UK, "syrup bread"). Lowering the screen terminated the trial. The rewarded object was pseudorandomly positioned over the left or right food wells. Subjects received up to 30 trials in a daily session during training. Criterion for all tasks was set at 90% correct responses within a 30 trial session (27/30). After completing 2 pairs of object discriminations, subjects progressed on to a discrimination task involving incongruent incentive objects.

Discrimination Task with Incongruent Incentive Objects

This task was identical to the object discrimination paradigm with the exception that the choice objects were now replaced by clear Perspex boxes of identical size (5 cm × 5 cm × 5 cm). One was half-filled with multicolored marshmallows (high incentive object) whilst the other was half-filled with dry food pellets (lab chow) of the type that they normally receive as daily feed (low incentive object). The food types were clearly visible within the boxes, but the animals did not have access to them. Animals were required to inhibit their prepotent response of reaching toward the high incentive food object and instead choose the low incentive food object in order to receive a food reward (Fig. 1B). Thus, the incentive values of the food objects were incongruent with the reward contingencies. A correct choice led to displacement of the object revealing a piece of syrup bread reward in the underlying food well, whereas an incorrect choice received no reward. The response (correct or error) was recorded. High and low incentive objects were allocated pseudorandomly to the left and right food wells and balanced across each block of 10 trials. If a subject displayed a significant side bias of 6 consecutive responses to one side, a correction procedure was implemented until the animal had made 3 correct responses on the non-preferred side.

Discrimination Task with Congruent Incentive Objects

On attaining criterion for the incongruent incentive reward contingency task, the following day, the contingencies were reversed so that the high incentive (marshmallow) foodbox now led to reward whereas the low incentive (pellets) foodbox was unrewarded, that is, the incentive values of the food objects were now congruent to the reward contingencies of the task (Fig. 1C).

Behavioral Assessment

Overall learning was measured by the total number of errors made before reaching criterion of 90% correct across thirty trials. In addition, signal detection theory (Macmillan and Creelman 1991) was used to classify the type of errors into "perseverative" (responding to the incorrect prepotent stimulus significantly above chance) and "non-perseverative" (responding to the correct stimulus at or above chance) for each block of 10 trials. For details of calculations, see (Clarke et al. 2004). In addition, the number of errors the animal made before making 2 correct responses was also determined. This is a measure of the ability to first initiate a response away from the high incentive stimulus and thus inhibit the prepotent response. It is a similar but not so stringent a measure to that used by Wallis et al. (2001) and Walker et al. (2006) in the detour reaching task, because the 2 correct responses in the present study, unlike in the previous studies, were not required to be consecutive. We chose 2 correct responses as opposed to a single correct response to be sure that the monkey really had

directed their attention away from the high incentive stimulus and had not touched the low incentive stimulus unintentionally. However, we did not require the 2 correct responses to be consecutive, as we had previously, because it seemed an unnecessarily stringent requirement. It should be noted that analyses of errors to 2 correct responses showed comparable patterns of statistical significance in the present study regardless of whether the responses were consecutive or not.

Lesion Assessment

All animals were euthanased with Euthatal (1 mL of pentobarbital sodium ph. Eur. 200 mg/mL solution i.p.; Merial Animal Health, Ltd, Essex, UK) and perfused transcardially with 500 mL of 0.1 M phosphate-buffered saline (pH 7.4) followed by 500 mL of 0.4% formaldehyde buffered solution over 10 min. The brain was removed and placed overnight in fixative before being transferred to a 30% sucrose solution (in 0.01 M phosphate-buffered saline) for at least 48 h. Coronal brain sections (60 µm) were cut on a freezing microtome and stained with Cresyl Fast Violet to assess the location and extent of the lesion. Brain sections were examined for presence of major neuronal loss and gliosis using a Leitz DMRD microscope. Areas of cell loss for each animal were represented on drawings of standard marmoset coronal sections and composite diagrams were produced to illustrate the degree of overlap between lesions.

Statistical Analysis

Behavioral data were analyzed using SPSS statistical package (SPSS version 12.0, Chicago, IL). Both the number of errors to 2 correct responses and the total errors to criterion were compared across the 4 groups using a one-way ANOVA. Significant effects of group ($P \leq 0.05$) were further investigated using Tukey tests for comparisons between lesion and control groups. Error types were analyzed using an ANOVA model of group (4; OFC, MS, amygdala, and control) by error type (2; perseverative and nonperseverative). Post hoc analyses of simple main and interaction effects involved ANOVA and independent samples *t*-tests. Where variances deviated significantly (as tested by Levene's test of homogeneity) data were square-root-transformed prior to ANOVA.

Results

Lesion Assessment

Orbitofrontal Cortex

Damage to the OFC (Fig. 2A) included dysgranular and agranular regions, extending from the posterior edge of the frontal pole to the genu of the corpus callosum. The highly granular cortex in the lateral convexity and frontal pole was spared. Variable cell loss occurred in the anterior portion of the left ventromedial convexity and one animal showed some additional damage in the anterior dorsal granular prefrontal cortex.

Medial Striatum

All animals showed neuronal loss encompassing the medial head of the caudate nucleus and nucleus accumbens (Fig. 2B). The lesion extended into the lateral head of the caudate in one animal, whilst the body of the caudate and putamen were largely spared in all animals. Additional damage to the subgenual cortex, posterior nuclei medialis, and dorsal septi as well as minor damage to the dorsal anterior cingulate occurred in one animal.

Amygdala

Two of the 3 animals revealed lesions which included the lateral, accessory, central and lateral basal nucleus components of the amygdala (Fig. 2C). The remaining animal showed damage to more medial basal components, medial and cortical nuclei, and medially along the accessory basal nucleus. One monkey sustained minor damage to the underlying entorhinal cortex.

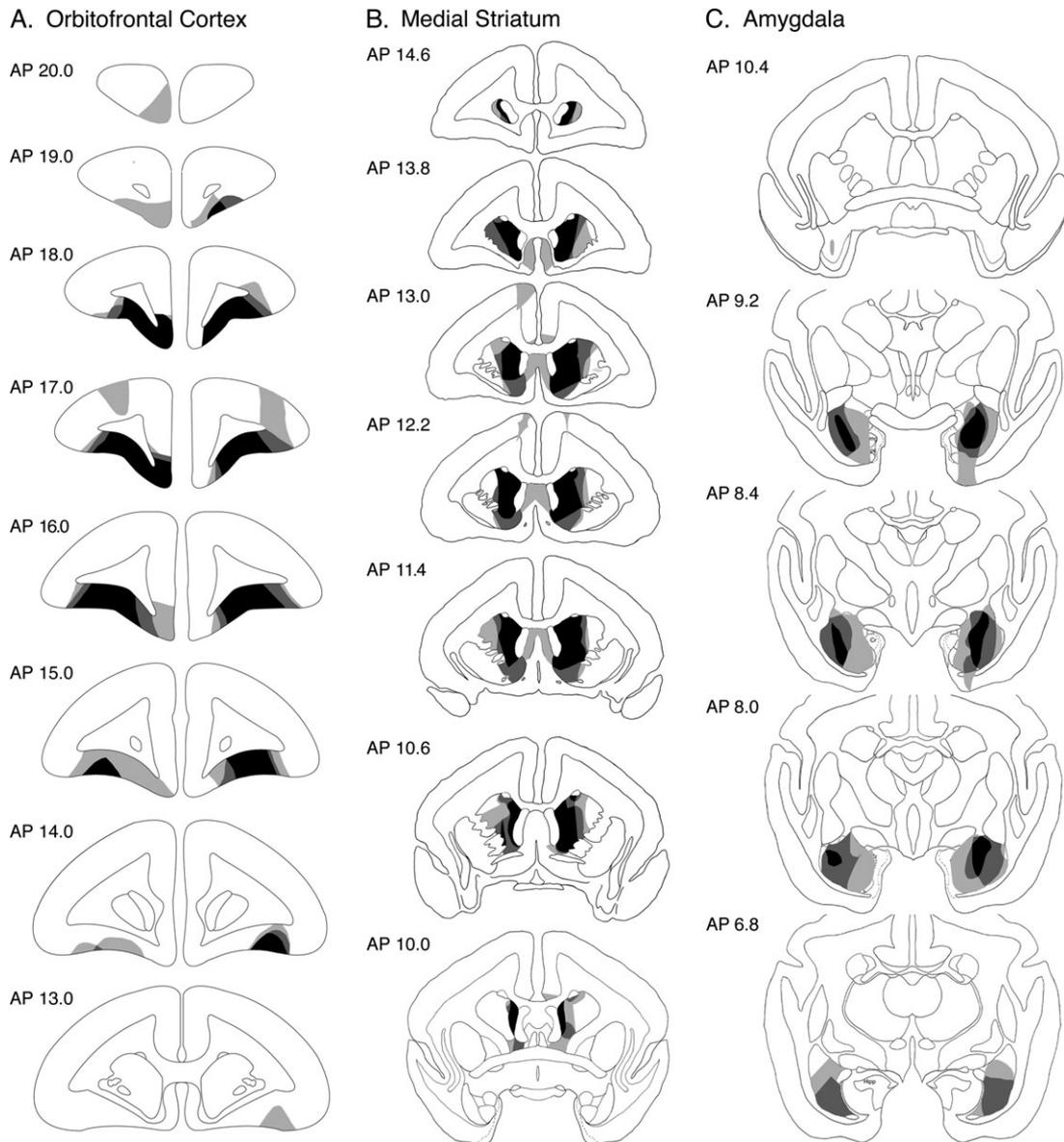


Figure 2. Schematic representations showing the extent of neuronal loss in all animals in the OFC, MS, and amygdala-lesioned groups. A series of coronal sections through the frontal lobes (A), anterior striatum (B), and anterior temporal pole (C) illustrating the extent of the lesions in the OFC, MS and amygdala, respectively. The different levels of shading (black to pale gray) indicate regions that were lesioned in all 3 monkeys, any 2 monkeys and just one monkey, respectively.

Preliminary Training Task

All animals successfully learnt 2 simple object discriminations and there were no differences in the performance of the groups. One-way ANOVA showed no effect of group on total errors to criterion or numbers of perseverative or non-perseverative errors for either discrimination (all $F < 1$). Therefore OFC, MS and amygdala lesions do not affect subsequent learning of 2-choice object discriminations.

Discrimination Task with Incongruent Incentive Objects

All animals showed an initial prepotent response tendency to select the high incentive (marshmallow) food object (Fig. 3A), making at least 3 errors before performing 2 correct responses to the low incentive food. However, although control and amygdala-lesioned monkeys very quickly learned to inhibit this prepotent tendency, this was not the case for OFC- and

MS-lesioned animals. One-way ANOVA revealed significant group differences in the number of errors to 2 correct responses ($F_{3,12} = 74.63, P < 0.001$). Post hoc analyses of multiple comparisons using Tukey's test found that the OFC-lesioned group made significantly more errors compared with controls, amygdala-lesioned and MS-lesioned groups (all $P > 0.001$). In addition, MS-lesioned animals made significantly more errors compared with controls and amygdala-lesioned animals (both $P = 0.001$), whereas amygdala-lesioned animals were no different from controls ($P > 0.1$).

Despite significant differences in the initial number of errors to 2 correct responses, there were no significant group differences in the total errors to reach 90% criterion within thirty trials ($F < 1, P > 0.05$). However, examination of error types across the entire discrimination, using signal detection analysis, revealed that overall, OFC-lesioned and MS-lesioned

animals made more perseverative errors (responding to the incorrect high incentive food object, i.e., marshmallow, significantly above chance across blocks of 10 trials) than either amygdala-lesioned and sham-operated control animals (Fig. 3B). ANOVA of square-root-transformed errors revealed significant effects of error type ($F_{1,9} = 15.7, P = 0.003$) and a group \times error type interaction ($F_{3,9} = 14.3, P = 0.001$). Analysis of the simple main effects showed a significant effect of group for perseverative ($F_{3,12} = 14.5, P = 0.001$), but not non-perseverative errors ($F_{3,9} = 2.45, P = 0.13$). Post hoc analyses using Tukey's test revealed that OFC-lesioned animals made significantly more perseverative errors compared with controls and the amygdala-lesioned group (both $P = 0.002$). MS-lesioned animals showed a similar pattern of perseverative errors to that of OFC-lesioned animals making significantly more errors than

amygdala-lesioned animals ($P = 0.038$) and just missing significance when compared with controls ($P = 0.052$). The number of perseverative errors made did not differ between OFC- and MS-lesioned groups, and amygdala-lesioned animals did not differ from controls.

In summary, OFC- and MS-lesioned subjects showed greater difficulty inhibiting the prepotent tendency to respond to the high incentive food object compared with control and amygdala-lesioned groups. This is illustrated graphically in Figure 4 which shows performance across blocks of 10 trials for each of the lesioned groups compared with controls using the d' value as calculated by signal detection theory. Data points that fall below the lower dotted line (gray shading) denote blocks of trials that were significantly perseverative ($P < 0.05$; performing significantly worse than chance). It can

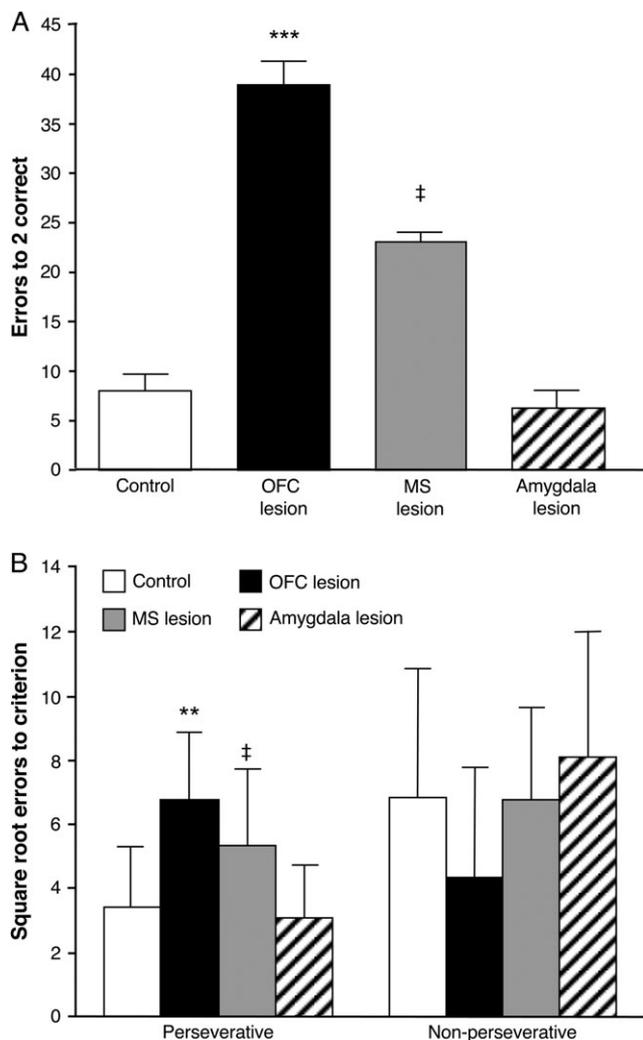


Figure 3. Mean number of errors (\pm SEM) in the incongruent incentive reward contingency task for control, OFC-lesioned, MS-lesioned, and amygdala-lesioned animals. (A) Mean number of errors to 2 correct responses. *** $P < 0.001$, OFC-lesioned animals made significantly more errors compared with animals with MS lesions, amygdala lesions and controls and ‡ $P = 0.001$, MS-lesioned animals made significantly more errors compared with amygdala-lesioned animals and controls. (B) Mean number of perseverative and nonperseverative errors made by each group before reaching criterion (square root total errors). ** $P < 0.01$, OFC-lesioned animals made significantly more perseverative errors compared with control and amygdala-lesioned animals and ‡ $P = 0.05$, MS-lesioned animals made significantly more perseverative errors than amygdala-lesioned animals.

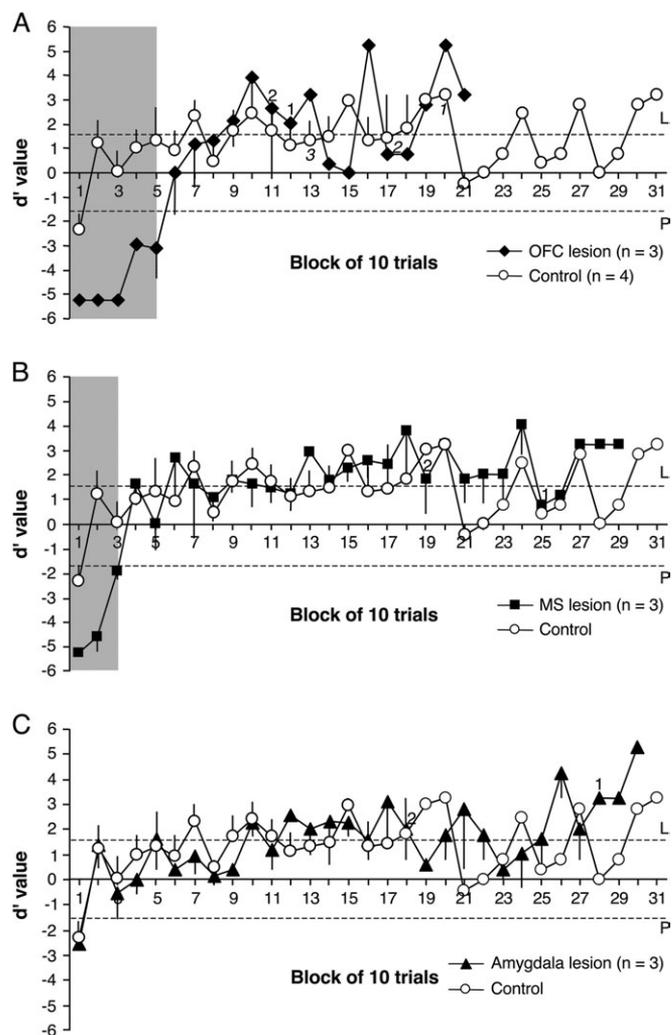


Figure 4. Learning curves for OFC-lesioned animals (A), MS-lesioned animals (B), and amygdala-lesioned animals (C) compared with controls (same control data plotted on each graph). " d' value" calculated using signal detection theory on blocks of 10 trials and averaged within groups per block of trials. Italicized numbers adjacent to data points represent the number of animals contributing to the mean d' value. Points within the upper and lower dotted lines reflect chance performance. Points below or above the dotted lines represent perseveration (P) or learning (L) stages, respectively. Gray shading denotes blocks of trials that were significantly perseverative in the lesioned groups ($P = 0.05$; performing significantly worse than chance).

be seen that initially OFC- and MS-lesioned animals were more perseverative and took longer to inhibit their prepotent tendency compared with controls, whilst the amygdala-lesioned group were no different from controls.

Discrimination Task with Congruent Incentive Objects

Reversal of the reward contingencies resulted in the high incentive food object becoming congruent with the reward contingencies. All animals showed at least one incorrect response (Fig. 5A) at the start of the reversal, but consistent with performance in the previous discrimination, control and amygdala-lesioned animals made fewer errors before making 2 correct responses compared with OFC- and MS-lesioned animals. One-way ANOVA on the “mean square root errors to 2 correct responses” revealed a significant group effect ($F_{3,12} = 4.86, P = 0.028$) which was due specifically to OFC- and MS-lesioned animals making more initial errors compared with controls ($t = 2.9, P = 0.034; t = 3.05, P = 0.028$, respectively).

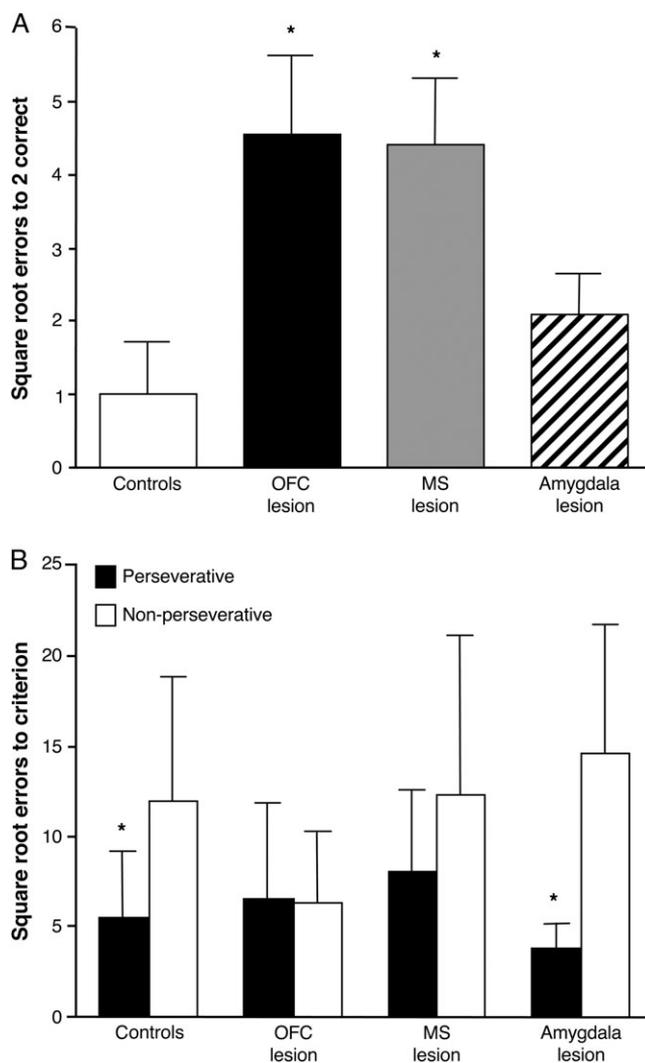


Figure 5. Mean number of errors (square root + SEM) during the congruent incentive reward contingency task. (A) Mean number of errors to 2 correct responses. $*P < 0.05$, OFC- and MS-lesioned animals made significantly more errors than controls. (B) Mean number of perseverative and nonperseverative errors to reach criterion. $*P < 0.05$, significant difference between perseverative and nonperseverative errors in controls and amygdala-lesioned animals.

Amygdala-lesioned subjects were no different from controls in this initial measure.

Separation of error type according to signal detection theory across the entire reversal revealed an error type ($F_{1,9} = 49.95, P < 0.001$) and group \times error type interaction ($F_{3,9} = 10.15, P = 0.003$). Figure 5B illustrates that the interaction effect is due to the controls ($P = 0.012$) and amygdala-lesioned groups ($P = 0.047$) making significantly more nonperseverative errors compared with perseverative errors. This difference in error types was not apparent in OFC- and MS-lesioned animals.

Discussion

Marmosets with lesions of the OFC or MS, but not the amygdala, made more prepotent responses to a high incentive food object (marshmallow) before learning to choose an incongruent, low incentive food object (lab chow) in order to gain access to food reward (syrup bread). However, when the reward contingencies were reversed and the high incentive food object became rewarding, OFC- and MS-lesioned marmosets continued to respond to the previously rewarded low incentive object for more trials than controls or amygdala-lesioned marmosets. Together, these results further specify the contexts under which the OFC is involved in behavioral flexibility, and in addition identify the critical contribution of the MS, but not the amygdala, in a neural network involved in such behavioral flexibility.

The current findings rule out 2 potential explanations for why OFC lesions were found to be without effect on the RRC task (Chudasama et al. 2007). First, that the OFC was more important in learning the changing associations between nonfood objects and their current biological value (object reversal) rather than comparable decisions based on food objects (RRC task). Here, it is shown that the OFC is equally important in learning comparable decisions based on food objects, for example, marshmallow and lab chow. Second, that the OFC was more important for substituting a different response to reach the same food (detour reaching) rather than selecting a smaller quantity of food (RRC task). Although the animals did not have to select a smaller quantity of visible food in the present study they did have to select a less preferred food, which, in both cases involves judgments based on the incentive value of the food. Thus, lesions of the OFC disrupt the animals ability to select an alternative nonfood or food object, regardless of whether the animal is required to inhibit responding to a nonfood object that has previously been associated with reward, or is inhibiting a prepotent bias toward a preferred food, neither of which involved a detour reach. Moreover, having overcome a prepotent bias and learned to select the less preferred food, OFC-lesioned animals are equally impaired at reversing their behavior and learning to select the preferred food. These findings demonstrate the important role of the OFC in enabling animals to continually update their behavior in line with changing reward contingencies.

It remains then to focus on the outstanding differences between the RRC task and the incongruent food reward task used in the present study to identify why the latter, but not the former is sensitive to OFC lesions. Sham-operated marmosets achieved successful performance on the incongruent food reward task within an average of 170 trials whilst unoperated rhesus monkeys required on average over 1000 trials to learn the RRC task (Murray et al. 2005). The increased difficulty experienced by the rhesus monkeys on the RRC task may be

due to 3 different aspects of the task design which may act independently or interactively to slow learning. First, both choices were rewarded, albeit one greater than the other, which may have helped to maintain the incorrect response (Silberberg and Fujita 1995). Second, obtaining reward at a location physically separate from the selected object would delay learning to select the smaller reward (Kralik 2005). Third, the presence of the larger food quantity as both a stimulus that should be avoided, and as the reward for making the correct choice, would also delay learning because memory of the preferred outcome would match the very stimulus that should be avoided when making a selection. This would make it more likely that the animal would attend to, and approach the remembered reward stimulus next time it was presented. We would argue that the psychological processes that allow these 3 sources of interference to be overcome are not orbitofrontal dependent and thus loss of orbitofrontal function would not be a rate-limiting factor in learning the RRC task. Indeed, OFC-lesioned animals may have actually been advantaged over controls in one respect when performing the RRC task. Neural activity in the OFC has been shown to represent expected outcomes (Schoenbaum et al. 1999; Hikosaka and Watanabe 2000) and OFC lesions disrupt performance dependent on outcome knowledge (Gallagher et al. 1999; Izquierdo and Murray 2005). Thus, memory of the outcome should only have acted as a source of interference, contributing to the bias to select the larger quantity of food, in controls, but not OFC-lesioned animals. In support of this, the biased responding to the larger food quantity present in the early stages of learning the RRC task appeared less in OFC-lesioned animals than controls (see Figs 2 and 3 in Chudasama et al. 2007), although such a difference was not significant, possibly due to a lack of statistical power.

These 3 sources of interference were not present in the current incongruent discrimination thereby enabling control subjects to learn the task relatively easily, and, we hypothesize, unmasking the important role played by the OFC in response control at the level of concrete stimuli based on the incentive value of both food and nonfood objects. Alternative and related explanations for the differences between the 2 studies are 1) species differences and 2) differences in lesion location. Neither of these seem very likely though because 1) there is considerable evidence for comparability between the effects of OFC lesions in marmosets, rhesus and human studies (Dias et al. 1996; Fellows and Farah 2003; Izquierdo et al. 2005) and 2) the cytoarchitecture and connections of the region of marmoset OFC targeted in the present study are relatively consistent with that region of the OFC targeted in the rhesus monkey (Carmichael and Price 1994; Roberts et al. 2007), although the marmoset OFC does not have so many distinct cytoarchitectonic divisions as the rhesus monkey.

The finding that amygdala-lesioned animals perform equivalently to controls on both the incongruent food reward task and its reversal, is consistent with the finding that these same amygdala-lesioned monkeys were unimpaired on the reversal of a visual discrimination task (Clarke et al. 2006). It is also consistent with previous findings that amygdala lesions in rhesus monkeys do not affect object reversal learning (Izquierdo and Murray 2007). Because the amygdala is implicated in Pavlovian, appetitive, conditioned responding (Hatfield et al. 1996) it might have been predicted that without an intact amygdala, animals would have had less of a prepotent

tendency to reach for the high incentive food object and thus would have performed better than controls. However, the initial response bias to the high incentive food displayed by amygdala-lesioned monkeys in the present study was no different to controls (see Fig. 4C). Moreover, whilst post-training amygdala lesions in marmosets disrupt conditioned increases in blood pressure to the sight of high incentive food reward, they do not alter the time spent looking and responding toward the food itself (Braesicke et al. 2005). Thus, a neural circuit, independent of the amygdala supports such food-elicited approach behavior.

Such a circuit is clearly modulated by the MS because lesions of the MS, like those of the OFC caused behavioral inflexibility on both the incongruent incentive discrimination task and its reversal. Like OFC-lesioned monkeys, MS-lesioned monkeys made significantly more prepotent responses to the high incentive food reward on the incongruent reward task, and also made more responses to the low incentive food object upon reversal, when responses to the high incentive food object became rewarded. A similar pattern of perseverative responding on the detour reaching task has been reported in baboons with selective neuronal degeneration in the caudate and putamen following chronic treatment with 3-Nitropropionic acid (Palfi et al. 1996). Although in the present study the lesion included the medial caudate and the nucleus accumbens (the striatal territory to which the OFC projects, Roberts et al. 2007) the more likely cause of the observed deficits is damage to the medial caudate. Thus, an early study of electrolytic lesions of the ventrolateral head of the caudate nucleus in macaques reported perseverative responding on an object discrimination reversal task (Divac et al. 1967); although the use of electrolytic lesions raised the possibility that damage to fibers of passage, rather than the caudate itself, contributed to the deficit. In addition, a number of more recent studies have shown that lesions restricted to the nucleus accumbens do not disrupt, selectively, the reversal of a sensory discrimination (Annett et al. 1989; Schoenbaum and Setlow 2003; but see Stern and Passingham 1995). In contrast, lesions of the dorsomedial striatum in rats do disrupt, selectively, both place and egocentric reversal (Ragozzino et al. 2002); although, unlike the present study, the deficits are not perseverative in nature. Finally, enhanced blood flow has been reported in the region of the medial caudate following reversal of a visual pattern discrimination in humans (Rogers et al. 2000).

In summary, the present experiments clearly demonstrate that OFC lesions disrupt the ability to inhibit a prepotent response tendency regardless of 1) whether the response is elicited by a nonfood object (visual discrimination reversal) or by food reward itself (incongruent incentive discrimination task; present study) or 2) whether the alternative response is to reach for a nonpreferred food (present study) or to acquire a new response to the preferred food (detour reaching task). The finding of a similar perseverative deficit following MS lesions highlights the critical importance of the striatum, as well as the OFC, in behavioral flexibility at the level of concrete stimuli, consistent with recent findings in humans (Cools et al. 2006). Whether the OFC and MS interact together within the same network to control such responding remains to be determined. Of relevance to this issue are recent findings showing that simultaneous lesions of the amygdala and OFC can abolish the discrimination reversal deficits in rats associated with OFC lesions alone (Stalnaker et al. 2007). If

these effects are corroborated in primates then it may suggest that the MS and OFC are components of separate, but overlapping circuits, making it important for future studies to determine the contexts under which these circuits interact.

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