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Interactions Between the Prefrontal Cortex and Amygdala During Delay Discounting and Reversal

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Abstract

Interactions between the prefrontal cortex and amygdala are thought to be critical for reward anticipation. Alterations in reward anticipation that lead to an inability to wait for rewards or a diminished capacity to change behavior when doing so would be optimal is often termed impulsivity and compulsivity, respectively. Distinct regions of the prefrontal cortex may support decreased impulsivity through self-control and decreased compulsivity through flexibility. However, both self-control and flexibility appear to involve the amygdala. Using a delay discounting paradigm, the present investigation found that inactivation and disconnection of the medial prefrontal cortex and basolateral amygdala led rats to become more impulsive by affecting preference for smaller immediate over larger delayed rewards. Conversely, inactivation and disconnection of the orbitofrontal cortex and amygdala led rats to become more compulsive as demonstrated by an inability to flexibly reverse stimulus reward relationships in an odor reversal task. The present findings support a double dissociation between orbitofrontal cortex - amygdala interactions for odor reversal and medial prefrontal cortex - amygdala interactions for delay discounting.

Keywords

Prefrontal; Amygdala; Decision; Reversal; Discounting

Introduction

Understanding the neurobiological basis of decision-making may give insight into a variety of pathological disorders observed in humans (Bechara, 2005; Belin, Mar, Dalley, Robbins, & Everitt, 2008; Cardinal, 2006; Cavedini, Gorini, & Bellodi, 2006; Everitt, et al., 2008; Robinson, et al., 2009; Schoenbaum, Roesch, & Stalnaker, 2006; Winstanley, Eagle, & Robbins, 2006). These disorders may be particularly influenced by alterations in reward anticipation that lead to an inability to wait for rewards or alter behavior when doing so would be optimal. Disorders marked by impaired decision-making, therefore, often involve impulsive and compulsive choice.

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Decision-making tasks informed by economic theory as well as standard behavioral tasks have been used to examine neural systems underlying decisions involving reward anticipation in humans, non-human primates, and rodents (Floresco & Ghods-Sharifi, 2007; Hahn, et al., 2009; Kable & Glimcher, 2007; Matsumoto, Suzuki, & Tanaka, 2003). Further, a number of studies across species suggest an important role for the prefrontal cortex (PFC) and basolateral amygdala (BLA) in decision processes (Baxter, Parker, Lindner, Izquierdo, & Murray, 2000; Cohen, Elger, & Weber, 2008; Floresco & Ghods-Sharifi, 2007; Murray & Izquierdo, 2007). The PFC is reciprocally connected to BLA and it has been argued that the PFC and BLA interact under changing stimulus, action, and reward contingencies (Floresco & Ghods-Sharifi, 2007; Holland & Gallagher, 2004; Murray & Izquierdo, 2007; Saddoris, Gallagher, & Schoenbaum, 2005; Schoenbaum, Setlow, Nugent, Saddoris, & Gallagher, 2003; Schoenbaum, Setlow, Saddoris, & Gallagher, 2003). It has also been suggested that distinct, but overlapping, circuits may mediate impulsivity and compulsivity (Chudasama, et al., 2003; Torregrossa, Quinn, & Taylor, 2008) and that medial PFC (mPFC) and orbitofrontal cortex (OFC) may differentially interact with the amygdala under different decision-making conditions (Murray & Izquierdo, 2007). Within the context of impulsive and compulsive choice, PFC regions may be dissociable, whereas the BLA is a common element to both types of decision processes (Chudasama, et al., 2003; Floresco, Zhang, & Enomoto, 2008; Schoenbaum, Setlow, Nugent, et al., 2003; Winstanley, Theobald, Cardinal, & Robbins, 2004).

The following experiments were designed to evaluate the hypothesis that two different regions of PFC, namely mPFC and OFC, interact with BLA through differential involvement in decision-making processes that can lead to impulsive and compulsive choice.

Impulsivity, while complex, may be defined as an inability to wait when doing so would be optimal (Evenden, 1999; Monterosso & Ainslie, 1999). Delay discounting involves choosing a small immediate reward over a larger delayed reward and is one way in which choice impulsivity has been operationalized (Cardinal, 2006). In studies using rodents, lesions of mPFC or BLA have been shown to increase impulsive choice or alter behavior during delay discounting and tests of motor impulsivity such as premature responding in reaction time tasks (Cardinal, Pennicott, Sugathapala, Robbins, & Everitt, 2001; Chudasama, et al., 2003; Mobini, et al., 2002; Narayanan, Horst, & Laubach, 2006; Winstanley, Theobald, et al., 2004). While investigations of the BLA in delay discounting have been limited, it has been suggested that disruption of BLA function may produce alterations in the incentive value of anticipated rewards, which may contribute to increased impulsive choice (Ghods-Sharifi, St Onge, & Floresco, 2009; Winstanley, Theobald, et al., 2004). There is also evidence that lesions comprising OFC or both mPFC and OFC can increase, decrease, or have no effect on impulsivity during delay discounting (Kheramin, et al., 2002; Mariano, et al., 2009; Rudebeck, Walton, Smyth, Bannerman, & Rushworth, 2006; Winstanley, Theobald, et al., 2004).

Compulsivity, the inability to change behavior under changing environmental, action, and reward outcomes, may be reflected in perseverative behavior observed in reversal tasks (Clarke, Robbins, & Roberts, 2008; Everitt & Robbins, 2005; Torregrossa, et al., 2008). Studies in rodents and non-human primates have shown that lesions of OFC or BLA impair performance of such reversals, yet inactivation of mPFC has no effect (Jones & Mishkin, 1972; Ragozzino, 2007; Schoenbaum, Setlow, Nugent, et al., 2003). Odor reversal is recognized as goal-directed behavior and neural recording studies have suggested that both OFC and BLA are involved in and interact during odor reversal through encoding of stimulus outcome relationships necessary for reward anticipation (Holland & Gallagher, 2004; Saddoris, et al., 2005; Schoenbaum, Setlow, Saddoris, et al., 2003).

Taken together, studies of delay discounting and reversal suggest a double dissociation of interactions between mPFC-BLA and OFC-BLA. The following experiments used temporary

pharmacological inactivation to evaluate (1) whether OFC and mPFC interact with BLA, (2) whether OFC and mPFC can be dissociated in their interactions with BLA based on decision-making contingencies, and (3) whether failures in each type of decision-making process associated with different contingencies may be a window into impulsive and compulsive choice.

The present results suggest a double dissociation of interactions in which each region of PFC differentially contributes to and interacts with BLA under different task conditions. The mPFC may act in concert with the BLA to mediate decisions based on temporal processing and incentive value during delay discounting. However, the OFC appears to be unnecessary for delay discounting in the present delayed reward task. Conversely, the OFC may be crucial for flexibility during reversal with OFC and BLA functioning conjointly to encode and maintain new contingences necessary for reward anticipation, whereas the mPFC appears unessential.

Methods

Subjects

All planned procedures and animal care were in accordance with the National Institute of Health and Institute for Animal Care and Use Committee guidelines and the Institutional Animal Care and Use Committee at the University of Utah. Seventy male Long Evans rats weighing 250-350 g were housed in individual plastic containers and kept on a 12/12 light/dark cycle. Their weight was maintained at 80-90% of free feed weight with water available ad libitum.

Surgery

Prior to surgery, subjects were deeply anesthetized using isoflurane gas, placed in a sterotaxic apparatus with a continuous flow of isoflurane, and prepared for the surgical procedure by applying a surgical drape and betadine antiseptic to the surgical site. An incision was made in the skin above the skull. The skin was retracted and burr holes were drilled in the skull to receive stainless-steel anchor screws and provide access to the regions intended for cannulation. Cannulas were inserted at the following coordinates: OFC: 3.0 mm anterior to bregma, +/_ 3.2 mm lateral from midline, 4.2 mm ventral from dura; mPFC: 25° from midline, 3.0 mm anterior to bregma, +/_ 2.0 mm lateral from midline, 4.6 mm ventral from dura; BLA: 3.1 mm posterior to bregma, +/_ 5.0 mm lateral from midline, 7.6 mm ventral from dura. Cranioplastic cement was applied around cannulas and screws to chronically anchor the cannulas in place. In experiment 1, subjects were implanted with cannulas bilaterally in both OFC and BLA (OFC-BLA group) or mPFC and BLA (mPFC-BLA group), which allowed for all possible inactivation patterns within a single subject (bilateral, contralateral, ipsilateral). In experiment 2, subjects were implanted with cannulas in either OFC, BLA, mPFC, or both OFC and BLA unilaterally in contralateral hemispheres or ipsilaterally.

Histology

After testing, subjects were euthanized with a lethal dose of sodium pentobarbital. Transcardial infusion of 0.9% saline was followed by infusion of 10% formaldehyde. Brains were refrigerated for 48 hrs at 4°C in a 10% sucrose and 30% formalin solution. Coronal sections of frozen brains were obtained using a cryostat and stained with cresyl violet to verify cannula placement (Fig. 1). Cannulas targeting the mPFC were found across the medial orbital, infralimbic, and prelimbic regions, but did not extend dorsally into cingulate cortex or laterally into ventrolateral or lateral orbitofrontal cortex. Cannulas targeting the mPFC were specifically placed at a 25° angle in order to decrease the likelihood that the more lateral regions of OFC would be affected. Cannulas targeting the orbitofrontal cortex extended laterally into the agranular insular cortex, dorsally to the border of motor cortex, and ventrally into lateral orbital and ventral orbital cortex. Cannulas targeting the BLA were observed across the basolateral

complex, bordering the central amygdala, and across the anterior-posterior axis of BLA. Additionally, cannulas targeting BLA were found at the most dorsal extent near the border of the caudate and at the most ventral extent near the border of the piriform cortex.

In addition to cannula placement, diffusion of muscimol is also likely a significant factor in the present investigation. Allen and colleagues (2008) introduced fluorophore-conjugated muscimol into the rat mPFC and BLA to determine the spread of muscimol and showed that a 0.5 μL infusion into BLA or mPFC had an approximate diffusion area of 0.5-1.0 mm. However, it was pointed out that the molecular weight of the fluorophore-conjugated muscimol is greater than muscimol proper and that the increase in weight may have restricted diffusion of the drug. In the present investigation we used a smaller volume (0.2 μL). Given the small volume injected, it is likely that the muscimol infusions were well restricted to the targeted regions. However, given the broad range of cannula placements, it is certainly possible that muscimol spread to unintended areas. For example, Allen and colleagues showed that muscimol tended to diffuse upward along the cannula in mPFC animals. It is possible that muscmiol may have diffused into more dorsal regions in our subjects as well. Moreover, given the proximity of BLA to the central amygdala and piriform cortex, it is also quite possible that these regions were affected by diffusion of muscimol. Lastly, it is possible that muscimol diffused into motor cortex from cannulas targeting OFC.

Experiment 1

Behavioral Apparatus

A T-maze was used for training in the delay discounting paradigm. The T-maze was fabricated from wood, painted black and elevated 91.4 cm from the floor. The arms were 15 cm high, 115 cm long and 15 cm wide. A 3 cm in diameter and 1.5 cm deep food well was located at the end of each arm.

Behavioral Training

Subjects learned to choose between two rewarded locations by entering the central alley of the T-maze. In the west arm of the T-maze a low value reward (LVR) was available in the food well (~ 0.25 Froot Loop cereal; Kellogg, Battle Creek, MI) and in the east arm a high value reward (HVR) was available in the food well (~ 1.25 Froot Loop Cereal). Once subjects learned to discriminate between the two arms, seeking the HVR, a gate was introduced in front of the arm with the HVR at the junction of the T-maze. Gates were remotely controlled from a room outside of the testing room. Subjects were shaped to wait at the gate in front of the arm with the HVR. Delays were increased in increments of 2 s until the subjects were willing to wait for 15 s, starting with a 1 s time delay. The LVR was always freely available (Fig. 2a). A 30 s intertrial interval was used. Subjects also received a forced choice trial prior to training each day. During the forced choice, subjects were not allowed to choose so that they were given the opportunity to visit each arm to be reminded of the different reward magnitudes. Prior to surgery, each subject received one session per day consisting of 10 trials. Once subjects selected the delayed HVR for 10 trials within one session, they received bilateral surgical cannulation of both mPFC and BLA (mPFC-BLA group) or both OFC and BLA (OFC-BLA group). The simultaneous bilateral implantation of mPFC and BLA or OFC and BLA allowed for the possibility of bilateral, contralateral, and ipsilateral inactivation patterns within subjects. After receiving surgery and recovering for at least 1 week, each subject was retrained. Once subjects reached a criterion of ≥ 80% selection of the HVR arm on a single day, on the following day they received an infusion consisting of 0.2 μL phosphate buffered saline in each hemisphere as a vehicle control. Once a criterion of ≥ 80% selection of the delayed HVR arm for two consecutive days was obtained, subjects received bilateral infusions of 0.2 μL muscimol, a $GABA_A$ agonist capable of temporarily and reversibly inactivating the targeted brain regions

(Allen, et al., 2008). Subjects began testing 10-15 min after receiving infusions of muscimol. Subjects in each group (OFC-BLA and mPFC-BLA) received counterbalanced bilateral inactivation of OFC or BLA and mPFC or BLA. Since there was an apparent effect of bilateral mPFC and BLA inactivation, counterbalanced ipsilateral and contralateral inactivation of structure pairs also was carried out in the mPFC-BLA group. After administration of the initial inactivation, each subject was retrained to a criterion of ≥ 80% selection of the HVR arm for two consecutive days prior to each subsequent inactivation pattern. Thus, as with the bilateral condition, after meeting the criterion of ≥ 80% selection of the HVR arm on a single day, on the following day subjects received an infusion consisting of 0.2 μL phosphate buffered saline in each hemisphere as a vehicle control. Once a criterion of ≥ 80% selection of the delayed HVR arm for two consecutive days was obtained, subjects received contralateral or ipsilateral infusions of 0.2 μL muscimol into mPFC and BLA. A final experiment was executed to evaluate whether bilateral mPFC or BLA inactivation impaired subjects' capacity to discriminate reward magnitudes (Fig. 2b).

In the reward magnitude discrimination task, subjects from the mPFC-BLA group were tested in the same manner as the delay task except both doors were gated for 15 s. At the end of the 15 s delay both gates were opened simultaneously and the subjects were allowed to choose. Subjects received either bilateral infusions of saline or muscimol into mPFC or BLA prior to testing.

Injection Procedure

Initially, subjects were submitted to a habituation procedure in which obdurators were tightened and loosened prior to training each day. Once the subjects reached ≥ 80% selection of the HVR on a single day, on the following day they received an infusion consisting of 0.2 μL phosphate buffered saline in each hemisphere as a vehicle control. Infusions were made with a Hamilton 10.0 μL syringe (Hamilton, Reno, NV) controlled by a microinfusion pump (Cole-Parmer, Vernon Hills, IL) at a rate of 0.1 μL/min. Once subjects obtained ≥ 80% selection of the HVR for two consecutive days they received infusions of 0.2 μL (1.0 μg/1.0 μL) muscimol into each hemisphere (Sigma Aldrich, USA) at a rate of 0.1 μL/min. Behavioral testing began 10-15 min after muscimol infusions were administered.

Statistical Analysis

All data were analyzed using SPSS 17 software for Macintosh. A two factor repeated measure design was used to examine choice and time spent waiting as a function of treatment (2 levels: saline; muscimol) and pattern of inactivation (2 levels: bilateral OFC; bilateral BLA) in experiment 1A and treatment (2 levels: saline; muscimol) and pattern of inactivation (4 levels: bilateral mPFC; bilateral BLA; contralateral mPFC-BLA; ipsilateral mPFC-BLA) in experiment 1B, and treatment (2 levels: saline; muscimol) and pattern of inactivation (2 levels: bilateral mPFC; bilateral BLA) in experiment 1C. Post hoc tests used the Bonferroni adjustment for comparisons of interest.

Results

Experiment 1

Rats chose between a small immediate reward and larger delayed reward in two different arms of a T-maze (Fig. 2a). Subjects were initially trained to wait for 15 s at a gate in order to obtain the HVR for 10 out of 10 trials within a session. After pretraining, subjects received surgical bilateral cannulation of both OFC and BLA (OFC-BLA; $n = 6$) or both mPFC and BLA (mPFC-BLA; $n = 7$). Therefore, there were two groups with the potential to have any pattern of inactivation executed among structure pairs (bilateral, contralateral, and ipsilateral). After recovering from surgery, subjects were retrained until they reached a criterion of ≥ 80%

selection of the HVR on a single day. The following day subjects received vehicle control infusions of phosphate buffered saline prior to testing. Initially, bilateral inactivation of OFC or BLA (OFC-BLA group) and mPFC or BLA (mPFC-BLA group) was performed.

Participation of at least two structures constitutes the sufficient condition to test for interactions (Floresco & Ghods-Sharifi, 2007). Accordingly, after subsequently meeting the criterion for two consecutive days, the mPFC-BLA group also received contralateral disconnection inactivation or ipsilateral control inactivation of mPFC and BLA on separate occasions. Two dependent measures were acquired during testing: frequency of choosing the delayed HVR arm and time spent waiting before choice.

In experiment 1A for the OFC-BLA group, inactivation of BLA, but not OFC, decreased selection of the HVR arm and decreased time spent waiting for the HVR, suggesting that inactivation of BLA increases impulsivity (Fig. 3a,b). The results for arm choice revealed a significant main effect for treatment $F(1, 5)= 89.28$, $p = 0.001$, pattern of inactivation $F(1, 5) = 30.55$, p = 0.003, and treatment × pattern of inactivation $F(1, 5) = 15.16$, $p = 0.011$. The results for time spent waiting showed a significant main effect for treatment $F(1, 5) = 104.24$, $p = 0.001$, pattern of inactivation $F(1, 5) = 26.12$, $p = 0.004$, and treatment × pattern of inactivation $F(1, 5) = 30.87$, $p = 0.003$. Post hoc tests demonstrated significant effects between bilateral BLA saline and bilateral BLA muscimol conditions for choice of the HVR arm, with subjects in the muscimol condition selecting the HVR arm less often $t(5) = 7.17$, $p = 0.005$ and waiting less time $t(5) = 7.41$, $p = 0.001$ than subjects in saline control conditions. However, bilateral OFC saline and muscimol treatments were not significantly different for either measure $t(5) = 1.22$, $p > 0.1$ and $t(5) = 1.11$, $p > 0.1$.

In experiment 1B for the mPFC-BLA group, bilateral inactivation of mPFC, BLA, and disconnection of mPFC and BLA led subjects to decrease selection of the HVR arm (Fig 4a). Bilateral inactivation of mPFC or BLA also led subjects to spend less time waiting for the HVR while disconnection of mPFC and BLA did not significantly decrease time spent waiting when compared to the ipsilateral inactivation condition (Fig. 4b). The results for arm choice revealed a significant main effect for treatment $F(1, 6) = 103.46$, $p = 0.001$, pattern of inactivation $F(3, 18) = 4.14$, $p = 0.02$, and treatment × pattern of inactivation $F(3, 18) = 7.61$, $p = 0.015$. Post hoc tests showed significant effects for choice of the HVR arm between bilateral mPFC saline and muscimol conditions $t(6) = 7.98$, $p = 0.001$, bilateral BLA saline and BLA muscimol conditions $t(6) = 3.76$, $p = 0.009$, contralateral mPFC-BLA muscimol and ipsilateral mPFC-BLA muscimol conditions $t(6) = -4.22$, $p = 0.006$. The results for time spent waiting showed a significant main effect for treatment $F(1, 6) = 74.38$, $p = 0.001$, but not pattern of inactivation $F(3, 18) = 3.71$, $p > 0.1$ or treatment × pattern of inactivation $F(3, 18) = 5.40$, $p > 0.1$. Post hoc tests showed significant differences between the bilateral mPFC saline and muscimol conditions $t(6) = 5.98$, $p = 0.001$, bilateral BLA saline and BLA muscimol conditions $t(6) = 3.77$, $p = 0.009$, contralateral mPFC-BLA saline and muscimol conditions $t(6) = 7.69$, $p = 0.001$, with subjects in the muscimol conditions waiting less time. There was no significant effect for time spent waiting between the ipsilateral and contralateral inactivation conditions $t(6) = 3.16$, $p = 0.02$.

In experiment 1C, a reward discrimination task was used to examine whether impairments observed in the mPFC-BLA group resulted from an inability to discriminate between reward magnitudes. After testing in the delay discounting task, the maze was altered so that both arms had gates in front of them and after a 15 s delay subjects had unobstructed free choice of HVR or LVR arm. Choice of the HVR arm after the removal of both gates was the dependent measure. Bilateral inactivation of mPFC or BLA did not result in an inability to discriminate between reward magnitudes (Fig. 5). The results for arm choice revealed no significant main

effect for treatment $F(1, 6) = 0.63$, $p > 0.1$, pattern of inactivation $F(1, 6) = 2.4$, $p > 0.1$, or treatment × pattern of inactivation $F(1, 6) = 0.01$, $p > 0.1$.

Experiment 2

Behavioral Apparatus

Subjects were trained in a rectangular red Plexiglas box (86.4 cm long × 30.5 cm wide × 30.5 cm high) with two bowls filled with sand and Froot Loops buried in each bowl (3 cm diameter × 3 cm high). The floor of the box was made from wood and painted grey (Fig. 6).

Behavioral Training

For the reversal experiments, subjects were shaped in their home cages to dig in two bowls filled with sand and a Froot Loop placed on top. The Froot Loop was then submerged in the sand until no longer visible on the following four days. After the shaping procedure, subjects were trained in a rectangular red Plexiglas box with two bowls filled with sand and Froot Loops buried in each bowl. During testing, odor pairs used in discrimination and reversal were pseudorandomly selected and paired as rewarded and non-rewarded odors. Suprathreshold olfactory stimuli consisted of powdered odorants (cocoa, vanilla, mustard, garlic, cinnamon, clove, coffee, and ginger) mixed in sand and presented in clear plastic cups. During acquisition subjects were trained to discriminate between the rewarded and non-rewarded odor. The location of the rewarded odor was pseudorandomly switched between two adjacent locations (with no more than three repetitions for a single location). An intertrial interval of 15-20 s was used. Testing occurred in two phases: acquisition (day 1), reversal (day 2). Acquisition was terminated when subjects made 9/10 consecutive correct choices in a moving block of 10 trials. On the following day, subjects were trained in the same manner except that the rewarded odor from the previous day was unrewarded and the previously unrewarded odor was rewarded. Errors were divided into perseverative and regressive types (Kim & Ragozzino, 2005). Generally, errors were counted as selecting the previously rewarded odor from acquisition during reversal testing. In order to be counted as perseverative errors, subjects were required to select the unrewarded odor on three trials in a four trial block. After subjects made less than three errors in a four trial block, subsequent errors were counted as regressive errors.

One week prior to testing, each subject received surgical bilateral cannulation of the mPFC, BLA, or OFC. Since bilateral inactivation of OFC and BLA led to impairments, another experiment was also carried out using contralateral and ipsilateral cannulation of OFC and BLA. Thus, there were four separate experiments to investigate the role of each structure and their interactions: bilateral mPFC, bilateral OFC, and bilateral BLA. There were two treatments in the first three experiments involving bilateral mPFC, OFC, and BLA: (1) saline-acquisition and saline reversal; (2) saline-acquisition and muscimol-reversal. There was a single treatment in the fourth experiment to test for interactions between OFC and BLA: OFC-BLA ipsilateral and OFC-BLA contralateral: (1) saline-acquisition and muscimol-reversal.

Injection Procedure

Infusion concentration, volume, rate, and methods were the same as experiment 1. All infusions were made prior to behavioral training. Behavioral training began 5-10 min after infusions were administered.

Statistical Analysis

All data were analyzed using SPSS 17 software for Macintosh. Separate ANOVA tests were used to determine differences in trials required to reach criterion between groups during acquisition and reversal as well as for evaluating differences in perseverative and regressive error types. Saline controls OFC ($n = 7$), mPFC, ($n = 7$) and BLA ($n = 6$) were collapsed into

a single group (n = 20) and used in all comparisons since an ANOVA revealed no significant difference between saline groups for mean number of trials required to reach criterion during acquisition $F(2, 19) = 1.54$, $p > 0.1$ or reversal $F(2, 19) = 1.81$, $p > 0.1$. Post hoc tests used the Bonferroni adjustment for comparisons of interest.

Results

Experiment 2

Initially, bilateral muscimol inactivation was used to assess the role of OFC (n = 8), mPFC, (n = 6) and BLA (n = 8) during odor reversal in experiments 2A-2C. In experiment 2D, subjects received contralateral disconnection (n = 7) or ipsilateral (n = 8) inactivation of OFC and BLA. Trials required to reach criterion during acquisition and reversal were the dependent measures for the odor reversal task. Errors were divided into perseverative and regressive types (see methods).

In experiment 2A-C, subjects that received bilateral inactivation of OFC or BLA required more trials to reach criterion during reversal than saline controls, but bilateral inactivation of mPFC did not result in impairments (Fig. 7a). Inactivation of OFC led to more perseverative and regressive errors while inactivation of BLA led subjects to only commit more regressive errors (Fig. 7b). During reversal there was a significant difference between groups for trials required to reach criterion $F(3, 41) = 13.01$, $p = 0.001$. Post hoc tests showed that muscimol inactivation led to an increased number of trials to reach criterion for the bilateral OFC group $t(26) = -5.28$, $p = 0.001$ and BLA group $t(26) = -4.02$, $p = 0.001$, compared to saline controls. Perseverative and regressive error patterns during odor reversal were also analyzed. There was a significant main effect for perseverative errors $F(3, 41) = 4.76$, $p = 0.006$ and regressive errors $F(3, 41) = 7.98$, $p = 0.001$. Post hoc tests demonstrated that inactivation of OFC led to more perseverative $t(26) = -3.30$, $p = 0.003$ and regressive errors $t(26) = -3.92$, $p = 0.001$, compared to saline controls. Inactivation of BLA or mPFC did not result in more perseverative errors $t(26) = -1.65$, $p > 0.1$ and $t(24) = -2.79$, $p > 0.1$, respectively. Inactivation of BLA $t(26) = -3.18$, $p = 0.004$, but not mPFC $t(24) = 0.51$, $p > 0.1$, led to significantly more regressive errors than saline controls.

In experiment 2D, subjects that received disconnection inactivation of OFC and BLA required more trials to reach criterion and committed more regressive errors than subjects receiving ipsilateral inactivation (Fig. 8a). There were no significant differences between disconnection saline control infusions of OFC and BLA or OFC and BLA ipsilateral saline infusions during acquisition $F(1, 14) = 1.96$, $p > 0.1$. However, for odor reversal there was a significant main effect for inactivation disconnection of OFC and BLA compared to ipsilateral inactivation of OFC and BLA $F(1, 14) = 11.13$, $p = 0.005$. Subjects in the disconnection OFC-BLA inactivation group required more trials to reach criterion than subjects in the ipsilateral OFC-BLA inactivation group. Perseverative and regressive error patterns during odor reversal also were analyzed. There were no significant differences between groups for perseverative errors $F(1, 14) = 2.0$, $p > 0.1$. However, there was a significant effect between groups for regressive errors $F(1, 14) = 11.6$, $p = 0.005$. Subjects in the contralateral OFC-BLA inactivation group committed more regressive errors than subjects in the ipsilateral OFC-BLA inactivation group (Fig 8b).

Discussion

The present investigation suggests both dissociations and interactions between the OFC mPFC, and BLA in decision-making during delay discounting and odor reversal. For each paradigm, pharmacological inactivation and disconnection of the OFC, mPFC, and BLA was performed.

It was expected that there would be a double dissociation of interactions for mPFC-BLA in experiment 1 during delay discounting and OFC-BLA in experiment 2 during reversal.

Initially, the experiments assessed whether bilateral inactivation of OFC, mPFC, or BLA produced disruptions in performance of the delay discounting and reversal tasks. For each task, when impairments were observed after separate bilateral inactivation of two different regions, a disconnection approach was used to determine whether the structures interact. The disconnection approach has been used to detect whether interactions between structures are necessary for performance (Baxter, et al., 2000; Floresco & Ghods-Sharifi, 2007).

In experiment 1, bilateral inactivation of mPFC or BLA decreased selection of the HVR arm and decreased the amount of time subjects spent waiting to obtain the HVR. Contralateral inactivation of mPFC and BLA also decreased selection of the HVR arm. However, bilateral inactivation of mPFC or BLA did not disrupt subjects' ability to discriminate between reward magnitudes. These observations support the idea that the mPFC and BLA participate in and interact in impulsive choice, but not in reward magnitude discrimination.

It has been suggested that various tasks may measure different dimensions of impulsivity and these dimension may be supported by distinct or overlapping neural systems, a relationship that may be modulated by task demands (Chudasama, et al., 2003; Dalley, Cardinal, & Robbins, 2004; de Wit, 2009; Reynolds, Penfold, & Patak, 2008; Robinson, et al., 2009). Specifically, choice impulsivity and motor inhibition are thought to be two distinct aspects of impulsive behavior and these distinct domains are often investigated using delay discounting and reaction time tasks, respectively (Dalley, et al., 2004; Reynolds, et al., 2008; Robinson, et al., 2009; Winstanley, Dalley, Theobald, & Robbins, 2004). There is evidence that mPFC may be involved in premature responding in some reaction time tests and also may be involved in delay discounting (Cardinal, et al., 2001; Chudasama, et al., 2003; Mobini, et al., 2002; Narayanan, et al., 2006; Winstanley, Dalley, et al., 2004). The present investigation found that inactivation of mPFC increased impulsive choice, but whether this was due to decreased behavioral inhibition or disrupted decision processes is unclear because the presently used task deviated from typical delay discounting paradigms.

In typical delay discounting tasks, subjects are required to commit to a choice prior to the delay and the delay period used during testing may vary within or between sessions (Cardinal, Winstanley, Robbins, & Everitt, 2004; Mar & Robbins, 2007; Rudebeck, et al., 2006). However, in the present investigation, subjects were free to select the small immediate reward at any time during the delay and delays remained constant throughout testing, an approach more akin to studies of delayed gratification in children (Mischel, Ebbesen, & Zeiss, 1972). Reynolds and colleagues (2002) demonstrated that rats show similar discounting functions using a delay discounting adjusting procedure or delay of gratification procedure involving a continuous choice. However, it was also shown that the group tested using the delayed gratification procedure switched responses less than the delay discounting group. Based on these findings they suggest that sustained choice required for delayed gratification might require increased behavioral inhibition. This raises the possibility that the presently employed paradigm may be more dependent on response inhibition and that mPFC may be necessary for inhibiting premature responses in order to obtain larger delayed rewards based on value signals from BLA (Chudasama, et al., 2003; Floresco, St Onge, Ghods-Sharifi, & Winstanley, 2008).

However, the relationship between delay and incentive value is also clearly important for delay discounting, insofar as motivation to wait decreases as delay to reward increases (Monterosso & Ainslie, 1999). Bilateral inactivation or disconnection of mPFC and BLA resulted in an increase in the percentage of discounted trials as well as decreasing the time subjects spent waiting, which are the results that might be expected if the delay had been increased or HVR

size decreased. However, it is evident that subjects did know the objective value of the rewards based on the reward magnitude discrimination test, indicating intact reward sensitivity. Additionally, subjects were required to wait for 15 s prior to choice during reward discrimination, showing that inactivating these structures did not cause an enhanced temporal decay of reward magnitude representations. An alternative interpretation of the present finding is that time estimation and incentive value are crucial variables for understanding choice behavior of animals with bilateral or disconnection inactivation of mPFC and BLA in this task (Barratt, 1983; Dickinson & Balleine, 1994; Evenden, 1999; Ghods-Sharifi, et al., 2009; Wittmann & Paulus, 2008). Several studies have shown that damage to a wide swath of human prefrontal cortex can result in impairments in time estimation, increasing estimates of elapsed time (Berlin, Rolls, & Kischka, 2004; Koch, Oliveri, Carlesimo, & Caltagirone, 2002). Single-unit activity recorded from the pigeon analog of prefrontal cortex has also been related to time discounting (Kalenscher, et al., 2005). Additionally, it has been demonstrated that lesions of frontal cortex disrupt dopamine modulated increases and decreases of time estimation in the rat (Meck, 2006). Thus, inactivation of mPFC in the present study may have led to overestimation of elapsed time and therefore a decrease in incentive value, while inactivation of BLA led to disruptions of incentive value that contribute to normal time estimation during reward anticipation. Therefore, these structures may interact through time estimation and incentive value representation in order to guide action selection. However, the present investigation does not provide unambiguous evidence for the role of mPFC in temporal discrimination, leaving open the question of whether observed impulsivity was actually due to failed behavioral inhibition or impaired temporal discrimination.

The role of OFC in delay discounting is of further importance. In the present investigation, inactivating OFC did not increase impulsive choice. However, several different studies have shown that lesions of OFC can produce increased impulsivity, decreased impulsivity, or have no effect on delay discounting (Kheramin, et al., 2002; Mariano, et al., 2009; Rudebeck, et al., 2006; Winstanley, Theobald, et al., 2004). Because unique approaches were used in these studies, observed differences may have been due to lesion size, location, or whether lesions were made before or after training (Cardinal, et al., 2004; Roesch, Calu, Burke, & Schoenbaum, 2007). Within or between session shifts in delay, degree of task experience, and environmental cues may also be significant factors in determining how OFC lesions modulate delay discounting (Floresco, St Onge, et al., 2008; Mariano, et al., 2009; Roesch, Taylor, & Schoenbaum, 2006). Single-unit recordings from the rat OFC have been shown to correlate with delay discounting and it has been suggested that lesions of OFC may disrupt these signals and lead to decreased impulsivity (Roesch, et al., 2007; Roesch, et al., 2006). In the present investigation it may have been possible that inactivation of OFC would have led to decreased impulsivity, but because delays were held constant within and between sessions, we can only speculate about this possibility.

In experiment 2, we examined the total trials required to reach criterion and divided errors into perseverative or regressive types (Kim & Ragozzino, 2005). It has been suggested that OFC may be necessary for both behavioral and cognitive flexibility through inhibition of a previously relevant strategy or response and the development of new strategies (Ragozzino, 2007). Inactivation and lesions of OFC have been shown to induce perseverative errors during reversal, which may provide a window into compulsive choice (Clarke, et al., 2008; Jones & Mishkin, 1972; Kim & Ragozzino, 2005; Ragozzino, 2007; Schoenbaum, Setlow, Nugent, et al., 2003). Regressive errors, on the other hand, occur after a subject has received reinforcement for the correct response, but returns to the previously acquired strategy or behavior that was previously unreinforced. Thus, perseverative errors are thought to be associated with an inability to shift away from an old strategy or response and regressive errors are thought to be associated with an inability to maintain a new choice (Kim & Ragozzino, 2005). Neural dissociations based on perseverative and regressive error types have been observed for the

mPFC, OFC and medial striatum, supporting the validity of these dimensions (Ragozzino, 2007). Reversal errors have also been analyzed using the overall number of trials to reach criterion, separate stages of learning, and on a trial-by-trial basis (Clarke, et al., 2008; Izquierdo & Murray, 2007; Jones & Mishkin, 1972; Kazama & Bachevalier, 2009; Rudebeck & Murray, 2008; Schwartzbaum & Poulos, 1965).

How the amygdala is involved in reversal learning is largely unclear. There are studies that suggest amygdala damage impairs reversal performance, has no effect, or enhances reversal performance (Clarke, et al., 2008; Izquierdo & Murray, 2007; Jones & Mishkin, 1972; Kazama & Bachevalier, 2009; Rudebeck & Murray, 2008; Schoenbaum, Setlow, Nugent, et al., 2003; Schwartzbaum & Poulos, 1965). Studies using combined lesion and electrophysiology approaches indicate that the BLA normally contributes to reversal and interacts with the OFC. For example, Schoenbaum and colleagues used single-unit recordings paired with lesions to show that lesions of OFC disrupt normal cue selectivity during reversal in cells recorded from BLA, whereas lesions of BLA disrupt the formation of stimulus-outcome representations in cells recorded from OFC during reversal (Saddoris, et al., 2005; Schoenbaum, Setlow, Saddoris, et al., 2003). Failure to find an effect of amygdala lesions during reversal has been attributed to compensation by other supporting neural structures (Holland & Gallagher, 2004; Kazama & Bachevalier, 2009; Stalnaker, et al., 2007). Consistent with this notion, temporarily inactivating the amygdala would likely prevent such compensation and may help to explain the present findings.

It was expected that OFC and BLA contribute to and interact during the accumulation of total errors during reversal as measured by trials required to meet criterion, a general index of inflexibility. Groups that received bilateral inactivation or disconnection of OFC and BLA in the reversal task required more total trials to reach criterion. Moreover, inactivation of OFC led subjects to commit more perseverative and regressive errors. There was a significant effect of BLA inactivation on regressive errors, but not perseverative errors. Disconnection of OFC and BLA led to an increase in the frequency of regressive, but not perseverative errors, suggesting that OFC and BLA may possibly interact to encode contingency changes. The observation that OFC inactivation led to more perseverative and regressive errors is in contrast to previous reports (Kim & Ragozzino, 2005). One possible explanation for the present finding is that during acquisition, subjects simultaneously acquire a goal-directed action-outcome representation and stimulus-response habit (Everitt & Robbins, 2005; Yin & Knowlton, 2006). Thus, during contingency reversal, it may be the case that subjects with inactivation of OFC cannot flexibly respond to changing contingencies and default to a habit acquired due to overtraining the previous day (Jones & Mishkin, 1972; Torregrossa, et al., 2008). Furthermore, flexible encoding and maintenance of the new contingencies may require the interaction of OFC and BLA, as implied by the increase in regressive errors committed by subjects with bilateral or contralateral inactivation of OFC and BLA in the present study. In support of this idea, it has been shown that rats with lesions of BLA were only initially and mildly impaired in acquiring a new contingency during reversal, whereas rats with lesions of OFC showed a general impairment in acquiring reversals (Schoenbaum, Setlow, Nugent, et al., 2003). Thus, OFC may possibly act as an interface for switching between stimulus-response habits and goal-directed action, requiring interactions between OFC and BLA for new contingency encoding and maintenance. However, there has been intensive investigation of the neural substrates involved in goal-directed behavior and specific tasks have been developed to analyze the structure underlying such behavior (Balleine & Ostlund, 2007). It has been suggested that goal-directed actions are characterized by both sensitivity to alterations in outcome value and changes in contingency between an action and outcome (Balleine & Dickinson, 1998; Yin, Ostlund, & Balleine, 2008). Thus, evidence for precisely how the OFC and BLA interact in goal-directed behavior may require studies that further decompose the associative structure underlying performance.

Self-control and flexibility are hallmarks of adaptive decision-making (Ainslie, 2001). The ability to wait when doing so will yield larger rewards and the ability to change in the presence of dynamic stimulus, action, and outcome relationships is essential for survival and prosperity. Distinct as well as overlapping neural systems may be necessary for optimal decision-making under different conditions (Murray & Izquierdo, 2007; Torregrossa, et al., 2008). By examining delay discounting as well as reversal it was possible to show the contributions of multiple interacting circuits within the frontal-limbic system that are dissociable. Moreover, the present findings are suggestive of particular ways in which neural structures contribute to decision-making processes. The integration of temporal information with incentive value is evidently necessary for delay discounting and this function may be supported by mPFC and BLA interactions. Additionally, flexible responding and encoding of stimulus reward associations is necessary for reversal, which may be mediated by OFC and BLA interactions. Dysfunctions within these systems may contribute to disruptions in reward anticipation and impaired decision-making.

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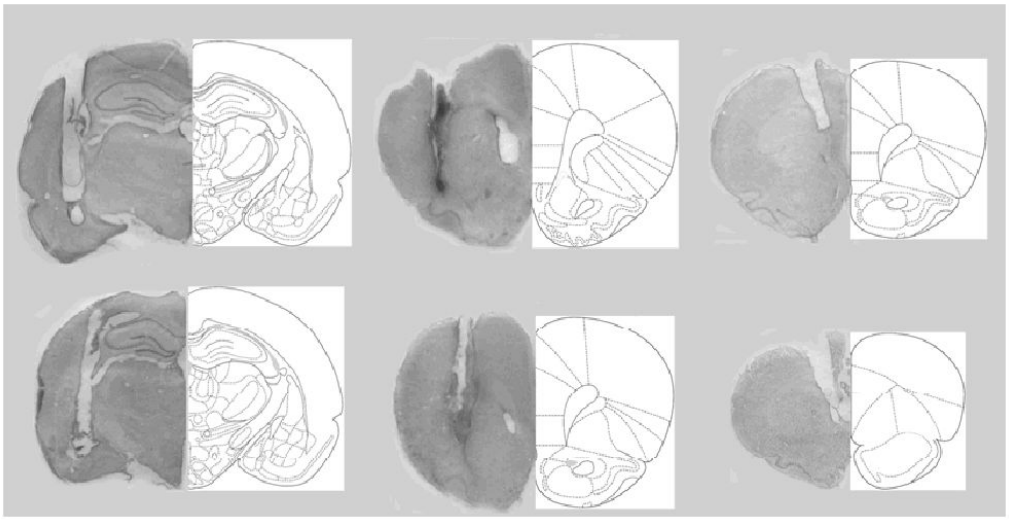

Figure 1.
Representative histological plates showing locations of cannula tips in BLA (Left), OFC (middle), and mPFC (right). Histological plates adapted from (Paxinos & Watson, 2005).

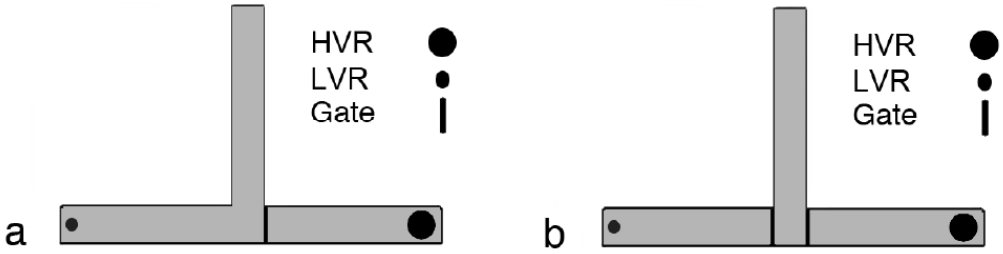

Figure 2.
Experimental apparatus used (a) for the delay discounting task in which subjects chose between two rewarded locations by entering the central alley of the T-maze. In one arm of the T-maze a low value food reward (LVR) was always freely available. In the opposite arm a high value food reward (HVR) was available. In order to obtain the HVR, subjects were required to wait in front of a gate for 15 s. (b) Subjects were also tested to evaluate whether they could readily discriminate between reward magnitudes. This was accomplished by allowing subjects to enter the central alley of the T-maze with both gates closed and waiting for 15 s to elapse prior to opening both gates simultaneously.

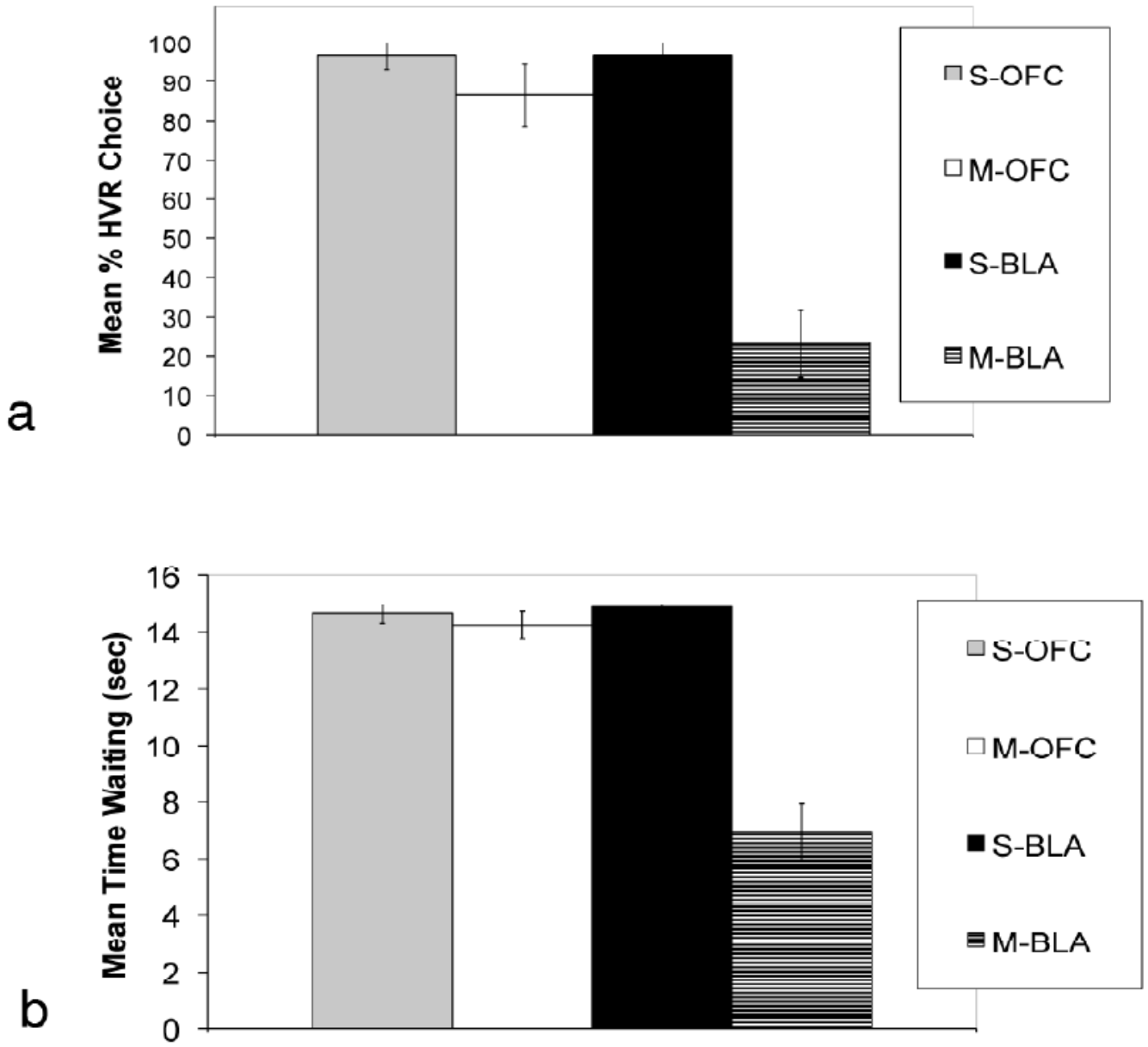

Figure 3.
Shows saline (S) and muscimol (M) conditions for (a) the mean percentage of selecting the delayed high value reward (HVR) arm for bilateral OFC and BLA and (b) the mean time spent waiting in seconds for the delayed HVR arm for bilateral OFC and BLA.

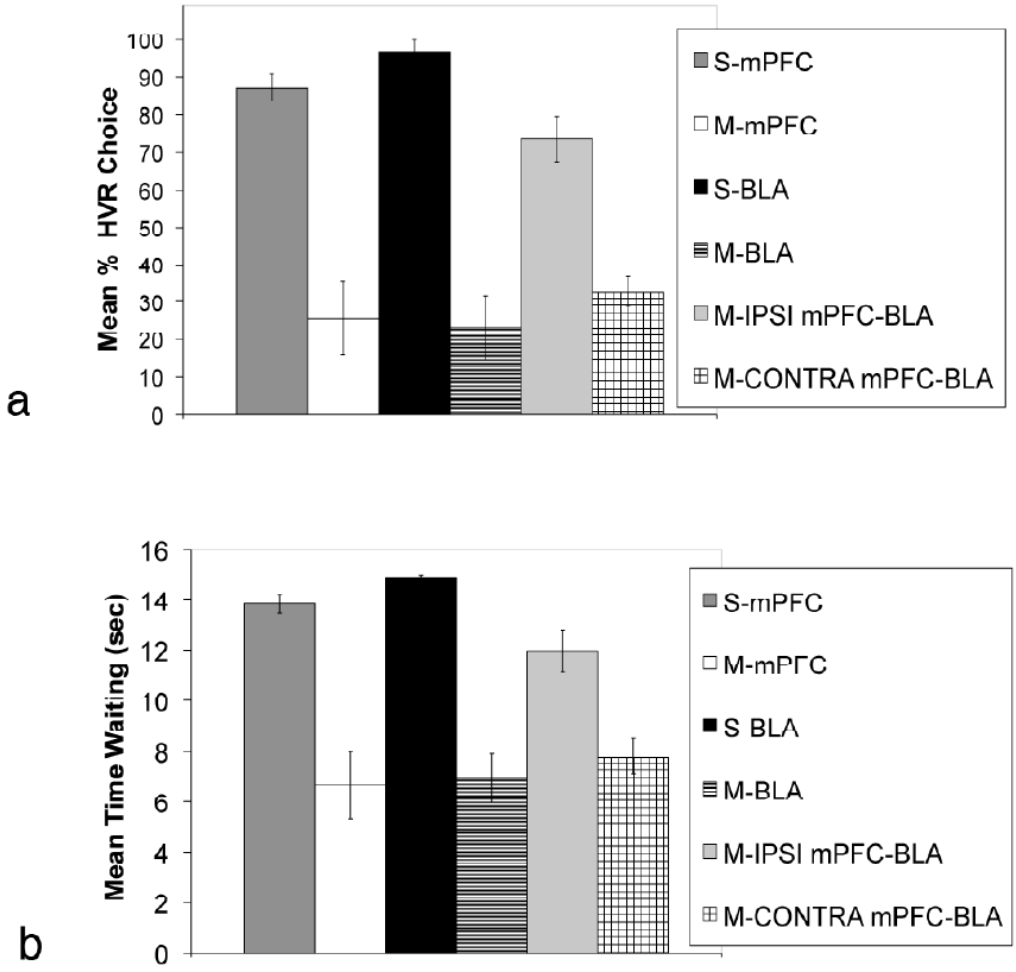

Figure 4.

Shows saline (S) and muscimol (M) conditions for (a) the mean percentage of selecting the delayed HVR arm for bilateral, ipsilateral (IPSI), and contralateral (CONTRA) mPFC and BLA and (b) mean time spent waiting in seconds for bilateral, IPSI, and CONTRA mPFC and BLA.

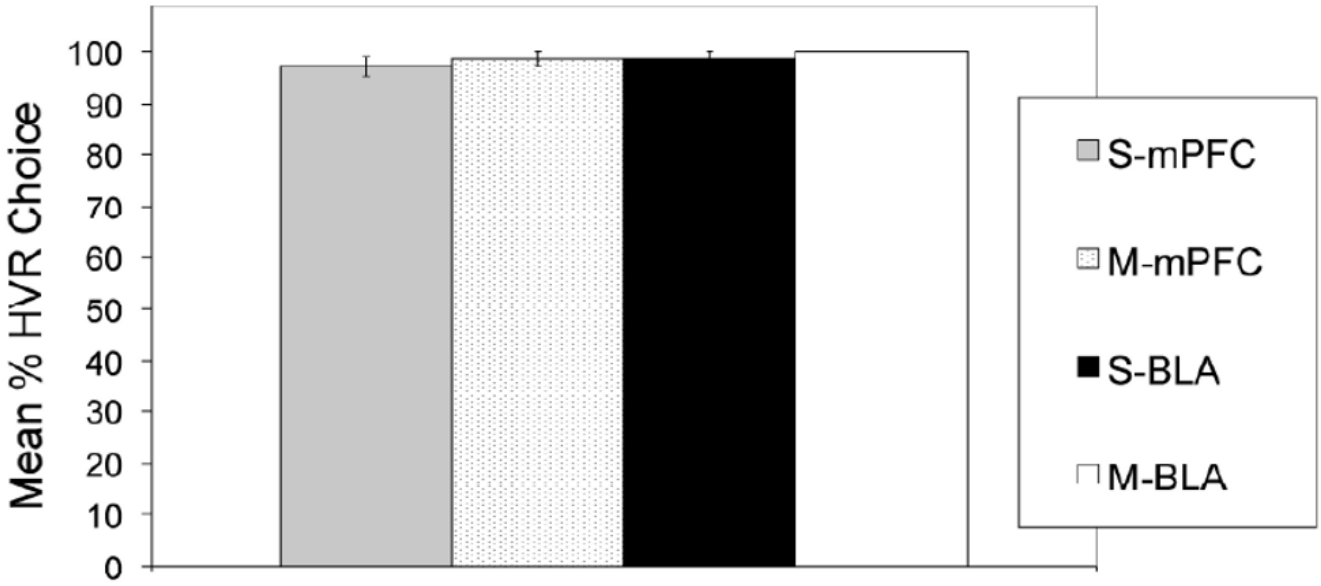

Figure 5.
Shows saline (S) and muscimol (M) conditions for the mean percentage of selecting the HVR for bilateral mPFC and BLA during discrimination between reward magnitudes.

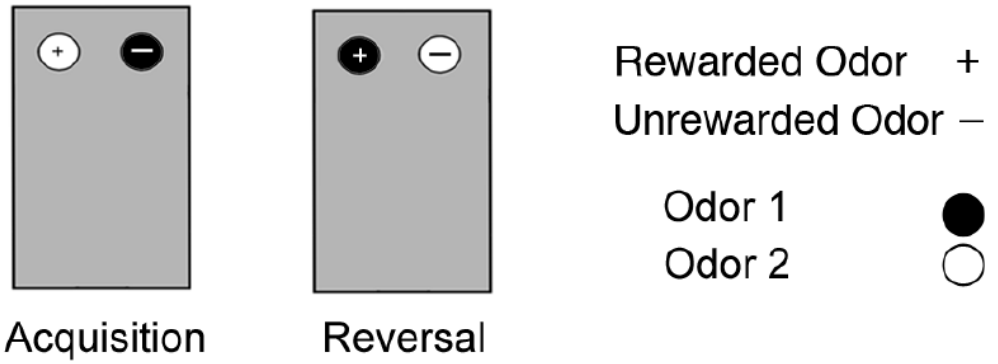

Figure 6.
Experimental apparatus used for the odor reversal task in which subjects were trained to discriminate between two different odors to obtain a food reward. On the day following discrimination training, the contingency was reversed so that the originally rewarded odor was unrewarded and the originally unrewarded odor became rewarded. The location of the rewarded odor was pseudorandomly switched between the two adjacent locations.

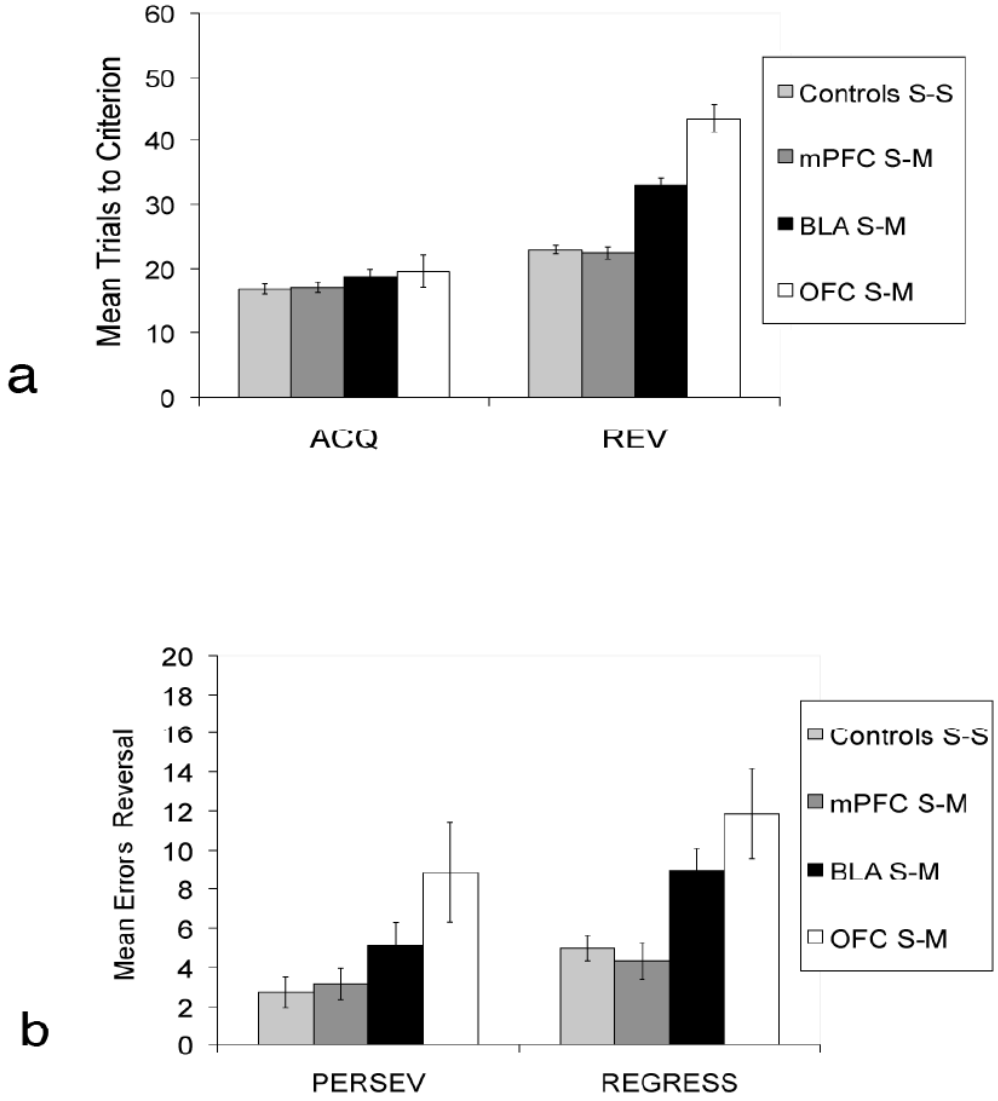

Figure 7.

Shows the mean number of trials to reach criterion during acquisition (ACQ) and reversal (REV) for saline-saline (S-S) and saline-muscimol (S-M) conditions across bilateral mPFC, BLA, and OFC groups and (b) the mean number of perseverative (PERSEV) and regressive (REGRESS) errors during reversal for bilateral mPFC, BLA, and OFC groups.

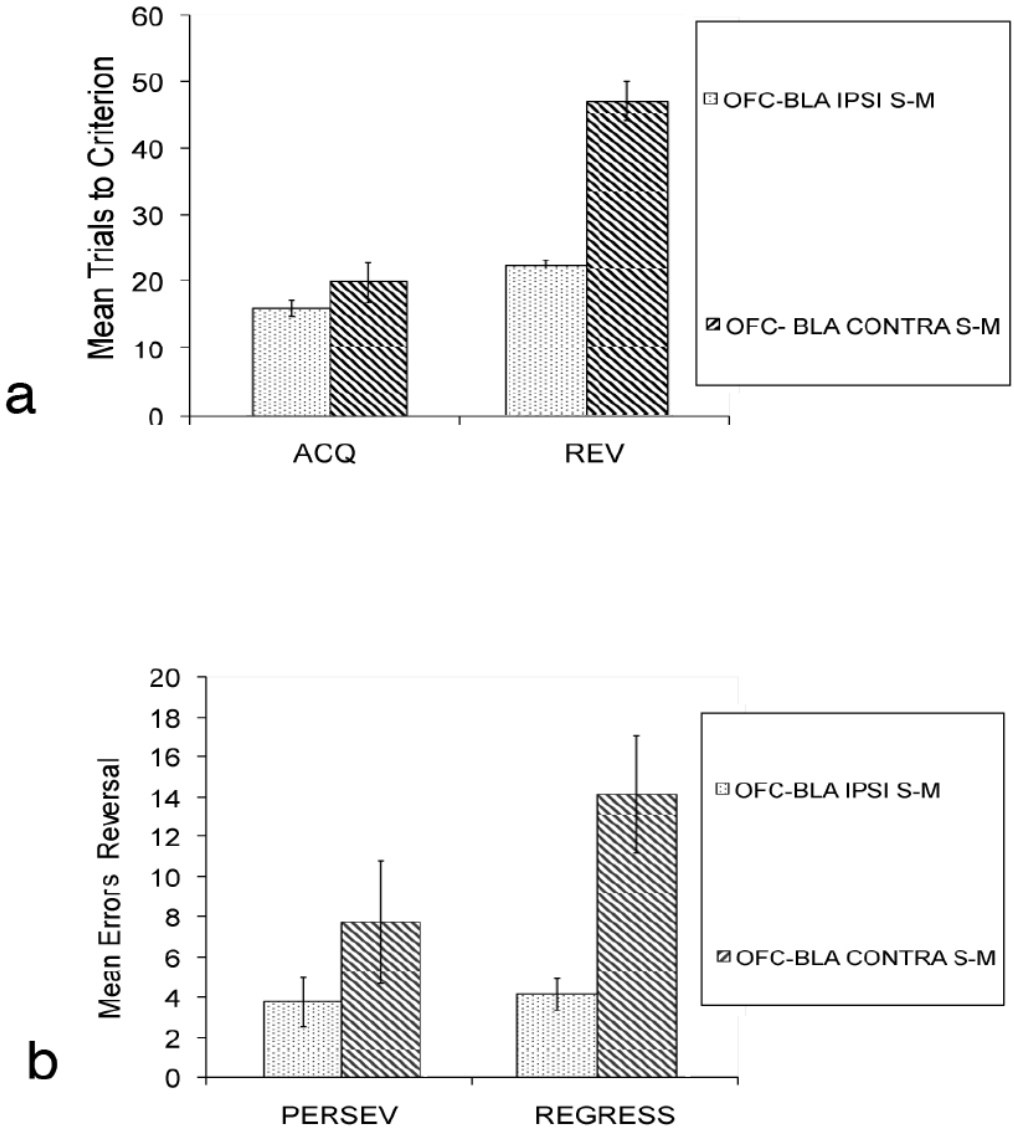

Figure 8.

Shows (a) the mean number of errors to reach criterion during acquisition and reversal for ipsilateral (IPSI) and contralateral (CONTRA) OFC and BLA and (b) the mean number of perseverative (PERSEV) and regressive (REGRESS) errors during reversal for IPSI and CONTRA OFC and BLA.