Differential effects of inactivation of the orbitofrontal cortex on strategy set-shifting and reversal learning

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Abstract

Different subregions of the rodent prefrontal cortex (PFC) mediate dissociable types of behavioral flexibility. For example, lesions of the medial or orbitofrontal (OFC) regions of the PFC impair extradimensional shifts and reversal learning, respectively, when novel stimuli are used during different phases of the task. In the present study, we assessed the effects of inactivation of the OFC on strategy set-shifting and reversal learning, using a maze based set-shifting task mediated by the medial PFC. Long–Evans rats were trained initially on a visual-cue discrimination to obtain food. On the subsequent day, rats had to shift to using a response strategy (e.g., always turn left). On Day 3 (reversal), rats were required to reverse the direction of their turn (e.g., always turn right). Infusions of the local anesthetic bupivacaine into the OFC did not impair initial visual discrimination learning, nor did it impair performance on the set-shift. In contrast, inactivation of the OFC did impair reversal learning; yet, these rats ceased using the previously acquired response rule as readily as controls. Instead, rats receiving OFC inactivations made a disproportionate number of erroneous arm entries towards the visual-cue, suggested that these animals reverted back to using the original visual-cue based strategy. These findings, in addition to previous data, further support the notion that the OFC and medial PFC play dissociable roles in reversal learning and set-shifting. Furthermore, the lack of effect of OFC inactivations on the set-shift indicates that this type of behavioral flexibility does not require cognitive operations related to reversal learning.
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1. Introduction

The ability to alter behavioral strategies and adapt to changes in one's environment is mediated by interactions between different cortical and subcortical brain regions. Studies across mammalian species have strongly implicated different subregions of the prefrontal cortex (PFC) in mediating distinct forms of behavioral flexibility. For instance, inactivations or lesions of the medial PFC in rodents disrupt the acquisition of novel strategies, rules or attentional sets, impairing the inhibition of a previously relevant strategy (Birrell & Brown, 2000; Floresco, Magyar, Ghods-Sharifi, Vexelman, & Tse, 2006a; Ragozzino, Detrick, & Kesner, 1999). In contrast, reversal learning, a simpler form of

behavioral flexibility that entails shifting between different stimulus-reward associations, is impaired by lesions of the orbitofrontal region of the PFC (OFC), while set-shifting remains unaffected with similar lesions (Birrell & Brown, 2000; Chudasasma, Bussey, & Muir, 2001; McAlonan & Brown, 2003).

A number of tasks have been developed to investigate the neural basis of these different types of behavioral flexibility in rodents, using different sensory modalities and classes of stimuli. For example, Birrell and Brown (2000) utilized a task in which rats discriminate between two bowls that can be distinguished based on a variety of features (digging media, odor, texture) to receive reinforcement. During the attentional (or extradimensional) set-shift, rats are presented with two novel bowls, and must attend to a previously irrelevant dimension (e.g., shift from texture to odor). Extradimensional shifts are unaffected with lesions of the OFC, however,

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reversal learning is impaired on this task (McAlonan & Brown, 2003). Another task used to assess set-shifting abilities is conducted on a cross-maze, whereby rats shift between visual-cue and egocentric spatial response based discrimination strategies. Initially, rats learn to enter an arm with a visual-cue to receive reinforcement. During the set-shift, animals must turn in a specific direction (e.g., always turn left) regardless of the position of the visual-cue. Manipulations of the medial PFC have been found to severely impair performance during the set-shift (Floresco et al., 2006a; Ragozzino et al., 1999; Stefani, Groth, & Moghaddam, 2003). An advantage of this task is that it can be used to distinguish between different types of impairments in set-shifting, such as perseverative deficits, or impairments in the ability to acquire and maintain a new strategy. We have used this task previously to delineate the distinct contributions that a number of brain regions connected with the PFC make to this form of set-shifting (Block, Dhanji, Thompson-Tardif, & Floresco, 2007; Floresco et al., 2006a; Floresco, Ghods-Sharifi, Vexelman, & Magyar, 2006b).

As opposed to other perceptual set-shifting tasks, where novel complex stimuli are used during initial discrimination training and extradimensional shifts (Birrell & Brown, 2000; McAlonan & Brown, 2003), the stimuli remain constant across different phases in this maze based procedure. As a consequence, this task places a heavier emphasis on response conflict because animals are presented with the same set of stimuli during the initial discrimination learning and during the set-shift, even though it requires complete shift of attention from one stimulus dimension to another (e.g. visual-cue to turn direction). It is possible, therefore, that in addition to an extradimensional shift, a partial reversal component may be embedded in this task. As such, it is not clear whether disruptions in behavioral flexibility observed after inactivation of the medial PFC or interconnected brain regions are due specifically to impairments in extradimensional shifting, or to disruptions in cognitive processes related to reversal learning. As noted above, lesions or inactivations of the OFC impair reversal learning, yet the role for this region of the PFC in this form of strategy set-shifting is unknown. Accordingly, the present study assessed the effects of reversible inactivations of the OFC on strategy set-shifting between visual-cue and response based strategies, as well as reversal learning of a response rule. If this set-shifting task engages cognitive operations related to reversal learning, then inactivation of the OFC should disrupt this form of behavioral flexibility. However, a lack of effect on the set-shifting component of this task would indicate that this type of extradimensional shift is not assessing cognitive processes related to reversal learning.

2. Methods

2.1. Subjects and surgery

Male Long–Evans rats weighing between 275 and 375 g (Charles River, Montreal, Quebec, Canada). were anesthetized with 100 mg/kg ketamine hydrochloride and 7 mg/kg xylazine. A pair of 23 gauge stainless

steel guide cannulas were implanted into the OFC [flat skull, from bregma: anteroposterior, +3.8 mm; mediolateral, ±2.6 mm dorsoventral, −2.9 mm from dura]. Thirty-gauge obdurators flush with the end of the guide cannulas were placed into the cannulas. Each rat was given at least 7 d to recover from surgery before training. During this recovery period, animals were single housed, food restricted to 85% of their free-feeding weight, and handled at least 5 min per day. All testing was in accordance of the Canadian Council of Animal Care and the Animal Care Committee of the University of British Columbia.

2.2. Apparatus

Rats were trained and tested on a four-arm cross maze which was used, and described previously (Floresco et al., 2006a, 2006b). The maze was constructed with 1.5-cm-thick plywood and painted white and was elevated 70 cm above the floor. Each arm was 60 cm × 10 cm × 20 cm with a cylindrical food well drilled 2 cm from the end wall. To create a "T" configuration, a removable piece of white opaque Plexiglas (20 × 10 cm) was used to block an arm.

2.3. Maze familiarization

The familiarization procedure has also been described in detail previously (Floresco et al., 2006a, 2006b). On the first day, a rat was allowed to freely navigate and consume 20 pellets placed throughout the 4 arms of the maze for 15 min. On the second day, arms were baited with three pellets each. To familiarize the rat to repeated handling, it was picked up and placed at the entrance of a different arm when it consumed all of the pellets in each of the other arms. Subsequent sessions were similar, except that only one food pellet was placed at the end of each arm. This procedure continued daily until the rat consumed all four food pellets on the maze at least four times during the 15 min period. Rats required an average of 3.2 ± 0.2 days of familiarization to reach this criterion. Once a rat achieved this criterion, its turn bias was determined by placing a Plexiglas insert at the entrance of an arm to form a "T" configuration. One reward pellet was placed in the food cup at the end of each of the "T" arms, and a black-and-white-striped laminated piece of posterboard (9 × 20 cm) serving as a visual-cue was placed in one of the arms. A rat was released from the stem arm and was allowed to choose between the two arms. Once the food pellet in one of the "T"-arms was consumed, the rat was picked up and placed back in the stem arm so it could consume the pellet in the opposite arm. This procedure was repeated as many times as necessary until it chose the arm it had not sampled from on that trial. After choosing from both arms, the rat was returned to the holding cage, the Plexiglas barrier and the visual-cue were moved to a different arm for the next trial. A total of seven trials were administered to determine the turn bias of the rat, using the initial choice (i.e., left or right) on each trial. Once the turn bias was determined, the rat underwent a mock infusion, whereby its obdurators were removed from the guide cannulas and two injection cannulas were inserted for 2 min and 12 s without being connected to the microinfusion pump.

2.4. Visual-cue to response-reversal strategy procedure

The set-shifting task used here was identical to procedures that have been previously employed (Floresco et al., 2006a, 2006b; Ragozzino, 2002). Training was conducted over 3 consecutive days. During these sessions, no trial limits were imposed, and a rat received as many trials as it needed to achieve criterion performance.

Day 1: Visual-cue learning (Fig. 1A). The day after the animal's turn bias had been determined, rats were trained to enter the arm containing the visual-cue, the location of which was pseudorandomly assigned with equal frequencies in the left and the right arms for every set of 12 trials. The stem arms were designated as North, South, East, and West. Each trial began with the placement of a rat in the stem of one of the arms (South, East or West). A rat was considered to have entered the arm only when it reached the food well at the end of the arm. If the rat entered the arm containing the visual-cue, it