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Pedunculopontine tegmental nucleus lesions impair probabilistic reversal learning by reducing sensitivity to positive reward feedback

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ABSTRACT

Recent findings indicate that pedunculopontine tegmental nucleus (PPTg) neurons encode reward-related information that is context-dependent. This information is critical for behavioral flexibility when reward outcomes change signaling a shift in response patterns should occur. The present experiment investigated whether NMDA lesions of the PPTg affects the acquisition and/or reversal learning of a spatial discrimination using probabilistic reinforcement. Male Long-Evans rats received a bilateral infusion of NMDA (30 nmoles/side) or saline into the PPTg. Subsequently, rats were tested in a spatial discrimination test using a probabilistic learning procedure. One spatial location was rewarded with an 80% probability and the other spatial location rewarded with a 20% probability. After reaching acquisition criterion of 10 consecutive correct trials, the spatial location – reward contingencies were reversed in the following test session. Bilateral and unilateral PPTg-lesioned rats acquired the spatial discrimination test comparable to that as sham controls. In contrast, bilateral PPTg lesions, but not unilateral PPTg lesions, impaired reversal learning. The reversal learning deficit occurred because of increased regressions to the previously 'correct' spatial location after initially selecting the new, 'correct' choice. PPTg lesions also reduced the frequency of win-stay behavior early in the reversal learning session, but did not modify the frequency of lose-shift behavior during reversal learning. The present results suggest that the PPTg contributes to behavioral flexibility under conditions in which outcomes are uncertain, e.g. probabilistic reinforcement, by facilitating sensitivity to positive reward outcomes that allows the reliable execution of a new choice pattern.

1. Introduction

A change in outcomes associated with a particular choice pattern can serve as a salient cue to switch an ongoing strategy. There is substantial evidence that prefrontal cortex, striatal and thalamic circuitry support a behavioral switch when there is a change in outcomes associated with a learned strategy (Birrell & Brown, 2000; Brown, Baker, & Ragozzino, 2010; Chudasama, Bussey, & Muir, 2001; Floresco, Block, & Tse, 2008; Kim & Ragozzino, 2005; McBride & Slotnick, 1997; Palencia & Ragozzino, 2006; Ragozzino, Kim, Hassert, Minniti, & Kiang, 2003; Ragozzino & Rozman, 2007). Less is known about how different brainstem structures may affect behavioral flexibility when a change in outcomes for a learned choice pattern occurs. The pedunculopontine tegmental nucleus (PPTg) is one brainstem structure that may play a role in behavioral flexibility. The PPTg projects to basal ganglia structures (Martinez-Gonzalez, Bolam, & Mena-Segovia, 2011) shown to be involved in behavioral flexibility including the dorsomedial striatum (Baker & Ragozzino, 2014b), nucleus accumbens core (Floresco, Ghods-Sharifi, Vexelman, & Magyar, 2006) and subthalamic nucleus (Baker & Ragozzino, 2014a). The PPTg also projects to thalamic subregions that support behavioral flexibility such as the mediodorsal thalamus (Chudasama et al., 2001; Oakman, Faris, Cozzari, & Hartman, 1999; Parnaudeau et al., 2015) and parafascicular thalamic nucleus (Brown et al., 2010). Further, the PPTg receives input from the medial prefrontal cortex (Holmstrand & Sesack, 2011; Kita & Kita, 2011; Semba & Fibiger, 1992) which is critical for behavioral switching (Baker & Ragozzino, 2014a,b; Floresco et al., 2008; Reichel et al., 2015). Thus, the PPTg is well positioned to contribute to switches in learned choice patterns with a change in reward contingencies.

Past studies suggest that the PPTg may support a flexible shift in choice patterns with a change in reward contingencies. For example, neurotoxic lesions of the PPTg impair win-shift performance in a radial-arm maze (Taylor, Kozak, Latimer, & Winn, 2004). In the delayed win-shift test, PPTg lesions led rats to commit more errors by choosing arms that were rewarded in the training phase, as well

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as repeat arms already entered in the test phase (Taylor et al., 2004). Even without a delay, PPTg lesions increased errors to maze arms that were never reinforced, as well as caused repeated entries to arms already selected in a session (Keating & Winn, 2002). Further, PPTg inactivation was shown to prevent a reduction in lever pressing when conditions switch such that a food reward is no longer contingent on a lever press (Maclaren, Wilson, & Winn, 2013). Thus, based on lesion or inactivation studies there is evidence that the PPTg supports a shift in choice patterns when there is a change in reward contingencies.

The correlated activity of PPTg neurons during different behavioral tasks also suggest that this brain area may support behavioral flexibility, in part, by the accurate processing of positive reward for a particular context (Hong & Hikosaka, 2014; Norton, Jo, Clark, Taylor, & Mizumori, 2011). In non-human primates required to exhibit flexible responding of different saccades, PPTg neurons preferentially respond to information related to positive rewards (Hong & Hikosaka, 2014). In rats performing a win-shift task, approximately one-third of PPT neurons exhibited elevated activity during consumption of a liquid reward (Norton et al., 2011). These same PPTg neurons displayed further enhanced activity to positive reward when the context was changed (Norton et al., 2011). This suggests that PPTg neurons integrate reward and context information and may facilitate a shift in choice patterns when a reward is associated with a particular context change. Because a significant proportion of PPTg neurons preferentially encode positive reward, PPTg lesions or inactivation may affect sensitivity to positive reinforcement that affects learning and/or reversal learning.

While these findings suggest that PPTg contributes to behavioral flexibility when learned choice patterns cease from being associated with a positive reward, unknown is whether PPTg supports specific behavioral processes that allow behavioral flexibility. Employment of behavioral paradigms such as set-shifting and reversal learning have been used to reveal whether a brain area supports behavioral flexibility, as well as specifically determine what processes neural systems support to enable behavioral flexibility, i.e. reduced perseveration of an initially learned choice pattern and/or maintenance of a new choice after initially selected (Baker & Ragozzino, 2014a; Brown, Amodeo, Sweeney, & Ragozzino, 2012; Castane, Theobald, & Robbins, 2010; Floresco et al., 2006; Ragozzino et al., 2003). Reversal learning that involves probabilistic reinforcement has the added advantage of determining whether a particular brain manipulation affects the sensitivity to positive and/or negative reinforcement (Amitai et al., 2014; Bari et al., 2010; Brown et al., 2012; Dalton, Phillips, & Floresco, 2014). Thus, manipulations of the PPTg during acquisition and reversal learning in a task with probabilistic reinforcement may reveal whether the PPTg supports initial learning and/or reversal learning through specific reward processes.

To examine whether the PPTg is involved in probabilistic reversal learning, the present study investigated the effects of NMDA lesions of the PPTg on acquisition and reversal learning of a spatial discrimination using an 80/20 reinforcement schedule. Specifically, rats chose between two different spatial locations in a modified T-maze such that the 'correct' location was reinforced on 80% of trials and the 'incorrect' location reinforced on 20% of trials. After learning this discrimination, the reinforcement contingences for the two spatial locations were reversed.

2. Materials and methods

2.1. Subjects

Adult male Long Evans rats weighing 330–425 g served as the subjects for this study. The rats were individually housed in plastic cages (26.5 × 50 × 20 cm) in a temperature controlled room at 21–23 °C and humidity 30%. They were on a 12 h light/dark cycle. The animals were food restricted to 85–90% of their body weight during the experiment. A total of 28 rats were used in this experiment. Animal care and use was in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and was approved by the Institutional Laboratory Animal Care and Use Committee at the University of Illinois at Chicago.

2.2. Apparatus

Behavioral testing occurred in a four-arm maze made from black acrylic. The maze had a center square base (10 × 10 cm) that connected all four arms. Each maze arm contained a base (10 × 55 cm), two side walls (15 × 55 cm) and a back wall (8 × 15 cm). There was a circular food well (3.2 cm in diameter and 1.6 cm deep) that was placed 3 cm away from the back wall. The maze was located in a room that had various extramaze cues that could be used to spatially navigate in the maze.

2.3. Surgery

Surgery was conducted during the light phase of the light/dark cycle. All rats were anesthetized with xylazine (10 mg/kg) and ketamine (100 mg/kg). The rats were randomly assigned to one of two groups; a sham group which received 0.25 μl of saline and a lesion group which received 0.25 μl of 4.2 μg of N-methyl-D-aspartate (NMDA) in saline. All surgeries were conducted in a stereotaxic frame with the following coordinates: anterior–posterior (AP) at −7.6 mm, mediolateral (ML) at ±1.6 mm from the midline, and dorsoventral (DV) −7.6 from the surface of the skull. The injections were made using a 28 gauge needle connected to a tube that was attached to a 10 μl syringe. The syringe was driven by a microinfusion pump that was programmed to infuse 0.25 μl of volume across 3 min. After infusion of saline or NMDA, the needle was left in position for 3 min to allow the drug to diffuse. After surgery, rats received a 1 mg/kg injection of meloxicam to reduce any pain from the surgery. Following surgery, rats were fed *ad libitum* for five consecutive days. Subsequently, rats were food restricted to reduce their weight to 85% of their free feed weight. Food restriction and stabilization to 85% of their free feed weight required approximately seven days. Rats were handled daily for 10 min during food restriction.

2.4. Maze training

After recovery from surgery and food restriction, rats received maze training. All training and testing occurred during the light phase of the light/dark cycle. Each rat was exposed to the cross-maze and trained to obtain a half piece of Froot Loops cereal (Kellogg, Battle Creek, MI, USA) from each food well. During training, a rat was also picked up after consuming a cereal piece and placed into a different maze arm. This acclimated a rat to being picked up in the maze as occurred in the test phase. After a rat consumed all four cereal pieces from each food well, it was placed in a holding chamber near the maze. The food wells were rebaited and a new trial was started. This phase of training continued until a rat completed a minimum of five trials in 15 min across two consecutive days. Subsequently, a final day of training occurred in which a black plastic block (9 cm wide × 13 cm high × 1 cm thick) was placed at the entrance of one arm, giving the maze a T-shape. A rat was placed in the stem arm and allowed to enter either choice arm to obtain a cereal piece. After the initial choice, a rat was placed back in the stem arm. If a rat chose the same arm as the initial choice, it was returned to the stem arm until it chose the other arm. Once a rat had selected both arms it was placed on top of its