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Acetylcholine activity in selective striatal regions supports behavioral flexibility

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ABSTRACT

Daily living often requires individuals to flexibly respond to new circumstances. There is considerable evidence that the striatum is part of a larger neural network that supports flexible adaptations. Cholinergic interneurons are situated to strongly influence striatal output patterns which may enable flexible adaptations. The present experiments investigated whether acetylcholine actions in different striatal regions support behavioral flexibility by measuring acetylcholine efflux during place reversal learning. Acetylcholine efflux selectively increased in the dorsomedial striatum, but not dorsolateral or ventromedial striatum during place reversal learning. In order to modulate the M2-class of autoreceptors, administration of oxotremorine sesquifumurate (100 nM) into the dorsomedial striatum, concomitantly impaired reversal learning and an increase in acetylcholine output. These effects were reversed by the m_2 muscarinic receptor antagonist, AF-DX-116 (20 nM). The effects of oxotremorine sesquifumurate and AF-DX-116 on acetylcholine efflux were selective to behaviorally-induced changes as neither treatment affected acetylcholine output in a resting condition. In contrast to reversal learning, acetylcholine efflux in the dorsomedial striatum did not change during place acquisition. The results reveal an essential role for cholinergic activity and define its locus of control to the dorsomedial striatum in cognitive flexibility.

1. Introduction

The ability to inhibit one strategy and learn a new strategy represents an essential form of adaptive behavior in daily living and often survival. Prefrontal cortex—basal ganglia circuitry plays a critical role in facilitating a shift in strategies or response patterns (Block, Dhanji, Thompson-Tardif, & Floresco, 2007; Monchi, Petrides, Petre, Worsley, & Dagher, 2001; Muhammad, Wallis, & Miller, 2006; Owen et al., 1993; Stefani & Moghaddam, 2006; Wise, Murray, & Gerfen, 1996). There is considerable evidence in different mammalian species that the basal ganglia nuclei support cognitive flexibility (Cools, Barker, Sahakian, & Robbins, 2001; Monchi et al., 2001; Owen et al., 1993; Ragozzino, Ragozzino, Mizumori, & Kesner, 2002). More specifically, several experiments have demonstrated that the striatum, the largest component of the basal ganglia, enables learning when conditions demand a shift in choice patterns, e.g. place reversal learning, as well as a shift in strategies, e.g. switch between basing a choice on visual object information to basing a choice on egocentric response information (Block et al., 2007; Ragozzino & Choi, 2004; Ragozzino, Ragozzino, et al., 2002).

At present, less is known about the specific circuitry and neurochemical processes in the striatum that may enable cognitive flexibility. One neurotransmitter in the striatum that may play a key role in facilitating cognitive flexibility is acetylcholine (ACh). The principle source of striatal ACh content originates almost entirely from interneurons (Bolam, Wainer, & Smith, 1984). The cholinergic interneurons are distinguished from the more plentiful projections neurons by their large somata, as well as extensive axonal fields (Bolam et al., 1984; Wilson, Chang, & Kitai, 1990). This anatomical feature suggests that cholinergic interneurons may be important for shaping the nature of striatal output to other brain regions also critical for cognitive flexibility. Furthermore, ACh in the striatum is critical for modulating synaptic plasticity that may underlie different forms of learning and memory (Calabresi, Centonze, Gubellini, Pisani, & Bernardi, 1998).

ACh actions at muscarinic cholinergic receptors in the striatum may alter synaptic plasticity that supports certain forms of learning and memory. In particular, several experiments have demonstrated that intra-cranial infusions of muscarinic cholinergic antagonists into the dorsal striatum prior to or after training impairs memory consolidation in rats (Diaz del Guante, Cruz-Morales, & Prado-Alcala, 1991; Giordano & Prado-Alcala, 1986; Solana-Figueroa & Prado-Alcala, 1990). Furthermore, cholinergic agents infused into the dorsal striatum also affect memory retrieval (Solana-Figueroa & Prado-Alcala, 1990). However, when muscarinic cholinergic receptors are blocked specifically in the dorsomedial striatum there is no effect on memory retrieval (McCool, Patel, Talati, & Ragozzino, 2008; Ragozzino, Jih, & Tzavos, 2002; Tzavos, Jih, & Ragozzino, 2004), suggesting that cholinergic actions at mus-

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carinic cholinergic receptors outside the dorsomedial striatum may affect memory processes.

In addition to mnemonic processing, there is indirect evidence that suggests that ACh actions in the striatum play a role in learning and possibly cognitive flexibility. For example, the activity of striatal tonically active neurons is correlated with the presentation of primary rewards or stimuli associated with reward (Aosaki et al., 1994). A significant proportion of the tonically active neurons are likely cholinergic interneurons and thus may represent plastic changes in these neurons during learning (Wilson et al., 1990). These neurons also exhibit changes in the temporal relationship between stimuli or events that may be critical when conditions require a shift in learned response patterns. (Apicella, 2002). Other experiments have found changes in striatal ACh efflux during learning and strategy switching, but have not demonstrated that changes in striatal ACh output are actually critical for specific aspects of learning (Chang & Gold, 2003; Ragozzino & Choi, 2004). Limitations of these past experiments have prevented a direct link between cholinergic activity and behavioral flexibility. Moreover, cholinergic interneurons are found throughout the striatum, thus these neurons may support behavioral flexibility in multiple striatal subregions.

The present experiments address the issues raised above by investigating whether ACh efflux in different striatal regions changes during reversal learning of a place discrimination. To more directly link a change in striatal ACh efflux in underlying a shift in choice patterns, the experiments also examined the effects of modulating striatal ACh efflux during place reversal learning.

2. Methods

2.1. Subjects

Male Long-Evans rats (Harlan, Indianapolis, USA) weighing between 350 and 400 g at the start of the experiment served as subjects. Rats were singly housed in plastic cages (26.5 cm wide × 50 cm long × 20 cm high) in a humidity (30%) and temperature (22 °C) controlled room with a 12-h light/dark cycle (lights on at 07:00 h). Rat received surgery approximately 5–7 days after arriving at the colony. 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. Surgery

Each rat received stereotaxic surgery to bilaterally implant cannula into the striatum. Before surgery each rat received an intraperitoneal (i.p.) injection of atropine sulfate (0.2 ml of a 250 μg/ml solution) followed 10 min later by an i.p. injection of sodium pentobarbital (50 mg/kg). In Experiments 1 and 2, twelve-mm guide cannula (CMA Microdialysis Inc., North Chelmsford, MA, USA) were bilaterally implanted and aimed toward the dorsomedial striatum (see Fig. 1). The cannulae were implanted at a 10° angle. The stereotaxic coordinates were 1.1 mm anterior to bregma, ±2.8 mm lateral to the midline and 4.0 mm below dura. In Experiment 3, cannulae were bilaterally implanted aimed at either the ventromedial striatum or dorsolateral striatum. The coordinates were based on Paxinos and Watson's (1996) rat brain atlas. An omega-shaped ring was placed behind the guide cannulae. The omega ring allowed the rat to be connected to the liquid swivel/balance arm by a wire attached with a hook that extended from the liquid swivel. This setup prevented the tubing from being twisted during microdialysis collection. Four jeweler's screws were positioned in the skull surrounding the guide cannulae. Dental acrylic (Stoelting, Wood Dale, USA) was used to secure the guide cannula. In Experiment 3, guide cannulae were bilaterally implanted aimed toward the dorsolateral striatum or ventromedial striatum (see Fig. 1). The stereotaxic coordinates for the dorsolateral striatum were 0.6 mm anterior to bregma, ±3.5 mm lateral to the midline and 4.0 mm below dura. The stereotaxic coordinates for the ventromedial striatum were 1.1 mm anterior to bregma, ±2.8 mm lateral to the midline and 6.0 mm below dura. The cannulae aimed for the ventromedial striatum were implanted at a 10° angle. After surgery, the rats received 6 ml of saline subcutaneously (s.c.) and fed ground rat chow and sugar mixed with water for 1 day. For 5–7 days after surgery the rats were allowed to recover and handled for 5 min each day. After 2 days of recovery all rats were food-restricted to maintain their weight at about 85% of their free-feed weight. Each rat had free access to water throughout the study. Behavioral training commenced 5–7 days after surgery.

2.3. Apparatus

Behavioral testing experiments were conducted in a black plastic four-arm cross maze. The height of the maze walls was 15.0 cm and each of the arms measured 55 cm long × 10 cm wide. There was a food well (3.2 cm diameter × 1.6 cm high) in each arm located 3 cm away from the end wall. The hole in the food well measures 2.3 cm in diameter and was 1.6 cm deep. The maze was elevated 72 cm above the floor in a room with extra-maze cues.

2.4. Pretraining

Except where noted, the experiments involved behavioral testing that required pretraining. On the first day of pretraining all arms of the maze were baited with a half piece of Froot Loops cereal (Kelloggs, Battle Creek, MI). Each rat was placed at the end of a maze arm and allowed to explore and consume the Froot Loop pieces. One pretraining trial was completed after a rat consumed all the cereal pieces. Between trials a rat was placed in a holding cage while the maze was rebaited with Froot Loops pieces. The pretraining session was terminated after 15 min had elapsed and the number of trials completed was recorded. If a rat did not complete 1 pretraining trial within 15 min it stayed in the maze until it consumed all cereal pieces or until 20 min had elapsed. During subsequent pretraining sessions, when a rat had consumed a piece of Froot Loops cereal from two baited arms it was picked up and placed in an unbaited arm. After eating from a third baited arm it was picked up and placed in another unbaited arm. After consuming cereal from the fourth arm, a rat was removed, the arms were rebaited and another trial was started. This procedure was used to acclimate a rat to being picked up in the maze after consuming a cereal piece. This procedure was continued until a rat was able to complete 5–7 trials in 15 min across 2 consecutive days. After reaching this criterion, a final day of pretraining occurred in which a black plastic block (9 cm wide × 13 cm high × 1 cm thick) was placed at the entrance of one arm so that it prevented entry, giving the maze a T-shape. Therefore, there were only two arm choices available to a rat. A rat was placed at the end of a 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. When a rat had chosen both arms it was placed in the holding cage while the two choice arms were rebaited. The session ended after a rat had completed 7 of these trials. Rats in all experiments required a total of 4–8 days of pretraining. In experiments 1 and 4, rats were pseudorandomly assigned to an experimental group such that all the groups received a similar amount of pretraining.