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Central cholinergic involvement in sequential behavior: Impairments of performance by atropine in a serial multiple choice task for rats

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ABSTRACT

Two experiments examined whether muscarinic cholinergic systems play a role in rats' ability to perform well-learned highly-structured serial response patterns, particularly focusing on rats' performance on pattern elements learned by encoding rules versus by acquisition of stimulus–response (S–R) associations. Rats performed serial patterns of responses in a serial multiple choice task in an 8-lever circular array for hypothalamic brain-stimulation reward. Two experiments examined the effects of atropine, a centrally-acting muscarinic cholinergic receptor antagonist, on rats' ability to perform pattern elements where responses were controlled by rules versus elements, such as rule-inconsistent "violation elements" and elements following "phrasing cues," where responses were controlled by associative cues. In Experiment 1, 3-element chunks of both patterns were signaled by pauses that served as *phrasing cues* before chunk-boundary elements, but one pattern also included a *violation element* that was inconsistent with pattern structure. Once rats reached a high criterion of performance, the drug challenge was intraperitoneal injection of a single dose of 50 mg/kg atropine sulfate. Atropine impaired performance on elements learned by S–R learning, namely, chunk-boundary elements and the violation element, but had no effect on performance of rule-based within-chunk elements. In Experiment 2, patterns were phrased and unphrased perfect patterns (i.e., without violation elements). To control for peripheral effects of atropine, rats were treated with a series of doses of either centrally-acting atropine or peripherally-acting atropine methyl nitrate (AMN), which does not cross the blood–brain barrier. Once rats reached a high criterion, the drug challenges were on alternate days in the order 50, 25, and 100 mg/kg of either atropine sulfate or AMN. Atropine, but not AMN, impaired performance in the phrased perfect pattern for pattern elements where S–R associations were important for performance, namely, chunk-boundary elements. However, in the structurally more ambiguous unphrased perfect pattern where rats had fewer cues and presumably relied more on S–R associations throughout, atropine impaired performance on all pattern elements. Thus, intact muscarinic cholinergic systems were shown to be necessary for discriminative control previously established by S–R learning, but were not necessary for rule-based serial pattern performance.

1. Introduction

Cholinergic systems relevant to learning, memory, and performance of previously learned behavior include the basal forebrain cholinergic system and brainstem cholinergic neurons (Everitt & Robbins, 1997; Gold, 2003; Maddux, Kerfoot, Chatterjee, & Holland, 2007; Sarter, 2007). Cholinergic systems play a complex role in Pavlovian conditioning (e.g., Carnicella, Pain, & Oberling, 2005a, 2005b), instrumental conditioning (Whitehouse, 1964), response timing (Meck, 1996; Meck & Church, 1987), and multiple types of attention (e.g., Maddux et al., 2007; Sarter, 2007). Muscarinic anticholinergic drugs such as the muscarinic acetylcholine receptor antagonists, atropine and scopolamine, have been shown to produce impairments in rats' retention performance on tasks such as single alternation (Heise, Hrabrich, Lilie, & Martin, 1975), go/no-go discrimination (Milar, Halgren, & Heise, 1978; Viscardi & Heise, 1986), delayed matching- and non-matching-to-position (Roitblat, Harley, & Helweg, 1989; Spencer, Pontecorvo, & Heise, 1985), and radial maze working memory (Beatty & Bierley, 1985; Okaichi & Jarrard, 1982). However, anticholinergic drugs do not seem to affect performance in some learning and retention tasks (Beatty & Bierley, 1985; Gonzalez & Altshuler, 1979) and, while they do affect attention, they may not impair learning *per se* in some types of sequential tasks such as serial reaction time (Nissen, Knopman, & Schacter, 1987).

The present studies employed a *serial multiple choice* (SMC) task to examine whether or not muscarinic cholinergic systems play a role in rats' ability to perform well-learned highly-structured serial patterns of behavior. The SMC task for rats is analogous to

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nonverbal pattern-learning tasks requiring human subjects to make responses in a particular sequential order according to a fixed and highly structured pattern (Restle & Brown, 1970a, 1970b). Rats in the SMC task learned to perform serial patterns by choosing from a circular array of 8 levers in the proper sequential order on successive trials (Fountain, 2008; Fountain, Benson, & Wallace, 2000; Fountain & Rowan, 1995a, 1995b). The measure of greatest interest on each element (trial) of the pattern was whether or not the first choice rats made was correct (Fountain, 1990; Fountain & Rowan, 1995a, 1995b). Which lever was the correct choice on any trial was predetermined by the programmed serial pattern designated for each group of rats to learn. Rats were trained on one of two 24-element serial patterns. One pattern was a *perfect* pattern, defined as a serial pattern that can be described by structure without exceptions, that is, the pattern had no *violation elements* (Fountain & Rowan, 1995b). The other was a *violation* pattern that contained an element that violated the simple structure (Fountain & Rowan, 1995a, 2000). The patterns were both composed of eight 3-element chunks:

- Perfect pattern: 123–234-345–456-567–678-781–812-
- Violation pattern: 123–234-345–456-567–678-781–818-

Digits indicate the clockwise position of correct levers in the circular 8-lever array on each trial, dashes indicate 3-s pauses that served as *phrasing cues* (Muller & Fountain, 2010; Stempowski, Carman, & Fountain, 1999), and all other intertrial intervals were 1 s. The first element of each 3-element chunk is termed the *chunk-boundary element*. In these patterns, chunk-boundary elements occurred every 3 elements (after dashes indicating phrasing cues) at serial positions 1, 4, 7, 10, 13, 16, 19, and 22. Thus, phrasing cues signaled chunk-boundary elements in these patterns. The second and third elements in each chunk are designated *within-chunk elements*.

The SMC task has been useful for assessing drug effects on cognition because it recruits multiple concurrent cognitive systems including discrimination learning based on associative stimulus–response (S–R) learning, serial position learning involving timing or counting processes, and hierarchical rule learning processes involving pattern chunking (Fountain, 2008; Fountain & Benson, 2006; Fountain, Rowan, & Carman, 2007; Fountain et al., 2012; Wallace, Rowan, & Fountain, 2008). Learning to anticipate chunk-boundary elements in a *phrased pattern* (a pattern with phrasing cues) has been shown to depend on both associative S–R learning and serial-position learning concurrently (Muller & Fountain, 2010; Stempowski et al., 1999). Earlier work has also shown that both rats and mice find violation elements unusually difficult to learn and that they learn to anticipate violation elements by associative discrimination learning involving multiple item cues from several preceding trials that signal the impending violation trial (Kundey & Fountain, 2010), In contrast, learning to anticipate within-chunk elements depends on learning a motor program or an abstract rule that is independent of external stimuli (Muller & Fountain, 2010).

Drug studies also provide evidence that the SMC task recruits multiple concurrent cognitive systems that depend on multiple brain systems. One set of studies were conducted to examine learning deficits when rats were trained under systemically administered MK-801, an N-methyl-D-aspartate receptor antagonist that blocks learning via long-term potentiation in hippocampus and other brain areas (Coan, Saywood, & Collingridge, 1987; Wong et al., 1986). MK-801 blocked learning to anticipate chunk-boundary elements and the violation element with virtually no disruption of acquisition of within-chunk elements (Fountain & Rowan, 2000). In addition, recent work with a nose poke version of the SMC task has shown that adolescent nicotine exposure causes sex-selective impairments of serial pattern learning in adult rats. Adolescent nicotine causes impairments of acquisition of chunk-boundary elements in male rats and violation elements in female rats, but spares within-chunk element acquisition in both male and female rats (Fountain, Rowan, Kelley, Willey, & Nolley, 2008; Pickens, Rowan, Bevins, & Fountain, 2013). Thus, both behavioral and pharmacological evidence from the SMC task indicate that learning to anticipate chunk-boundary elements, within-chunk elements, and violation elements depends on different underlying cognitive systems and that these dissociable cognitive systems likely depend on dissociable neural systems (Fountain, 2008; Fountain & Rowan, 2000; Fountain et al., 2012).

Two experiments examined the effects of atropine, a centrally-acting muscarinic cholinergic antagonist, on rats' ability to perform well-learned serial patterns. The studies examined the effects of atropine on elements controlled by rules, such as the elements within chunks, versus elements controlled by discriminative cues through S–R learning, such as violation elements and chunk-boundary elements with and without signaling phrasing cues (Kundey & Fountain, 2010; Muller & Fountain, 2010; Stempowski et al., 1999). In both experiments, rats were first trained to a high criterion before drug challenge. In Experiment 1, rats were first trained on a phrased perfect pattern or phrased violation pattern. Once they reached criterion, rats were injected with either vehicle or atropine prior to testing on 1 day only. In Experiment 2, rats were first trained on a phrased perfect pattern or an unphrased perfect pattern. Once they reached criterion, 1 group of rats was injected with a series of 3 doses of atropine alternating with saline treatment days. To determine whether any observed effects of atropine were caused by central versus peripheral effects of the drug, 1 additional group of rats was injected with a series of 3 doses of the peripherally-acting atropine methyl nitrate (AMN) alternating with saline treatment days. Thus, half the rats in each phrasing condition received systemic injections of atropine, a drug which acts both peripherally and centrally because it readily crosses the blood–brain barrier, and half received AMN, a drug that has the peripheral effects of atropine but cannot cross the blood–brain barrier. Drug effects associated with atropine but not AMN would indicate effects attributable to involvement of central rather than peripheral muscarinic acetylcholine receptor systems. The results of these manipulations were expected to provide new information regarding the extent to which muscarinic cholinergic systems are involved in rat sequential behavior and the extent to which serial pattern performance in this SMC task depends on multiple dissociable psychological and brain systems.

2. Methods

2.1. Subjects

All procedures were conducted in accordance with the "Principles of laboratory animal care" (NIH publication No. 86-23, revised 1985) and were approved by the Institutional Animal Care and Use Committee of Kent State University. Male hooded rats bred in-house were at least 90 days of age at the time of surgery. Rats that were successfully shaped to lever press (see Section 2.3 below) served as subjects, totaling 12 rats for Experiment 1 and 24 rats for Experiment 2. All rats were implanted unilaterally on the left side with bipolar electrodes (MS301, Plastic Products, Roanoke, VA) for hypothalamic brain-stimulation reward (BSR) (coordinates, skull level: 4.5 mm posterior, 1.5 mm lateral, 8.5 mm below the surface of the skull). Prior to surgery, rats were deeply anesthetized by 35.56 mg/kg ketamine and 3.56 mg/kg xylazine i.p. injection. After surgery, the wound was treated with a topical antiseptic ointment (Furaderm) and rats received antibiotics (60,000 units