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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

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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 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-second pauses that served as *phrasing cues* (Muller & Fountain,

2010; Stempowski, Carman, & Fountain, 1999), and all other intertrial intervals were 1 second. 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 one 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 penicillin i.m.) following surgery to reduce the chance of infection. They were carefully monitored for infection following surgery and were allowed at least 1 week for recovery from surgery. Rats were housed in individual cages with food and water freely available. They were maintained on our colony’s standard 15–9-hr light-dark cycle. To our knowledge, all behavioral serial patter learning research in rats to date has been conducted during the light phase of the cycle, thus testing was likewise conducted during the light portion of the cycle in the present study to facilitate comparison to earlier work. Both food and water were freely available in the home cage.

2.2. Apparatus

Two Plexiglas operant chambers (30 × 30 × 30 cm), each equipped with a single retractable response lever mounted 5.0 cm above the floor of stainless steel rods and a commutating

device centrally located in the ceiling, were used for shaping the lever-press response for BSR. Each was enclosed in a sound-attenuating shell made of particleboard (20 × 60 × 65 cm).

Two octagonal operant chambers, each with 15 cm wide × 30 cm tall Plexiglas walls and hardware cloth floors, were used for serial pattern training in the SMC task. A retractable response lever was centered on each wall 5.0 cm above the floor. Each lever required approximately 0.15-N force for activation. Rats were connected to a stimulator by a flexible cord (Plastic Products MS304) and a commutating device centrally located in the ceiling of the chamber. Chambers were in separate testing rooms (approximately 2.0 × 2.6 m) illuminated by fluorescent lighting. Cues outside the chamber were minimized to encourage rule learning rather than spatial learning; the most prominent remaining room cues were electrical outlets on two walls and a door on a third wall. The experiment was controlled by a microcomputer and interface (interface and Med-State Software, Med Associates, Inc., St. Albans, VT) and monitored remotely by closed-circuit video cameras.

2.3. Procedures

Throughout all phases of the experiment, rats received reinforcement consisting of a single 250-ms BSR *pulse* of a 60-Hz sinusoidal pulse train from a constant current source of 40–100 μ A. After at least 1 week's recovery from surgery, rats were shaped to lever press for BSR in a shaping chamber. At the beginning of each shaping session, the lever was inserted into the chamber and remained inserted throughout the 30-min session. Rats were required to make at least 1000 lever press responses within a 30-min session and received up to 2 sessions to meet criterion. Approximately 10% of rats failed to meet criterion and were excluded before those that were successfully shaped were randomly assigned to groups in one of several ongoing experiments.

2.4. Experiment 1: Atropine effects on performance of phrased perfect and phrased violation serial patterns

During the serial pattern learning phase using the SMC task, at the beginning of each trial, all 8 levers were inserted into the chamber. If the rat's first lever choice was correct, all levers were retracted and BSR was delivered. If instead the rat's first response was incorrect, a correction procedure followed; other levers were retracted leaving only the correct lever and the rat was required to choose it to receive BSR. This correction procedure assured that rats received feedback regarding the correct response on each trial. Six rats were randomly assigned to learn a *phrased perfect* versus a *phrased violation* serial pattern composed of 24 elements phrased as eight 3-element *chunks*:

Phrased perfect pattern: 123–234–345–456–567–678–781–812- (...repeat pattern)

Phrased violation pattern: 123–234–345–456–567–678–781–818- (...repeat pattern)

Digits indicate the clockwise position of the correct levers on successive trials. An intertrial interval of 1 sec was imposed between elements within 3-element chunks. Dashes indicate 3-s pauses that served as *phrasing cues* positioned at transitions between chunks. Rats in Experiment 1 had previous experience with serial pattern learning without drug exposures in another study. For this study they were trained on the foregoing patterns for 50 patterns per

day until they reached a criterion of no more than 10% errors on any trial of the pattern. Rats in the phrased perfect pattern condition took a mean of 8.33 days to reach criterion whereas rats in the phrased violation pattern condition took a mean of 9.67 days, which were not significantly different ($t(10) = 1.69, p = 0.122$). The day after rats reached criterion, they received an i.p. injection of 50 mg/kg atropine in saline vehicle in a volume of 1.0 ml/kg administered 30 minutes before testing. On the next day, that is, on the first post-injection day, rats were again tested without injections.

2.5. Experiment 2: Atropine and AMN effects on performance of phrased perfect and unphrased perfect serial patterns

All rats were trained using the same SMC task described above. Twelve naïve rats were randomly assigned to *phrased perfect* versus *unphrased perfect* pattern conditions:

Phrased perfect pattern: 123–234–345–456–567–678–781–812- (...repeat pattern)

Unphrased perfect pattern: 123234345456567678781812 (...repeat pattern)

where digits indicate the clockwise position of the correct levers on successive trials and dashes in the phrased perfect pattern indicate 3-s pauses that served as phrasing cues. All other intertrial intervals for both conditions were 1-s intervals. All rats were trained on the foregoing patterns for 50 patterns per day until they reached a criterion of no more than 10% errors on any element of the pattern. Rats in the phrased perfect pattern condition took a mean of 6.67 days to reach criterion whereas rats in the unphrased perfect pattern condition took a mean of 8.33 days, which were not significantly different ($t(22) = 1.29, p = 0.210$). Half of each pattern group was randomly assigned to atropine versus AMN groups. Beginning on the day after rats reached criterion, all rats received i.p. injections 30 min prior to testing on 6 consecutive days with injections of drugs or saline in the following order for all rats: saline (vehicle), 50 mg/kg atropine or AMN (according to group assignment), saline, 25 mg/kg atropine or AMN (according to group assignment), saline, and 100 mg/kg atropine or AMN (according to group assignment). It should be noted that rats received 50 mg/kg of either atropine or AMN as their first drug injection in Experiment 2 to facilitate comparisons of the results with those of Experiment 1 with the same dose.

3. Results

3.1. Experiment 1: Effects of atropine on performance of perfect and violation serial patterns with phrasing cues

The main results of Experiment 1 were that atropine injections impaired performance on chunk-boundary and violation elements, but largely spared performance on within-chunk elements of the pattern. The foregoing conclusions were based on the results of a pattern group X day X chunk of the pattern X element position within chunks (viz., a $2 \times 3 \times 8 \times 3$ design, where only pattern group was a between-subjects factor) analysis of variance (ANOVA) conducted on rats' daily mean trial-by-trial error rates for each element of the phrased perfect pattern and phrased violation pattern for the criterion day, injection day, and post-injection day, where pattern group was the only between-subjects factor. Main effects and interactions were considered significant if $p < 0.05$. The ANOVA revealed significant main effects for day of the experiment, $F(2,8) = 13.10, p = 0.003$, chunk of the pattern,

$F(7,28) = 2.40, p = 0.047$, and element position within chunks, $F(2,8) = 23.77, p < 0.001$. Significant interactions included pattern group $\times$ chunk, $F(7,28) = 2.44, p = 0.044$, day $\times$ element, $F(4,16) = 9.60, p < 0.001$, chunk $\times$ element, $F(14,56) = 3.45, p < 0.001$, pattern group $\times$ chunk $\times$ element, $F(14,56) = 4.33, p = 0.001$, day $\times$ chunk $\times$ element, $F(28,112) = 2.22, p = 0.002$, and pattern group $\times$ day $\times$ chunk $\times$ element, $F(28,112) = 3.50, p < 0.001$. No other significant main effects or interactions were indicated, including interactions involving both drug and phrasing conditions ($ps > 0.05$). Planned comparisons for comparing pattern element means were based on the appropriate error term from the ANOVA and were considered significant if $p < 0.05$.

Figure 1 shows rats' group mean percent error rates presented for each element of the phrased perfect pattern (Figure 1a) or the phrased violation pattern (Figure 1b), for the criterion day (the day before atropine injection), atropine injection day, and post-injection day. Breaks in the curves indicate the locations of pauses that served as phrasing cues between logical 3-element chunks. As shown in Figure 1a, on the atropine injection day rats in the phrased perfect pattern group made significantly more errors on chunk-boundary elements (element 1 of each 3-element chunk) than they did on the criterion or post-injection days ($ps < 0.05$). They also made more errors on these elements than for any other elements of their patterns ($ps < 0.05$). However, atropine did not increase error rates for within-chunk elements (elements 2 and 3 of chunks) compared to criterion and post-injection days ($ps > 0.05$), though some nonsignificant inflation of error rates was observed for within-chunk pattern elements. Examination of intrusions, that is, the kinds of errors rats made, on chunk-boundary elements showed that atropine-induced errors were not random. Although there are 7 possible incorrect choices rats could have made on any trial, in the phrased perfect pattern group whose data are shown in Figure 1a, atropine-induced intrusions at chunk boundaries (i.e., on the first element of each 3-element chunk) tended to be perseveration errors (repeating the last correct response, e.g., after 234, responding on lever 4 again), rule-overextension errors (e.g., after the 234 chunk, turning right to respond on lever 5 instead of turning left to lever 3), or "back 2" inaccuracy errors involving turning left as required but moving too many levers to the left (e.g., after the 234 chunk, correctly turning left but going too far to lever 2 instead of lever 3). For chunk boundaries of the phrased perfect pattern, perseverations accounted for approximately 50% of intrusions, rule-overextensions accounted for 33% of intrusions, and inaccuracy errors accounted for 9% of intrusions.

A similar pattern of results was observed for the phrased violation pattern group as shown in Figure 1b. On the atropine injection day rats in the phrased violation pattern group made significantly more errors on chunk-boundary elements (element 1 of each 3-element chunk) than they did on the criterion or post-injection days ($ps < 0.05$), much as the phrased perfect group did. In addition, rats in the phrased violation pattern group also made significantly more errors on the violation element, the last element of the pattern, than they did on the criterion or post-injection days ($ps < 0.05$). On the atropine injection day, rats in the phrased violation group made significantly more errors on the violation element than on any other element in their pattern ($ps < 0.05$). It is also interesting to note that the inflated rate of errors on the first element of the phrased violation pattern ("1" of the chunk, "123") was nevertheless a significantly lower rate than on any other chunk boundary of the phrased

violation pattern. Examination of intrusions on chunk-boundary elements of the phrased violation pattern revealed that perseverations accounted for approximately 55% of intrusions, rule-overextensions accounted for 27% of intrusions, and inaccuracy errors accounted for 14% of intrusions. The overall highest rate of atropine-induced errors was observed for the violation element, the last element of the phrased violation pattern. On the violation element, the most frequent intrusion was a rule-overextension; instead of responding 818, rats extrapolated the rule to produce 812. This rule-overextension error accounted for 88% of intrusions. Perseverations on lever 1 on the violation element accounted for 12% of intrusions, and no other type of intrusion appeared on the violation element during the criterion, atropine injection, or post-injection days.

Comparing atropine effects on performance of phrased perfect patterns and phrased violation patterns in Figures 1a and 1b, atropine-injected rats in both groups had significantly inflated errors for chunk-boundaries. However, the highest error rate on the atropine injection day was observed for the violation element in the phrased violation pattern; rats in the phrased violation group made significantly more errors on the violation element than on any other element in either the phrased violation pattern or the phrased perfect pattern ($p < 0.05$). In contrast, for both the phrased perfect and phrased violation pattern groups, error rates did not increase significantly for elements 2 and 3 of 3-element chunks compared to criterion and post-injection days, though nonsignificant inflation of error rates was observed for those pattern elements. For chunk boundaries of both the phrased perfect pattern and the phrased violation pattern, perseverations accounted for approximately 50% and 55% of intrusions, respectively, rule-overextensions accounted for 33% and 27% of intrusions, respectively, and inaccuracy errors accounted for 9% and 12% of intrusions, respectively. That is, atropine caused similar patterns of intrusion errors for chunk-boundary elements in the two pattern types. In contrast, for the violation element, rule-overextension errors accounted for 88% of intrusions, well over twice the proportion observed for chunk-boundary elements. Thus, performance on chunk-boundary elements (element 1 of each chunk) and the violation element was severely impaired by atropine, whereas performance on within chunk elements (elements 2 and 3 of each chunk) was largely spared by atropine. Because atropine increased the rate of errors at chunk boundaries without changing the relative proportions of the types of intrusion errors, the effect of atropine on chunk boundary performance can be characterized as a performance deficit rather than a shift in strategy. However, the different patterns of errors produced on chunk boundaries versus on the violation element suggest that performance under atropine was guided by different behavioral mechanisms for chunk-boundary elements and the violation element.

3.2. Experiment 2: Effects of atropine and AMN on performance of phrased perfect and unphrased perfect serial patterns

The first main result of Experiment 2 was that injections of AMN, which does not cross the blood-brain barrier, had no significant effect on pattern performance relative to saline injection. This conclusion was based on the results of a phrasing condition X drug group X drug manipulation (saline vs. drug injections) ANOVA (viz., a $2 \times 2 \times 2$ design), where phrasing condition and drug group were between-subjects factors. The ANOVA revealed

significant main effects of drug group, $F(1,20) = 10.12$, $p = 0.005$, and drug manipulation, $F(1,20) = 14.23$, $p = 0.001$, and a significant interaction of drug group x drug manipulation, $F(1,20) = 14.42$, $p = 0.001$. Planned comparisons for comparing means were based on the appropriate error term from the ANOVA and were considered significant if $p < 0.05$. Planned comparisons showed that atropine produced elevated error rates with respect to saline and AMN ($ps < 0.05$) and will be analyzed in greater detail below. Planned comparisons also showed that performance on saline injection days never differed between atropine and AMN groups ($ps > 0.05$), performance on later saline injection days never differed from the first saline injection day in either group ($ps > 0.05$), and AMN error rates were never significantly different compared to saline error rates ($ps > 0.05$). Thus, the results indicated that no drug carry-over effects were ever observed on the days after drug exposure. Thus, given that there were no carry-over drug effects observed on saline days and AMN error rates were never significantly different compared to saline error rates, data for AMN injection groups were used as controls for comparison to atropine injection groups in subsequent analyses reported below.

The second main result of Experiment 2 was that all 3 doses of atropine affected rats' errors in both phrased perfect patterns and unphrased perfect patterns relative to AMN injection controls. The foregoing conclusions were based on the results of a phrasing condition X drug group X dose X chunk of the pattern X element position within chunks ANOVA (viz., a $2 \times 2 \times 3 \times 8 \times 3$ design), where only phrasing condition and drug group were between-subjects factors. The ANOVA was conducted on rats' daily mean trial-by-trial error rates for each element of the phrased perfect pattern and unphrased perfect pattern comparing each atropine dose to the corresponding AMN dose. The ANOVA revealed significant main effects for drug, $F(1,20) = 12.24$, $p = 0.001$, and element position within chunks, $F(2,40) = 23.47$, $p < 0.001$. Significant interactions included drug x dose, $F(2,40) = 3.39$, $p = 0.044$, drug x element, $F(2,40) = 11.94$, $p < 0.001$, drug x dose x element, $F(4,80) = 2.61$, $p = 0.042$, and phrasing condition x dose x element, $F(4,80) = 3.45$, $p = 0.012$. No other significant main effects or interactions were indicated, including interactions involving both drug and phrasing conditions ($ps > 0.05$). Planned comparisons for comparing pattern element means were based on the appropriate error term from the ANOVA and were considered significant if $p < 0.05$.

Figure 2 shows rats' group mean percent error rates presented for each element of their phrased perfect pattern (Figure 2a) or the unphrased perfect pattern (Figure 2b), for the 3 drug-injection days. As before, breaks in the curves indicate the locations of pauses that served as phrasing cues between logical 3-element chunks. As shown in Figure 2a, for the phrased perfect pattern, all doses of atropine caused significantly inflated errors for chunk-boundary elements (element 1 of each 3-element chunk) in the already well-learned phrased perfect pattern compared to when rats received equivalent doses of AMN ($ps < 0.05$), but atropine did not significantly affect performance on within-chunk elements, that is, elements 2 and 3 of each 3-element chunk ($ps > 0.05$). The 50 mg/kg dose of atropine, the first dose rats experienced, caused higher error rates on all chunk boundary elements than did 25 and 100 mg/kg doses ($ps < 0.05$) that were administered later as the second and third atropine doses rats experienced, respectively. Examination of intrusion errors showed, as in

Experiment 1, that atropine-induced errors were not random. In the phrased perfect pattern group whose data are shown in Figure 2a, rats under atropine produced high rates of perseverations (repeating the last correct response, e.g., after 234, responding on lever 4 again), which accounted for 43–62% of intrusion responses across different doses of atropine. Atropine caused relatively fewer rule overextension errors (e.g., after the 234 chunk, responding on lever 5 instead of turning left to lever 3), accounting for only 5–16% of intrusions, and inaccuracy errors (e.g., after the 234 chunk, correctly turning left but going too far to lever 2 instead of lever 3), accounting for 17–33% of intrusions. These results thus paralleled those of the phrased perfect pattern group of Experiment 1 in that atropine in Experiment 1 caused relatively high rates of perseveration errors on chunk-boundary elements and lower rates of rule overextension and inaccuracy errors.

As shown in Figure 2b, for the unphrased perfect pattern, all doses of atropine caused significantly inflated errors for chunk-boundary elements (element 1 of each 3-element chunk) compared to when rats received equivalent doses of AMN ($p < 0.05$). Also, 50 and 25 mg/kg doses of atropine caused inflated errors for within-chunk elements (elements 2 and 3 of each chunk) compared to when rats received equivalent doses of AMN ($p < 0.05$). For 50 and 25 mg/kg doses of atropine, rats made more errors on element 3 of chunks than on element 2 for chunks 1–6 ($p < 0.05$). In the unphrased perfect pattern, all doses of atropine caused significantly higher error rates on all chunk-boundary elements (element 1 of chunks) than on both within-chunk elements in the same chunk ($p < 0.05$). Finally, atropine caused significantly higher error rates on element 3 of chunks than on element 2 in the same chunk ($p < 0.05$) in chunks 1–6. Examination of intrusion errors showed that in the unphrased perfect pattern group, at chunk boundaries rule overextension errors were the most frequent type of intrusion, accounting for 68–75% of intrusions across different doses of atropine, whereas perseveration errors accounted for only 2–33% of intrusions and inaccuracy “back 2” errors for only 3–5% of intrusions. Error rates were also inflated by the 50 and 25 mg/kg doses of atropine on elements 2 and 3 of chunks of the unphrased perfect pattern. These errors were most frequently anticipations of the chunk-boundary response to turn left (e.g., instead of 234, turning left prematurely on the second element to produce 21 or on the third element to produce 232). Anticipation errors accounted for 50–54% of intrusions on these elements of the pattern whereas perseverations accounted for only 29–41% of intrusions.

Comparing atropine effects on performance of chunk-boundary elements as a function of dose, as shown in the dose-response curves depicted in Figure 3, all atropine doses caused significantly increased errors on chunk-boundary elements (element 1 of each chunk) for both the phrased perfect pattern and the unphrased perfect pattern ($p < 0.05$) as shown in Figure 3a and 3b, respectively. In addition, 50 and 25 mg/kg doses of atropine also caused significantly increased errors on within-chunk elements (elements 2 and 3 of each chunk) for the unphrased perfect pattern ($p < 0.05$), an effect not observed in the phrased perfect pattern ($p > 0.05$). For the unphrased perfect pattern, atropine increased element 3 errors significantly more than element 2 errors for the 25 and 50 mg/kg atropine doses ($p < 0.05$). Because 25 and 50 mg/kg doses caused significantly inflated element 2 and 3 errors whereas 100 mg/kg atropine did not significantly increase element 3 error rates ($p > 0.05$), it can be

concluded that the effects of the later 100 mg/kg dose, which was the last dose rats experienced, were attenuated relative to the effects of the earlier 50 and 25 mg/kg doses that were the first and second drug exposures rats experienced, respectively. Finally, it should also be noted that 25 and 50 mg/kg doses of atropine caused significantly higher error rates on all elements of chunks in the unphrased perfect pattern compared to the corresponding elements in the phrased perfect pattern, whereas the 100 mg/kg dose of atropine caused significantly higher error rates only on element 1 of chunks in the unphrased perfect pattern compared to the corresponding elements in the phrased perfect pattern. A comparison of chunk-boundary intrusion errors observed for the phrased perfect pattern and the unphrased perfect pattern showed that perseverations accounted for approximately 43–62% versus 2–33% of intrusions, respectively, rule-overextensions accounted for 5–16% and 68–75% of intrusions, respectively, and inaccuracy errors accounted for 17–33% and 3–5% of intrusions, respectively. That is, atropine revealed very different patterns of intrusion errors for chunk-boundary elements in phrased and unphrased versions of the same pattern.

4. Discussion

A general goal of these two studies was to determine the extent to which muscarinic cholinergic receptor systems play a role in performance of well-learned highly-structured serial response patterns. The results clearly demonstrated that atropine produced significant impairments in performance in both experiments. In Experiment 1 where serial patterns had phrasing cues signaling chunk boundaries, atropine impaired performance in both patterns, both the phrased perfect pattern and the phrased violation pattern. However, impaired performance was restricted to chunk-boundary elements and the violation element; no impairment was observed for performance on within-chunk elements. In Experiment 2, atropine, but not AMN, impaired performance for chunk-boundary elements in the perfect phrased pattern, but spared performance on within-chunk elements in much the same manner as in Experiment 1. In the unphrased perfect pattern of Experiment 2, atropine caused impairments on all pattern elements, not just on chunk-boundary and violation elements. Thus, the general outcome was that atropine caused impairments that were selective to some but not all pattern elements in phrased patterns in both Experiments 1 and 2, but atropine-induced impairments were more generalized in the unphrased pattern in Experiment 2.

The most coherent explanation for the impairments observed in the current studies is that atropine impaired rats' ability to use the compound associative cues that would be required to anticipate chunk boundary elements and the violation element but spared rats' ability to use previously-learned abstract rules. This view depends on past research which showed that when rats learn to anticipate chunk-boundary elements they encode compound cues, where the stimulus compound they learn is composed of both the temporal pause itself and the serial position of the pause in the chunk (Muller & Fountain, 2010). This view also depends on past research showing that when rats learn to anticipate the violation element they encode compound cues, where the stimulus compound they learn is composed of multiple pattern elements preceding the violation element that uniquely predict it (Muller & Fountain, 2010). Evidence indicates that learning to anticipate within-chunk elements, on the other hand, depends on learning a motor program or abstract rule that is independent of external stimuli,

as shown by nearly perfect performance for within-chunk elements after transferring rats to a novel operant chamber (Muller & Fountain, 2010). In a well-learned pattern, removing or blocking phrasing cues or pattern element location cues causes rats to fail to anticipate chunk-boundary or violation element trials, but within-chunk element performance is not affected by cue-removal (Kunder & Fountain, 2010; Muller & Fountain, 2010). Thus, evidence supports the view that rats' ability to produce chunk-boundary and violation element responses in the SMC task depends on encoding and using multiple discriminative cues concurrently, but the ability to produce within-chunk element responses does not. The atropine-induced impairments in phrased perfect and phrased violation patterns in both experiments map onto this dichotomy well, with atropine producing a pattern of effects similar to cue removal in phrased patterns. Although error rates increased for some elements of phrased perfect and phrased violation patterns but not for other elements, atropine did not change the types and relative proportions of intrusion errors rats made on elements where error rates increased. To the extent that intrusion patterns reveal the underlying cognitive representation guiding rats' performance, we can conclude that atropine increased rats' errors on these pattern elements without appreciably altering rats' pattern-tracking strategy in phrased perfect and phrased violation patterns in both experiments.

The effects of atropine on performance for the unphrased perfect pattern of Experiment 2 differed greatly from effects observed on the 3 phrased patterns in both experiments. Prior research has supported the view that phrasing cues can bias serial pattern perception and encoding in both humans and rodents (Boltz & Jones, 1986; Bower, 1970; Fountain, 1990; Fountain et al., 2007; Restle, 1972). In the absence of phrasing cues in the unphrased perfect pattern of Experiment 2 where pattern structure was relatively more ambiguous (Fountain et al., 2007; Restle, 1972), atropine-induced impairments were observed on all pattern elements, not just on chunk-boundary and violation elements. Without unique phrasing cues to differentiate chunk-boundary elements from within-chunk elements, rats learning the unphrased perfect pattern had to depend more on acquiring multiple-element compounds to cue differentially forthcoming pattern elements. That is, rats in the unphrased condition would have to rely more on discriminative control than rats in the structurally less ambiguous phrased perfect and phrased violation patterns, especially for within-chunk elements (elements 2 and 3 of chunks). The analysis of rats' intrusion errors were consistent with this view in that while under atropine, rats in unphrased perfect pattern and phrased perfect pattern groups produced a different distribution of perseverations, rule-overextensions, and inaccuracy intrusion errors at chunk boundaries compared to that observed under all phrased patterns. Given the powerful effects of phrasing on serial pattern perception and encoding, it is not clear whether atropine simply revealed encoding differences caused by differences in phrasing during acquisition (prior to atropine exposure) or instead that atropine caused a shift in pattern tracking performance through an interaction with the phrasing manipulation at the time of atropine treatment. Unfortunately, within-group comparisons of intrusion rates on control versus atropine injection days which would be helpful in answering this question were not possible due to the very low rate of intrusions of any sort on control injection days. Future work examining acquisition rather than performance of phrased and unphrased patterns with daily control versus atropine injections may help clarify the role of atropine in these unphrased perfect pattern results. What is clear,

however, is that atropine-treated rats' performance of the phrased perfect pattern and unphrased perfect pattern was not equivalent; that is, atropine did not simply reverse the effects of prior training with phrasing cues; if it had, performance for the phrased perfect pattern and unphrased perfect pattern would have been identical under atropine, but they were clearly different.

The foregoing view that atropine impairs discriminative control by compound cues is also appealing because it provides an explanation for two important features of the results. First, why did atropine-treated rats have greater difficulty anticipating the violation element compared to other elements? According to the hypothesis that atropine-treated rats had impaired discriminative control, on the violation element treated rats should fail to anticipate the violation and instead extrapolate the pattern according to the spared rule common to all other chunks, namely, "+1" or move "one receptacle to the right." This would produce high frequency intrusion errors at receptacle "2" on the violation element, a strong effect observed in the phrased perfect pattern. Second, why did atropine-treated rats have less difficulty anticipating chunk-boundary elements than violation elements? According to the hypothesis that atropine-treated rats had impaired discriminative control, the violation element should produce very high error rates because it is not predicted at all by pattern structure alone. In contrast, rats' with a spared ability to use pattern structure should have been able to partially anticipate chunk-boundary elements based on higher-order pattern structure relating chunks (Fountain & Rowan, 1995b) even though phrasing cue discriminative control would have been impaired. The result would be somewhat better performance at chunk-boundary elements than at the violation element, as was observed. The results thus fit with prior evidence indicating that rats concurrently encode both S-R (associative) and rule-based information from serial patterns and that different brain systems underlie these behavioral systems (cf. Fountain, 2006; Fountain et al., 2012).

Alternative hypotheses to account for these data can be entertained. Earlier work with another widely-used sequential behavior task, the repeated acquisition task, suggests that the muscarinic antagonist scopolamine causes rats to "skip" elements in learned sequences of behavior (e.g., Cohn, Zirias, Cox, & Cory-Slechta, 1992). In the data reported for the phrased violation pattern in Experiment 1, the most common error on the violation element was "2," which cannot be due to a tendency of the rat to "skip" a trial in the pattern, 123–234–345–456–567–678–781–818, where the violation element is underlined. It should also be noted that from an associative perspective, "2" errors on the violation should be infrequent since "1" is followed by "2" only once in the pattern but it is followed by "8" twice. Associative mechanisms thus predict low levels of errors on the violation element because "8" would be a correct response, yet "2" errors were observed as greater than 80% of responses on the violation element under atropine challenge. Finally, the fact that rats performed well under atropine on the trial immediately before the violation and with relatively few errors on the last element of other chunks suggests that the deficit was neither generalized nor due to the rat's "losing track" of its position within the pattern. Instead, the results fit better with the view that high frequency "2" errors were the result of control by the spared rule-based system, whereas failures to perform the correct violation element response and chunk-boundary element responses, where performance has already been shown to depend on S-R associations (cf. Kundey & Fountain, 2010; Muller & Fountain,

2010; Stempowski et al., 1999), were the result of impaired production of responses based on previously-learned S-R associations.

The basis of the inverted U-shaped dose-response function observed in Figure 3 is not known and was not explicated in this study. In fact, those results may not accurately depict the actual function for several reasons. First, the Experiment 2 dose-response data were collected in a within-subjects daily-injection design with the 3 atropine doses presented in the same order for all rats rather than following the more traditional method of presenting the doses in counterbalanced order across rats. We chose to present doses in a fixed order with the 50 mg/kg dose first for all rats in Experiment 2 to facilitate comparisons to Experiment 1, where a 50 mg/kg dose was the only dose rats experienced. Second, it is also the case that the dose-response data may not accurately depict the actual function because of the possibility that atropine might accumulate over time or affect receptor sensitivity with repeated exposures. It should be noted that data from other studies suggest little tolerance or similar effects should be expected (cf. Whishaw, 1989). In addition, our failure to detect changes in performance on saline injection days between atropine exposures suggest that such effects were small at best, but such effects cannot be rule out. However, it is not unusual to observe an inverted U-shaped function of learning or performance effects with muscarinic anticholinergic drugs such as atropine and scopolamine (e.g., Carnicella et al., 2005a, 2005b; Rasmussen & Fink-Jensen, 2000; Stewart & Blain, 1975), and such effects have been attributed to drug effects on cortical arousal measured by EEG and concomitant cognitive arousal that align with the Yerkes-Dodson law (Graef, Schoknecht, Sabri, & Hegerl, 2011).

The muscarinic cholinergic system plays a role in a number of brain, behavioral, and cognitive systems, including reward systems, attention, behavior sequencing, response timing, working memory, and other forms of memory, and executive function (Graef et al., 2011; Meck & Church, 1987; Singh, Desiraju, & Raju, 1997; Willingham, 1999). Most, if not all, of these systems are likely relevant to rats' serial pattern performance in the SMC task. Thus, there are several alternative accounts that need to be addressed. For example, perhaps atropine impaired performance in the SMC task in the present study by reducing the reinforcing value of BSR (e.g., Singh et al., 1997), though this idea suggests a more general effect that would not account for spared performance on within-chunk elements or differential effects on the violation element versus chunk-boundary elements with the violation pattern. Perhaps atropine impaired serial pattern performance in the SMC task by interacting with perception of temporal intervals between trials or using temporal intervals as discriminative cues (e.g., Meck & Church, 1987; Meck, Church, Wenk, & Olton, 1987). This idea would not account for poor performance on the violation element and unphrased chunk boundaries which followed short intertrial intervals and, in the case of the violation element, has been shown not to depend on timing or serial position in the phrased violation pattern (Muller & Fountain, 2010). Thus, follow-up work should also examine the effects of muscarinic antagonists on rats' ability to anticipate violation elements in different positions in the serial pattern, especially given that our research shows that anticipation of a violation element depends on encoding serial position information if the element occurs earlier in a pattern but depends solely on discriminative control by S-R associations later in patterns (Kundey & Fountain, 2010; Muller & Fountain, 2010). Such a manipulation might reveal

performance impairments early in the pattern due to impairments in timing or serial position coding (Meck & Church, 1987; e.g., Meck et al., 1987). In addition, although atropine did cause rats to make perseveration errors, consistent with the tendency of muscarinic antagonist drugs to increase some types of perseveration (e.g., Giardini, Amorico, De Acetis, & Bignami, 1983; Ragozzino, 2003; Soffie & Lamberty, 1987) but not others (e.g., Heise et al., 1975), this was not the most frequent type of error for any pattern element type. Nonetheless, although none of the foregoing ideas alone accounts for the results of the current experiments, that is not to say that they played no role or that they would not play a role in somewhat different patterns. The role of these factors in rats' performance in this very complex serial pattern learning and performance task, designed as a rat model of similarly complex cognitive tasks in humans, deserves further scrutiny. The extent to which the observed effects are due to impairment of attention, working memory failure related to using compound cues concurrently, memory impairment such as retrieval failure, or impairment in motor or cognitive sequencing is still to be determined.

It is interesting to note that in an earlier study a systemic injection of 0.0625 mg/kg MK-801, an *N*-methyl-D-aspartate (NMDA) receptor antagonist, produced similar impairments of performance to those reported in Experiment 1 above for rats performing the same phrased violation pattern (Fountain & Rowan, 2000, Experiment 2). That is, although the observed effects were smaller, MK-801 treatment impaired anticipation of chunk-boundary elements and the violation element in a well-learned serial pattern much as atropine did in the present studies. Given the correspondence between rats' performance deficits under atropine, a muscarinic cholinergic blocker, and MK-801, an NMDA glutamatergic blocker, perhaps it would be worthwhile to examine possible interactions between cholinergic and glutamatergic systems as a possible factor in the observed performance deficits in the studies reported here. Similarly, given that MK-801 produces profound acquisition deficits for the violation element and chunk-boundary elements in the SMC task used here, a parallel acquisition study with muscarinic receptor blockade should be undertaken for comparison.

More work is needed to better characterize the processes involved in performance of highly-structured patterns in the SMC task, with particular attention to the distinction between responses guided by associative discriminative control versus responses guided by rules. Such work would be strongly motivated by a significant body of prior behavioral research that has appealed to this distinction repeatedly in the rat serial pattern learning literature, specifically paralleling analogous theoretical concerns and assertions in the human serial pattern learning literature. Whereas the hunt is still on for clues to the neural basis of rule learning and rule-based performance in this paradigm – that is, for the functions that were not impaired by atropine – the results of the current study suggest that future experiments with the SMC task might profitably be directed toward assessing the differential contributions of distinct cholinergic neural systems that may collectively or independently contribute to discriminative control in serial pattern performance.

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Highlights

- Two experiments examined atropine effects on rats' performance of serial patterns.
- Atropine, but not atropine methyl nitrate, impaired serial pattern performance.
- Atropine impaired serial pattern performance dependent on using associative cues.
- Atropine spared rule-based serial pattern performance.

Fig. 1.

Rats' group mean element-by-element percent error rates for (a) the phrased perfect pattern and (b) the phrased violation pattern on criterion day (open diamonds), the 50 mg/kg atropine injection day (filled squares), and the post-injection day (open triangles).

Fig. 2.

Mean element-by-element percent error rates for (a) the phrased perfect pattern and (b) the unphrased perfect pattern on days rats received either atropine (filled symbols) or AMN (open symbols) with drug doses that were presented in the order: 50 mg/kg (squares), 25 mg/kg (triangles), and 100 mg/kg (circles).

Fig. 3. Dose response curves as mean percent error rates as a function of atropine dose for elements 1, 2, and 3 (circles, triangles, and squares, respectively) of 3-element chunks in the (a) phrased perfect pattern and (b) unphrased perfect pattern.