The present study examined the effects of systemically administered atropine sulfate, a muscarinic cholinergic antagonist, on a series of probe tests in the retention of a highly-structured serial pattern in a serial multiple choice (SMC) task. Rats were trained on a 24-element pattern composed of eight 3-element chunks ending with a violation element: 123-234-345-456-567-678-781-81 where the digits represent the clockwise position of levers in an octagonal chamber, dashes indicate 3-s pauses termed “phrasing cues,” and other intertrial intervals were 1 s. In daily acquisition trials rats were given either 50 mg/kg of atropine sulfate or an equivalent amount of saline (Chenoweth & Fountain, 2015). Following acquisition, rats were given a series of drug challenges, and the present study reports a series of Phrasing Cue Removal Probes that tested rats’ abilities to make correct responses in the absence of phrasing cues. Rats tested under atropine demonstrated more difficulty in recalling encoded responses in these probe trials than did rats tested under saline. The results indicate that intact central muscarinic cholinergic systems were needed for rats to display efficient adaptive response strategies under conditions where some features of the previously-learned pattern change.

Evidence has established the importance of temporal phrasing cues in facilitating the learning of sequential patterns in rats (Fountain, Benson, & Wallace, 2000; Fountain, Henne, & Hulse, 1984; Fountain, Rowan, & Carman, 2007; Muller & Fountain, 2010; Stempowski, Carman, & Fountain, 1999; for review see Fountain et al., 2012). A temporal phrasing cue is a pause between sets of discrete events which typically serves as a discriminative cue contributing to chunking of sequential patterns (Fountain et al., 1984). Further, if rats trained with temporal phrasing cues are later presented the same pattern without phrasing cues or with cues shifted to other points in the pattern, performance is severely disrupted (Fountain et al., 2000; Muller & Fountain, 2010, 2016; Stempowski et al., 1999). Recently, we reported on the role central muscarinic cholinergic systems play in the acquisition (Chenoweth & Fountain, 2015) and performance (Fountain, Rowan, & Wollan, 2013) of sequential patterns in the serial multiple choice (SMC) task. In this task rats are presented with a circular array of 8 levers on which they learn to respond in a sequential pattern. The typical pattern consists of 24 elements:

123-234-345-456-567-678-781-81

where the numerals represent the position of the levers (clockwise), dashes represent 3-s temporal pauses, or “phrasing cues,” that divide the pattern into eight 3-element chunks. All other intertrial intervals (ITIs) are 1 s. Each chunk begins with a “chunk-boundary” element, with the remaining elements in the chunk termed “within-chunk” elements. The last item of the pattern (8) is designated as a “violation” element as it violates the rules set forth earlier in the pattern, i.e., turn left after a phrasing cue.

Using this paradigm, Chenoweth and Fountain (2015) established that intact central muscarinic cholinergic systems are critical in acquiring and maintaining the correct discriminative responses required at chunk-boundaries. Further, Chenoweth and Fountain (2015) were able to replicate the finding by Fountain et al. (2013) that atropine impairs performance at the chunk-boundary elements of a well-learned pattern. The present study sought to determine the extent of the role of central muscarinic cholinergic systems in performance following the removal of phrasing cues if rats acquired the pattern while treated with atropine.

In the present study, rats from the prior Chenoweth and Fountain (2015) study received two types of probe patterns inserted into the training procedure. The “Extended Chunk Probe” presented a simple pattern: 12345678, with a 1-s ITI throughout the pattern. The “Phrasing Cue Removal Probe” presented the Training Pattern with the exception that all ITIs throughout the
pattern were 1 s. In both probes, the element of interest was in Serial Position 4 (SP4), the first element following a phrasing cue in the Training Pattern. Based on prior research, it was expected to see performance disrupted at chunk-boundary elements in our control group (Fountain et al., 2000; Muller & Fountain, 2010, 2016; Stempowski et al., 1999). However, for the atropine-treated group with already impaired performance at these transitions, this manipulation also provided us with a unique opportunity to further examine the effects of a central muscarinic cholinergic antagonist on performance and response strategy on an uncued chunk-boundary that had been previously cued.

Subjects. Eleven Long Evans hooded rats (Rattus norvegicus) previously used in Chenoweth and Fountain (2015) were at least 90 days old at the time of bipolar electrode implant (MS301, Plastics One, Roanoke, VA; coordinates, skull level: 4.5 mm posterior, 1.5 mm lateral, 8.5 mm below skull surface, Paxinos & Watson, 2005) surgery served as subjects. Rats were individually housed with free access to food and water in their home cages throughout the experiment. They were kept on a 15:9-h light–dark cycle with testing during the light cycle. All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Kent State University Institutional Animal Care and Use Committee.

Apparatus. Four octagonal test chambers (40 cm between parallel walls × 30 cm tall; Fountain & Rowan, 1995a, 1995b) were constructed of clear Plexiglas with a response lever mounted on each wall and a commutating device located centrally on the ceiling of the chamber, and a floor of stainless steel hardware cloth. Rats were connected to the stimulator via a flexible cord (Plastic Products MS304) attached to the commutator. Chambers were located in pairs separated by a melamine board in two separate rooms and controlled from an adjoining room with a microcomputer and interface (Med Associates, Inc., Fairfield, VT). All test chambers were monitored using closed circuit cameras mounted above the chambers.

Drugs. Atropine-treated rats received intraperitoneal (i.p.) injections of 50 mg/kg atropine sulfate (Sigma) dissolved in bacteriostatic water in a volume of 1.0 ml/kg. Saline-treated rats received i.p. injections of an equivalent volume of saline. All injections were given 30 min prior to daily training and probe sessions.

Procedure. Rats received 1–3 250-ms BSR pulses of a 60-Hz sinusoidal pulse train from a constant current source of 20–80 μA for correct responses throughout the experiment. Rats successfully shaped to lever press were then trained on the serial pattern per the procedures described by Chenoweth and Fountain (2015). Rats were then trained on a discrete trial procedure with correction for a 24-element pattern with a violation (123–234–345–456–567–678–781–812–), and were required to produce 50 patterns a day for 12 daily sessions. Once rats in the control group had reached a criterion of no more than 10% errors on any element type within a daily session, they then received one day each of drug challenge transfers in which atropine-treated rats (N = 6) were tested without atropine and saline-treated rats (N = 5) were tested with atropine (Chenoweth & Fountain, 2015). Following the drug challenges, rats returned to their original training procedure and drug condition to establish the return of baseline performance. Note that saline control rats experienced 3 more sessions of training than atropine-exposed rats during the previous experiment.

In the current study, the same rats then experienced the addition of one of two probes inserted into the training procedure every 5 patterns (i.e., 5 Training Patterns, 1 Probe Pattern, etc.), for a total of 55 Training Patterns and 10 Probe Patterns each day for two consecutive days. On Day 1 rats experienced the Extended Chunk Probe Pattern (12345678–), and on Day 2 rats experienced the Phrasing Cue Removal Probe Pattern (123234345456567678781818–), where digits represent the levers numbered clockwise in the chamber, and all ITIs were 1 s. The interpattern interval between the end of the Training Pattern immediately preceding and the Probe Pattern was the same as between Training Patterns (3 s), therefore a rat would not know that it was in a probe trial until it reached the serial position where a phrasing cue would have occurred in the Training Pattern. The location of the first lever response rats produced was recorded for each trial. In both probes the element of interest was in Serial Position 4 (SP4), the first element that followed a phrasing cue in the Training Pattern.

**Atropine produced more errors in the Extended Chunk Probe.** Fig. 1 shows the mean percentage of errors for atropine-treated and saline-treated rats on the Extended Chunk Probe Pattern compared to performance on the Training Pattern, revealing increased errors at SP4 in both groups in the Extended Chunk Probe. A group × pattern × element (viz., a 2 × 2 × 8 design, where group was the between-subjects factor) mixed-design analysis of variance (ANOVA) was conducted on rats’ mean number of errors, pooled across all 10 Extended Chunk Probe Patterns and all 50 Training Patterns from the last day of acquisition. Main effects and interactions were considered significant if p < 0.05. Significant main effects were found for pattern, F(1, 9) = 26.60, p = 0.001, and element, F(7, 63) = 15.92, p = 0.001, and a marginal main effect was found for group, F(1, 9) = 4.94, p = 0.053. Significant interactions were found for Group × Element, F(7, 63) = 8.04, p < 0.001, Pattern × Element, F(7, 63) = 13.54, p < 0.001, and Group × Pattern × Element, F(7, 63) = 2.38, p = 0.032. Fisher’s Least Significant Differences tests indicated all rats produced a significantly higher number of errors at SP4 in the Extended Chunk Probe than in the Training Pattern, with atropine-treated rats having produced significantly more errors than saline-treated. An examination of the types of errors made revealed that atropine-treated rats were more disorganized in their responses than saline-treated rats (see Table 1).

**Atropine produced more errors in Phrasing Cue Removal Probe.** Fig. 2 shows the mean percentage of errors for atropine-treated and saline-treated rats on the Phrasing Cue Removal Probe Pattern compared to performance on the Training Pattern, and revealed similar differential responses to the Phrasing Cue Removal Probe. A group × pattern × chunk × element (viz., a 2 × 2 × 8 × 3 design, where group was the between-subjects factor) mixed-design ANOVA was conducted on rats’ mean percentage of errors, pooled
One particularly relevant finding by Muller and Fountain (2010, 2016) was that when chunks were longer than rats expected from prior training, rats increased errors at the serial position within the chunk where they had experienced a chunk-boundary element in the original Training Pattern. Thus, even when phrasing cues were not encountered, rats used timing or counting processes to anticipate where the chunk-boundary would occur. Similarly, Muller and Fountain (2016) trained rats on serial patterns composed of 3-element, 4-element, or 5-element chunks with terminal violation elements and showed results similar to those reported here in the same Extended Chunk Probe Pattern test. In each case, even though no phrasing cue was presented in the probe trial at the proper location, rats still showed significantly higher rates of left-turn responses that anticipated the prior location of the first chunk-boundary. Taken together, the results of the current study are consistent with those of Muller and Fountain (2016) and indicate that in the present study rats were able to use timing or counting processes to anticipate the location of a former chunk-boundary trial in an uncued pattern, even under the influence of atropine. The latter conclusion, that atropine does not block timing/counting processes used for anticipating an uncued chunk-boundary element, is consistent with other research showing that exposure to cholinergic antagonists such as atropine slow the internal clock in temporal interval timing estimation in rats but do not block timing processes (Meck, 1996).

The results for the saline-treated rats in the probe conditions used in this experiment were not surprising given the prior research showing a disruption in performance following phrasing cue removal but evidence of some anticipation of chunk-boundary elements even when uncued (Fountain et al., 1984; Muller & Fountain, 2010, 2016; Stempowski et al., 1999). However,
the further impairment observed in the atropine-treated group indicates that atropine-treated rats partially encoded the phrasing cues as discriminative cues marking a change in the pattern, namely, a chunk-boundary. Thus, both groups showed evidence of encoding the serial positions at which the phrasing cues occurred, as evidenced by the increased number of errors at SP4 when the phrasing cues were removed as compared to when those cues were present in the Training Pattern. However, the types of errors committed at chunk-boundaries when the cues were removed indicate that saline-treated rats were more strategic compared to atropine-treated rats in their responses in probe trials in which phrasing cues were removed. Saline-treated rats responded strategically by limiting their responses to a range of locations demarcated by lever locations to the left and right of the last correct response, whereas atropine-treated rats failed to do so.

This is not the first report of an inability to develop and use effective response strategies due to atropine treatment in rats. Similar conclusions were drawn by Whishaw and colleagues after observing rats in a place navigation task (Sutherland, Whishaw, & Regehr, 1982; Whishaw, 1985). Atropine-treated rats were shown to use a less-efficient strategy, learning “how” to make the correct response rather than the more efficient strategy of learning “where” to make the correct response as saline-treated rats did. Mendez, Gilbert, Bizon, and Setlow (2012) found that atropine reduced rats’ ability to make decisions in a cost-benefit decision making task. Mendez et al. (2012) attributed this deficit to an increase in impulsive choice rather than on task motivation. Thus, responses of atropine-treated rats appeared impulsive rather than strategic. It is possible that the results we observed that suggested rats were less strategic when confronted with ambiguous cues may be related to a more general effect of atropine on discrimination, attention, or impulsivity. Further psychopharmacological research should examine this question more closely in the current paradigm.

An alternative hypothesis is that atropine disrupted probe trial performance in the current study, which was manifested as less strategic response choices, by interfering with pattern completion processes previously shown to be dependent on hippocampal function (Hoang & Kesner, 2008). Although pattern completion processes would seem to be necessary for completing or, in our terms, “extrapolating” (cf. Fountain & Hulse, 1981) a known sequence to predict unexpected pattern elements, it is not clear how this idea accounts for the rats’ specific strategy and error patterns observed in the current experiment. Additional work is necessary to elucidate the neurobiological basis of the results reported here to determine what role hippocampal function plays in what we have described as strategic response choices.

The evidence from the current study indicates that when rats performing well-learned serial patterns were faced with probe trials in which phrasing cues were removed, saline-treated rats responded strategically by limiting their responses to a range of locations demarcated by lever locations that had previously been correct, whereas atropine-treated rats failed to do so. However, these data represented only one day of testing with each of two probes. Data from acquisition, as presented by Chenoweth and Fountain (2015), indicated that atropine-treated rats were capable of learning to make correct responses pattern structure changes when those locations were consistently cued, albeit at an impaired rate of acquisition compared to saline control rats. Therefore, one important question is whether a central muscarinic cholinergic antagonist may impair but not entirely block acquisition of efficient strategies in sequential learning when training is extended.

Acknowledgments

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References


