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RESEARCH NOTE

Adolescent exposure to nicotine impairs adult serial pattern learning in rats

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Abstract In the present study investigating the effects of adolescent nicotine exposure on adult serial pattern learning, adolescent rats received daily i.p. injections of either 1.0 mg/kg nicotine or saline for 5 days per week for 5 weeks beginning on postnatal day 25 (P25), then were allowed 35 days drug free. Rats then began training on P95 as adults on a 24-element serial pattern composed of eight 3-element chunks. Adolescent exposure to 1.0 mg/kg nicotine produced persistent retardation of learning for the first element of each 3-element chunk of the pattern, that is, for chunk boundary elements, and transient retardation of learning for elements 2 and 3 of each chunk of the pattern, that is, for the within-chunk elements. Deficits at chunk boundaries were interpreted as deficits of phrasing cue discrimination learning whereas deficits for learning responses for elements within-chunks (elements 2 and 3 of chunks) were interpreted as deficits of rule learning. These results indicate that the effects of adolescent nicotine exposure on adult learning and cognitive capacity deserve further scrutiny.

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Introduction

Recent research in animal models has shown that adolescent nicotine exposure can cause long-lasting changes in brain physiology, brain reward systems, susceptibility to later addiction, and simple behavior (e.g., Abreu-Villaca et al. 2003a, b; Kelley and Middaugh 1999; Kelley and Rowan 2004). Animal models of the effects of adolescent nicotine exposure on neural and behavioral functioning in adulthood have been used to characterize the effects of adolescent drug exposure on a broad range of biological and behavioral processes including genetic expression, apoptosis, synaptogenesis, cell replication, receptor expression in neurotransmitter systems, and the functional programming of simple behavioral responses (Abreu-Villaca et al. 2003a, b; Kelley and Middaugh 1999; Kelley and Rowan 2004; Trauth et al. 1999a, b, 2000a, b, c, 2001). Animal adolescent exposure protocols have been used to study the effects of a variety of drugs including nicotine, alcohol, and cocaine, to name but three common drug threats for adolescents, though almost no work has examined the effects of adolescent nicotine exposure on animal models of complex learning. The research described in this article employed a serial pattern learning paradigm for studying rat complex cognitive processes that is tightly modeled after nonverbal paradigms for studying higher-level cognitive functions in humans. This task can be used to study higher-level cognitive functions because the rule-based serial patterns that rats are required to learn in this paradigm appear to recruit

both simpler stimulus-response (S-R) learning processes and more abstract rule learning processes that appear to be employed concurrently (Fountain and Rowan 2000; Stempowski et al. 1999; Fountain 2006; Fountain and Benson 2006; Fountain et al. 2007). In this article, we applied the serial pattern learning method to detect cognitive dysfunction in adult rats that earlier received adolescent exposure to nicotine.

The adolescent exposure paradigm that we used in the present study has been used by our laboratories and others to study the effects of adolescent nicotine exposure on adult neurobiology, motivation, and reward, and simple forms of behavior (Abreu-Villaca et al. 2003a, b; Kelley and Middaugh 1999; Kelley and Rowan 2004; Trauth et al. 1999a, b, 2000a, b, c, 2001). In this procedure, weanling rats are exposed daily to nicotine for several days to weeks (Kelley and Middaugh 1999; Kelley and Rowan 2004; Trauth et al. 2000a). In the present study, we used the same 5-week adolescent exposure period that has been used in prior studies in rodents (Kelley and Rowan 2004; Kelley and Middaugh 1999). This exposure period is followed by 5-week of no exposure to the drug, which is followed by assessment of long-lasting changes due to adolescent exposure. The 5-week no-drug period allows the drug to completely clear the rat's system so that any changes in the exposure group relative to controls indicate a long-lasting developmental effect of the drug rather than direct effects of the drug itself. Early exposure to nicotine by such methods causes alterations in serotonergic, dopaminergic, noradrenergic, and cholinergic systems (Abreu-Villaca et al. 2003a, b; Kelley and Middaugh 1999; Trauth et al. 1999a), though almost no work has examined the effects of adolescent nicotine exposure on animal models of complex learning.

Serial pattern learning is the process of learning to organize behavior through time, typically by learning to anticipate or respond to a sequence of events or to learn to properly organize a sequence of behavior. Recent studies from our labs show that serial pattern learning recruits multiple learning and cognitive systems concurrently (Fountain 2006; Fountain and Benson 2006; Fountain and Rowan 2000), so this general behavioral paradigm is sensitive enough to detect neurobehavioral dysfunction and sophisticated enough to characterize the nature of the cognitive deficits caused by the biological challenge (Fountain 2006; Fountain and Benson 2006; Fountain and Rowan 2000).

Our method for studying serial pattern learning in rats is a functional analogue of nonverbal human pattern learning tasks that require subjects to learn to choose items from an array in the proper sequential order, thus producing a repeating response pattern (e.g., Nissen et al. 1987; Reber 1989; Restle 1972, 1973; Restle and Brown 1970). In our method, rats learn to perform complex serial patterns that involve choosing manipulanda in a circular array in the proper sequential order. Rats are trained in an octagonal Plexiglas box equipped with either nose poke receptacles or retractable levers, one mounted on each wall (Fountain and Rowan 1995a, b). In the procedure requiring nose poke responses used in the present study, each of the eight receptacles is equipped with a light and all eight receptacle lights are illuminated at the beginning of each trial. A water-deprived rat may then choose (i.e., nose poke) any of the eight receptacles. The task for the rat is to learn to choose the eight receptacles in the proper sequential order for water reinforcement on the successive trials that comprise the serial pattern. The first choice on each trial is recorded as correct or incorrect. Correct responses are rewarded and incorrect responses are corrected via a forced-choice procedure. The measure of interest is how often rats are correct on their first choice in this 8-choice task on each of the 24 trials that comprise each serial pattern as rats acquire the task over many repetitions of the 24-element serial pattern.

The present study examined the effects of adolescent exposure to 1.0 mg/kg nicotine for 5 weeks, a daily dose that rats have been shown to self-administer in both intravenous and oral self-administration models (DeNoble and Mele 2006; Glick et al. 1996; Valentine et al. 1997). In the present study investigating the effects of adolescent nicotine exposure on adult serial pattern learning, adolescent rats received daily i.p. injections of either 1.0 mg/kg nicotine or saline for 5 days per week for 5 weeks beginning on postnatal day 25 (P25), then were allowed 35 days drug free in an analogue of a nicotine exposure method used previously to study adolescent nicotine exposure effects on adult cocaine sensitivity in mice (Kelley and Middaugh 1999; Kelley and Rowan 2004). Rats then began training on P95 as adults on a 24-element serial pattern composed of eight 3-element chunks, 123-234-345-456-567-678-781-812, where digits indicate the clockwise position of the correct response on successive trials and dashes indicate brief pauses that serve as "phrasing cues." It should be noted that "8" is adjacent to "1" in the circular array, so that a 7-8-1 or 8-1-2 sequence of responses requires the same direction of movements on successive trials as a 1-2-3 sequence. Nicotine-exposed and control rats were trained on five patterns per day for 21 days in this serial pattern learning paradigm. The results were expected to provide new information regarding the extent to which adolescent nicotine exposure is a threat to adult cognitive capacity.

Method

Subjects and adolescent drug administration

This experiment was conducted in accordance with the "Principles of laboratory animal care" (NIH publication

No. 86-23, revised 1985). The 14 Long Evans male rats were received on postnatal day 21 (P21) and were individually housed in stainless steel hanging cages throughout the experiment with free access to food and water.

After a 3-day acclimation period, rats were randomly assigned to two treatment groups. Beginning on P25, eight rats received intraperitoneal injections of 1.0 mg/kg nicotine bitartrate (Sigma Chemical, Saint Louis, MO) expressed as the weight of the free base and six received saline vehicle as 0.01 ml/g body weight. Subjects were weighed and injected Monday through Friday for 5 weeks and then had 35 days drug free prior to testing as adults.

Apparatus

Four Plexiglas shaping chambers (30 × 30 × 30 cm) had stainless steel mesh floors and a single nose poke receptacle 5 cm above the floor on one wall. Nose poke receptacles were 3.0-cm diameter PVC pipe end caps painted flat black with infrared emitter-detector pairs mounted on the sides and a cue light in the rear. A solenoid (General Valve Corp., 20 psig., 24 V) was attached by tubing to a water opening at the bottom of the receptacle. A 20 ml syringe served as a water reservoir.

Four clear Plexiglas test chambers were octagonal in shape (15 cm wide × 30 cm tall walls with 40 cm between opposite walls) with a stainless steel mesh floor. One nose poke receptacle described above was centered 5 cm above the floor on each chamber wall. Each shaping chamber and test chamber was housed in a separate particleboard sound attenuating shell.

Procedure

Rats were water deprived for 48 h before initial nose poke training. After every second day of training, rats drank freely until satiated (about 5 min). In 1-h nose poke shaping sessions, the receptacle light was illuminated at the beginning of each trial and, when the rat responded, was extinguished and a water droplet delivered. A 1-s intertrial interval separated trials. Training continued until each rat responded 240 times for two consecutive days.

During the testing phase, at the beginning of each trial, all eight receptacle lights were illuminated. If the rat's first response on a given trial was at the correct receptacle, the response was recorded as correct, all receptacle lights were extinguished, and a droplet of water was administered. If instead the rat's first response on a trial was an incorrect response, the response was recorded as incorrect for that trial. In the correction procedure that followed incorrect responses, only the correct receptacle light was illuminated and water reward was administered only after the rat chose the correct receptacle. This correction procedure assured that rats received feedback regarding the correct response on each trial. All rats learned the same 24-element (trial) serial pattern of responses composed of eight 3-element "chunks":

123-234-345-456-567-678-781-812- (…repeat pattern)

where digits indicate the clockwise position of the correct receptacle response on successive trials and dashes indicate brief pauses that served as "phrasing cues." An intertrial interval of 1 s was imposed between elements within 3-element chunks and 3 s phrasing cues were positioned at transitions between chunks and as the inter-pattern interval between patterns. Rats were tested on five patterns per day for 21 days.

Results

As shown in Fig. 1, correct choice data showed that adolescent exposure to 1.0 mg/kg nicotine produced persistent retardation of learning for the first element of each 3-element chunk of the pattern and transient retardation of learning for elements 2 and 3 of each chunk of the pattern. Response latency data were not analyzed because drinking time and variable 1-3-s intertrial intervals were interposed between successive trials in this paradigm. An analysis of variance (ANOVA) was conducted on rats' daily mean trial-by-trial correct/incorrect response rates for each element of the pattern across 21 days of the experiment. Main effects and interactions were considered significant if $p < 0.05$. The ANOVA revealed significant main effects for day of the experiment, $F_{(20,240)} = 67.04$, $p < 0.001$, element position within-chunks, $F_{(2,24)} = 302.53$, $p < 0.001$, and significant interactions for day × element position within-chunks, $F_{(40,480)} = 3.21$, $p < 0.001$, and drug group × day × element position within-chunks, $F_{(40,480)} = 2.52$, $p < 0.001$. As the ANOVA did not indicate significant differences across chunks of the pattern, planned comparisons based on the appropriate error term of the ANOVA were conducted on daily mean error rates for the three elements that composed chunks (i.e., element 1 vs. element 2 vs. element 3 of chunks) to identify significant differences indicated in Fig. 1 and the text below.

Results for the first element of chunks, namely, the chunk-boundary elements that immediately followed phrasing cues, are shown in the top panel of Fig. 1. Results showed that adult rats previously receiving 1.0 mg/kg adolescent nicotine exposure retarded learning, that is, nicotine-exposed rats produced fewer correct responses than controls on days 8 and 11–21 of the experiment. The results clearly showed that adolescent nicotine exposure retarded later adult learning (i.e., it decreased daily mean correct responses in the figure) for chunk-boundary elements of

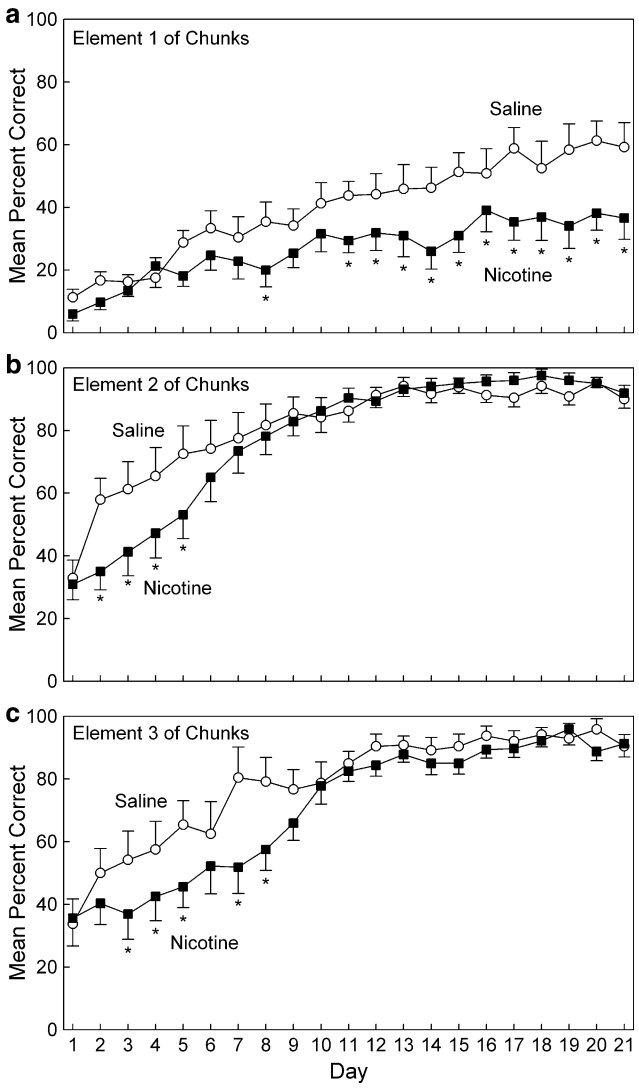

Fig. 1 Adult rats' acquisition curves for elements 1, 2, and 3 of chunks (panels **a**, **b**, and **c**, respectively) over 21 days of training. Rats received prior adolescent exposure to either 1.0 mg/kg nicotine or saline. Error bars: ±SEM. *Asterisks* indicate significant differences relative to controls ($p < 0.05$)

rats' pattern that represented transitions between "chunks" of the pattern.

Results for elements 2 and 3 of chunks, namely, within-chunk elements, are shown in the middle and bottom panels of Fig. 1, respectively. For element 2 of chunks (middle panel), results showed that adult rats previously receiving 1.0 mg/kg adolescent nicotine exposure produced fewer correct responses than controls on days 2–4 of the experiment. For element 3 of chunks (bottom panel), results showed that adult rats previously receiving 1.0 mg/kg adolescent nicotine exposure produced more errors than controls on days 3–5 and 6–7 of the experiment. These results showed that adolescent nicotine exposure transiently retarded later adult learning for within-chunk elements of the pattern that represented rule-governed elements of their pattern, but adolescent nicotine exposure did not affect asymptotic levels of acquisition by the end of the experiment.

Discussion

These results provide evidence that adolescent exposure to 1.0 mg/kg nicotine per day for 35 days produces deficits in adult rat sequential learning that can be detected and characterized with the serial pattern learning paradigm. Although our data show that adolescent nicotine exposure can impair adult cognitive processes in rat serial pattern learning, a number of important questions remain. In future studies, it will be important to conduct dose–response studies with larger groups of animals and to follow acquisition to asymptote. Adolescent nicotine exposure clearly retarded adult rat acquisition of chunk boundary elements that followed phrasing cues, but the rats were not trained to asymptote, so it is not clear whether this deficit should be characterized as a transient retardation of learning or a more persistent and profound deficit like that observed when rats learn this type of serial pattern after injections of MK-801, an *N*-methyl-D-aspartate receptor antagonist that blocks synaptic plasticity and learning in the hippocampus and other structures (Fountain and Rowan 2000). In addition, we do not have any data on effects of adolescent nicotine exposure on adult rat acquisition of elements that violate the inherent structure of the pattern. Violation elements of this type are known to be particularly sensitive to other biological challenges and have properties dissociable from those of other element types (cf. Fountain 2006; Fountain and Rowan 2000). Such studies will be helpful in characterizing the nature of the effects reported in the present study.

It should be noted in this context that the results from the serial pattern learning paradigm can already suggest hypotheses regarding the nature of the learning deficits and possible psychological processes impacted by adolescent nicotine exposure based on our current model of rat serial pattern learning. Briefly, our research to date indicates that rats employ concurrently both S-R discrimination learning processes and more abstract rule learning processes to learn serial patterns in this paradigm. Evidence for S-R learning includes the fact that training with phrasing cues, like the temporal pauses employed as phrasing cues in this study, followed by transfer to phrasing cue removal shows that rats use phrasing cues as simple or compound discriminative cues that control responses at pattern chunk boundaries (Fountain et al. 2000; Stempowski et al. 1999; Wallace et al. 2008). Evidence for concurrent rule learning processes comes from other studies indicating that phrasing cues can also bias pattern perception in rat

serial pattern learning, suggesting that phrasing cues play a role in pattern perception and rule learning (Fountain and Rowan 1995b; Fountain et al. 2007). In addition, behavioral studies manipulating pattern structure indicate that within-chunk element performance (elements 2 and 3 of 3-element chunks in the current study) should be attributed to motor or cognitive rule learning (Fountain and Rowan 1995a, b; Fountain and Rowan 2000). Results of psychobiological studies are consistent with the idea of concurrent S-R learning and rule learning in this paradigm; the effects of MK-801 showed that discrimination learning processes important for learning at chunk-boundaries appear to be dissociable from the rule learning processes involved in learning within-chunk responses (Fountain and Rowan 2000). Rule learning appears to be resistant to disruption by MK-801 treatment, whereas discrimination learning at chunk boundaries was profoundly impaired (Fountain and Rowan 2000). Based on the foregoing information, one possible interpretation of the data reported here is that adolescent nicotine exposure may have differentially affected discrimination learning processes that were necessary for using phrasing cues at chunk boundaries but left rule learning relatively spared, thus resulting in a more profound and longer-lasting retardation of learning at chunk-boundaries (element 1 of chunks) but only a brief period of mild retardation of learning for within-chunk elements (elements 2 and 3 of chunks). The results reported here thus fit with our recent conclusion based on a variety of behavioral and neurobiological evidence that multiple concurrent psychological and neural processes are recruited in rat serial pattern learning (Fountain 2006; Fountain and Benson 2006; Fountain et al. 2007; Wallace et al. 2008). However, other interpretations are possible, such as the view that adolescent nicotine exposure produced a global learning deficit that was expressed as a transient deficit for the easily-learned within-chunk elements and as a deficit with much longer time course for the more difficult chunk-boundary elements, and additional studies will undoubtedly clarify the nature of the deficits reported here.

The principal conclusion from this study, however, is that nicotine that was experienced only in adolescence produced significant deficits in adult rat serial pattern learning even after several intervening weeks of nicotine-free recovery time. The results thus show that adolescent nicotine exposure may be a threat to adult cognitive capacity. These results indicate that the effects of adolescent nicotine exposure on adult learning and cognitive capacity deserve further scrutiny.

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