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Research report

# The effects of amphetamine on working memory and locomotor activity in adult rats administered risperidone early in life

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ABSTRACT

Antipsychotic drugs are used to manage symptoms of pediatric psychiatric disorders despite the relative absence of research regarding the long-term effects of these drugs on brain development. Using rats as a model, research has demonstrated that administration of the antipsychotic drug, risperidone, during early postnatal development elevates locomotor activity and sensitivity to the locomotor effects of amphetamine during adulthood. Because risperidone targets neurotransmitter receptors and forebrain regions associated with working memory, the present study determined whether early-life risperidone altered working memory during adulthood and its sensitivity to amphetamine-induced impairment. Female and male rats received subcutaneous (sc) injections of risperidone daily on postnatal days 14-42. Early-life risperidone increased spontaneous locomotor activity and amphetamine-induced hyperactivity during adulthood, although the effects were significantly greater in females. Working memory was tested in an operant-based, delayed non-matching-to-sample task. Early-life risperidone did not affect the percentage of correct choices observed during sessions with 0–8 second delays but impaired performance during sessions with 0–24 second delays. In a subsequent set of tests using 0–24 second delays, amphetamine (0.75 and 1.25 mg/kg, sc) significantly reduced the percentage of correct choices at most delays, but risperidone did not exacerbate this effect. These data suggest that early-life risperidone leads to modest deficits in working memory during adulthood, but does not alter the perturbation of working memory by amphetamine.

## 1. Introduction

Antipsychotic drugs are used in the treatment of pediatric psychiatric disorders, such as attention deficit hyperactivity disorder, disruptive behavioral disorder, and autism [1–3]. Boys are more likely to receive these drugs than girls [2], and the most widely prescribed antipsychotic drug to children in the United States is risperidone [4]. Drugs such as risperidone produce their clinical effects via the blockade of dopamine and serotonin receptors in various regions of the forebrain [5]. One concern regarding the prolonged use of these drugs in children is that activity at these receptors may be critical for postnatal brain development, and that the extended receptor blockade imposed by drug treatment early in life may undermine behavioral and cognitive competence during adulthood. In rodents, some neurotransmitters receptors are not uniformly mature or expressed across early development [6,7], but early-life manipulations directed at specific dopamine or serotonin receptors can lead to a variety of neural and behavioral changes during adulthood [e.g., 8–12].

Recent studies in young rats have shown that risperidone administration at ages analogous to early childhood through early adolescence in humans leads to locomotor hyperactivity later in life [13–15]. This outcome raises the question as to whether early-life risperidone administration modifies other behavioral and cognitive functions linked to brain regions targeted by risperidone. For example, the frontal cortex has been implicated in a myriad of behaviors related to impulse control, decision-making, and working memory [16], and appears to be a primary site of risperidone's action in the brain (see Kuroki, Nagao, & Nakahara [17] for review). Daily risperidone administration for 3–4 weeks after weaning in rats modifies neurotransmitter receptor density in the frontal cortex, including increases in dopamine $D_2$, serotonin $5HT_{1A}$, and glutamatergic AMPA receptors, and decreases in $D_1$ and $5HT_{2A}$ receptors [18–21]. If these changes in receptor number persist into adulthood, or simply alter the subsequent course of frontal cortical development, they could possibly weaken cognitive functions such as working memory.

In adult rats, the effects of chronic risperidone administration on working memory have been mixed, with results demonstrating positive [22], negative [23], and no effects [24]. To date, no study has assessed the effects of developmental risperidone administration on working memory in rats, although Frost and colleagues [25] reported that developmental olanzapine administration disrupts performance in a delayed non-matching-to-sample task during adulthood. A study by

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Mandell, Unis, and Sackett [26] compared the effects of early-life risperidone and quetiapine administration on perseverative errors made within a trial set by juvenile macaque monkeys. While performance was not significantly affected during an eight-week period of drug administration, risperidone-treated animals made more perseverative errors over a four-week period following the cessation of drug administration. Whether this effect persisted beyond this period and into adulthood was not determined.

Even if developmental risperidone administration does not affect working memory in rats, it could alter the sensitivity of forebrain systems that subserve this function to disruption. Given the affinity of risperidone for dopamine and serotonin receptors, it merits consideration that drug challenges known to produce memory deficits via their actions on dopamine and serotonin may have a greater impact on adult rats administered risperidone early in life. For example, acute administration of D-amphetamine, a psychostimulant that increases extracellular dopamine and norepinephrine levels, decreases the percentage of correct choices in an operant-based, delayed non-matching-to-sample task [27]. Our lab has recently reported that rats administered risperidone early in life are more sensitive to the locomotor-activating effects of amphetamine [15]. Whether adult rats administered risperidone early in life are more sensitive to the memory-impairing effects of amphetamine remains unknown.

One of the goals of this study was to characterize the effects of early-life, subcutaneous (sc) administration of risperidone on working memory during adulthood in rats. Working memory was assessed in an operant-based, delayed non-matching-to-sample task using two different sets of delay intervals (0–8 and 0–24 second range). Following the assessment of working memory at these intervals, performance was then measured using the latter delay intervals after acute administration of two doses (0.75 and 1.25 mg/kg, sc) of amphetamine. Locomotor activity and locomotor responses to a single dose (1.0 mg/kg, sc) of amphetamine were measured prior to memory testing. These latter experiments were included to confirm our previous results regarding the effects of early-life risperidone on spontaneous and drug-induced locomotor activity, and to consider the possibility of sex differences in these effects.

## 2. Materials and methods

### 2.1. Animals and housing

Forty Long-Evans rats were used with 18 females and 22 males. These rats were derived from five litters born at our animal facility. Litters were culled on postnatal day 8 to eight total pups comprised of three-four pups of each sex (based on the availability of female and male pups from each litter). Rats were weaned on postnatal day 21. Upon weaning, rats were housed two per cage with continuous access to food and water, except where noted for the working memory testing. The lights in the housing room were on between 6:30 a.m. and 6:30 p.m. All experimental procedures were carried out according to the Current Guide for the Care and Use of Laboratory Animals (USPHS) under a protocol approved by the Northern Kentucky University Institutional Animal Care and Use Committee.

### 2.2. Drugs

The dose of risperidone used (3.0 mg/kg, sc) was based on our previous behavioral work [13–15,28] and reports demonstrating the effects of early-life risperidone on neurotransmitter receptor levels [18,19,21]. Risperidone was dissolved in a small volume of 10% glacial acetic acid, brought to volume with 0.9% saline, and adjusted to a pH ˜6.2 with 1 M sodium hydroxide. Control rats were administered the vehicle solution only. Injections were administered at a volume of 2.0 ml/kg of body weight. The National Institute of Mental Health's Chemical Synthesis and Drug Supply program kindly provided the risperidone.

Rats were weighed and administered risperidone or vehicle daily from postnatal day 14 through 42. This developmental period in the rat has been considered analogous to the time between early childhood and late adolescence in humans [7,29]. Given that many young children receive antipsychotic drugs continuously over long periods of time across this age period [30,31], the timing of the injections was meant to mimic prolonged antipsychotic drug exposure during childhood and early adolescence in humans.

D-amphetamine (Sigma) was dissolved in 0.9% saline. A dose of 1.0 mg/kg of amphetamine was chosen for study in the locomotor experiment because we had recently reported that adult male rats administered risperidone early in life were more sensitive to the locomotor-activating effects of that specific dose administered subcutaneously [15]. In the working memory experiments, two doses of amphetamine (0.75 and 1.25 mg/kg) were chosen for study. They were selected because Gulley and colleagues [27] reported deficits in delayed non-matching-to-sample performance in adult rats challenged with these doses. Saline served as the control solution in all experiments involving amphetamine administration, and all injections in these experiments were subcutaneous and occurred at a volume of 1 ml/kg.

### 2.3. Locomotor activity

Locomotor activity was measured in clear polypropylene cages (51 cm long x 26.5 cm wide x 32 cm high) covered with wire tops and inserted into *SmartFrame* cage racks (Kinder Scientific, Poway, CA). Locomotor activity was defined by the number of photobeam breaks generated within a given time period. All locomotor testing occurred in a dark room.

Locomotor activity was recorded for one hour on four consecutive days beginning on postnatal day 54. Testing occurred between 9:00 a.m. and 4:00 p.m. each day. These tests determined if rats administered risperidone early in life were more active as young adults, as reported previously [13,14].

On postnatal days 61 and 64, locomotor activity was measured for three hours after a sc injection of saline or 1.0 mg/kg of amphetamine. The order of saline or amphetamine administration was balanced across days within sex and risperidone/vehicle groups. These tests were intended to confirm recent work [15] showing that male rats administered risperidone early in life demonstrated a greater locomotor response to amphetamine, and to extend this line of inquiry by determining whether this effect was sex-dependent.

### 2.4. Delayed non-matching-to-sample working memory task

Eight operant-conditioning chambers (28 × 21 × 21 cm; ENV-008; MED Associates, Fairfax, VT) located inside sound attenuating cubicles (ENV-018 M; MED Associates) were used. The front and back walls of each chamber were aluminum, and the sidewalls were Plexiglas. Each chamber contained a recessed food tray (5 × 4.2 cm) located 2 cm above the floor in the bottom-center of the front wall. A 28-V white stimulus light was located 6 cm above each of the two retractable response levers. A 28-V white house light was mounted in the center of the back wall of the chamber. A nose poke aperture was located 2 cm above the floor in the bottom-center of the back wall. All responses and scheduled consequences were recorded and controlled by a computer interface and a computer running Med-PC IV software (Med-Associates). Rats were tested each week on Monday, Tuesday, Thursday, and Friday between 8:00 a.m. and 4:00 p.m.

Beginning on postnatal day 65, female and male rats were respectively given access to ˜11 and 15 g of rat chow per day. All rats were weighed twice a week. On postnatal day 68, rats were trained to press each response lever on a FR1 reinforcement schedule. The presentation of the lever switched between the left and right lever on each trial, and each lever press was followed by delivery of a 45 mg food pellet (F0021 dustless precision pellet, Bio-Serv, Frenchtown, NJ). There was a 5 s interval between food delivery from the last trial and lever extension for the next trial. Rats were tested for 30 min a session for a total of eight

sessions. Over four subsequent sessions, all experimental parameters were the same except that rats were required to place their nose into the nose poke aperture in order to activate lever extension. By the end of these FR1 schedule sessions, all rats were generating at least 75 responses per session.

Rats were then trained on a discrete-trial, delayed non-matching-to-sample task. Each trial began by extinguishing the house light, and presenting a single lever and its respective cue light (forced choice) 5 s later. A response on the lever led to the cue light being extinguished, lever retraction, and illumination of the nose poke light. When the rat placed its nose into the nose poke receptacle, the nose poke light extinguished, both levers were extended, and both cue lights illuminated (free choice). If the rat pressed the lever that was not extended prior to the nose poke, it was considered a correct choice. After such a choice, a food pellet was delivered, the cue lights extinguished, the levers retracted, and the house light illuminated 5 s later. An incorrect choice occurred if the rat pressed the same lever that was extended prior to the nose poke. After such a choice, the same changes to the cue light, lever, and house light happened as described above, but no food pellet was delivered. Between each trial, the house light was illuminated for 45 s. Each testing session lasted 40 min. For the first six days of testing, there was no delay between sample lever presentation and illumination of the nose poke aperture.

After six days of no delay testing, delays of 0, 1, 2, 4, and 8 s between forced lever choice and the activation of the nose-poke light were used. The delay order was randomized with the constraint that all six delays occurred within each sequence of six trials. This testing occurred for 14 sessions spanning a period of 18 consecutive days. Rats were then tested on delays of 0, 3, 6, 12, and 24 s. Thirteen sessions were conducted over a period of 18 consecutive days. The percentage of correct choices recorded during the first two daily sessions were averaged as a measure of initial learning, and the same data from remaining sessions were averaged as a measure of working memory performance.

Following these sessions, rats were tested for four additional weeks using the 0–24 second delays to ascertain the effects of 0.75 and 1.25 mg/kg of D-amphetamine or saline. Rats were tested five minutes after sc injection on Tuesdays and Fridays, and tested without injection on Mondays and Thursdays in the no-delay version of the task. Rats were administered either amphetamine dose or saline on two occasions and the data averaged for sessions following the administration of each dose.

### 2.5. Statistical analyses

Locomotor activity, as defined as photobeam breaks, was averaged across the hour-long sessions conducted between postnatal days 54-57. A two-way analysis of variance (ANOVA) was used to compare the data with antipsychotic drug administration (saline and risperidone 3.0 mg/kg) and sex serving as between-groups factors. In this and all analyses, $p < .05$ (two-tailed) was considered statistically significant, and post-hoc testing was performed using a Tukey/Kramer test.

A three-way ANOVA was used to compare the total number of photobeam breaks recorded for three hours after saline and amphetamine injections in each rat. Antipsychotic drug administration and sex served as between-groups factors, and saline-amphetamine injection served as a within-subject factor.

For the delayed non-matching-to-sample testing, the dependent measures of interest were: 1) the latency to press the forced choice sample lever, 2) the number of trials completed per session, and 3) the percentage of correct choices. For the first two measures, the data were averaged across all trials for each rat and analyzed using a two-way ANOVA with antipsychotic drug treatment and sex serving as between-group factors. The percentage of correct choices recorded during the first two daily sessions was analyzed separately from the same data recorded during the remaining sessions. These data were analyzed using delay as a repeated measure, and antipsychotic drug treatment and sex as between-groups factors. For all measures, the data from the 0–8 and the 0–24 second delay testing sessions were analyzed separately.

The data from the working memory tests conducted after amphetamine administration were analyzed using ANOVA with antipsychotic drug administration and sex as between-group factors, and amphetamine drug dose and delay as repeated measures. An ANOVA was also used to compare the effects of antipsychotic drug administration and sex on the average number of trials completed and the average latency to press the forced choice sample lever as a function of each amphetamine dose.

## 3. Results

Rats were tested for locomotor activity once a day for one hour on four consecutive days beginning on postnatal day 54. There was a significant main effect of testing day, $F(3, 108) = 11.2$, $p < .0001$, with declines in activity seen mainly on the last three days of testing relative to the first test day. Since there were no interactions between sex or risperidone with test day, the remaining analyses focused on the main effects of the former two variables on the hourly activity averaged across the four test days. Females were found to be significantly more active than males, $F(1, 36) = 42.3$, $p < .0001$, and rats administered risperidone early in life were more active than those administered vehicle, $F(1, 36) = 12.6$, $p = .001$(Fig. 1). The interaction between sex and risperidone was not significant.

On postnatal days 61 and 64, locomotor activity was tested for three hours after injection of saline or 1.0 mg/kg of amphetamine in a counterbalanced order across days within sex and risperidone groups. A three-way ANOVA using sex and risperidone administration as between-subjects factors and amphetamine as a within-subjects factor did not indicate any overall interaction between all three variables (Fig. 2). However, there were significant interactions between amphetamine x sex, $F(1, 36) = 15.8$, $p = .0003$, amphetamine x risperidone, $F(1, 36) = 8.8$, $p = .005$, and sex x risperidone, $F(1, 36) = 6.8$, $p = .01$, as well as main effects of sex, risperidone, and amphetamine, $F(1, 36) = 52.1$, 26.8, & 326.7, $p < .0001$ respectively. Subsequent post-hoc analyses of these interactions indicated that activity levels did not differ between risperidone and vehicle rats after saline injection, but were greater in the risperidone groups relative to the vehicle rats after amphetamine injection ($p = .003$). Female rats were more active than male rats after both saline ($p = .006$) and amphetamine ($p < .0001$) injections, with a larger magnitude difference observed between the sexes after amphetamine injection. Finally, when the data generated after saline and amphetamine injections were aggregated, it was found that females administered risperidone early in life were more active than vehicle females ($p < .0001$), whereas the same comparison between male rats did not yield a statistically significant difference ($p = .06$).

In the delayed non-matching-to-sample tasks, rats were trained and then tested for three weeks using delays that varied between 0, 1, 2, 4,

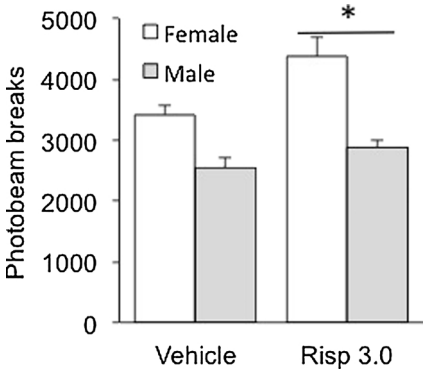


**Fig. 1.** Locomotor activity summed over 1 h and averaged across postnatal days 54 - 57. Females were more active than males ($p < .0001$). * $p = .001$ vs. vehicle rats collapsed across sex. Each bar represents group mean + S.E.M. n = 9 females and 11 males in each Vehicle or Risp 3.0 group.

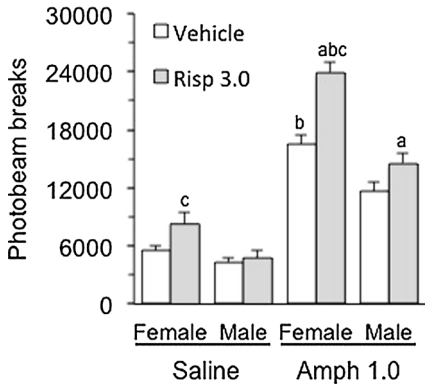


**Fig. 2.** Total locomotor activity summed over three hours after injection of saline or 1.0 mg/kg of amphetamine. [a] $p = .003$ vs vehicle females and vehicle males administered amphetamine, [b] $p < .006$ - $.0001$ vs respective vehicle and risperidone males after amphetamine injections, and [c] $p < .0001$ vs vehicle female rats after saline or amphetamine injections. Each bar represents group mean + S.E.M. n = 9 females and 11 males in each Vehicle or Risp 3.0 group.

and 8 s, and for another three weeks using delays that varied from 0, 3, 6, 12, and 24 s. To assess the potential non-mnemonic effects of early-life risperidone and sex in this task, the number of trials completed within a session was recorded for each rat, as well as the latency to press the lever during the forced choice phase of each trial (Table 1). Averages were generated based on data aggregated across all trials separately for the 0–8 and the 0–24 second delay sessions. There were no statistically significant effects of risperidone administered early in life on the number of trials completed during either the 0–8 or 0–24 second delay sessions. Males completed significantly more trials than females, $F(1, 36) = 8.2$ & $20.8$, $p = .007$ & $.0001$, respectively, during the 0–8 and 0–24 second delay sessions. The latency to press the lever during each forced choice lever presentation was not altered by early-life risperidone administration during the 0–8 second delay sessions, but the risperidone groups pressed the lever significantly faster during the 0–24 second delay sessions, $F(1, 36) = 5.5$, $p = .02$. Male rats exhibited quicker latencies than female rats, $F(1, 36) = 20.6$ & $36.9$, $p < .0001$, respectively, during the 0–8 and 0–24 second delay sessions.

To account for initial learning versus more stable memory performance, the average number of correct choices was analyzed separately for the first two daily sessions and then for the remaining sessions for the 0–8 and 0–24 second delay tests. A three-way ANOVA using sex and risperidone administration as between-subjects factors and delay as a within-subjects factor did not indicate any significant main effects or interactions during the first two sessions of the 0–8 second delay test (data not shown). The same analysis of the first two sessions of the 0–24 second delay test did not indicate any main effects of risperidone or sex, but did reveal a significant delay effect, $F(4, 144) = 20.6$, $p < .0001$. Overall, rats exhibited more correct choices at the 0 and 3 s delays relative to the 12 and 24 s delays ($p < .0003$ - $.0001$) during the first two sessions of testing using these delays (data not shown).

The analysis of the subsequent daily sessions assessing correct choices at the 0–8 second delays revealed significant main effects of sex, $F(1, 36) = 4.51$, $p < .04$, and delay, $F(4, 144) = 10.15$, $p < .0001$ (Fig. 3A). Female rats made more correct choices than males, and rats made more correct choices at the 0, 1, 2, and 4 s delays relative to the 8 s delay ($p < .0001$ for each comparison to the 8 s delay). The analysis of the remaining daily sessions assessing performance at the 0–24 second delays again indicated significant main effects of sex, $F(1, 36) = 5.31$, $p = .03$, and delay, $F(4, 144) = 127.51$, $p < .0001$, but also a significant interaction between risperidone administration and delay, $F(4, 144) = 4.02$, $p = .004$ (Fig. 3B). Female rats demonstrated more correct choices than male rats, and the number of correct choices observed at each delay differed from all other delays ($p < .0001$ for each comparison), with the exception of the similarity of correct choices made at 0 and 3 s. When compared to rats in the vehicle group, rats administered risperidone early in life made fewer correct choices at the 0 s delay ($p = .04$). There were no significant effects of risperidone observed at any other delay.

During a subsequent set of tests using 0–24 second delays, the effects of acute administration of amphetamine (0.75 and 1.25 mg/kg, sc) were tested on the number of trials completed, the latency to press the lever during the forced choice portion of each trial, and the percentage of correct choices during the free choice portion of each trial. In all of these analyses, there was not a main effect of sex or any interaction between sex and the other independent variables – as a consequence, all of the reported analyses, tables, and figures focus solely on the effects of amphetamine and risperidone. One female rat in the risperidone group was excluded from these analyses since it failed to complete more than two trials during the sessions in which it received either dose of amphetamine.

Acute amphetamine administration decreased the number of trials completed in a dose-dependent manner, $F(2, 74) = 15.1$, $p < .0001$, and increased the latency to press the lever, $F(2, 74) = 7.3$, $p = .001$ (Table 2). In the analysis of the number of correct choices, there were no main effects of sex or risperidone, or interactions between these factors with amphetamine dose or delay. However, there was a significant interaction between amphetamine dose and delay, $F(8, 280) = 5.17$, $p < .0001$. Subsequent analyses of the effects of amphetamine at each delay indicated that there was a significant effect of amphetamine at each delay, $F(2, 74) = 24.5, 15.5, 16.9, 11.2,$ & $3.5$, $p < .04$ - $.0001$ for the 0, 3, 6, 12, and 24 s delays respectively. Both amphetamine doses decreased the percentage of correct choices during the 0, 3, and 12 s delay trials relative to the effects of saline ($p < .001$ - $.0001$). The percentage of correct choices was significantly different between all three groups at the 6 s delay ($p < .02$ - $.0001$), while only the high dose of amphetamine decreased the percentage of correct choices at the 24 s delay ($p = .01$).

When the effects of delay were analyzed separately for each amphetamine dose, rats that received saline injections made significantly fewer correct choices at each longer delay ($p < .005$ - $.0001$) with the exception of the comparison between correct choices made at 3 and 6 s (Fig. 4A). After injection of the 0.75 mg/kg dose of amphetamine, rats made significantly fewer correct choices at the two longest delays relative to the three shortest ones ($p < .001$ - $.0001$)(Fig. 4B). Finally, after injection of the 1.25 mg/kg dose of amphetamine, rats made fewer correct choices at the 12 and 24 s delays relative to the 0 s delay ($p = .005$ & $.001$ respectively) and at the 24 s delay relative to the 3 s delay ($p = .004$) (Fig. 4C).

**Table 1**
Effects of early-life risperidone on trials completed and latency to press the sample lever in the delayed non-matching-to-sample task.

| | | 0-8 second delay task | | 0-24 second delay task | |
|---|---|---|---|---|---|
| Group | *n* | Mean # trials completed | Mean lever latency (sec) | Mean # trials completed | Mean lever latency (sec) |
| Vehicle - female | 9 | 45.3 + 0.5* | 2.86 + 0.2* | 39.4 + 0.5* | 3.52 + 0.3* |
| Vehicle - male | 11 | 47.5 + 0.5 | 1.67 + 0.2 | 42.3 + 0.4 | 1.95 + 0.2 |
| Risp 3.0 - female | 9 | 47.1 + 0.4* | 2.27 + 0.2* | 41.0 + 0.4* | **2.76** + **0.3*** |
| Risp 3.0 - male | 11 | 47.9 + 0.6 | 1.65 + 0.1 | 42.2 + 0.4 | **1.68** + **0.2** |

Data represent mean + s.e.m. Within each column, * denotes sex main effect, and **bold** denotes drug main effect.

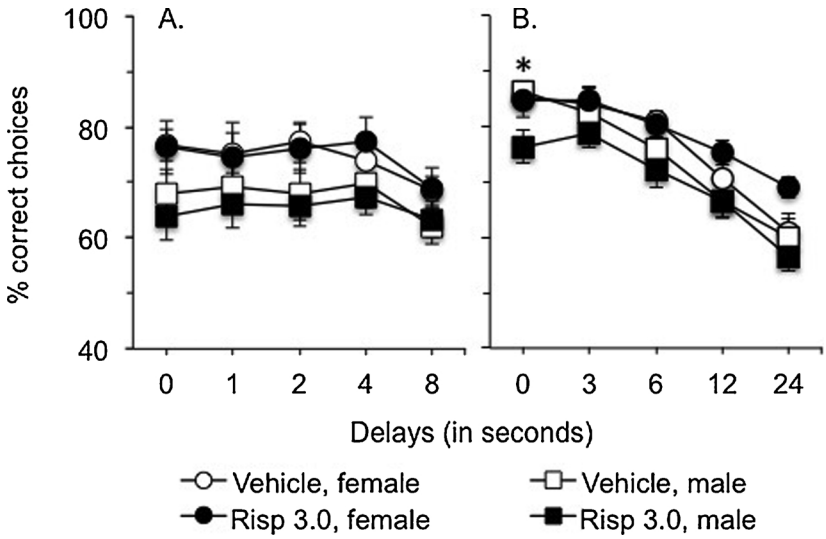


**Fig. 3.** Correct choices averaged across the 0–8 and 0–24 second delay sessions in the delayed non-matching-to-sample task (excluding the first two days of testing for each delay session). Female rats made more correct choices during each set of delay sessions ($p$ = .04 & .03 for the 0–8 and 0–24 second sessions respectively). * $p$ = .04 vs. vehicle rats collapsed across sex at the 0 s delay. Each marker represents group mean + S.E.M. n = 9 females and 11 males in each Vehicle or Risp 3.0 group.

**Table 2**
Effects of acute amphetamine on trials completed and latency to press the sample lever in the delayed non-matching-to-sample task.

| | Mean # of trials completed | | Mean lever latency (seconds) | |
|---|---|---|---|---|
| *Drug dose* | Vehicle | Risp 3.0 | Vehicle | Risp 3.0 |
| Saline | 43.0 + 0.2 | 42.6 + 0.3 | 1.5 + 0.1 | 1.7 + 0.2 |
| AMPH 0.75 mg/kg | **40.1 + 1.3** | **40.7 + 1.3** | **3.4 + 0.8** | **3.0 + 0.9** |
| AMPH 1.25 mg/kg | **35.0 + 2.3*** | **37.6 + 2.3*** | **5.34 + 1.2** | **3.2 + 1.0** |

Data represent mean + s.e.m. n = 20 and 19 respectively for the vehicle and risperidone groups. For each measure, **bold** denotes difference from saline, and * denotes difference from AMPH 0.75.

## 4. Discussion

Rats administered risperidone early in life demonstrated modest yet significant changes in working memory as adults. When working memory was tested using shorter delays during the initial test sessions, the risperidone group performed the same as the vehicle group. However, when longer delays were built into the testing, the risperidone-administered rats made significantly fewer correct choices during the no delay trials. It is not obvious why these rats performed worse during the no delay trials as opposed to longer delay trials within these sessions, or why these same deficits were not apparent at the same delay during the initial testing or the saline trials from the amphetamine experiments. One possible explanation is that the increased cognitive load imposed by the longer delays during the first three weeks of 0–24 second delay testing created a slight but significant disruptive effect on memory processing in the absence of delay. Chronic risperidone administration early in life may have altered subsequent functioning of neural pathways that process working memory such that the ability of these pathways to maintain optimal processing in the face of large delay interval variation is weakened. Alternatively, working memory performance in the absence of delay peaked during the last three weeks of testing prior to examining the effects of amphetamine on memory. It is possible that early-life risperidone may simply limit the level of optimal performance achievable in this task during such trials.

Before commenting further on working memory performance, it should be recognized that the groups differed in non-mnemonic behaviors recorded during the test sessions. Risperidone administration was associated with quicker latencies to press the sample lever relative to the vehicle group during the initial set of 0–24 second delay sessions. Sex differences were also observed during the 0–8 and 0–24 second delay sessions, with male rats completing more trials and responding more quickly to sample lever presentation in a manner independent of developmental drug treatment. The effect of risperidone on the latency measure, while inconsistent across each set of sessions, could be interpreted as an indirect manifestation of the increased motor activity reported in our

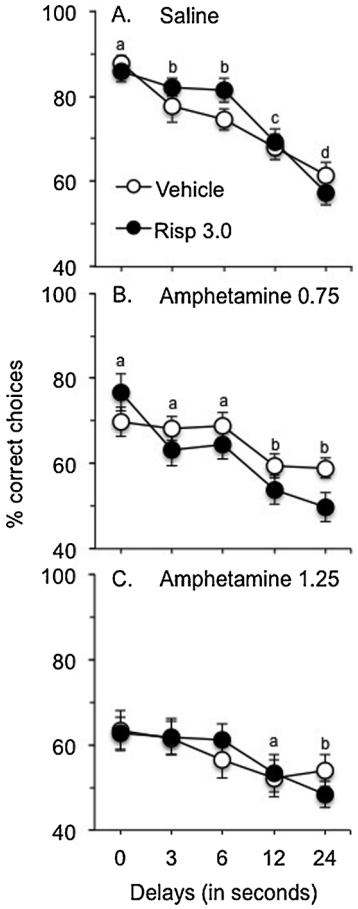


**Fig. 4.** Effects of amphetamine on correct choices in the 0–24 second delay version of the non-matching-to-sample task. In A. and B., markers with different letters are significantly different from one another ($p$ < .005 - .0001). In C., [a] $p$ < .005 vs rats at the 0 s delay, and [b] $p$ < .001 & .004 vs rats at the 0 and 3 s delays respectively. Each marker represents group mean + S.E.M. n = 20 and 19 rats in the Vehicle or Risp 3.0 groups respectively.

previous studies [13,14] of early-life risperidone as well as the present one. This interpretation is a bit tenuous given that female rats were more active than males in the locomotor studies but completed fewer trials and demonstrated longer lever response latencies. Changes in these non-mnemonic behaviors may account for some proportion of the working memory differences found between the groups yet the inconsistencies, differing patterns, and modest degree of change in some of these measures makes it difficult to gauge the extent of their impact on working memory.

Another caveat to this study is that it did not include an adult comparison group. As such, the effects of risperidone described here may not be the specific aftermath of developmental administration. However, we have previously shown that early-life risperidone produces some of the same long-term locomotor effects seen here that are not observed after adult administration [14]. Others have also reported that peripubertal risperidone administration affects neurotransmitter receptor binding differently than chronic administration during adulthood [18,19,21]. In the present study, we were interested in simply determining whether early-life risperidone administration had any effect on the behaviors in question – a goal with translational relevance to the use of this drug in pediatric populations but also one that does not clarify the possible temporal dependence of risperidone's impact across the lifespan. Thus, interpretations of the effects reported here as being specific to early postnatal development should be met with caution.

Previous research in male rats has demonstrated that developmental exposure to the second generation, atypical antipsychotic drug, olanzapine, [25] leads to spatial working memory deficits in a T-maze task during adulthood. Our work parallels and extends these results by showing that early-life administration of another atypical antipsychotic drug, risperidone, can lead to similar impairments in spatial working memory in female and male rats. Our findings are also consistent with the persistence in perseverative errors reported in juvenile macaques administered risperidone during development [26]. In this latter study, animals were presented with a two-choice discrimination task in which one object was paired with reward, and object pairings were changed every six trials. Animals treated with risperidone who chose the incorrect object on the first trial were more likely than controls to repeat the same mistake on the second trial. As was the case for the delayed non-matching-to-sample task used here, the stimulus associated with reward changed often over the course of the entire testing session, and animals exposed to risperidone early in life demonstrated subtle yet significant decrements in their ability to process such information. Together, these findings offer more solid support for the conclusion that chronic antipsychotic drug administration may adversely impact brain pathways involved in working memory.

Psychostimulant drugs, such as D-amphetamine, have been shown to improve working memory at low doses [32] but impair the same function at higher doses [27,33]. At a neurochemical level, the ability of amphetamine to block and reverse dopamine reuptake (see Spencer et al. [32], for brief review) has been implicated in its cognitive effects. Risperidone administration early in development has been associated with significant changes in dopamine and glutamate receptor density in the frontal cortex and striatum after treatment cessation in rats [18–21]. In light of this literature, we determined if rats exposed to risperidone during development displayed a differential sensitivity to the cognitive effects of amphetamine. Our choice of amphetamine doses came from a previous study of working memory [27] that used intraperitoneal injections. We chose to study the effects of subcutaneous amphetamine injections in order to allow a better comparison of its effects in this test with the locomotor effects of subcutaneous amphetamine injections reported here and in our previous work [15].

Amphetamine challenge significantly decreased working memory performance consistent with an earlier report [27]. It also reduced the number of trials completed and increased the latency to respond to sample lever presentation. The effects of amphetamine on these behaviors were not modified by early-life risperidone administration. Whatever mechanism accounts for the slight but significant changes in working memory observed in adult rats administered risperidone early in life, this same mechanism does not appear to yield greater sensitivity to disruptive effects of amphetamine on working memory performance.

While early-life risperidone did not alter the cognitive effects of amphetamine, it did enhance sensitivity to the locomotor-activating effects of amphetamine in female and male rats. This latter finding extends our recent work [15] that revealed the same outcome in a male-only group of rats administered risperidone early in life. Moreover, it was discovered that the overall effects of early-life risperidone on activity during adulthood are greater in female rats than males. Using lower but more frequent doses of risperidone, De Santis et al. [34] reported that juvenile risperidone administration led to greater activity in males relative to females in adulthood. They have also reported sex-specific alterations in regional dopamine and serotonin receptor density after juvenile risperidone administration [20,35]. While we observed sex differences in behavior that were the opposite of those reported by DeSantis et al. [34] it is possible that these differences still emanate from sex-specific modifications in dopamine and serotonin neurotransmission induced by chronic risperidone administration.

Since risperidone enhanced amphetamine-induced elevations in activity but not amphetamine-induced deficits in working memory, it is possible that distinct neural pathways modulate amphetamine's effects on locomotor activity [36] versus working memory [32]. However, given the slight but significant effects of early-life risperidone on working memory alone, it is possible that the development of some nodes within the neural circuitry that processes working memory are compromised by early-life risperidone, but that these nodes are not the locus of amphetamine's action on working memory. Finally, it may be worth considering whether other aspects of amphetamine's action in the brain, such as its reinforcing properties, are altered by early-life risperidone. Along these lines, it should be noted that developmental exposure to olanzapine enhances amphetamine-induced conditioned place preference during adulthood in rats [37].

The behavioral data gathered here when combined with neurochemical findings from the literature may offer some insights into the biological mechanism responsible for early-life risperidone's negative impact on working memory during adulthood. A likely site of risperidone-induced modulation of working memory is the prefrontal cortex. As discussed earlier, developmental risperidone administration in rats has been associated with decreases and increases, respectively, in prefrontal $D_1$ and $D_2$ receptors, although some of these effects are sex-specific and independent of age of administration [20,21]. Nonetheless, these reported changes are intriguing since insufficient activity at prefrontal $D_1$ receptors as well as over-stimulation of prefrontal $D_2$ receptors have been associated with working memory deficits in rats (see Puig et al. [38] for review). That risperidone-exposed rats did not show an exacerbated deficit in working memory after amphetamine administration complicates this interpretation given amphetamine's effects on synaptic dopamine levels. But amphetamine interacts directly and indirectly with a number of neurotransmitter systems - perhaps most notably with norepinephrine (see Hutson et al. [39], for review). It is possible that amphetamine disrupts working memory by acting on neurotransmitter systems not significantly impacted by early-life risperidone.

Antipsychotic drugs are used for a host of disorders in children; mainly to manage behavioral symptoms such as irritability and aggressiveness [2,3]. Clinical studies have also considered the relatively short-term effects of antipsychotic drugs alone or in combination with other drugs, on cognition in such children, with generally mixed findings of no effect or slight improvements in attention and working memory [40,41]. However, clinical investigation of the effects of developmental risperidone administration on cognition is problematic since it is difficult to disentangle the effects of drug from disorder or other circumstances on measures such as working memory. Basic studies such as the present one are useful in this regard since one can isolate the effects of developmental drug administration alone on later behavioral and cognitive processing, albeit in an animal model. Our results raise concerns about the long-term consequences of such drug

administration on working memory during adulthood, as well as the possibility that some effects of early-life risperidone are accentuated in females. These concerns suggest that antipsychotic drug use in children should be approached in a cautionary manner.

## Conflict of interest

All authors declare no conflict of interest.

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## References

[1] W.D. Lohr, R.T. Chowning, M.D. Stevenson, P.G. Williams, Trends in atypical antipsychotics prescribed to children six years of age or less on Medicaid in Kentucky, J. Child Adolesc. Psychopharmacol. 25 (2015) 440–443, https://doi.org/10.1089/cap.2014.0057.

[2] M. Olfson, M. King, M. Schoenbaum, Treatment of young people with antipsychotic medications in the United States, JAMA Psychiatry 72 (2015) 867–874, https://doi.org/10.1001/jamapsychiatry.2015.0500.

[3] B. Vitiello, C. Correll, B. van Zwieten-Boot, A. Zuddas, M. Parellada, C. Arango, Antipsychotics in children and adolescents: increasing use, evidence for efficacy and safety concerns, Eur. Neuropsychopharmacol. 19 (2009) 629–635, https://doi.org/10.1016/j.euroneuro.2009.04.008.

[4] M. Olfson, C. Blanco, S. Liu, S. Wang, C.U. Correll, National trends in the office-based treatment of children, adolescents, and adults with antipsychotics, Arch. Gen. Psychiatry 69 (2012) 1247–1256, https://doi.org/10.1001/archgenpsychiatry.2012.647.

[5] R.B. Mailman, V. Murthy, Third generation antipsychotic drugs: partial agonism or receptor functional selectivity? Curr. Pharm. Design 16 (2010) 488–501, https://doi.org/10.2174/138161210790361461.

[6] T. Der-Ghazarian, C.B. Widarma, A. Gutierrez, L.R. Amodeo, J.M. Valentine, D.E. Humphrey, A.E. Gonzalez, C.A. Crawford, S.A. McDougall, Behavioral effects of dopamine receptor inactivation in the caudate-putamen of preweanling rats: role of the D2 receptor, Psychopharmacology 231 (2014) 651–662, https://doi.org/10.1007/s00213-013-3280-9.

[7] L.P. Spear, The adolescent brain and age-related behavioral manifestations, Neurosci. Biobehav. Rev. 24 (2000) 417–463.

[8] C. Alexandre, D. Popa, V. Fabre, S. Bouali, P. Venault, K.P. Lesch, M. Hamon, J. Adrien, Early life blockade of 5-hydroxytryptamine 1A receptors normalizes sleep and depression-like behavior in adult knock-out mice lacking the serotonin transporter, J. Neurosci. 26 (2006) 5554–5564.

[9] R.W. Brown, D.J. Peterson, Applications of the neonatal quinpirole model to psychosis and convergence upon the dopamine D(2) receptor, Curr. Top. Behav. Neurosci. 29 (2016) 387–402, https://doi.org/10.1007/7854_2015_394.

[10] D.O. Frost, S.C. Page, C. Carroll, B. Kolb, Early exposure to haloperidol or olanzapine induces long-term alterations of dendritic form, Synapse 64 (2010) 191–199, https://doi.org/10.1002/syn.20715.

[11] S.L. Moon, Prenatal haloperidol alters striatal dopamine and opiate receptors, Brain Res. 323 (1984) 109–113.

[12] C.H. Vinkers, R.S. Oosting, M.J. van Bogaert, B. Olivier, L. Groenink, Early-life blockade of 5-HT(1A) receptors alters adult anxiety behavior and benzodiazepine sensitivity, Biol. Psychiatry 67 (2010) 309–316.

[13] M.E. Bardgett, J.M. Franks-Henry, K.R. Colemire, K.R. Juneau, R.M. Stevens, C.A. Marczinski, M.S. Griffith, Adult rats treated with risperidone during development are hyperactive, Exp. Clin. Psychopharmacol. 21 (2013) 259–267, https://doi.org/10.1037/a0031972.

[14] R.M. Stevens, M.A. Gannon, M.S. Griffith, M.E. Bardgett, Delayed yet persistent effects of daily risperidone on activity in developing rats, Behav. Pharmacol. 27 (2016) 460–469, https://doi.org/10.1097/FBP.0000000000000230.

[15] B.L. Stubbeman, C. Brown, J.R. Yates, M.E. Bardgett, Early-life risperidone enhances locomotor responses to amphetamine during adulthood, Eur. J. Pharmacol. 812 (2017) 256–263, https://doi.org/10.1016/j.ejphar.2017.07.025.

[16] S.F. Logue, T.J. Gould, The neural and genetic basis of executive function: attention, cognitive flexibility, and response inhibition, Pharmacol. Biochem. Behav. 123 (2014) 45–54, https://doi.org/10.1016/j.pbb.2013.08.007.

[17] T. Kuroki, N. Nagao, T. Nakahara, Neuropharmacology of second-generation antipsychotic drugs: a validity of the serotonin-dopamine hypothesis, Prog. Brain Res. 172 (2008) 199–212.

[18] Y.K. Choi, M.P. Gardner, F.I. Tarazi, Effects of risperidone on glutamate receptor subtypes in developing rat brain, Eur. Neuropsychopharmacol. 19 (2009) 77–84, https://doi.org/10.1016/j.euroneuro.2008.08.010.

[19] Y.K. Choi, T. Moran-Gates, M.P. Gardner, F.I. Tarazi, Effects of repeated risperidone exposure on serotonin receptor subtypes in developing rats, Eur. Neuropsychopharmacol. 20 (2010) 187–194, https://doi.org/10.1016/j.euroneuro.2009.09.002.

[20] J. Lian, B. Pan, C. Deng, Early antipsychotic exposure affects serotonin and dopamine receptor binding density differently in selected brain loci of male and female juvenile rats, Pharmacol. Rep. 68 (2016) 1028–1035, https://doi.org/10.1016/j.pharep.2016.06.003.

[21] T. Moran-Gates, C. Grady, Y. Shik Park, R.J. Baldessarini, F.I. Tarazi, Effects of risperidone on dopamine receptor subtypes in developing rat brain, Eur. Neuropsychopharmacol. 17 (2007) 448–455.

[22] E.P. Lim, V. Verma, R. Nagarajah, G.S. Dawe, Propranolol blocks chronic risperidone treatment-induced enhancement of spatial working memory performance of rats in a delayed matching-to-place water maze task, Psychopharmacology 191 (2007) 297–310.

[23] H. Rosengarten, D. Quartermain, The effect of chronic treatment with typical and atypical antipsychotics on working memory and jaw movements in three- and eighteen-month-old rats, Prog. Neuropsychopharmacol. Biol. Psychiatry 26 (2002) 1047–1054.

[24] E.J. Hutchings, J.L. Waller, A.V. Terry Jr, Differential long-term effects of haloperidol and risperidone on the acquisition and performance of tasks of spatial working and short-term memory and sustained attention in rats, J. Pharmacol. Exp. Ther. 347 (2013) 547–556, https://doi.org/10.1124/jpet.113.209031.

[25] J.A. Milstein, A. Elnabawi, M. Vinish, T. Swanson, J.K. Enos, A.M. Bailey, B. Kolb, D.O. Frost, Olanzapine treatment of adolescent rats causes enduring specific memory impairments and alters cortical development and function, PLoS One 8 (2013) e57308, , https://doi.org/10.1371/journal.pone.0057308.

[26] D.J. Mandell, A. Unis, G.P. Sackett, Post-drug consequences of chronic atypical antipsychotic drug administration on the ability to adjust behavior based on feedback in young monkeys, Psychopharmacology 215 (2011) 345–352, https://doi.org/10.1007/s00213-010-2147-6.

[27] L.K. Sherrill, J.J. Stanis, J.M. Gulley, Age-dependent effects of repeated amphetamine exposure on working memory in rats, Behav. Brain Res. 242 (2013) 84–94, https://doi.org/10.1016/j.bbr.2012.12.044.

[28] M.A. Gannon, C.J. Brown, R.M. Stevens, M.S. Griffith, C.A. Marczinski, M.E. Bardgett, Early-life risperidone administration alters maternal-offspring interactions and juvenile play fighting, Pharmacol. Biochem. Behav. 130 (2015) 90–96, https://doi.org/10.1016/j.pbb.2015.01.007.

[29] S.L. Andersen, Stimulants and the developing brain, Trends Pharmacol. Sci. 26 (2005) 237–243.

[30] R.J. Constantine, S. Jentz, M. Bengtson, M. McPherson, R. Andel, M.B. Jones, Exposure to antipsychotic medications over a 4-year period among children who initiated antipsychotic treatment before their sixth birthday, Pharmacoepidemiol. Drug Saf. 21 (2012) 152–160, https://doi.org/10.1002/pds.2189.

[31] L.J. Kalverdijk, H. Tobi, P.B. van den Berg, J. Buiskool, L. Wagenaar, R.B. Minderaa, L.T. de Jong-van den Berg, Use of antipsychotic drugs among Dutch youths between 1997 and 2005, Psychiatr. Serv. 59 (2008) 554–560, https://doi.org/10.1176/appi.ps.59.5.554.

[32] R.C. Spencer, D.M. Devilbiss, C.W. Berridge, The cognition-enhancing effects of psychostimulants involve direct action in the prefrontal cortex, Biol. Psychiatry 77 (2015) 940–950, https://doi.org/10.1016/j.biopsych.2014.09.013.

[33] D.N. Harper, R. Wisnewski, M. Hunt, S. Schenk, (+/-)3,4-methylenedioxymethamphetamine, d-amphetamine, and cocaine impair delayed matching-to-sample performance by an increase in susceptibility to proactive interference, Behav. Neurosci. 119 (2005) 455–463.

[34] M. De Santis, J. Lian, X.F. Huang, C. Deng, Early antipsychotic treatment in childhood/adolescent period has long-term effects on depressive-like, anxiety-like and locomotor behaviours in adult rats, J. Psychopharmacol. (Oxford) 30 (2016) 204–214, https://doi.org/10.1177/0269881115616383.

[35] M. De Santis, X.F. Huang, C. Deng, Early antipsychotic treatment in juvenile rats elicits long-term alterations to adult serotonin receptors, Neuropsychiatr. Dis. Treat. 14 (2018) 1569–1583, https://doi.org/10.2147/NDT.S158545.

[36] P.H. Kelly, P.W. Seviour, S.D. Iversen, Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum, Brain Res. 94 (1975) 507–522.

[37] M. Vinish, A. Elnabawi, J.A. Milstein, J.S. Burke, J.K. Kallevang, K.C. Turek, C.S. Lansink, I. Merchenthaler, A.M. Bailey, B. Kolb, J.F. Cheer, D.O. Frost, Olanzapine treatment of adolescent rats alters adult reward behaviour and nucleus accumbens function, Int. J. Neuropsychopharmacol. 16 (2013) 1599–1609, https://doi.org/10.1017/S1461145712001642.

[38] M.V. Puig, J. Rose, R. Schmidt, N. Freund, Dopamine modulation of learning and memory in the prefrontal cortex: insights from studies in primates, rodents, and birds, Front. Neural Circuits 8 (2014) 93, https://doi.org/10.3389/fncir.2014.00093.

[39] P.H. Hutson, F.I. Tarazi, M. Madhoo, C. Slawecki, A.A. Patkar, Preclinical pharmacology of amphetamine: implications for the treatment of neuropsychiatric disorders, Pharmacol. Ther. 143 (2014) 253–264, https://doi.org/10.1016/j.pharmthera.2014.03.005.

[40] G.J. Pandina, Y. Zhu, B. Cornblatt, Cognitive function with long-term risperidone in children and adolescents with disruptive behavior disorder, J. Child Adolesc. Psychopharmacol. 19 (2009) 749–756, https://doi.org/10.1089/cap.2008.0159.

[41] C.A. Farmer, J.N. Epstein, R.L. Findling, K.D. Gadow, L.E. Arnold, H. Kipp, D.J. Kolko, E. Butter, J. Schneider, O.G. Bukstein, N.K. McNamara, B.S. Molina, M.G. Aman, Risperidone added to psychostimulant in children with severe aggression and attention-deficit/hyperactivity disorder: lack of effect on attention and short-term memory, J. Child Adolesc. Psychopharmacol. 27 (2017) 117–124, https://doi.org/10.1089/cap.2016.0040.