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Sex differences in serial pattern learning in mice

Shannon M.A. Kundey[a,*], Aahana Bajracharya[b], Holly Boettger-Tong[c], Stephen B. Fountain[d], James D. Rowan[c]

[a] Hood College, Department of Psychology, 401 Rosemont Avenue, Tatem 324, Frederick, MD, 21701, United States
[b] Washington University in Saint Louis
[c] Wesleyan College
[d] Kent State University

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ABSTRACT

Numerous investigators have examined the hypothesis that males and females learn or perform differentially on various tasks. However, many of the behavioural investigations with nonhuman animals (e.g., rats) have used paradigms that do not permit the exploration of complex learning and memory between the sexes. To this end, we explored the ability of male versus female mice to learn three different patterns in succession in three separate experiments: single alternation (e.g., right, left, right, left), double alternation (e.g., right, right, left, left), and runs (e.g., 123, 234, 345, 456, 567, 678, 781, 812, where digits represent locations within a circular array in the counterclockwise direction). We hypothesized that sex differences, if they existed, would be most likely to appear as the pattern to be learned became more complex (required more rules to capture how elements relate to one another). The results indicated that mice can learn all three pattern types, but learning was more difficult as pattern complexity increased. Males learned the runs pattern significantly more quickly than females did; no significant differences were found between males and females for acquisition of the single-alternation or double-alternation patterns. These results suggest that sex differences in serial pattern learning within rodents are not unique to rats and are more likely to be seen during acquisition of more complex patterns.

1. Introduction

Many researchers have explored whether the sexes learn or perform differentially across a variety of tasks (e.g., Andreano and Cahill, 2019). For example, Andreano and Cahill (2019) noted sexual dimorphism based on volume in the hippocampus, caudate nucleus, anterior cingulate gyrus, dorsolateral prefrontal cortex, planum temporale, amygdala, and paracingulate gyrus in a recent review. These behavioural differences may be due to the numerous examples of neuroanatomical sexual dimorphism noted by Andreano and Cahill (2019). With discoveries of structural dimorphism based on sex, investigators are better equipped to approach understanding the neural basis of learning and memory mechanisms.

However, while such neurobiological sex differences are uncovered frequently, it is often unclear how these dissimilarities impact behaviour more generally, especially as behaviour increases in complexity (Andreano and Cahill, 2019). Such differences have been observed across common learning tasks in both human and nonhuman animals. For example, evidence suggests differential performance by males and females in spatial navigation tasks (e.g., Kimura, 1999) and object recognition tasks (e.g., Frick and Gresack, 2003). Species' ecology often relates to both the existence and scale of sex differences (e.g., Gaulin and Wartell, 1990). Differences across the sexes may be more common than previously accepted and likely extend beyond purely verbal and spatial domains to include various memory abilities (Andreano and Cahill, 2019). Additionally, such differences could be impacted by stress (Luine, 2002).

Unfortunately, many of the behavioural investigations of sex differences to date, especially those utilizing nonhuman animals, have not employed paradigms permitting the exploration of complex learning and memory (as noted by Pickens et al., 2013; Sharp et al., 2018). This is unfortunate, as paradigms geared toward more complex forms of learning and memory are likely to be especially revealing regarding behavioural differences based on sex. More specifically, as tasks become more complex, they become more difficult; when a task is more difficult, differences in performance are likely to be more pronounced. Additionally, the cognitive tasks species engage in during everyday life are unlikely to involve only one cognitive process; thus, tasks tapping into only one cognitive process may not mirror behaviour in more complex circumstances where multiple cognitive processes are

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[*] Corresponding author.
E-mail address: kundey@hood.edu (S.M.A. Kundey).

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involved.

One such study targeting the effect of adolescent nicotine exposure on rats employed a serial learning paradigm geared toward evaluating more complex behaviour (Pickens et al., 2013). The researchers found that exposure impaired the ability of adult serial pattern learning differentially across the sexes both in the experimental and control groups. In this task, rats were required to arrange their behaviour sequentially in time to choose correct nosepoke receptacles in a predetermined order that could be described using a set of rules. This task is similar to nonverbal tasks used with humans where the participants are required to respond in a particular order according to a predetermined and ordered pattern (e.g., Restle and Brown, 1970). Since this task requires the utilization of multiple concurrent cognitive systems (e.g., associative stimulus-response learning, serial position learning involving timing and/or counting processes, and rule abstraction) to be performed correctly, it is of particular interest to explorations of behavioural differences across the sexes (e.g., Fountain and Benson, 2006; Kundey and Fountain, 2010).

Although a number of studies have explored the ability of rats to learn complex patterns of information, few prior studies have explored this ability in mice (Fountain et al., 1999). In several experiments utilizing a paradigm similar to that used by Pickens et al., Fountain et al. (1999) explored whether mice could learn a structured pattern, the effect of a violation of pattern structure, and whether phrasing cues (e.g., a pause signalling when to change from using one rule to using another) facilitated learning. The results showed that, like rats and humans, mice learned faster when no violation of pattern structure was present. Additionally, the experiments indicated that mice have difficulty utilizing phrasing cues in learning structured patterns.

Here, we evaluated whether mice could learn several patterns of escalating complexity and whether sex differences across pattern learning and performance would emerge. As in rats, there is no obvious selective pressure for strong cognitive sex differences in mice. Additionally, such explorations are needed in mice because they are often used as an animal model to evaluate the role of candidate genes in biological and behavioural processes (e.g., Wehner and Silva, 1996; Schimanski and Nguyen, 2004). Mice are often utilized for such models because of the greater availability of genetic information for mice compared to many other species and the relative ease of genetic modifications in mice (Silver, 1995). A variety of studies across species indicate that as pattern complexity increases, such as when more than one rule is needed to describe how the elements of the pattern relate to one another or when an element violates the rules of the pattern, the pattern becomes more difficult to learn and more errors are likely to be committed (see Fountain et al., 2012).

To this end, mice learned three different patterns in succession in separate experiments: single alternation (e.g., right, left, right, left), double alternation (e.g., right, right, left, left), and runs (e.g., 123, 234, 345, 456, 567, 678, 781, 812, where digits represent locations within a circular array in the counterclockwise direction). If sex differences existed, we hypothesized that they would be most apparent as the pattern to be learned became more complex. Restle's (Restle and Brown, 1970) hierarchical model suggests that as patterns become more complex, more rules are necessary to adequately capture pattern structure; when more rules are required, pattern difficulty increases.

We can use Restle's hierarchical model to describe the rules that might be used to encode these patterns. The single-alternation pattern can be described by these rules: (1) start by choosing the receptacle on the right, (2) choose the receptacle on the left, and (3) repeat steps 1-2. The double-alternation pattern can be described by these rules: (1) start by choosing the receptacle on the right, (2) repeat that choice, (3) choose the other receptacle, (4) repeat steps 2-4. The runs sequence can be described using these rules: (1) start by choosing receptacle 1, (2) move one circle in the counterclockwise direction, (3) move one circle in the counterclockwise direction again, (4) move one circle in the clockwise direction, (5) repeat steps 2-5. Of the patterns employed, the fewest rules are required to represent single-alternation patterns and the most rules are required to represent runs patterns. Thus, we would posit that sex differences would be most likely to appear and would be of the highest magnitude in the runs pattern, with respect to the patterns we utilized.

2. Experiment 1

Previously, Kundey and Rowan (2009) showed that rats can learn various alternation patterns, including single alternation. In Kundey and Rowan's study, rats' performance was significantly facilitated by correction trials when incorrect responses were made.

The goal of Experiment 1 was to explore the ability of mice to learn a single-alternation pattern and to evaluate the existence of any sex differences. As in Kundey and Rowan (2009), we utilized a correction procedure to provide mice with feedback about their performance on each trial, as this was found to be crucial with rats. Fountain et al. (1999) found that phrasing cues did not facilitate mouse performance of runs patterns; consistent with this work, we did not employ phrasing cues in Experiment 1.

2.1. Method

2.1.1. Subjects

The subjects for this experiment were 12 C57BL/6 (Black 6) mice (6 females, 6 males; Charles River Laboratories, Inc.). All the mice were housed individually in a satellite room of the vivarium at Wesleyan College. Throughout the experiment, mice were exposed to a light/dark cycle of 14 h/10 h with free access to food and were checked daily for any signs of ailments.

2.1.2. Apparatus

Four operant chambers (MedAssociates, Inc.) were used. These chambers included two nosepoke receptacles side by side. The nosepoke receptacles were 4 cm from the floor to the centre of the nosepoke receptacle; the distance between the centres of the two nosepoke receptacles was 16 cm. Each nosepoke receptacle was equipped with an infrared switch to detect a nosepoke, a 1-W indicator white LED light, and a water delivery system. The floor of the chamber consisted of metal bars spaced approximately 1 cm apart. A speaker emitting a white noise of 65 dB was turned on throughout the duration of the experiment to counter any extraneous noise from outside the chamber. Data collection from the apparatus was facilitated by MED-PC software, which monitored the performance of the mice based on their responses.

2.1.3. Procedure

Mice were deprived of water during the experiment, as described below. After each day's trials, access to water was provided for five minutes. All procedures for all experiments were approved by Wesleyan College's Institutional Animal Care and Use Committee (IACUC) in 2016 as part of a larger experimental proposal.

All mice completed shaping to nosepoke for a droplet of water reward for 4 days. At the beginning of each trial, the lights were off. After the mouse poked its nose in the receptacle, a 0.025-ml droplet of water was delivered at the bottom of the receptacle. Which nosepoke receptacle was reinforced varied randomly across trials such that subjects received shaping training for both receptacles. Each trial was separated by a delay of 1 s after the correct response was made. The purpose of this was to prepare the mice to make the required association to perform subsequent pattern learning task.

Following shaping, all mice learned a single-alternation (SA) pattern (12 12 12 12 12 12 12 12), where 1 and 2 indicated the left-right pattern to which the mice should respond (counterbalanced across subjects). At the beginning of each trial, the lights in both nosepoke receptacles were dark. A correct response resulted in reward with a droplet of water, and the lights in the nosepoke receptacles remained dark. Subsequently, there was a 2 s delay prior to the next trial.

A correction procedure was used when an incorrect response was made. When a mouse poked its nose in an incorrect receptacle, the LED in the correct receptacle would light up and remain lit until the correct response was performed. Making the correct response in the lit receptacle resulted in water reward and the extinguishing of the light. The mouse then moved on to the next element in the sequence. This correction procedure assured that mice could only start a new trial after a correct response was made on the current trial. Mice completed 10 patterns each day for 18 days.

2.1.4. *Data analysis*

Appropriate *t*-tests and repeated measures Analysis of Variance (ANOVA) were employed to evaluate potential group differences. Comparisons were only viewed as significant if $\alpha < 0.05$, and all tests were two-tailed. Both Statistica and SPSS were employed. In this experiment, chance performance equalled 50% errors because there were two nosepoke receptacles and choosing one randomly would result in an incorrect response one out of two times. When appropriate, we also calculated a Bayes Factor (http://pcl.missouri.edu/bayesfactor).

2.2. *Results*

In total, five female and six male mice finished the experiment. One mouse died prior to the end of the experiment, and those data are not included in any analyses. First, we determined the mean percentage errors that mice of each sex committed over the entire 18 days of training. Females committed 19.00% errors (SEM = 1.96), and males committed 17.80% errors (SEM = 3.09). As indicated in Fig. 1, more errors were committed on the first day of training than on later days of training.

Then, we evaluated whether mice learned the single-alternation pattern at a level greater than would be expected by chance (50%) using a one-sample *t*-test. To do this, we determined the mean percent error across all trials for each sex. The analysis showed that both males and females did learn the pattern significantly better than would be expected by chance alone (females: $t(4) = 15.81, p < 0.001, d = 7.08$; males: $t(5) = 10.43, p < 0.001, d = 4.26$).

Next, we examined whether learning differed across acquisition based on sex by evaluating whether the mean percent error rate for each day differed across sexes. We accomplished this by conducting a sex (female, male) X day (1–18) repeated measures ANOVA on the mean percent error rate per day (correction trials were not included). Days was treated as a factor because it was not known when asymptote would be reached. This indicated a significant effect of day ($F(17,153) = 81.69, p < 0.001$, partial eta squared = 0.90). No other significant main effects or interactions were detected. This suggested that both males and females improved their performance significantly across the days of the experiment but did not significantly differ in the way that their performance changed based on sex.

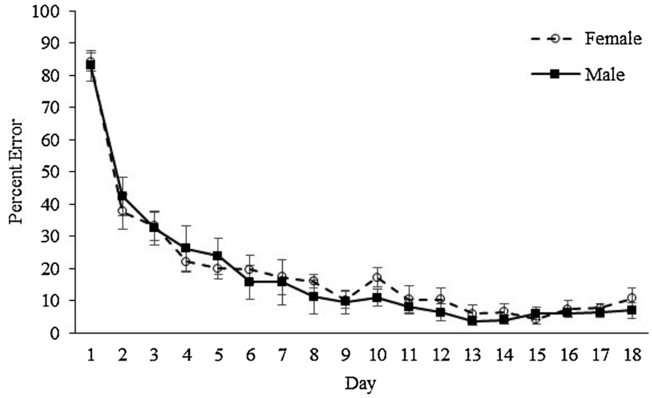

Fig. 1. Mean percentage of errors committed across acquisition by males and females exposed to the single-alternation pattern across the 18 days of the experiment (Experiment 1). The error bars indicate SEM.

Lastly, to evaluate potential differences between males and females at the conclusion of the experiment further, we used an independent-samples *t*-test to compare performance over the last seven days of training. This revealed no significant difference in performance ($t(9) = 0.76, p = 0.47$). To support the notion that males and females did not significantly differ in their performance further, we calculated a Bayes Factor (http://pcl.missouri.edu/bayesfactor). This indicated a Bayes Factor in support of the null (Scaled JZS Bayes Factor = 1.74, Scaled-Information Bayes Factor = 1.29).

In this single-alternation pattern, mice were limited in the types of errors they could make due to the presence of only two nosepoke receptacles in the apparatus. Thus, when mice committed an error, it resulted from picking the only possible incorrect receptacle in the apparatus.

2.3. *Discussion*

In this experiment, both male and female mice learned to produce a single-alternation pattern. No significant sex differences were observed in their performance. However, the single-alternation pattern used was relatively simple. We hypothesized if sex differences existed in the ability of mice to learn serial patterns, they would be more likely to be evidenced as pattern difficulty escalated.

3. Experiment 2

The goal of Experiment 2 was to evaluate the ability of mice to learn a more complex double-alternation pattern as well as to explore whether any sex differences existed. We hypothesized that mice would be more likely to evidence differences between sexes, if they existed, on this more complicated pattern than on the single-alternation pattern used in Experiment 1. As in Experiment 1, we utilized a correction procedure to give mice feedback regarding their performance on each trial; no phrasing cues were utilized.

3.1. *Method*

3.1.1. *Subjects*

The same mice used in Experiment 1 were used in Experiment 2. The mice were housed and kept in the same conditions as in Experiment 1.

3.1.2. *Apparatus*

The same equipment used in Experiment 1 was used in Experiment 2.

3.1.3. *Procedure*

Mice were deprived of water in a similar manner as in Experiment 1; as in Experiment 1, after each day's trials, access to water was provided for five minutes.

All mice learned a double-alternation (DA) pattern (11 22 11 22 11 22 11 22), where 1 and 2 indicated the left and right nosepokes (counterbalanced across subjects) of an operant chamber. The correction procedure and timing between trials was identical to Experiment 1. Mice completed 10 patterns each day for 18 days.

3.1.4. *Data analysis*

Data analysis proceeded similarly to Experiment 1. As in Experiment 1, chance performance equalled 50% errors because there were two nosepoke receptacles and choosing one randomly would result in an incorrect response one out of two times.

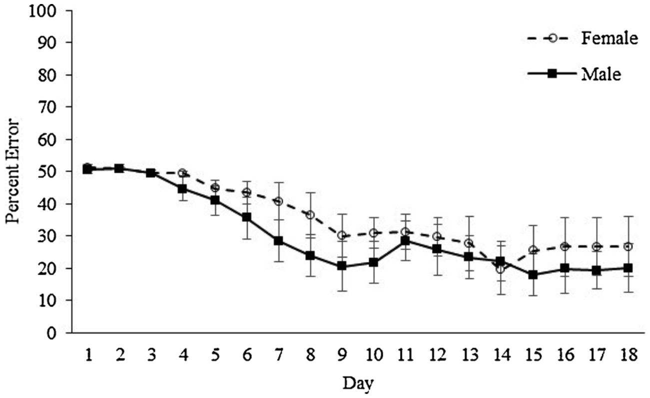

Fig. 2. Mean percentage of errors committed across acquisition by males and females exposed to the double-alternation pattern across the 18 days of the experiment (Experiment 2). The error bars indicate SEM.

3.2. Results

As in Experiment 1, we first determined the mean percentage errors that mice of each sex committed over the entire 18-day training period. Females committed 35.65% errors (SEM = 4.18), and males committed 30.23% errors (SEM = 4.86). As indicated in Fig. 2, more errors were committed on the first day of training than on later days of training.

Then, we evaluated whether mice learned the double-alternation pattern at a level greater than would be expected by chance (50%) using a one-sample *t*-test. To do this, we determined the mean percent error across all trials for each sex. The analysis showed that both males and females did learn the pattern significantly better than would be expected by chance alone (females: $t(4) = 3.44$, $p = 0.03$, $d = 1.54$; males: $t(5) = 4.07$, $p = 0.01$, $d = 1.67$).

Next, we examined whether learning of the double-alternation pattern differed across acquisition based on sex by evaluating whether the mean percent error rate for each day differed across sexes (correction trials were not included). Days was treated as a factor because it was not known when asymptote would be reached. We accomplished this by conducting a sex (female, male) X day (1–18) repeated measures ANOVA. This indicated a significant effect of day ($F(17,153) = 14.56$, $p < 0.001$, partial eta squared = 0.62). No other significant main effects or interactions were detected. This suggested that both males and females improved significantly in their performance across the days of the experiment but did not significantly differ in the way that their performance changed based on sex.

Lastly, to evaluate potential differences between males and females at the conclusion of the experiment further, we used an independent-samples *t*-test to compare performance over the last seven days of training. This revealed no significant difference in performance ($t(9) = 0.48$, $p = 0.64$). To support the notion that males and females did not significantly differ in their performance further, we calculated a Bayes Factor (http://pcl.missouri.edu/bayesfactor). This indicated a Bayes Factor in support of the null (Scaled JZS Bayes Factor = 1.93, Scaled-Information Bayes Factor = 1.43).

In this double-alternation pattern, mice were limited in the types of errors they could make due to the presence of only two nosepoke receptacles in the apparatus. Thus, when mice committed an error, it resulted from picking the only possible incorrect receptacle in the apparatus. The correct pattern required performing the series 11 22 repeatedly (performing two responses to receptacle 1 followed by 2 responses to receptacle 2 repeatedly).

When the data are collapsed across day and across element, in the series of 1 s for the double-alternation pattern, males and females overall chose receptacle 1 most of the time for the first choice to receptacle 1; (females 14.00% errors, males 16.76% errors). For the second choice to receptacle 1, females committed 29.78% errors while males only committed 16.76% errors. For the first choice to receptacle 2, females committed 31.78% errors while males committed 45.60% errors. For the second choice to receptacle 2, females committed 67.06% errors while males committed 41.81% errors. This suggests that females were more likely than males to alternate between receptacles rather than perform the 11 22 double alternation pattern.

Next, we examined the errors committed during the first and last day of the experiment. On the first day of the experiment, males and females overall chose receptacle 1 most of the time for the first choice to receptacle 1; (females 10.00% errors, males 5.83% errors). For the second choice to receptacle 1, females committed 74.00% errors while males only committed 67.50% errors. For the first choice to receptacle 2, females committed 27.00% errors while males committed 33.33% errors. For the second choice to receptacle 2, females committed 94.00% errors while males committed 95.83% errors. This pattern of errors suggests that during the first day of the experiment, mice were attempting to perform the single-alternation pattern.

On the final day of the experiment, males and females overall chose receptacle 1 most of the time for the first choice to receptacle 1; (females 6.00% errors, males 9.17% errors). For the second choice to receptacle 1, females committed 17.00% errors males committed no errors. For the first choice to receptacle 2, females committed 26.00% errors while males committed 39.17% errors. For the second choice to receptacle 2, females committed 58.00% errors while males committed 31.67% errors.

3.3. Discussion

In this experiment, both male and female mice learned to produce a double-alternation pattern. No significant differences in sex were observed in their performance. Although the double-alternation pattern used in Experiment 2 was more complex than the single-alternation pattern used in Experiment 1, we posit that it is possible that it still was not complex enough to show the potential sex differences we hypothesized initially.

It is possible that a different pattern of results would have been seen with naïve mice. For example, our data suggest that the mice's prior knowledge gleaned from Experiment 1, where they learned a single-alternation pattern, interfered with their performance of the double-alternation pattern employed in Experiment 2. This is because using the rule learned to describe the single-alternation pattern employed in Experiment 1 to perform the double-alternation pattern utilized in Experiment 2 would have led to 50% errors. Naïve mice would not have shown such potential interference.

4. Experiment 3

The goal of Experiment 3 was to explore the ability of mice to learn a runs serial pattern, which is more complex than either the single-alternation pattern used in Experiment 1 or the double-alternation pattern used in Experiment 2, as described above. We posited that mice would be more likely to evidence differences in learning between sexes, if they existed, as pattern difficulty escalated. Thus, we hypothesized that we would be most likely to see potential differences between learning across the sexes for the runs pattern relative to the other patterns we utilized. As in the first two experiments, we used a correction procedure to give mice feedback about their performance on each trial. Phrasing cues were included in Experiment 3, as described below, to heighten the difficulty of the pattern to increase the likelihood of detecting potential sex differences, if they existed.

4.1. Method

4.1.1. Subjects

The same mice used in Experiments 1 and 2 were used in Experiment 3. The mice were housed and kept in the same conditions as in Experiment 1.

4.1.2. Apparatus

The test chamber used was octagonal in shape, with each wall being 14 cm high and 7 cm wide. The distance between opposite walls was 18 cm. One nosepoke receptacle was located on each wall and centred 2.25 cm from the right and left edge of either wall. The distance from the floor to the bottom of each nosepoke receptacle was 2 cm. The floor was made of stainless steel mesh. Nosepoke receptacles were constructed from 3.0-cm diameter PVC pipe end caps painted flat black. Infrared emitter-detector pairs were mounted on the sides, and a 1-W indicator white LED light was mounted in the rear of the receptacle. A solenoid (General Valve Corp., 20 psig, 24 V) was attached by tubing to a water opening at the bottom of each receptacle. A 20-ml syringe served as a water reservoir for each receptacle. When activated, the solenoid delivered a 0.02-ml droplet of water at the bottom of the receptacle to which it was attached. The test chamber was housed in a separate particleboard sound-attenuating shell. A speaker emitting a white noise of 65 dB was turned on throughout the duration of the experiment to counter any extraneous noise from outside the chamber. As in Experiments 1 and 2, data collection from the apparatus was facilitated by MED-PC software, which monitored the performance of the mice based on their responses.

4.1.3. Procedure

No pretraining was required for the new apparatus. Mice were deprived of water during the experiment in the same manner as in Experiments 1 and 2. After the conclusion of the day's trials, access to water was provided for five minutes.

All mice learned a runs pattern (123 234 345 456 567 678 781 812), where digits indicate the counter-clockwise position of the eight nosepoke receptacles and spaces indicate 3-s intertrial intervals (phrasing cues). All other intertrial intervals were 2 s.

At the beginning of the day's trials, no lights were illuminated. A correct response led to reward with a droplet of water and all lights remained off. Subsequently, the mouse moved on to the next trial.

A correction procedure was used when an incorrect response was made. When the mouse poked its nose in an incorrect receptacle, all lights were illuminated except the correct one. After the mouse nosepoked in the correct nosepoke (the unlit one), all lights were extinguished, the mouse received water reinforcement, and the mouse continued to the next trial. For each of 40 days, mice completed a total of five patterns. More days were included in acquisition because of the increased complexity of the pattern.

4.1.4. Data analysis

Data analysis proceeded similarly to Experiments 2 and 3. However, chance performance equalled 87.5% errors because there were eight nosepoke receptacles and choosing one randomly would result in an incorrect response seven out of eight times.

4.2. Results

In total, 5 females and 4 males completed all 40 days of Experiment 3; two male mice did not complete all training days of the experiment, and their data are not included in the analyses. As in Experiments 1 and 2, we first found the mean percentage errors that mice of each sex committed over the entire 40-day training period. Females committed 64.50% errors (SEM = 1.53), and males committed 49.64% errors (SEM = 4.64). As indicated in Fig. 3, more errors were committed on the first day of training than on later days of training.

Then, we evaluated whether mice learned the runs pattern at a level greater than would be expected by chance (87.5%) using a one-sample *t*-test. To do this, we determined the mean percent error across all trials for each sex. The analysis showed that both males and females did learn the pattern significantly better than would be expected by chance alone (females: $t(4) = 14.94, p < 0.001, d = 14.94$; males: $t(3) = 8.17, p = 0.004, d = 8.16$).

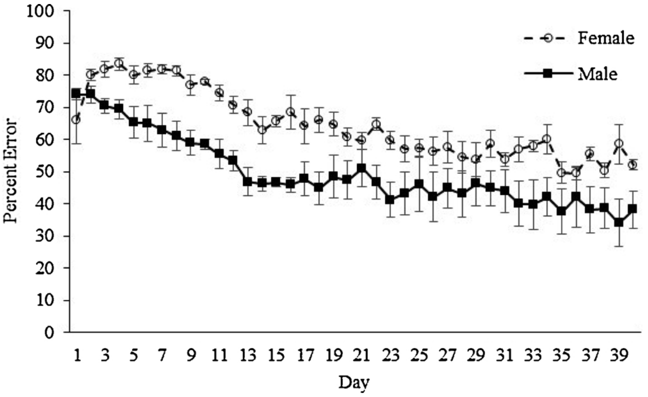

Fig. 3. Mean percentage of errors committed across acquisition by males and females exposed to the runs pattern across the 40 days of the experiment (Experiment 3). The error bars indicate SEM.

Next, we examined whether learning of the runs pattern differed across acquisition based on sex by evaluating whether the mean percent error rate for each day differed across sexes (correction trials were not included). Days was treated as a factor because it was not known when asymptote would be reached. We accomplished this by conducting a sex (female, male) X day (1–40) repeated measures ANOVA. This indicated a significant effect of day ($F(39,273) = 18.88, p < 0.001$, partial eta squared = 0.73) and sex ($F(1,7) = 11.26, p = 0.012$, partial eta squared = .62). Additionally, a significant sex X day interaction was found ($F(39,273) = 1.46, p = 0.044$, partial eta squared = 0.17).

Next, we examined whether learning of the runs pattern differed across the elements of the pattern based on sex by evaluating whether the mean percent error rate for each chunk and each element within each chunk differed for the final five days of the experiment (see Fig. 4). Here, a chunk is defined as a meaningful group of elements within the pattern (Fountain and Annau, 1984); for the pattern in question, there were eight chunks of three elements each. We accomplished this by conducting a sex (female, male) X chunk (1–8) X element within chunk (1–3) repeated measures ANOVA. This indicated a significant effect of chunk ($F(7,49) = 15.93, p < 0.001$, partial eta squared = 0.70), element ($F(2,14) = 53.22, p < 0.001$, partial eta squared = 0.88), and sex ($F(1,7) = 5.72, p = 0.048$, partial eta squared = 0.45). Additionally, a significant chunk X element interaction was also found ($F(14,98) = 4.87, p < 0.001$, partial eta squared = 0.41). No other interactions were significant. This suggests that males outperformed females over the course of the experiment; however, the lack of

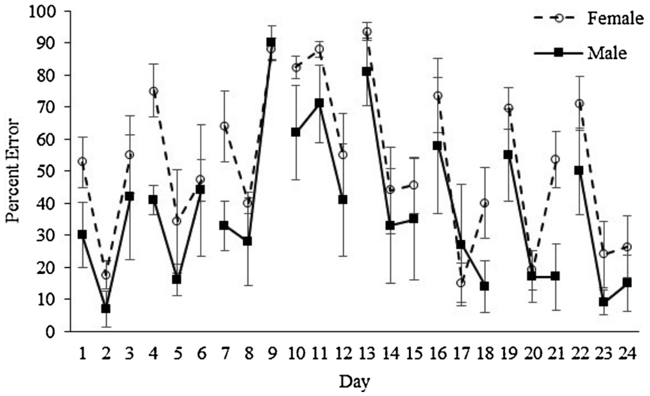

Fig. 4. Mean percentage of errors committed across acquisition by males and females exposed to the runs pattern across the last five days of the experiment by pattern element (Experiment 3). The error bars indicate SEM.

interaction suggests that females showed an overall decrement in performance relative to males. Subsequent Fisher's LSD tests with Bonferroni correction indicated significantly worse performance on chunk 4 than chunks 1 and 5–8 and significantly worse performance on chunk 5 than chunk 8. Additionally, subsequent Fisher's LSD tests with Bonferroni correction indicated significantly worse performance on element 1 of each chunk relative to elements 2 and 3 as well as significantly worse performance of element 3 relative to element 2.

Although there are a number of procedural and apparatus differences across the three experiments, we sought to obtain a gross measure of whether pattern complexity and sex interacted over the three experiments. Thus, we performed a sex (female, male) X experiment (1–3) repeated measures ANOVA on the overall percentage correct across all days of each experiment. This indicated a significant effect of experiment ($F(2,14) = 155.87$, $p < 0.001$, partial eta squared = 0.96) and sex ($F(1,7) = 12.29$, $p = 0.010$, partial eta squared = 0.64 as well as a significant experiment X sex interaction ($F(2,14) = 4.95$, $p = 0.024$, partial eta squared = 0.41). Although this result should be interpreted cautiously due to the differences in apparatus and procedure, this suggests that performance was influenced both by sex and pattern complexity.

4.3. Discussion

In this experiment, both male and female mice did learn to produce a runs pattern at above chance levels. However, they were not as proficient at doing so as mice in Experiments 1 and 2. This supports the notion that the runs pattern used in Experiment 3 was more complex, and thus more difficult to learn, than the single-alternation and double-alternation patterns used in Experiments 1 and 2. However, it is possible that mice's prior learning of the single-alternation and double-alternation patterns in Experiments 1 and 2 interfered with their ability to learn the runs pattern as trying to perform either of the previously-learned patterns would have led to a high number of errors.

While we did not find evidence of sex differences in performance of the single-alternation and double-alternation patterns used in Experiments 1 and 2, we did find a significant difference between males and females in their performance of the runs serial pattern learned in Experiment 3. The significant interaction in the ANOVA across experiments supports our hypothesis that as pattern difficulty escalates, sex differences, if they existed, would become more apparent.

However, it is also possible that the mice's learning of the single-alternation and double-alternation patterns in the first two experiments and/or the procedural differences between the experiments led to the observation of sex differences in the third experiment. Notably, the octagonal operant chamber used in the third experiment requires mice to move among nosepoke receptacles to a greater degree than was required in Experiments 1 or 2 since there were only two nosepoke receptacles available in the first two experiment and eight available in Experiment 3. Thus, it is possible that the poorer performance by females compared to males in Experiment 3 is due to this greater spatial component inherent in the apparatus used in Experiment 3 rather than pattern complexity. Future experiments manipulating pattern complexity within the same apparatus might aid in further disentangling this issue.

5. Conclusion

While Fountain et al. (1999) previously demonstrated that mice can learn runs patterns, we provide evidence that they can also learn single-alternation and double-alternation patterns in addition to runs serial patterns. However, as pattern complexity increased, mice committed more errors, with the highest number of errors committed for the runs pattern.

Historically, double-alternation patterns have been more difficult for a variety of species to learn than single-alternation patterns (for review, see Kundey and Rowan, 2009). Although Kundey and Rowan (2009) did not find an appreciable increase in difficulty for double-alternation patterns compared to single-alternation patterns in rats, we found that both male and female mice committed more errors on the double-alternation than the single-alternation pattern in Experiments 1 and 2, respectively. This suggests that the double-alternation patterns were more difficult for the mice to learn than the single-alternation patterns. However, it is also possible that mice's prior learning of the single-alternation pattern interfered with their ability to learn the double-alternation pattern later as trying to perform the double-alternation pattern according to the rule learned in Experiment 1 would have led to 50% errors.

As hypothesized based on pattern complexity, mice showed the most difficulty learning the runs pattern. Additionally, it is also possible that mice's prior learning of the single-alternation and double-alternation patterns interfered with their ability to learn the runs pattern, as trying to perform the runs pattern according to either the single-alternation or double-alternation pattern rules would have led to a high error rate. It is also possible that the difference in apparatus and procedure could have led to differential performance between the sexes in Experiment 3.

Interestingly, mice in Experiment 3 did not learn the runs pattern as well by the end of the training period as mice in Fountain et al. (1999) did. In the current experiment, although mice were performing the runs pattern at above chance levels, males committed approximately 53% errors on day 21 of training and females committed approximately 59% errors on day 21 of training. In contrast, the male mice in Fountain et al. (1999) committed approximately 35% errors at the same period in their training. As indicated above, it is possible that mice's prior learning of the single-alternation and double-alternation patterns disrupted their later learning of the runs serial pattern. Additionally, it is possible that a difference in strain between contributed. While we used BL6 mice, Fountain et al. (1999) used CF1 mice. Future experiments should investigate this possibility more closely by attempting to understand the relationship between genetics and serial pattern learning performance. Additionally, future experiments might investigate the role of hormones in differential performance of serial patterns across males and females as well as when such differences emerge in ontogeny. Interactions of stress and sex on serial pattern learning should also be investigated.

In conclusion, we have shown that mice can learn several types of patterns, including single-alternation, double-alternation, and runs serial patterns. Additionally, our results suggest that males learned the more complex runs serial patterns more quickly than females did, although no such difference was found for single-alternation or double-alternation patterns. This fits with our hypothesis that sex differences would be most likely to be found as pattern complexity increased.

Author note

These studies comply with the ethical standards of the United States for the use of nonhuman animals as research subjects. We have no conflicts of interest to report.

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