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PATTERN LEARNING IN LONG EVANS RATS (*RATTUS NORVEGICUS*): 3-ITEM SERIAL REACTION TIME TASKS

By

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PATTERN LEARNING IN LONG EVANS RATS (RATTUS NORVEGICUS): 3-ITEM SERIAL REACTION TIME TASKS

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ABSTRACT

Rats have been shown to be capable of learning and using rules to accomplish serial pattern tasks. The aim of this project was to broaden our understanding of serial pattern learning in rats and to determine whether a basic serial reaction time task (SRTT) is sufficient for investigations of this nature. The three main objectives of this thesis were to determine (1) whether rats can recognize patterns; (2) how switching between different patterns within sessions affects performance; and (3) performance differences between subjects who are given fixed inter-signal intervals (ISIs) and those given varied ISIs on rule-governed sequences. The results indicate that rats can learn basic response patterns, but become poor at switching from one pattern to another over time. ISI consistency did not affect performance, but amount of pattern learning experience did.

DEDICATION

I would like to dedicate this thesis to my friends and family who have helped me along this journey. I would also like to dedicate this to the many rats who devoted their lives to my experiments. I love you all and I couldn’t have done it without you!

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TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

Chapter 2:

Chapter 3:

Chapter 4:

CHAPTER ONE:

GENERAL INTRODUCTION

Serial Pattern Learning

In the earlier literature discussing serial learning in rats, a debate broke out. What exactly about a serial pattern does a rat learn? Two opposing views on this question were formed. According to one position, rats form associations between pairs of elements of serial patterns; a second position suggests rats develop an overall representation or *abstract rule.* Capaldi (1980) was a strong proponent for the former view, referring to it as the *memory generalization view*; however, Hulse and Dorsky (1979) strongly supported the latter view, calling it the *rule-encoding model*.

In 1979, Hulse and Dorsky presented data on decreasing-monotonic pattern learning in rats. A straight runway apparatus was used, at the end of which, varying amounts of food pellets could be retrieved by subjects. To complete a pattern, subjects were required to make several runs, with each one rewarded by different amounts of food (10, 5, 3, 1, or 0 pellets). In the beginning of the experiment, rats were given one of two conditions: 1) decreasing monotonic patterns (M) of runs (e.g., 10-3-0), or 2) entirely random (R) patterns (e.g., 3-5-1). In the second part of the experiment, the transfer phase, rats from both conditions were required to learn either a novel monotonic (M; 16-9-3-1-0) or a non-monotonic pattern (NM; 16-1-3-9-0). Subjects were given seven 5-run trials of their assigned pattern each day for ten days. Researchers measured subjects’ running speed in the runway until all food was eaten. Rats had to wait in the runway’s start chamber 10-15sec (the inter-run-interval) between runs and wait 10-20 min outside the runway between trials (the inter-trial-interval). During training, both monotonic and random patterns resulted in the slowing of speeds from one run to the next. During testing, this decline in speed on the 0 element was found to be even larger in the M-M, R-M and R-NM transfer groups. Hulse and Dorsky (1979) concluded that this was evidence that the rats' behaviour was being modulated by the monotonic pattern's "less than relationship" – the rule.

Capaldi and colleagues (1980) argued that the findings in Hulse and Dorsky's (1979) study could be explained by simple associations between elements in the sequences (i.e., stimulus generalization). To support their argument, Capaldi et al. (1980) attempted to create a test that could evaluate both the memory generalization and rule-encoding explanations. To do this, a straight runway, similar to that of Hulse and Dorsky (1979), was used. Rats were divided into four groups, with each group required to complete a particular sequence of runs (4-0, 16-4, 16-4-0, 16-4/4-0). The numbers in the sequences refer to the number of pellets that could be found at the end of the runway on that particular run. Groups 4-0, 16-4, and 16-4-0 received only one trial per day, whereas the last group (16-4/4-0) received two trials – one each of the two sequences. Capaldi stated that, according to the rule-encoding view, more instances of the rule should result in the rule being encoded more quickly. In his experiment then, group 16-4/4-0 should track the best as those subjects received twice the number of trials with the rule. However, the memory-generalization view suggests that more instances of a rule would hinder tracking if this led to stimulus generalization of rewarded to unrewarded runs. The findings were consistent with the memory generalization view. Group 4-0 produced better tracking of the 0 pellet runs than group 16-4/4-0. Capaldi suggested that the 16 pellet runs acted as positive cues for reward and that may have been generalized to the 4 pellet runs. In group 4-0, though, no positive cues were available to be generalized with another run in the sequence.

Hulse (1980) responded to Capaldi's findings by stating that the sequences Capaldi used were not long enough to promote rule learning. He argued that stimulus generalization does occur in short sequences of two runs, but when longer patterns are used, an overall rule must and does form. Hulse further argued that finding's produced by Capaldi's methods did not apply to the area of rule-learning.

Roitblat et al. (1983) attempted to reconcile the procedural differences between the two and determine which of the two views remained valid. A runway apparatus was once again used with varied amounts of pellets at the end. A monotonic (14-7-3-1-0) and a non-monotonic (14-1-3-7-0) sequence were used for these experiments. Inter-run intervals (short: 10-15s; long: 4-5min) and number of trials per day (1 or 4) were manipulated in the first three experiments. In the last experiment, the monotonic sequence was tested with probe trials, wherein a normally rewarded run becomes a 0 pellet run. The overall results indicated that the monotonic runs were more easily anticipated by subjects than non-monotonic sequences consisting of the same runs. Inter-run, but not inter-trial, intervals appeared to affect rats' ability to learn a sequence. That is, shorter inter-run intervals promoted easier acquisition of a sequence. Probes were found to have no effect on speeds. Where in the sequence the probe was located also did not seem to make a difference. These findings were most consistent with Hulse and Dorsky's (1979) rule-encoding view. Roitblat concluded that Capaldi's (1980) generalization view was not sufficient to explain the results of this study but neither were his own nor those of Hulse and Dorsky (1979) able to account for Capaldi's (1980) findings. Roitblat concluded that the procedural differences between the two researchers' studies made comparisons between their findings difficult and possibly their very different sets of findings could not be explained by one model.

Recent studies have approached serial pattern learning in rats with a mind-set consistent with the rule encoding model (e.g., Fountain, 2008; Fountain & Benson, 2006; Kundey & Fountain, 2001). However, I also accept the idea that paired associations, as suggested by the memory generalization view (Capaldi, 1980), do occur in serial patterns, and may actually contribute to the overall governing rules formed of these patterns. The goal of this project is not to continue this debate, or provide support for either side of it, but I do wish to recognize the importance of the research behind the arguments. It is this literature, after all, that prompted me to ask specific questions about rule learning and how it occurs in rats. Now I will address the reasons behind my use of a free operant-style of testing.

The Use of My Free Operant Procedure

I would like to note that the early serial pattern sequence learning runway tasks described in the preceding section follow manual discrete trial procedures. Discrete-trial procedures have been described as those in which the responding of the subject is restricted by certain "externally controlled stimulus events," such as, removal from the conditioning apparatus (i.e, the runway or maze), or even 'time-outs' such as the retraction of a levers in the free-operant conditioning chamber. Free operant procedures, however, are considered those in which the subject's responses are not restricted, and may be made at any time (Hachiya & Ito, 1991). In my apparatus, the continuous availability of all five nose-poke keys allows the rat select any of them (lit or unlit). This condition makes my procedure a more automated, free operant-like task. I have two methodological reasons for using a free operant procedure to examine serial pattern learning.

First, a situation which encourages/ results in variable responding would make learning a pattern more difficult than a situation in which responding is more consistent. It is important that the procedure used for pattern learning in rats, nurtures a more consistent responding style. A study by Morris (1989) examined the differences in effects of lag values on the control of response variability between discrete trial and free operant procedures. Pigeons were given three sessions wherein two keys (R-right, L-left) were available and pecks to these keys were reinforced on an FR-4 (fixed-ratio-4 reinforcement) schedule. Two baseline sessions were given to each subject: first, a free-operant baseline where all keys were illuminated throughout each trial, followed by a discrete-trial baseline, wherein 2-sec time-outs occurred after each peck. In both baseline sessions, pecks remained reinforced on the FR-4 schedule. After all subjects received these baseline sessions, they were given lag contingencies (2, 4, & 6) - where reinforcement only occurred if the response sequence differed from those response sequences made on the previous 2, 4, or 6 trials. For example, in a lag-2 contingency the pigeon might initially make two different 4-peck sequences (e.g., RLRL and RRLL). The pigeon's third sequence must then differ from both of these previous sequences in order to receive reinforcement (e.g., LLRR). Fifteen sessions under each procedure were given, with 5 sessions for each lag contingency. It was concluded that the discrete trial procedure resulted in greater response variability for all subjects and lag values than the free operant procedure. As less variable responding is equated with more accurate performance on complex discrimination tasks, and serial pattern learning is a type of complex discrimination task, this reduction in variable responding can be seen as an advantage in the free operant procedure.

Second, in my pattern learning tasks, rats will be required to complete 3-item sequences by nose-poking keys that become lit in particular patterns (i.e., the governing pattern). Each pattern will govern multiple sequences; that is, *sequences* under the same pattern will differ in respect to where they start (signal 1). Where the 2nd and 3rd signals will occur depends on the 1st signal, with the relationship consistent across sequences under the same pattern. It is fair to say, then, that in order for rats to learn these patterns, they may have to remember what they have done on previous sequences. McDevitt and Bell (2008) conducted a study investigating whether stimulus preference during probe trials was due to differential stimulus value or generalization of behaviour patterns developed during training. Pigeons were given a discrete trial procedure of alternating concurrent schedules, where the side keys varied in reinforcement probabilities (.05 & .10, and .10 & .20). In this procedure, a 6sec inter-trial interval was given after each peck. Subjects were then trained on a free-operant procedure consisting of the same contingencies as the discrete trial; however, each response was no longer followed by an inter-trial interval. Two types of probe trials were presented at the end of training: one consisting of two .10 alternatives and one with .20 and .05 alternatives. During training, both procedures resulted in a preference for the 'richer' stimulus. Both procedures showed preference for the .20 over the .05 alternatives in probe sessions. A difference between the two procedures occurred in probe tests with the two .10 alternatives. The discrete trial procedure resulted in no preference; whereas, the free-operant procedure resulted in preference for the .10a alternative (previously associated with the .05 during training) in all subjects. These findings suggest that the free operant procedure may have made it easier for subjects to recall information from previous trials in order to make future choices, further supporting the value of free operant procedures for serial pattern learning. Here, I would like to point out that pigeons and rats are different species. However, as pigeons have actually been shown to have poorer memory capacity than rats (Bond, Cook & Lamb, 1981) I believe that a procedure that aids in memory, as the free operant task has been shown by McDevitt and Bell (2008) to do, will not negatively affect rats' performance.

Finally, Roitblat et al. (1983) provides a theoretical reason for using the free operant procedure in serial pattern learning. It is better to have short ISIs for serial pattern learning and as mentioned above, Roitblat et al (1983) has shown that inter-signal (or inter-run) intervals affect rats' performance. They found that longer IRIs reduced prediction accuracy (i.e., the task was more difficult). These results show that massed runs (short inter-run intervals) lead to better sequential learning than more distributed runs (longer inter-run intervals). This is also supported by earlier data presented by Winz (1931), where massed practice was shown to produce better recall than distributed practice. Winz concluded that during the periods of no practice in a distributed condition, some of the material becomes forgotten. It is reasonable to say, then, that during longer inter-run (or inter-signal in our case) intervals, the previous run may become forgotten by the animal, which will affect performance. In my procedure, remembering a previous press, within a sequence, can aid the rats' performance.

Rules and Incidental Learning

In the literature, cognitive processing, including detecting, learning and using *rules* or *patterns*, is referred to in terms of two categories: *automated* (a.k.a. automatic) and *deliberate* (a.k.a. controlled) processing. Automated processes can be thought of as sequences of behaviours activated in the presence of particular stimuli. These processes begin and continue without the subject's attention or control. When a cognitive process becomes automated, it has been found to be a long-lasting and persistent process. A deliberate process, on the other hand, is a more temporary sequence of behaviours requiring control and attention on the part of the subject (Shiffrin & Schneider, 1977; Ben-Zur, 1998; Schneider & Chein, 2003).

It is possible for a rat to learn a rule without actively attempting or intending to do so; that is, rule learning can be *incidental* (McGeoch & Irion, 1952; Tresselt & Mayzner, 1960). When rule learning is incidental, it is often also implicit. *Implicit learning* has been described as when an organism learns, without the awareness of doing so and in the absence of "explicit instructions to learn" (Locurto, Gagne & Nutile, 2010; Locurto, Dillion & Collins, 2013); the subjects are not explicitly informed of the fact that similarities between the sequences exist. In my experiments, learning is assumed to be both incidental (unintentional) and implicit (unconscious). This is opposed to the incidental but *explicit* serial pattern learning used by Fountain and colleagues (Fountain, 2008). When rules are learned and executed incidentally and implicitly, they can be considered as automated processes.

Studies by Fountain and colleagues (Fountain, 2008; Fountain & Benson, 2006; Fountain & Rowan, 1999; Kundey & Fountain, 2010; Kundey & Fountain, 2011; Kundey & Fountain, 2014; Kundey et al., 2014) have investigated rats' ability to explicitly learn sequential patterns. In early studies on serial patterns, the animal simply had to run down a runway, and whether the animal had learned the rule/pattern was determined by its variations in running speed (Capaldi & Molina, 1979; Hulse & Dorsky, 1979). In Fountain's experiments, however, the animal must *show* that they know the rule by producing it. The majority of his studies have made use of octagonal chambers consisting of 8 levers arranged in a circular array (*Figure 2*). In most of the experiments, rats have been required to learn and execute long, clockwise serial patterns of lever presses (e.g. "123-234-345-456-567-678-781-812" – a "*run*" pattern). The serial patterns contain "chunks" of 3 elements, with each chunk typically sharing a common rule (e.g., "+1"). These sequences are typically 24 responses long and often contain a “violation” element – a response that “blatantly violates the base within-chunk rule”- e.g., “123-234-345-456-567-678-781-81*8*” (Fountain, 2008). A rat must produce the sequence by pressing the levers in the correct order. Each corrected response is reinforced, but incorrect responses result in immediate correction (all levers except correct one retracted until the correct press made) before the rat can continue on through the sequence. The rat must then repeat the sequence multiple times (e.g., 20 trials) per day. Data from the acquisition of these long sequences is recorded and often involves long periods of time – e.g., 28, 35, 41 days (Fountain & Rowan, 1999; Fountain & Benson, 2006; Fountain, 2008; Kundey & Fountain, 2011). Mean percent error for each element of the sequence is analyzed across trials. When a decline in mean percent errors is seen across chunk elements (i.e., fewer errors on 2nd than 1st and on 3rd than 2nd), the chunk-governing pattern is said to have been learned.

Fountain’s research on rule learning has provided strong evidence to support the idea that rats are capable of learning and using basic rules in sequential tasks. As interesting and versatile as his procedures of investigating rule learning are, these procedures can require extensive training of the animals. In one study, for example, subjects were exposed to interleaved patterns for 35 days (Fountain & Benson, 2006). Subjects are not just required to recognize the pattern/rule/sequence, but to recall it on their own. As some of the patterns are quite long (e.g., 60 items), it is clear why so many training trials are necessary. The experimental procedures used in my investigations are simplified versions of the tasks used by Fountain and colleagues, requiring significantly less training. This simplification is achieved through three important differences between the tasks. First, in my studies the animals do not necessarily have to memorize the patterns in order to complete the task; they can simply follow the light. In the

Fountain and colleagues' tasks, rats must *explicitly* use the pattern – they are constrained to make specific responses by the correction procedure. Second, the sequences used in my tasks consist of only three responses, with each sequence being governed by the same overall pattern. Subjects, then, must only remember where they began and which signal they are on; whereas in Fountain's patterns, subjects must remember the pattern as well as which chunk and which signal within the chunk they are at. Third, my operant chamber contains a five signal panel along one wall (*Figure 1*) in contrast to the eight signals or levers in the octagonal chamber; thus, the probability of making an incorrect response is only 4/5 (80%) as opposed to 7/8 (87.5%). The linear array of my panel also allows for each signal to be easily distinguished from one another. The eight walls of the octagonal chamber, though, are identical and can only be distinguished by cues outside of the chamber (*Figure 2*).

If animals aren't required to use the rules in order to complete my tasks, then why should they bother to learn them? One possible answer is that using the rule makes the animals' performance more efficient; they no longer need to pay attention to visual information (i.e., track the light) except for first signal, and make fewer errors (Fountain, 2008). Thus, using the governing rule will assist rats in completing sequences and, therefore, acquire reinforcement, more quickly.

Summary and Objectives

This master's thesis is a systematic study of some of the kinds of possible sequences that a rat can learn and the types of violations that affect their performance on these sequences. I sought to determine what exactly it is about the patterns that the animals are learning. The first objective of this project was to determine if rats could incidentally recognize patterns and if so, whether they then use a response pattern (i.e., a global three element rule) - as opposed to a signal pattern (i.e., simply following the light) or response association pattern (i.e., paired associations between responses of the sequence). Next, I set out to determine how switching between three different patterns within a session affects performance overall. Finally, I investigated whether subjects who are given three-signal sequences with fixed inter-signal intervals (ISIs) – the time between each individual signal within a sequence – produced performance differences from those with varied ISIs.

Three experiments were conducted to tackle these three objectives. All three experiments required subjects to complete 3-item sequences that followed governing patterns, in order to receive reinforcement. In my first experiment, subjects were given experience with three different pattern types. This experiment was divided into three phases, with each phase corresponding to a pattern type and all subjects receiving the phases in the same order. A phase consisted of ten training and six probe sessions. Probe sessions contained both *normal* (those that followed the governing pattern) and *violation* (those that did not follow the pattern) sequences. In the second experiment, all three pattern types from the first experiment were then given within each session, occurring in blocks of 22 sequences (one block for each pattern type). This experiment consisted of four 6-session blocks. Each session within a block contained a different order of the three patterns than the other five sessions. My final experiment made use of a novel pattern type. Subjects were divided into two groups: a fixed inter-signal interval group and a varied inter-signal interval group. The groups both received sessions of the same sequences, but with different intervals between the signals of these sequences. The animals were given fifteen training sessions, followed by six probe sessions containing violation sequences.

It is important to point out that this project is not just a replication of past studies on sequence learning, but is an extension of these studies. Previous experiments (e.g., Domenger & Schwarting, 2005; Schwarting, 2009) only looked at response times of sequences that were performed perfectly (as animals were required to restart a sequence when an error was made). In this project, performance was also analyzed on sequences where mistakes were made. This provides information as to whether a "decision" is being made by the animal before making a response, both correct and incorrect. This is particularly vital information when considering probe sequences.

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CHAPTER TWO:

ARE RATS CAPABLE OF ACQUIRING AND USING 3-ITEM GOVERNING PATTERNS?

“Human beings are pattern-seeking animals. It is part of our DNA…we always look for the wider, bigger explanations for things” (Adrian McKinty, *The Cold Cold World*).

Introduction

Serial reaction time tasks (SRTTs) require subjects to perform sequences of behaviours in response to the presentation of stimuli, such as presses, in order to receive reinforcement; reaction times of responses are recorded (Nissen & Bullemer, 1987; Christie & Dalrymple-Alford, 2004; Christie & Hersch, 2004; Domenger & Schwarting, 2005; Schwarting, 2009). SRTTs have shown to be useful tools in the analysis of learning and performing sequential tasks in rats. The use of patterns in serial reaction time tasks has recently become more common in the literature (Fountain, 2008; Muller & Fountain, 2010; Kundey et al., 2014; Kundey & Fountain, 2014). Why the interest in pattern learning? In addition to the information patterns provide that allows us to find explanations for occurrences, patterns are also important tools for acquiring more basic needs. The use of patterns can allow animals to survive and thrive in many ways. Social patterns can influence reproductive success in guppies (James, 1980), and the waggle dance (a pattern of movements) in honey bees communicates food location to others in the hive (Dornhaus & Chittka, 2003). In some species, such as the Allegheny Dusky salamander (Mead & Verrel, 2002), failure to perform certain behavioural patterns may lead to reproductive isolation.

Domenger and Schwarting (2005) developed a 4-choice SRTT variant to investigate serial reactions of random and sequential sequences of stimuli in rats. The training apparatus used in this task contained four light-equipped holes for nose-poking, arranged in a square, with a food pellet cup located in the center of the array (*Figure 1*). Rats were required to complete a series of nose pokes (on Fixed Ratio-6 or FR-12 reinforcement schedules) consisting of either a random set of signals or a fixed, repeating set of signals. To complete these sequences, a hole would become illuminated, prompting the animal to nose poke it. If the sequence was correctly completed, the animal would be reinforced and the reinforcement would be followed by the illumination of the next hole in the sequence. If a rat made a mistake (i.e., poked an unlit hole), the fixed ratio sequence would restart. Response times were measured on each nose poke in the sequence. These data revealed that rats reliably responded faster on their last (reinforced) nose poke in the fixed, rather than random, sequences. This reaction time advantage was mirrored by an accuracy advantage in the fixed over the random sequence condition. Both conditions, however, resulted in an overall negative correlation between reaction time and sequence signal (i.e., RTs to signal 2 were slower than RT to the last signal of a sequence, 6 or 12).

The procedure followed by Domenger and Schwarting (2005) demonstrated that rats performed better on sequences of a fixed, repeated pattern of response locations than on sequences of random response locations. However, it did not provide information as to *what* exactly it is about the fixed sequence that is being learned. Is there some basic underlying rule, or is a simple response chain being developed? A compilation of studies conducted by Fountain and colleagues (2008) investigated whether rats were capable of learning and using sequential rules. These studies made use of an octagonal operant chamber (*figure 2*), within which a lever was located on each of the eight walls. Reinforcement occurred after each correct response. Unlike the nose-poke SRTT tasks, the correct response was not signaled, and the rat was required to remember a pattern to choose the correct responses and receive reinforcement. If an incorrect response was made, all incorrect levers were retracted, leaving only the correct choice. In one of

Fountain’s (2008) experiments, rats were given two patterns to learn, each consisting of a long chain of responses with short pauses after each third response: one with a simple structure (123-234-345-456-567-678-781-812) and one that contained a violation element – a response that did not follow the overall simple pattern (123-234-345-456-567-678-781-81**8**). As expected, the pattern containing a violation element was more difficult to learn, resulting in more errors than the one without a violation element.

In a similar study, Kundey et al. (2014) reported that both humans and rats were capable of performing two sequences - “runs” (i.e., 123-234-345-456-567-678-781-812) and “trills” (i.e., 121-232-343-454-565-676-787-818) - over the same locations with accuracy above chance. In this study, subjects were provided with eight response choices positioned in a circle (touch screen computer screen for humans; octagonal operant chamber with levers for rats). In each trial, subjects were required to perform a 24-item sequence based on one of the two patterns (trill or run). A cue was given immediately before the start of a sequence to indicate which pattern to follow. Both rats and humans produced fewer errors than chance and reportedly improved their performance on trill and run sequences throughout the experiment. Rats appeared to perform run patterns better than the trills, but this difference was not observed in humans. These results further support Fountain’s (2008) findings that rats are capable of acquiring and using cognitive representations (rules) of sequential responses.

The one drawback to having rats learn and remember 24-item sequence patterns is the amount of time it takes to do so. This obstacle can be overcome by adapting this procedure to one that can be used in a basic SRTT. In the current study, I combined the simplicity of Domenger and Schwarting’s (2005) rat SRTT variant with the pattern-based component of Fountain (2008) and Kundey’s (2014) experiments in order to investigate pattern learning in rats.

I was interested in uncovering whether rats can recognize basic sequence patterns and, if they then develop a response pattern (i.e., a global three element rule), as opposed to a signal pattern (i.e., following the signal light) or response association pattern (involving pairs of associations between individual responses in the sequence; i.e., “after response x, make response y”) to remember these patterns. As opposed to requiring the animals to *use* a rule to perform a sequence, as the procedures used by Fountain (2008) and Kundey (2014) do, in my task the rats must simply learn to press a lit key. Any other information that the animals learn (such as the governing pattern) is incidental.

In order to answer my question, I gave subjects three different pattern types to learn in an SRTT, using a basic operant chamber (*figure 3*). The first pattern was simple, making use of only two keys on the panel: “forward 1, back 1” (e.g., 121; *figure 4a)*. The second pattern covered more distance on the panel, consisting of three keys: “forward 1, forward 1” (e.g., 123; *figure 4b*). Finally, the last pattern was a more complex, blend of the first two patterns: “forward 2, back 1” (e.g., 132; *figure 4c*). In contrast to Domenger and Schwarting’s (2005) 6- or 12-item sequences and Fountain (2008) and Kundey’s (2014) 24-item sequences, I used these smaller, 3-item sequences on a fixed-ratio reinforcement schedule. Another difference to be noted is that while the fixed sequences used in Domenger and Schwarting’s (2005) study consisted of one repeated series of responses, my three fixed patterns each govern (i.e., rule or define) multiple sequences. These two differences allowed me to examine the ability of rats to learn an overall pattern but vary where each individual sequence began and ended across trials (e.g., 121-343-010-232-343-010-121…).

Animals were trained on patterns during training days that were followed by probe days. Training day sessions consisted of sequences that all followed the basic governing pattern. Probe day sessions also presented the animals with the regular sequences but interspersed these with violation sequences that did not follow the pattern. It was predicted that subjects would show a decline in reaction times across the three signals of sequences after training on each of the three patterns. It was also predicted that violation sequences would lead to an increase in reaction times on 2nd and/or 3rd signals (depending upon the violation) if learning had occurred. If the animals had not learned the patterns, the decline in reaction times would be seen in both normal and probe sequences (no disruption of performance). These predictions were based on the knowledge of fixed-ratio reinforcement schedules – as each response occurs closer to the reinforced response, animals respond more vigorously (Sidman & Stebbins, 1954; Mechner, 1958).

It was also predicted that animals would make, on average, fewer errors on pattern-based sequences than probe sequences (high number of errors indicates disruption in performance). Furthermore, it was expected that most of the errors made on violation sequences would be "correct errors" (i.e., responses that indicate anticipation of signals that follow the pattern). These predictions are based on findings noted by Fountain (2008) that, 1) in one experiment subjects made the highest number of errors on responses where the sequence changed direction (disrupting their performance), and (2) in a second experiment, most of the errors being made by subjects on violations were responses that obeyed the structure of the overall governing pattern. I also expected to see higher proportions of sequences that contain no errors (*perfect sequences*) in the 2nd block than the 1st block of training for each pattern. Lastly, I expected higher proportions of pre-violation sequences, than violation sequences, on probe days to be performed without errors. This prediction was an extrapolation of the two previous expectations.

Methods

Subjects:

Seven male, one-year old Long Evans rats (*Ratus norvegicus*) were used for this experiment. Subjects were housed in two group cages within a larger colony room in which a 12/12 hour light and dark cycle was maintained. Humidity (~50%) and temperature (~20-25°C) were monitored daily to ensure appropriate living conditions. All group cages were equipped with a running wheel and PVC pipe to enrich the environment as well as bedding, a salt stone, and unlimited drinking water. Animals were placed in individual feeding cages with 20-25g rat chow for two hours each day and weights were monitored to prevent the occurrence of under- or over-eating. Subjects were thoroughly tamed prior to this experiment. Each experimental session was run at midday, before feedings.

Apparatus and Materials:

Three identical, basic operant conditioning chambers were used for this experiment (*Figure 3a.*). All were well ventilated and sound proof. Each chamber contained a curvilinear five-key signal panel along one wall (*Figure 3b*). The centers of each key are exactly 4cm in distance from one another. The middle key is located 23cm across from the reinforcement wall of the chamber, whereas the end keys are only 21cm away from this wall. These keys would individually light up, indicating which to nose poke. Directly across from the signal panel was a pellet dispenser through which reinforcement pellets were delivered to and received by the animal upon completion of a correct sequence. Each reinforcement pellet, therefore, was provided after the animal successfully nose poked the three lit keys. Each chamber was also equipped with a tone that would come on immediately before a reinforcement was released for 1000ms, acting as a secondary reinforcer.

The software program Labview was used to record data from the operant chambers and convert them into an excel file for analysis. The software allowed for the recording of reaction times (in milliseconds) – the time it took the animal to press the key after it had become lit – as well as which key had been pressed (including correct and incorrect responses). This allowed for the collection of both error type and error trials data.

Standard, dustless precision pellets, ordered from Bio-Serv, were used as reinforcers in the chambers. When an animal successfully completed a full session, 64 pellets were received each day. These pellets contain casein, sucrose, fiber, dextrose, D1-methionine, L-cystine, choline, corn oil, corn syrup, mineral mix, and a vitamin mix.

Procedure:

Pretraining: The seven subjects used for this experiment were all used in previous serial reaction time task procedures in which they were required to complete three-item sequences. These previous tasks required the subjects to complete three-item sequences on a VR-2 (variable ratio two) reinforcement schedule. The sequences were either governed by a basic direction rule ("move one to the right" or "move one to the left", depending on the subject) or the direction was random. All subjects received both conditions. The animals, therefore, had some, albeit limited experience with a form of rule learning and consequently, received no pre-training immediately before the start of this experiment.

Performing a Sequence:

This experiment required the animals to complete three-item sequences in order to receive reinforcement. Each session consisted of 64 sequences, resulting in a total of 192 responses. Each individual nose-poke response required the animal to wait for a key to light up, then immediately select this key. An inter-signal interval of 10ms occurred between each signal in a sequence. The entire task of completing a sequence went as follows: the animal starts in the chamber facing the signal panel, a key lights up, the animal nose-pokes this key and the light turns off, 10ms after this light turns off, the second signal becomes lit until the animal nose pokes it, followed by another 10ms interval before the third and final signal. Once the final signal was poked, a 1000ms tone sounded, indicating that reinforcement would be released once the rat nose poked food hopper (or after 1000ms had passed). If at any time the subject selected an incorrect key (i.e. one that was not lit), the correct key would turn off and immediately turn back on until it was successfully chosen.

Experimental Schedule: This experiment was conducted in three phases. Each phase corresponded to a particular basic governing pattern being used. Phase A consisted of sequences all using the basic pattern of "Forward 1 key, backward 1 key," or "F1B1" for short. An example sequence of this pattern (*Figure 4)* would be "1-2-1." Phase B consisted of sequences governed by the "Forward 1 key, forward 1 key" pattern ("F1F1"). As depicted in *Figure 2a*, an example sequence of this pattern is "1-2-3." Finally, in phase C of the experiment, the sequence "1-3-2" is an example following the pattern of "Forward 2 keys, backward 1 key" ("F2B1" – *Figure 4c*). Each phase was comprised of two blocks, with seven training sessions followed by three testing sessions making up each block.

Training:

Each of the training sessions consisted of 64 sequences. The “forward” direction referred to moving right across the signal panel for half of the animals, whereas it referred to moving left for the other half. All six animals received the three phases in the same order: A (“F1B1”) – B (“F1F1”) – C (“F2B1”).

Testing:

The three testing sessions in each experimental block were made up of 64 sequences, 58 of which were normal sequences (i.e. they followed the governing pattern) and six of which were “probe” sequences. These probe sequences violated the governing pattern and were divided into two types: mirror and other. The mirror probes violated the direction of the pattern – the sequence was a mirror-image of the pattern (ex. The mirror probe of 1-2-1 would have been 1-0-1). The other probes depended on what the governing pattern was: for phase A and C patterns, the other probes were “F1F1” (e.g., The other probe of 1-2-1 or 1-3-2 was 1-2-3); for phase B patterns, the other probes were “F1B1” (e.g., Other probe of 1-2-3 was 1-2-1). The six probe sequences were found at every tenth sequence within the testing sessions.

Statistical Analyses:

A three-way (trial type X probe type X signal) within subjects ANOVA was conducted for each of the three phases to analyze reaction time data for pre-violation and violation sequences. Paired samples t-tests were conducted to compare reaction times of 2nd and 3rd signals of both pre-violation and violation sequences across the two probe types. A two-way (training block X session) within subjects design was used to analyze perfect proportion data for each phase. A three-way (trial type X testing block X phase) ANOVA was conducted to compare all three phases' proportions of perfect pre-violation and violation sequences. Finally, a three-way (trial type X error type X probe type) repeated measures design was used to analyze errors. Error type was assumed to be independent for the purpose of comparing the two categories. A two-way design (error type X probe type) was also used to analyze the error data from violation sequences only. Each phase was analyzed separately.

Results

Reaction Time Analysis of Correct Presses for All Sequences:

Figure 5 shows mean reaction time results for pre-violation and violation sequences across all three signals for probe days. Due to their high reaction times, 1st signals were removed from analysis in order to focus on 2nd and 3rd signals (*figure 6*). Effects of trial type were found for all three phases, with pre-violation sequences resulting in faster reaction times than violation sequences (A: *F* (1,6)= 11.95, *p*<.05; B: *F* (1, 6)= 14.80, *p*<.01; C: *F* (1, 6)= 9.95, *p*<.05).

In phase A (pattern F1B1), mirror type violations did not affect reaction times on either the 2nd (t (6)= 1.62, *p*=.16) or 3rd signal (t (6)= -.95, *p*=.38), as seen in *figure 6a*. F1F1 type violations did not cause disruption on the 2nd signals (t (6)= .45, *p*=.67) of this pattern either, but did increase reaction times on the 3rd signals (t (6)= -6.24, *p*<.01). Reaction times declined across the two signals of the F1F1 type pre-violation sequences (t (6)= 4.55 p<.01); however, this decline was not significant on any other sequence type.

As shown in *figure 6b*, mirror type violations disrupted performance on both signals 2 (t (6)= -3.33, *p*<.05) and 3 (t (6)= -4.40, *p*<.01) in phase B (pattern F1F1); however, neither signals were disrupted by F1B1 violation sequences. A decline in reaction times across signals was not observed in pre-violation or violation sequences of either probe type on this pattern.

In phase C (pattern F2B1), performance on both 2nd (t (6)= -2.70, p<.05) and 3rd (t (6)= -2.76, p<.05) signals were disrupted on mirror violation sequences. The expected disruption of reaction time on the 3rd signal of F1F1 type violation sequences was also found and this increase was significant (t (6)= -5.93, p<.01). Surprisingly, F1F1 type violations actually lead to faster reaction times than pre-violations on the 2nd signals in this phase (t (6)= 5.01, p<.01). The overall predicted decline in reaction times across signals was observed in both pre-violation and the mirror violation sequences, but not in the F1F1 type violation sequences.

Reaction time Analysis for Correct Presses on Errorless Sequences Only. Results of this analysis were consistent with the findings from the previous analysis (*Figure 7*).

Proportions of Perfect Sequences:

Training. As seen in *figure* 8, no effect of block was found in phase A (F (1, 6)= 4.88, p=.07); however, a trend was noted, with block 2 resulting in a higher mean proportion of perfect sequences than block 1. In phase B, an effect of block was found F (1, 6)= 36.00, p<.01), with block 2 resulting in better performance (i.e., higher proportion of perfect sequences) than block 1. Phase C did not show an effect of block (F (1, 6)= .28, p= .62) and no trend was noted either.

Probes. *Figure* 9 compares mean proportions of perfect sequences of pre-violations and violations across the phases. The data is collapsed over probe types due to small proportion values when separated. Collapsing the probe types reduced variability, strengthening the power behind my analysis. A main effect of trial type was observed (F (1, 6)= 31.20, p<.01) with pre-violation sequences resulting in a higher proportion of perfects than violation sequences across all three phases. An overall main effect of block was also found (F (1, 6)= 11.24, p<.05), with a higher proportion of perfect sequences occurring in blocks 2 compared to blocks 1. No differences were found between the three phases (*F* (2, 12)= .22, *p*= .81).

Errors:

Figure 10 illustrates the mean number of “correct” and “other” errors for pre-violation and violation sequences of all three phases. Repeat errors were considered to be unintentional errors (< 100ms reaction time) and were removed from statistical analyses. In phase A (*Figure 10a*), an effect of trial type on number of errors occurred (*F* (1, 6) =10.28 p<.05), with pre-violation sequences resulting in fewer total errors than violation sequences. No difference was observed between the mirror and F1F1 probe types (F (1, 6)= 3.03,p=0.13) in this phase. The total number of “correct” and “other” errors were not different for this pattern (*F*(1, 6)= 2.68, p=0.15). A trial type by probe type interaction was noted in phase A (*F* (1, 6)= 10.54, *p*<.05), with pre-violation sequences of the F1F1 probe type resulting in the least errors and pre-violation sequences of the mirror probe type resulting in the most. Subjects made a moderate number of errors on violation sequences of the mirror and F1F1 probe types.

No effects of trial type (*F* (1, 6)= 3.44, *p*=.11) was identified in phase B. The two probe types were found to be different (*F* (1, 6)= 6.83, *p*<.05) on this pattern, with mirror type sequences resulting in more errors than F1B1 type sequences (*Figure 10b*). The number of “other” errors were higher than “correct” errors in this phase (*F* (1, 6)= 13.46, *p*<.05). A trial type by error type interaction was also observed (*F* (1, 6)= 12.61, *p*<.05). Subjects made more “other” errors on pre-violation than violation sequences. Although the trial type by probe type interaction was not significant in this phase (*F* (1, 6)= 5.07, *p*=.07), a trend was observed, with more errors in general being made on mirror probe type sequences than F1F1 probe type sequences.

The results of phase C (*Figure 10c*) showed no differences between pre-violation and violation sequences (*F* (1, 6)= 2.78, *p*=.15) or between the two probe types (*F* (1, 6)= .29, *p*=.61) in total number of errors. However, there was an effect of error type noted in this phase (*F* (1, 6)= 13.91, *p*<.05), with more "other" errors being made than "correct" errors. A trial type by error type interaction was also found in (*F* (1, 6)= 7.28, *p*<.05). Subjects made more "other" errors on pre-violation than violation sequences. The interaction between trial type and probe type was not significant in phase C (*F* (1, 6)= 1.48, *p*=.27).

Violation sequences only. Violation sequences were looked at separately as "correct" errors could not be made in pre-violation sequences. These findings can also be observed in *Figure 10*.

There were no differences in the total number of "correct" errors versus "other" errors in phase A (*F*(1, 6)= .08, *p*=0.79) on violation sequences. However, a difference between the two error types was found on violation sequences in phases B (*F* (1, 6)= 7.27, *p*<.05) and C (*F* (1, 6)= 7.48, *p*<.05). In phase B, more "other" errors were being made than "correct" errors. In phase C, the opposite was true. Mirror and other (F1F1 or F1B1, depending on pattern) violations resulted in different numbers of errors in both phases A (*F* (1, 6)= 11.49, *p*<.05) and B (*F* (1, 6)= 27.87, *p*<.01). In phase A, subjects made more errors on the F1F1 probe types than mirror probe types. However, in phase B, subjects made more errors on the mirror violations than the F1B1 violations. In phase C, the two probe types produced similar differences to phase A, however, not significantly (*F* (1, 6)= 3.27, *p*=.12).

Finally, a non-significant trend for error type by probe type interaction occurred in phases A (*F*(1, 6)= 5.51, *p*=0.06) and B (*F* (1, 6)= 5.35, *p*=.06). In phase A, the most errors subjects made were "correct" errors on mirror violations, and the least were "correct" errors on F1F1 violations. In phase B, the most errors were "correct" errors on mirror violations and the least were "correct" errors on F1B1 violations. This interaction was found in phase C (*F* (1, 6)= 15.08, *p*<.01), with more the most errors being "others" on F1F1 violations, and the least being "correct" errors made on F1F1 violations.

Discussion

I examined whether experience with sequences that consistently follow governing patterns would result in incidental 'rule' learning in rats. Violation sequences were used to test whether learning had occurred. It was expected that a decline in reaction times would occur across the three signals of sequences following the governing patterns, but not in those that violated the pattern. It was also predicted that presentation of pattern-violating sequences would lead to a disruption in performance (i.e., increase in reaction times, decrease in proportion of perfect sequences and increase in number of errors). These predictions were based on standard knowledge of reinforcement schedules (Sidman & Stebbins, 1954; Menchner, 1958) and recent findings of violation element effects (Fountain, 2008; Kundey, 2014). Overall, I have concluded that the rats were capable of recognizing all three basic patterns used in this experiment; however, what was incidentally learned about each pattern differed. As evidenced by comparisons of pre-violation and violation sequence data and further supported by proportion data, the F1B1 (phase A) and F1F1 (phase B) patterns seemed to show response rule patterns had been learned, whereas the F2B1 (phase C) pattern results suggest a response association pattern.

Although rats did not show significant improvement in proportion of perfect sequences on pattern-governed sequences across blocks of training in phase C as predicted, this improvement was significant in phase B and a trend in this direction was found in phase A. It appears that the animals were getting better at performing these pattern-based sequences without mistakes, at least in the first two phases. As predicted, animals made significantly higher proportions of perfect sequences on pre-violation than on violation probe trials for each FR-3 pattern. This provides support for the notion that violations in general were significantly disruptive to performance, indicating that the animals may have been attempting to use the basic governing patterns.

The predicted overall decline in reaction time over the last two signals was supported by my results on the pre-violation sequences of the F1B1 pattern. This decline was not observed on the F1F1 violation sequences, suggesting that learning had occurred. The F1B1 pattern used in phase A did not show disruption in reaction times on either the 2nd or 3rd signals by the presentation of mirror type violations. There are two different possible response rule patterns that could have been learned about this pattern. The first possibility is that the animals have learned “move forward 1 key, then move back 1 key.” The second possible response rule pattern is “the 1st and 3rd responses occur at the same location, which is the location that is reinforced.” The data suggests the rats had learned the second response rule pattern. If the direction/location of the 2nd signal were important to the underlying rule, its violation by the mirror sequence would have caused disruption in performance. This conclusion may also be supported by the reaction time data of F1F1 violation sequences. Only the 3rd signal of the pattern is violated by these sequences. If the response rule pattern simply involved understanding that the 1st and 3rd signal are at the same location, changing the location of the 3rd signal should significantly disrupt performance on this response (Fountain, 2006), which is exactly what I find. The prediction that more total response errors would occur on violation than pre-violation sequences was also supported; though no significant differences in the number of “correct” versus “other” errors was found. These results suggest that the animals did in fact recognize and incidentally learn the basic F1B1 pattern.

The overall decline in reaction time across signals did not occur on sequences following the F1F1 pattern. However, this decline was not produced by the violation sequences of this pattern either. The prediction that violation sequences would negatively affect reaction times was supported though. Mirror violations significantly increased reaction times to both signals. Once again, there are two possible underlying response rule patterns the animals may have developed. One suggested rule would be “continue to move forward along the panel.” The second possibility is similar to the first, but involves direction: “always move along the panel in one particular direction.” The data appears to point to the direction-specific response rule pattern. If a response-association (also referred to as response-based association) pattern had been developed, instead of an overall response pattern, I would not have seen an increase in reaction time on signal 3 as the 2nd signal would prime the next response. A response association pattern develops when subjects form associations between the individual responses, not the sequence as a whole (Schwarb & Schumacher, 2012). The F1B1 probe sequences only violated the 3rd signal of the governing pattern, by switching direction. This is the only signal I observed to be disrupted, supporting the importance of direction in this pattern. Subjects did not make more response errors on violation sequences than pre-violation sequences in this phase. No significant differences in total number of errors were found between the pre-violation and violation sequences, but an overall trend was noted with more errors being made on violation sequences.

The reaction time data for pattern F2B1 suggests that the animals had learned the pattern, but the perfect proportions and error data contradict this. These findings lead me to believe that the animals may not have learned an overall response pattern, but instead developed a response association pattern. The predicted decline in reaction times across signals was observed in all pre-violations as well as mirror violations, but an increase occurred in F1F1 violation sequences. Mirror violations did increase reaction times on both signals, as both were violations of the pattern. Though, the decline in reaction time from 2nd to 3rd signals of these violation sequences suggest that after the 2nd signal proved to be inconsistent with the governing pattern, subjects may have adapted to the change, and were able to quickly respond to the 3rd signal. In order for this to be the case, an association between the individual responses within the pattern would have to have been developed. The response-association pattern learned may be along the lines of "the 2nd signal is two positions away from the 1st, and the 3rd signal is 1 position away from the 2nd, in the opposite direction." Each prior response dictates what to do next, as opposed to following what the overall pattern says to do next.

The lower reaction time on the 2nd signal in F1F1 violation than on pre-violation F2B1 sequences may be accounted for by the distance involved in the transition from signal 1 to signal 2 on the pre-violation sequences. Rats had to move twice the distance to make a 2nd signal response on pre-violation than violation sequences. Also, the 2nd signal of the F1F1 violation sequences is the location where the animal would receive reinforcement if the sequence had obeyed the pattern. Perhaps the importance rats place on reinforced signals may have led them to make these quick reaction times to those keys (Sidman & Stebbins, 1954; Menchner, 1958). As the 3rd signal was also violated by F1F1 sequences, the increase in reaction time supports my expectations. The lack of difference in total number of errors between the pre-violation and violation sequences did not support my prediction, suggesting the animals may not have learned the pattern. However, when investigating the violation sequences only, more “correct” errors were observed than “other” errors. The high occurrence of “correct” errors suggests that the rats were attempting to follow the pattern even when completing violation sequences – they were making predictions about future responses.

The findings in this study provide further evidence for the notion that rats are capable of learning and using pattern-based rules to accomplish sequential tasks. If rats were simply following the light to perform these tasks, errors and changes in reaction times would not be expected to occur when the response patterns are violated. It has also shown that basic SRTT procedures can be used to investigate this learning. Future studies should seek to determine how the presence of multiple basic patterns within a single task may affect subjects’ performance. I tackle this question in the next chapter.

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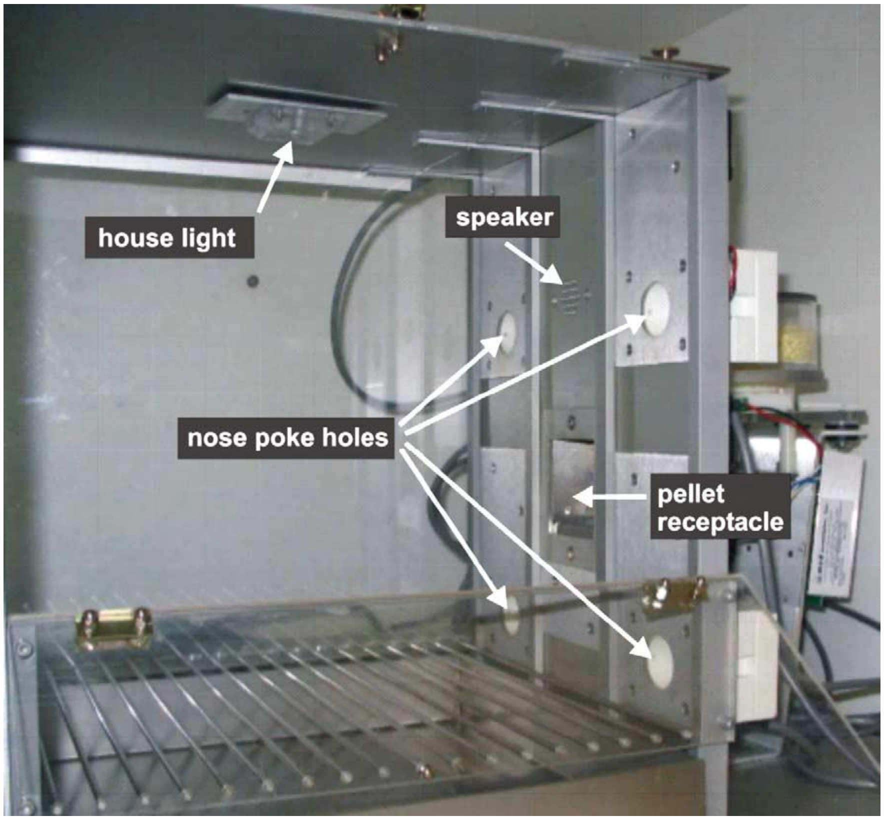

Figure 1. Apparatus used by Domenger & Schwarting (2005)’s 4-choice SRT variant.

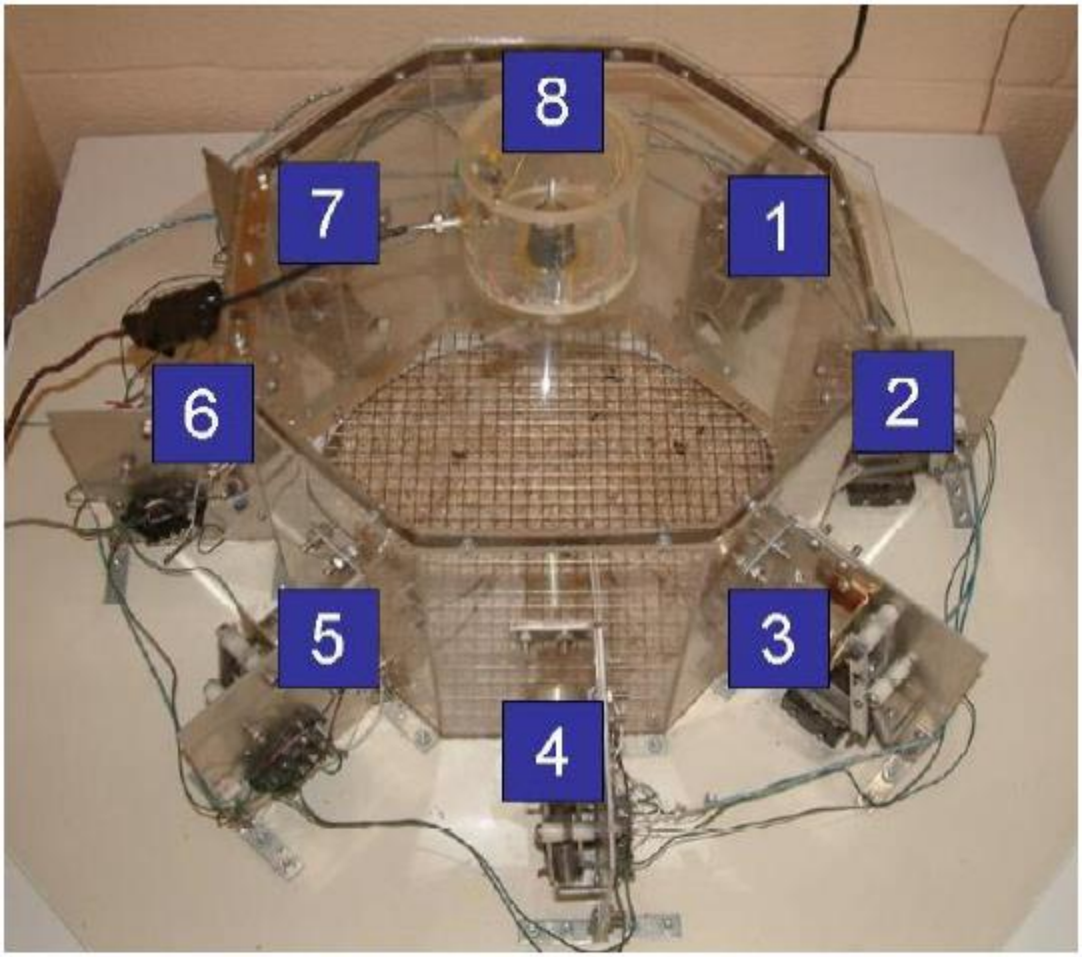

Figure 2. Octagonal operant chamber equipped with retractable levers, used by Fountain (2008). A similar apparatus was used by Kundey (2014) also. Numbers identify lever positions.

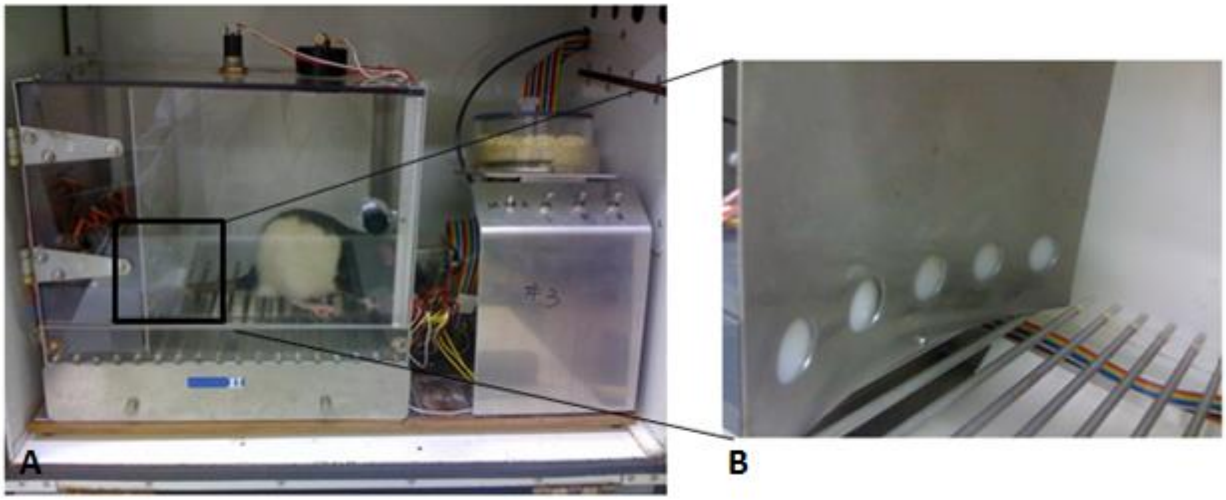

Figure 3. Basic operant conditioning chamber with subject retrieving reinforcement pellet (A) and close up view of signal key panel (B).

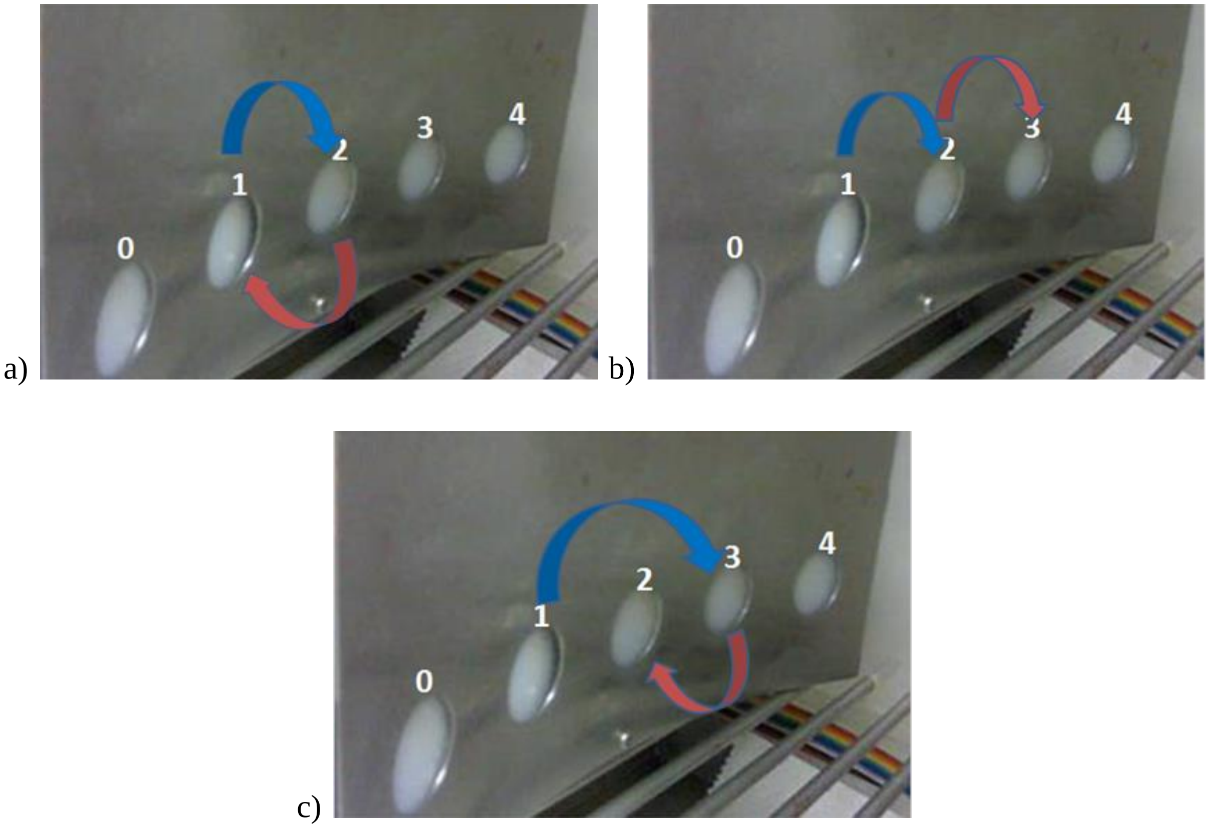

Figure 4. Linear signal panel illustrating example sequence of governing pattern from a) phase A ("F1, B1") – 1-2-1; b) phase B ("F1,F1") – 1-2-3; and c) phase C ("F2, B1") – 1-3-2. Blue arrows represent1st-2nd signal transition, red arrows represent 2nd-3rd signal transition.

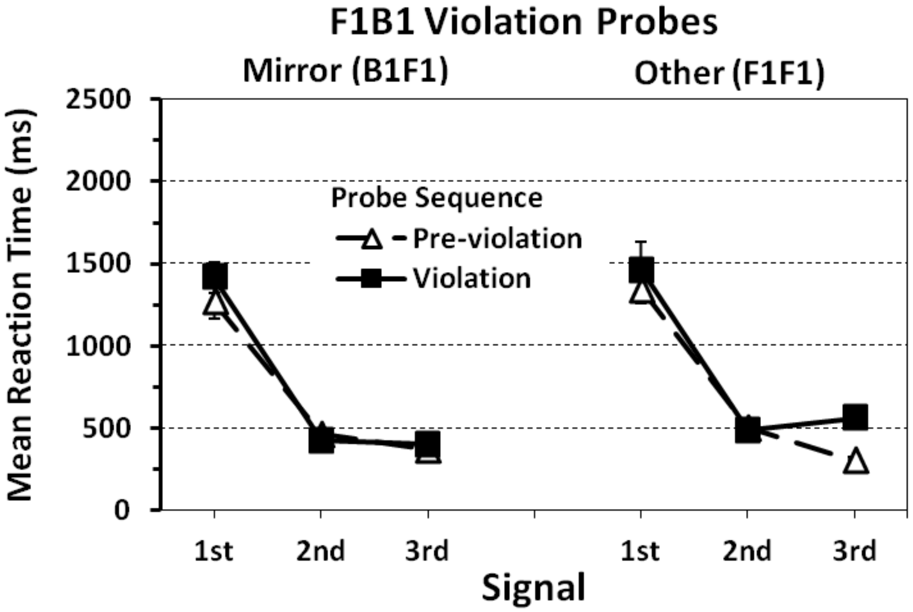

Figure 5. Mean reaction times of all three signals comparing pre-violation and violation sequences for both probe types – Phase A.

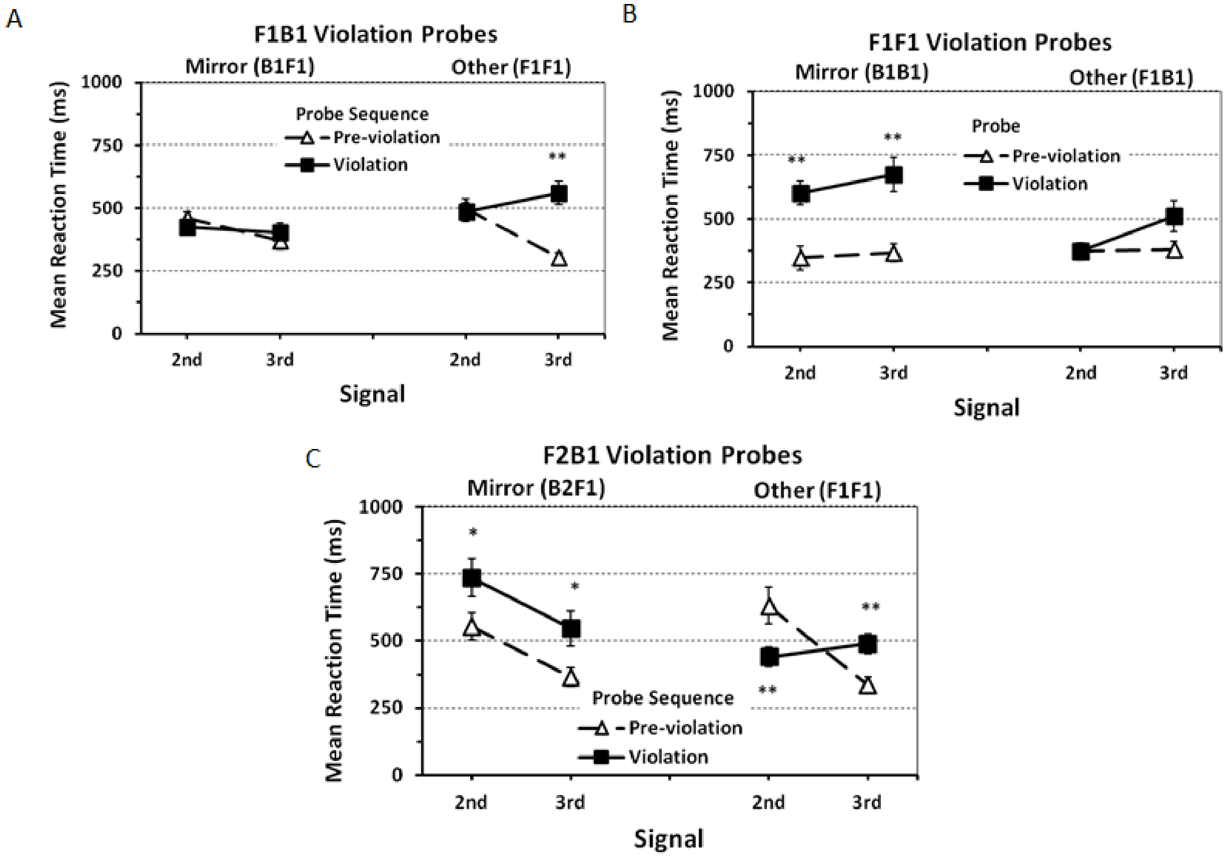

Figure 6. Mean reaction times for signals 2 and 3 comparing pre-violation and violation sequences for both probe types: A) Phase A; B) Phase B; C) Phase C. 1st signal was removed for clearer inspection of 2nd and 3rd signals.

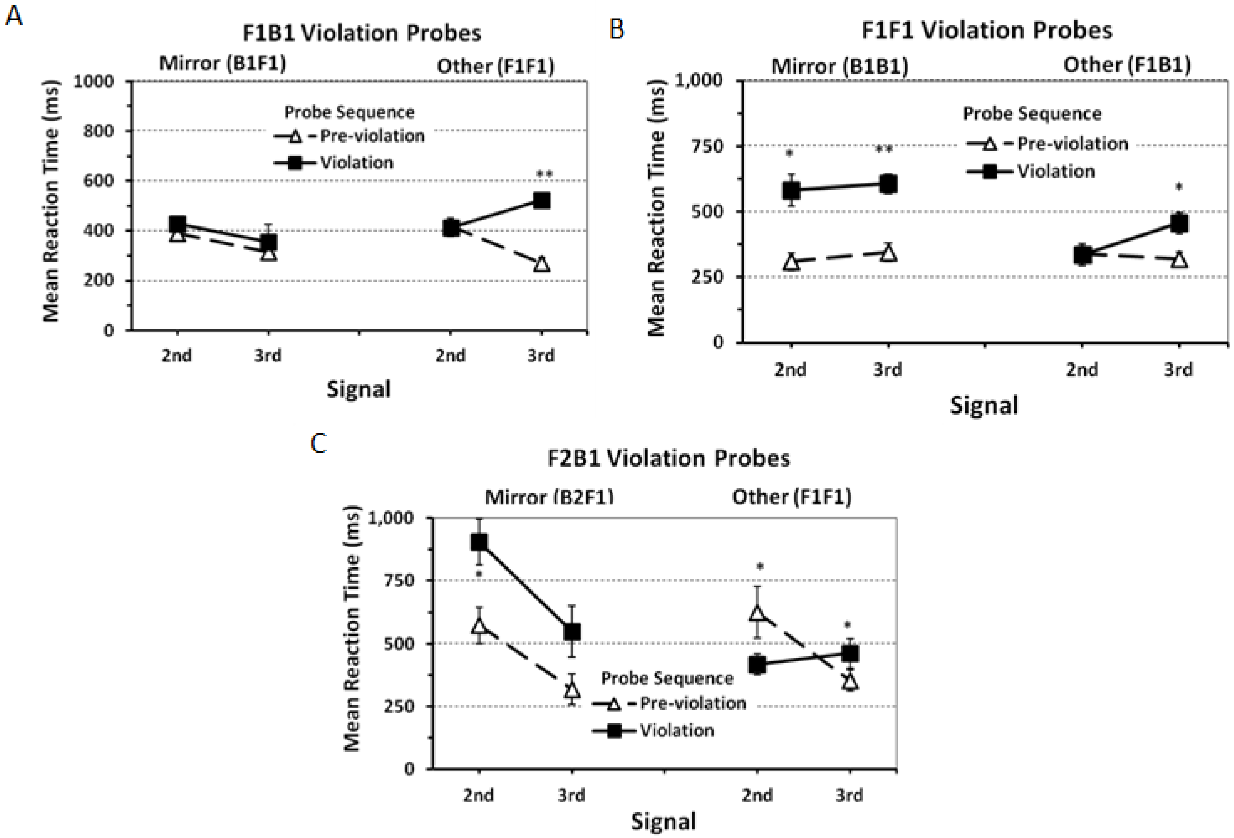

Figure 7. Mean reaction times for perfectly performed sequences only. A) Phase A, B) Phase B, and C) Phase C. 1st signal was removed for clearer inspection of 2nd and 3rd signals.

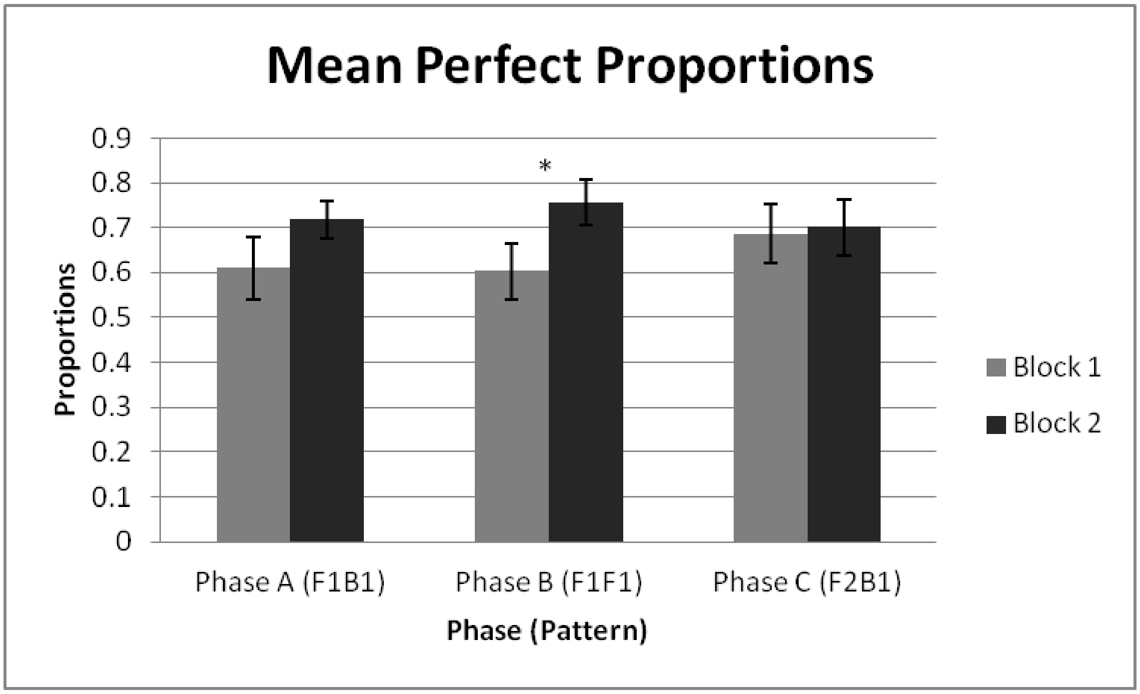

Figure 8. Proportions of training sequences performed perfectly for each phase between blocks.

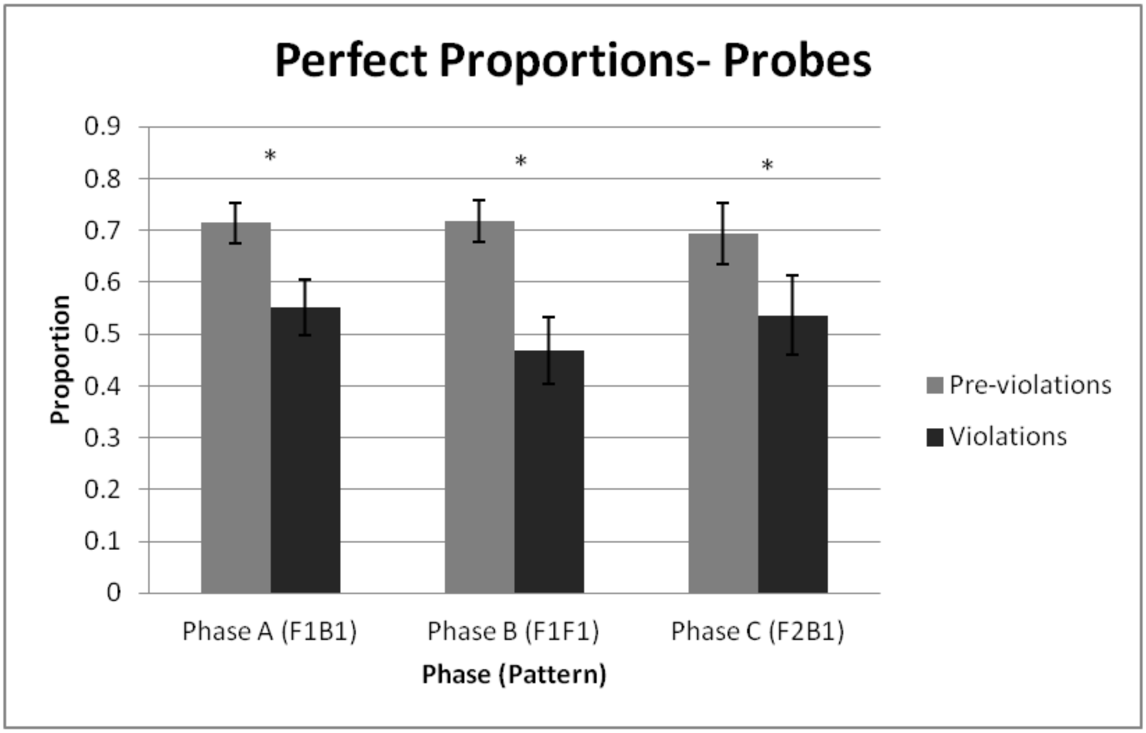

Figure 9. Proportions of probe sequences performed perfectly across testing sessions.

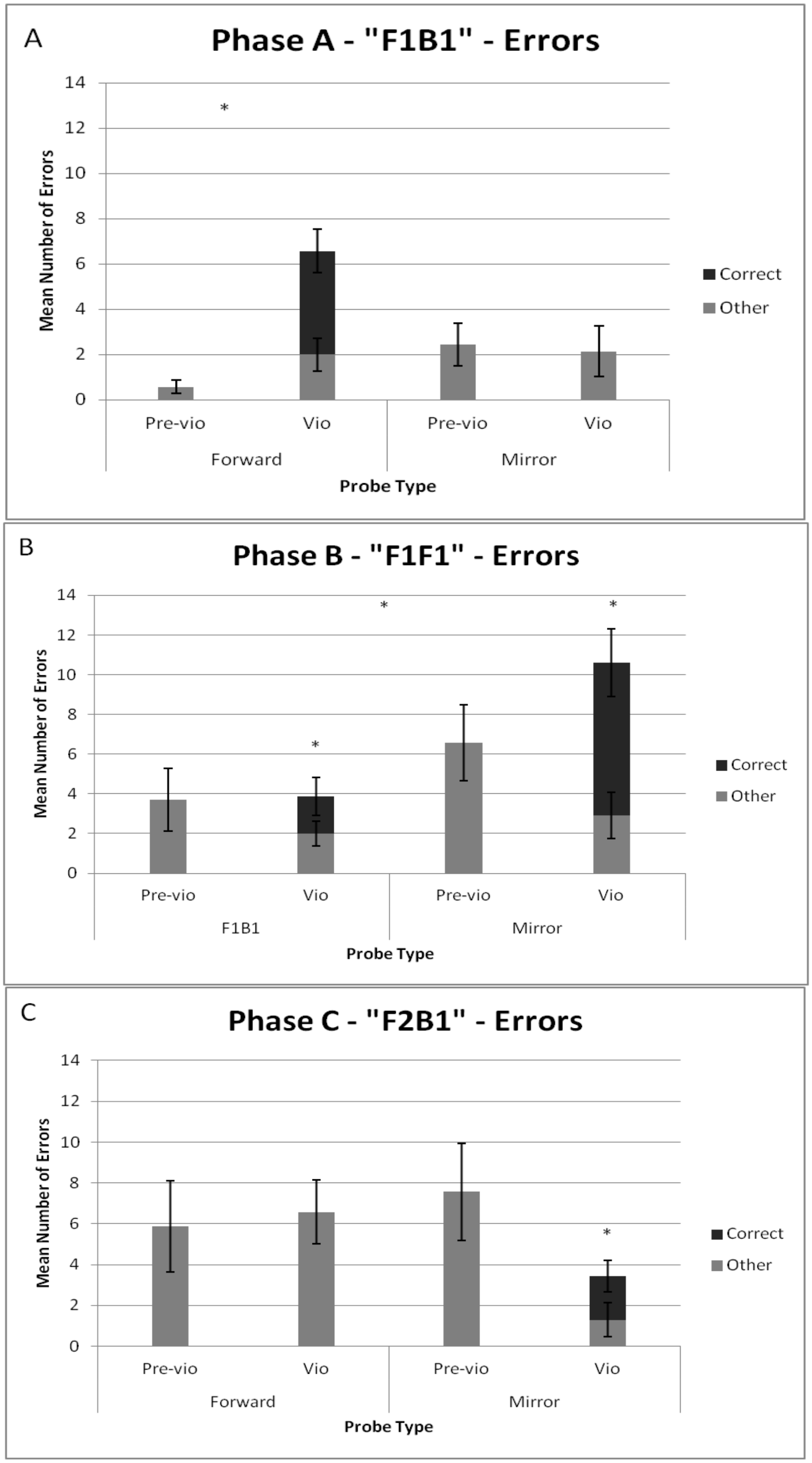

Figure 10. Mean number of errors made during each phase by probe type; pre-violation vs. violation sequences.

CHAPTER THREE:

HOW SWITCHING BETWEEN DIFFERENT 3-ITEM PATTERNS WITHIN A SESSION AFFECTS PERFORMANCE

Introduction

To date, rats' acquisition of multiple sequence pattern rules within a session has only been directly investigated by two research groups: Fountain and colleagues at Kent State University, and Kundey and her team at Hood College. Of the studies on rats' abilities to learn and use pattern rules in the octagonal chamber (*Figure 2*) (Fountain & Rowan, 1995; Fountain & Rowan, 1999; Fountain & Benson, 2006; Fountain, 2008; Kundey & Fountain 2010; Kundey & et al, 2014), one required rats to use sequence pattern rules comprised of two interleaved subpatterns (Fountain & Benson, 2006), and another required rats to switch between two sequence pattern rules within the same session (Kundey et al., 2014).

A study by Fountain and Benson (2006) suggests that rats are capable of learning patterns that contained two interleaved sub-patterns. That is, they can sort information from sequences to differentiate the sub-patterns. Octagonal operant chambers (*Figure 2*) were used in this experiment, each equipped with retractable levers on each of the eight walls. The subjects were divided into 3 groups, each receiving a different pattern composed of two sub-patterns: 1) Structured/Structured (SS) pattern (**1**5**2**6**3**7**4**8**5**1**6**2**7**3**8**4), 2) Two violation/Structured (2VS) pattern (**1**5**2**6**4**7**3**8**5**1**6**2**7**3**8**4; underlined elements indicate *violation*), or 3) Four violation/Structured (4VS) pattern (**1**5**2**6**4**7**3**8**6**1**5**2**7**3**8**4) – *the elements of the first sub-patterns are bolded*. In order to complete a session, a rat was required to produce its given pattern by pressing the correct sequence of levers, being reinforced for each correct response, 20 times.

Incorrect responses lead to the retraction of all but the correct lever. The rat had to press this lever in order to receive reinforcement and continue on with the sequence. The inter-trial and inter-pattern intervals were all 1s. Overall, results indicated that the animals were able to successfully learn these interleaved patterns; with different degrees of difficulty. Rats in all three groups showed declines in mean percent errors over sessions. Group 1 (SS pattern) rats appeared to learn their pattern more easily (i.e., made fewer errors) than the other two groups. It was also discovered that the first sub-pattern of each group was more easily learned than the second sub-pattern. This finding indicated that the rats were capable of identifying the presence of more than one sub-pattern within each overall serial pattern, as subjects were performing differently on the separate sub-patterns.

A study by Kundey et al. (2014) provided evidence that both humans and rats are capable of using more than one pattern rule within a single serial task. Once again using the octagonal operant chambers (rats) and a computer analog of these chambers (humans), subjects were required to complete sequences corresponding to either a “runs” (123-234-345-456-567-781-812) or “trills” (121-232-343-454-565-676-787-818) pattern. Sequences of both pattern types were interspersed within each session. Human subjects were instructed to select the correct circle on the screen by touching it with a finger; rats were required to press the correct keys. Subjects were informed of which pattern the following sequence would obey by the presence of a discriminative stimulus (symbols for humans; light or tone for rats); however, what each discriminative stimulus indicated was never explicitly explained to the subjects. All correct responses were reinforced (i.e., humans- the word “CORRECT” and a happy face on the screen; rats- 0.2mL of water). Incorrect responses resulted in all keys/circles, except the correct one, being extinguished until the correct press was made. Subjects of both species made fewer errors than predicted by chance and showed improvement of performance on both patterns across trials. Moreover, in most cases, the errors both species made while performing either sequence type could be labeled as *pattern* errors (i.e., when performing runs sequences, the errors made indicated the subject was using the trills pattern, and vice versa). This suggests that subjects were recalling a pattern even when it was not governing that particular sequence. These findings show further support for the ability of rats to recognize and use multiple pattern types within a session of a serial pattern task.

There are also data to suggest that switching between pattern rules will result in impairments in performance (at least for responses directly after the switch). A study by Fountain and Rowan (1995), looking at structural importance in patterns, found that more errors were made on responses within the sequence where changes in one element of the pattern changed. Once again, octagonal chambers were used (*Figure 2*). Subjects were placed in one of two groups, with each group given a pattern with a different structure. The first group was given a hierarchical structure (123-234-345-456-567-876-765-654-543-432) and the second group was given a linear structure - created by rearranging the hierarchical pattern (123-234-543-456-567-876-765-654-345-432). The linear structured pattern contained two "chunks" (i.e., 3 responses grouped together by the presence of a pause before and after the group) that violated the simple structure from the first group's pattern. Subjects were reinforced for correct responses and given correction procedures (wherein only the correct lever remained until pressed) when incorrect responses were made. On hierarchical structured patterns, rats were found to make the most errors on chunks 1 and 6 of the sequence (**123**-234-345-456-567-**876**-765-654-543-432). On the linear structured patterns, most errors were found on chunks 3 and 6 (123-234-**543**-456-567-876-765-654-**345-**432). As these chunks were those in which direction of the pattern was switched within the sequence, this finding suggests that changing the pattern (or even just an element of the pattern) disrupts performance. However, over sessions, rats were able to learn both patterns, as indicated by overall declines in mean percent of errors. This suggests that the rats were capable of learning these directional change components.

Based on the preceding, it appears that rats can handle two patterns or sub-patterns within a session. This led me to wonder what would happen if I included three patterns. Could rats successfully learn to switch from one pattern to another to another within a session? To answer this question, I utilized the same subjects and the three patterns from experiment 1. All three patterns types were given within each session, with a block of 22 sequences for each pattern type. The order that each particular pattern was presented was varied across trials in each session-block. It is important to point out that, unlike the Kundey et al. (2014) study, I did not use any discriminative stimuli to cue which pattern would govern the following sequence. This study may provide insight into whether rats can recognize changes and successfully switch governing patterns. As in experiment 1, I expected to see a decline in reaction time on the last two signals (Sidman & Stebbins, 1954; Mechner, 1958). It was predicted that the change to a new pattern would result in disrupted performance (i.e., slower reaction times and fewer proportions of perfect sequences). This prediction was based on Fountain and Rowan's (1995) finding that when a pattern sequence chunk switched to the opposite direction from the previous chunk, significantly more errors were made than on other chunks of the sequence. I also predicted that performance on these switching trials would improve with experience – that rats would perform better in the 4th 6-session block than the 1st, 2nd, and 3rd. This prediction is based on the finding in Kundey's (2014) experiment that subjects showed improvement on both patterns in the task across trials.

Method

Subjects:

The same seven subjects from experiment one were used for this experiment. All living and experimental conditions remained identical. These animals were age 1.5 years at the time of this experiment.

Apparatus and Materials:

The same operant conditioning chambers and associated software used in experiment 1 were also used in this experiment. However, subjects now received 66 pellets per day if sessions were successfully completed. This number of sequences was chosen as it was easily divisible by three.

Procedure:

Training and Testing: No additional training was given before the start of this experiment as all three patterns used (A- “F1B1”; B- “F1F1”; and C- “F2B1”) came from experiment 1. This experiment involved 24 sessions of 66 sequences. Within each session, the subjects were required to make two switches between pattern types. The 66 sequences were divided into three groups of 22, with each group being governed by one of the three patterns (A, B or C). The 24 sessions were broken down into four blocks of six. Each 6-session block ensured that every animal encountered all six orders of switching - ABC, ACB, BAC, BCA, CBA, and CAB – once (*Table 1*). Since all subjects had previous experince with the three governing patterns, their task here was to switching between using one pattern for the first 22 sequences to another pattern for the next 22 sequences and finally to the third pattern for the remaining 22 sequences. *Analyses:*

Repeated measures ANOVAs were conducted for all four session blocks to compare mean reaction times of the last two signals in each of the three pattern-blocks; one including session (2X3X6) and one with sessions collapsed (2X3). 3X6 repeated measures ANOVAs were also run for each session-block to compare the proportion of perfect sequences performed in 1st, 2nd, and 3rd pattern-blocks across sessions. The dependent variables in this study were reaction time and proportion of perfect sequences. The independent variables were session-block, pattern-block, signal (with signal 1 removed) and session. Analysis was also run on the data from the last block of training from experiment 1 (refer to previous chapter) in order to test for possible fatigue effects on performance. A 2X3X6 repeated measures ANOVA was conducted to study reaction times from this data, with signal, pseudopattern-block and session being the independent variables. Pseudopatterns refer to the division of sequences from experiment-1 sessions into thirds. These psuedopatterns were used as controls to compare with the pattern-blocks in this experiment.

Results

Reaction Time Data:

Sessions included. An effect of signal was found in the first 6-session block (*F* (1, 6)= 7.14, *p*<.05), with slower responses on the 2nd than the 3rd signal. This effect disappeared in the last three 6-session blocks. No differences were found between the three pattern-blocks in the first 6-session block; however, it appeared in 6-session block 2 (*F* (1 6)= 7.57, *p*<.01) and remained in 6-session blocks 3 (*F* (1, 6)= 13.24, *p*<.01) and 4 (*F* (1, 6)= 9.28, *p*<.01). In the last 3 6-session blocks, reaction time on the 1st pattern-block was faster than the 2nd and 3rd pattern-blocks within trials. No difference was observed between sessions in the 1st, 3rd or 4th 6-session blocks, but differences did occur in trial block 2 (*F* (1, 6)= 3.69, *p*<.05).

Sessions collapsed across blocks. *Figure 11* illustrates reaction time data for 2nd and 3rd signals of the three pattern-blocks for all four 6-session blocks of the experiment. When sessions were removed as a variable, again, the effect of signal was only found in the 1st 6-session block (*F* (1, 6)= 7.13, *p*<.05), with the 2nd signal being pressed slower than the 3rd signal. Also consistent with the results above, a main effect of pattern was not found in the 1st 6-session block, but appeared in the 2nd (*F* (2, 12)= 7.60, *p*<.01), 3rd (*F* (2, 12)= 13.13, *p*<.01), and 4th (*F* (2, 12)= 9.34, *p*<.01). The 1st pattern-block was performed the fastest in the last three 6-session blocks, with the 3rd pattern-block performed the slowest.

Proportion Data:

Figure 12 illustrates the proportions of perfect sequences for each pattern-block across the four 6-session blocks. No differences between the three pattern-blocks were observed in the first two 6-session blocks; however, effects of pattern were found in trial blocks 3 (*F* (2, 12)= 7.52, *p*<.01) and 4 (*F* (2, 12)= 7.85, *p*<.01). In both of these 6-session blocks, the 1st pattern produced the highest proportion of perfect sequences. No differences across sessions were observed in any of the 6-session blocks. A pattern by session interaction did occur in 6-session block 4 though (*F* (10, 60)= 2.09, *p*<.05). The 1st pattern in this 6-session block produced a higher proportion of perfect sequences than the 2nd pattern, but the 1st and 3rd, and 2nd and 3rd patterns were not different from one another.

Pseudopattern Data (Exp. 1):

Figure 13 illustrates reaction time data for the last two signals of 1st, 2nd and 3rd pseudopatterns of trials from the last block of training from experiment 1. These reaction times were used as a baseline-type set of data. Overall, signal 2 was slower than signal 3 (*F* (1, 6)= 26.51, *p*<.01) trials. However, no effect of pseudopattern was observed (*F* (2, 12)= 3.41, *p*=.07).

Discussion

I investigated how requiring rats to switch between three, previously learned, pattern-rules within trials affected their performance. A decline in reaction times from the 2nd to the 3rd signal was expected to occur on sequences of all pattern-blocks, as suggested by standard knowledge of fixed-ratio reinforcement schedules (Sidman & Stebbins, 1954; Menchner, 1958). I predicted that improvements in performance across blocks would be observed. Performance was measured by reaction time on the 2nd and 3rd signals of sequences as well as the proportion of sequences that were performed perfectly in each pattern-block. The loss of performance differences between the three pattern-blocks was used to define "improvement." I have concluded that, contrary to my predictions, rats appeared to be getting *worse,* across 6-session blocks, at switching between patterns.

The predicted decline in reaction time across the last two signals of sequences was observed only in the first 6-session block of the experiment. The loss of this effect indicates that subjects were not responding faster on the last signal of sequences as experience with multiple patterns increased. It appears that performance was not affected by pattern switching during the first 6-session block of the experiment. However, rats' performance on the 2nd and 3rd pattern-blocks in later trials was poorer than on the 1st pattern-block. The proportion data all supports my conclusion that the ability to switch between patterns declined with experience. The last two 6-

session blocks resulted in significantly lower proportions of perfect sequences on the 2nd and 3rd patterns than the 1st.

In order to determine if fatigue, due to long sessions, was a cause of this decline in performance, I analyzed data from the last block of training in experiment 1. The sequences of each session were divided into three pseudopattern-blocks in order to compare performance at the beginning, middle and end of trials. No significant differences between the three pseudopatterns were observed on either of the last two signals of sequences, ruling out fatigue as a possible cause for the poorer performance seen on the 2nd and 3rd patterns of switching trials. Frustration is another possible explanation for the proposed abandonment of the pattern-rules. Frustration has been suggested as a probable cause of the substitution of a constructive behaviour by a nonconstructive one (Maier, 1959). However, I were unable to test this hypothesis. The average *frustration threshold* for subjects is required in order to validate when frustration should occur (Maier, 1959). We, therefore, propose a more likely explanation: the rats simply learned to abandon response rule pattern use after the first sequence occurs that violates the 1st pattern from the trial. The animals learned that this 1st pattern would consistently govern sequences for a portion of the trial. The first sequence that did not obey that pattern then indicated that it was now easiest to revert to a signal pattern of responding. This reversion to the signal pattern of responding may be a result of the difficulty of the task. This suggested explanation is based on the general findings of serial pattern learning that more difficult patterns result in more errors and require more trials to learn (Fountain & Rowan, 1995; Fountain & Rowan, 1999; Fountain & Benson, 2006; Fountain, 2008; Kundey & Fountain 2010; Kundey & Fountain, 2014).

Another possible explanation for the difference between the current study and those of Fountain's and Kundey's research groups is the number of serial patterns used in the various tasks. In my experiment, the rats were given three patterns in each session, whereas in the Fountain and Benson (2006) and Kundey et al (2014) studies, rats were only required to use two patterns/subpatterns within a session. Perhaps two patterns is the maximum information load for rats under these conditions.

The findings in this study provide insight into how tasks requiring the recognition and use of multiple pattern-rules can affect performance over time. This experiment has shown that when rats are exposed to this task, the animals begin to show differences in performance between the 1st pattern and all subsequent patterns in later trials. This decline in performance does not appear to be a result of fatigue; it may be due to another motivational variable, such as frustration or difficulty. Future research should investigate the conditions under which rats use these automated response rule patterns and under what conditions they instead use a deliberate, signal pattern of responding, even when the pattern is consistent. I look at this in the following chapter.

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Block	**Session 1**	**Session 2**	**Session 3**	**Session 4**	**Session 5**	**Session 6**
1	CBA	BAC	CAB	ACB	BCA	ABC
2	BCA	CBA	ACB	BAC	CAB	ABC
3	BAC	ACB	CBA	CAB	BCA	ABC
4	CAB	CBA	ACB	BCA	ABC	BAC

Table1. Experimental switching schedule for subject 16-3, depicting example of testing blocks.

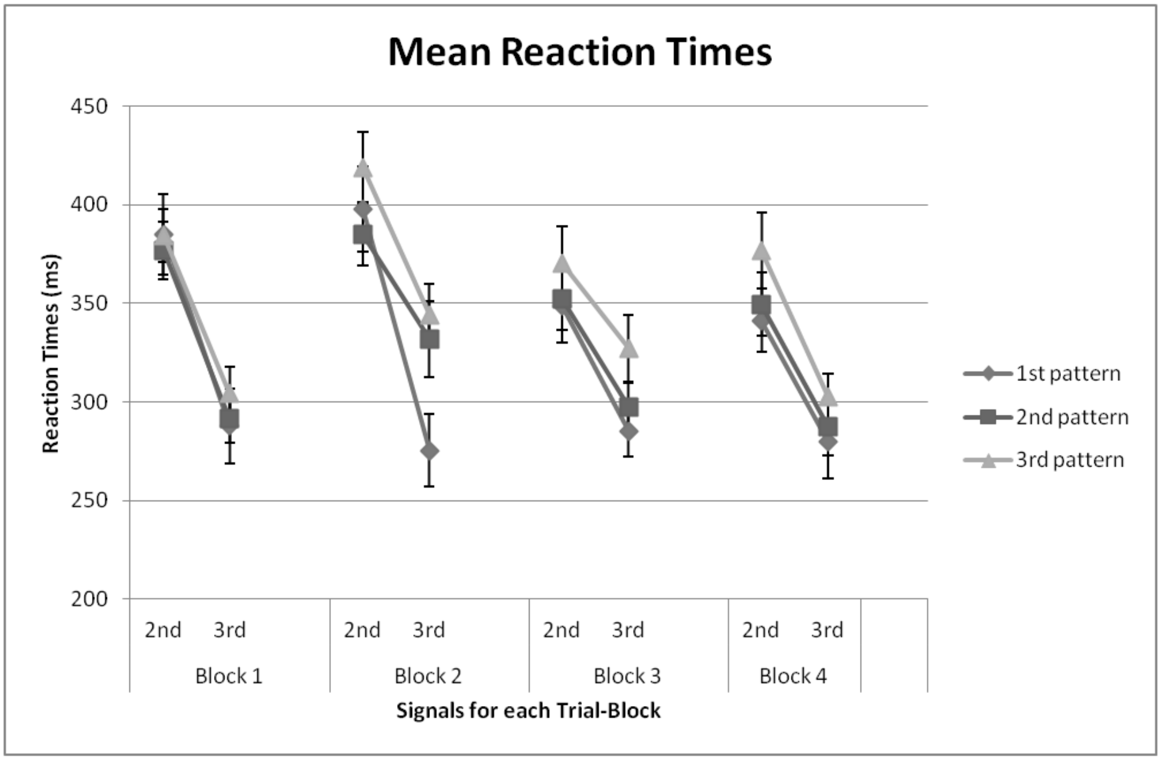

Figure 11. Mean reaction time data for each of the four 6-session blocks, comparing performance of the three pattern-blocks on the last two signals.

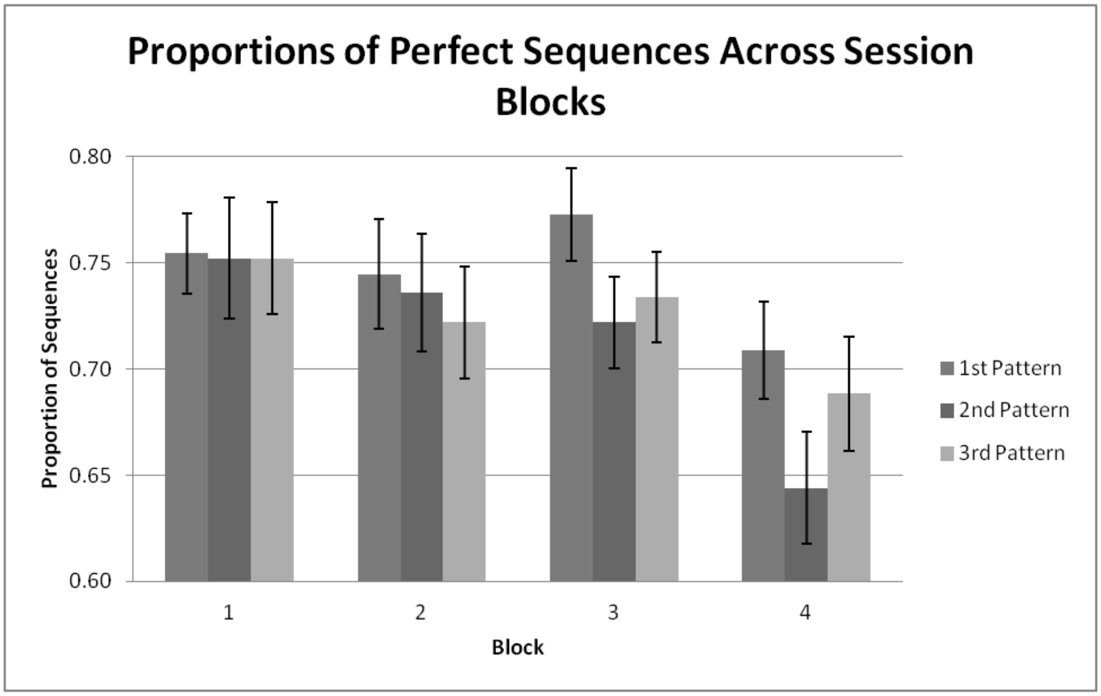

Figure 12. Proportions of perfect sequences comparing the three pattern-blocks across the four 6-session blocks.

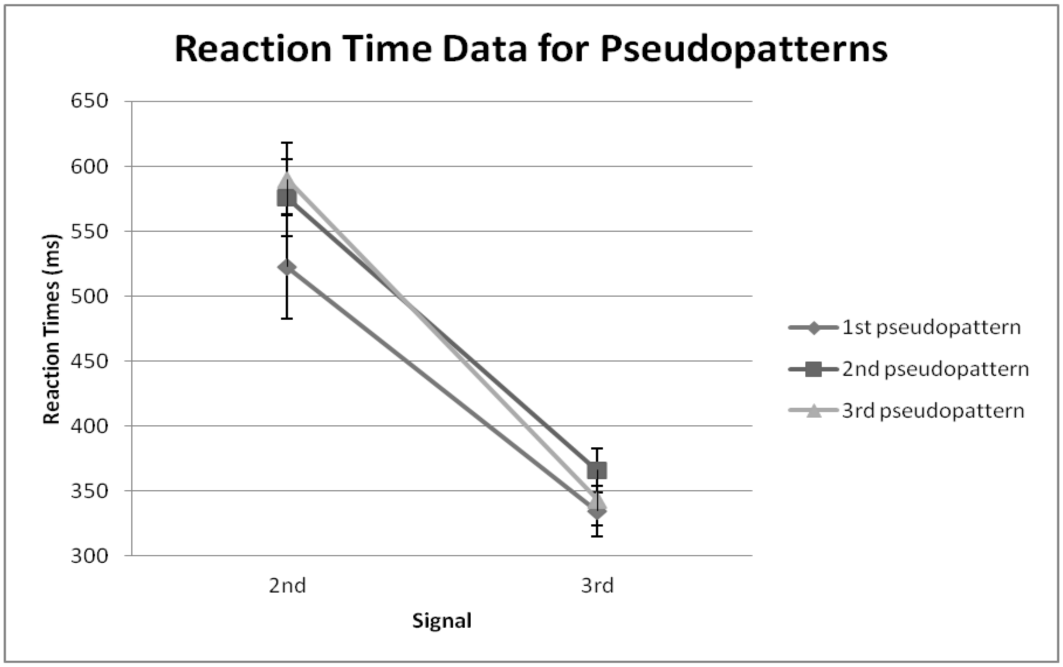

Figure 13. Pseudopattern reaction time data for last block of training from experiment 1.

CHAPTER FOUR:

FIXED VERSUS VARIED INTER-SIGNAL INTERVALS: EFFECTS ON PERFORMANCE

Introduction

The literature on sequence/serial pattern learning is limited in respect to the direct investigation of the importance of temporal consistency within these sequences. The following three studies provide insight into the importance of temporal information in general and how it affects performance on sequences/serial patterns. This experiment aims to answer questions relating to temporal information *within* sequences and how this information affects learning of the patterns that govern these sequences.

Tanaka & Watanabe (2013) report an experiment with humans looking at the effects of altering intervals between stimuli and/or responses in a sequential task. This study made use of a panel with 16 buttons set up in a 4 X 4 array. Participants were required to select a separate "home" button for 0.5s in order to begin a trial. A trial consisted of 9 or 6 "sets" of signals – multiple signals simultaneously illuminated; this was referred to as a *hyperset*. When a hyperset was presented, participants were required to determine the correct order of buttons through trial and error. Two hypersets of responses were presented: a learning block, followed by a transfer block. Two types of hypersets were used: one in which two buttons came on at the same time (2X9) and one where three buttons came on at the same time (3X6); with both types resulting in 18 buttons total. The switch between the two hyperset types resulted in interval changes caused by where the sets were "sectioned" – the *number* of intervals changed. The hyperset used for the training block determined which hyperset was used for the transfer block – the two were always opposing (e.g., if 2X9 was used for training, 3X6 was used for transfer).

The participants were assigned to one of two groups. The *identical* group was so called because the two hyperset types (2X9 and 3X6) had identical sequences. In the *random* group, the two hypersets consisted of random sequences. Participants were then further divided into 4 groups: identical 2 x 9 (learning) to 3 x 6 (transfer), identical 3 x 6 to 2 x 9, random 2 x 9 to 3 x 6, and random 3 x 6 to 2 x 9. Data from participants in the identical groups who later reported noticing this identical quality of the blocks were removed from analysis. It was determined that the number of intervals within sequences had significant effects on both implicit and explicit learning. Here, explicit learning was considered to be the recognition of regularity by participants. The researchers concluded that this was due to the strong relationship between intervals and chunking. This experiment illustrated how intervals between sets of stimuli can be an important factor in the learning and performance of subjects on sequential tasks and, therefore, serial reaction time tasks.

Church and Lacourse (1998) investigated rats' ability to adjust their responding based on repeating sequences of inter-food intervals. Their study used sequences of intervals in differing orders (random, ascending, and descending). Rats were tested in operant boxes that contained a lever and pellet dispenser. All correct lever presses were reinforced. The most important finding of this study was that when the sequences of intervals were ordered in ascending or descending order, rats were capable of tracking/predicting future intervals; however, when the sequences were random, the rats could not do this. These findings suggest that when rats are given simple temporal information that follows a pattern or rule, they are capable of learning this pattern or rule, but this is not the case when the temporal information is random. In other words, the reduction of temporal sequence predictability impedes learning of this sequence.

In 2002, Shin and Ivry explored the importance of temporal information on learning sequences in humans. A computer screen upon which an *X* could be presented in four locations horizontally across the center was used for this study. The response to stimulus interval (RSI) – time between a participant's press and the next stimuli- was varied. Responses were made on a response board consisted of 4 horizontally aligned keys – similar to the stimuli display. The location of *X* changed either following a fixed repeated sequence – called the *sequenced-location* blocks – or randomly – called the *random-location* blocks. The RSI was also either repeating (*sequenced-RSI*) or random (*random-RSI*). This resulted in four possible blocks: sequenced-location/sequenced-RSI, sequenced-location/random-RSI, random-location/sequenced-RSI, and random-location/random-RSI.

Two groups of participants were formed, differing in the length of their repeating RSI sequences during sequenced-RSI blocks. One group was given *matched-length* blocks, where the RSI sequence was equal in length to the location sequence. The second group was given *mismatched-length* blocks, where the RSI sequence differed from the location sequence. Each experimental session contained 4 sequenced-learning probes, consisting of 4 blocks each. It was discovered that the temporal sequence was only learned when it matched the spatial sequence. This suggests that spatial information (*where*) was important for the learning of temporal information (*when*). It was also noted that performance was *better* when the RSI and location sequence lengths matched, indicating that participants were using temporal information to improve their performance. These findings suggest that participants can use temporal information to improve their performance on spatial sequences.

The goal of my experiment was to investigate performance differences between subjects who were given fixed inter-signal intervals and those who were given varied inter-signal intervals. The underlying question I was asking with this task was whether manipulating ISI consistency would affect rats' ability to form automated responses. To answer this question, one novel pattern type ("Forward 1, back 2" – "F1B2"; refer to *figure 14*) was used for all subjects. My task is a simplified version of the task used by Shin and Ivry (2002). In my procedure, the inter-signal intervals (ISIs) – times between each signal in a sequence - differed between groups. In one group, both inter-signal intervals within sequences were 250ms (i.e., the fixed group). In the other group, the inter-signal intervals in each sequence were 50ms & 450ms, 450ms & 50ms, or both 250ms (i.e., the varied group). The governing pattern informed both groups of *where* a signal would occur; however, only the fixed group knew *when* a signal would appear.

Two assumptions were made about the groups in this experiment. First, it was assumed that the fixed group would develop *automated* response patterns - due to the reliability of signals and repetition of response behaviour, little attention was required from subjects in order to perform well. The second assumption was that the varied group would develop more *deliberative* response patterns – as the signals were not reliable, the animals had to pay close attention to the temporal information before responding or performance would be poor. Based on these assumptions and the findings by Church and Lacourse (1998) ─ that when intervals are fixed, rats can track them and respond accordingly ─ I predicted that the fixed ISI group would be more disrupted by violation sequences than the varied group. Specifically, fixed subjects would produce slower reaction times, fewer perfect sequences and make more errors on violation sequences than on pre-violation sequences. These indicators of disruption were not expected from varied ISI subjects.

Method

Subjects:

Six of the subjects from the previous two experiments were also used in this experiment. However, due to the nature of this experiment (i.e., between subjects design), a larger sample size was necessary. Another “batch” of six animals was trained and run on this experiment. Batch 1 (i.e., the experienced animals) were ~1.5 years old at the start of this experiment; batch 2 (i.e., less experienced animals) were ~ 4 months old. Living conditions for all subjects were identical to those described in experiment 1. The twelve animals were assigned to two separate groups (Fixed ISI and Varied ISI), with six animals in each group – three from each “batch.”

Apparatus and Materials: Chambers and software from the previous two experiments were also used for this experiment.

Procedure:

Pretraining: No additional training was given to the six animals from the first two experiments. However, the six naïve animals from the second “batch” required training with the apparatus. This pre-training consisted of five stages. In the first three stages, I directly observed each animal and controlled the chambers. The last two stages made use of the automated software program, as used for the actual experimental sessions. Stage one involved habituation to the chambers, wherein the rats were placed in a chamber for 15 minutes and allowed to explore. Several dustless precision pellets were left placed in the food hopper before each rat entered the chamber in order to encourage exploration. Each animal received two-three habituation trials, depending on the individual. Stage two consisted of classical conditioning. In this stage, an association between a tone and a reinforcement pellet was developed. The tone would later act as a secondary reinforcement. To do this, a 2-second tone was sounded immediately before the presentation of a pellet. One trial per day of this stage was given until the subject would turn to the hopper as soon as the tone was presented, regardless of where the animal was in the chamber. The third stage used a shaping procedure to train nose-poking of keys. This shaping procedure began with animals being reinforced for facing the signal panel. Once animals were consistently facing the signal panel, they were required to approach/investigate/sniff keys for reinforcement. Once the subject nose-poked a key for the first time, only direct nose-poking was reinforced from then on. In the final step of this shaping stage, the middle key was lit and nose-poking only this lit key resulted in reinforcement.

Stage four was a follow-the-light procedure, where subjects were reinforced for lit-key nose-poking. Each signal occurred at a random location along the panel. This stage consisted of 11 trials, progressively increasing in reinforcement ratio (i.e., continuous reinforcement → FR-2 reinforcement → FR-3 reinforcement schedules). The final stage was step up as a supplement to the pattern-learning experience of the first “batch” of animals. All six animals of the second “batch” were given four trials each of the three patterns from experiment 1. This difference in pattern-experience between the two “batches” of animals formed two pseudo-groups: batch 1- with extensive exposure to patterns – and batch 2- with minimal exposure to patterns prior to this experiment. The result was four quasi-groups: batch 1 fixed, batch 1 varied, batch 2 fixed, and batch 2 varied (n = 3 in each batch/ISI group).

Training: All twelve animals received fifteen days of training with the sequence pattern “342/324.” Each day’s session consisted of 60 3-item sequences, all of which obeyed the sequence pattern. As previously mentioned, two experimental groups, differing in ISI consistency, were formed: Fixed and Varied. The ISIs for the fixed group was 250ms between the first and second signal as well as between the second and third signal. The varied group consisted of three possible sets of ISIs: 50/450ms, 250/250ms, or 450/50ms. All three sets occurred with the same frequency within each daily session. These ISIs were chosen to ensure that the total ISI time within every sequence will be the same. A total of 500ms allows ISIs to be kept short enough to prevent many ISI nose-pokes, but is also long enough to be manipulated for the varied group.

Probe Trials: Six days of testing sessions immediately followed the fifteen training trials. These testing sessions each contained six violation sequences: two “mirror” type probes, two “forward” type probes and two “backward” type probes. “Mirror” probes refer to sequences in which the pattern is violated by the signals appearing in the reverse direction than the normal sequences would (“324” = the mirror of “342”). “Forward” probes refer to the sequences in which the pattern is violated by the signals continuing forward in one direction (“345” = the forward of “342”). “Backward” probes are the opposite of forward probes (“321” = the backward of “342”). These probe sequences occurred at regular intervals throughout the session (sequences 11, 22, 33, 44, 55, and 66). Due to the addition of these six probes, the testing sessions contained 66 sequences each.

Analyses: Repeated measures ANOVAs were used to analyze reaction time, proportion of perfect sequences and errors. The independent variables used were group, batch, signal, trial type and session. Paired samples t-tests were also conducted to directly compare signals of pre-violation and violation sequences in cases where there appeared to be a significant difference.

Results

Reaction Time Data:

Figure 15 compares mean reaction time data for pre-violation and violation sequences across the last two signals for probe sessions. Due to high reaction times on the 1st signal of each sequence, those RTs were removed from analysis and figures. *Figure 15A* looks at the forward probe type ("F1F1"), between groups collapsed over batches *Figures 15B* and *15C* look at the backward ("B1B1") and mirror ("B1F2") probe types in the same manner, respectively. No main effects or interactions were found for any of the three probe types when group was the between subjects factor. Only when batch was the between subjects factor did I find an effect for the batch factor. Therefore I report statistical findings with batch as the between subjects factor and trial type (pre-violation trial sequence vs. violation probe trial sequence) and key light signal (signal 2 and signal 3) as within-subjects factor).

No main effect of trial type (pre-violation vs. violation sequence) was observed for the forward probe type. A main effect of signal, $F(1, 8)= 42.78$, $p<.01$, was found, with rats reacting to 2nd signals faster than to 3rd signals. A trial type by batch interaction occurred, $F(1, 8)= 6.61$, $p<.05$, due to pre-violation sequences being completed faster than violation sequences in the more experienced batch of rats, but with the opposite occurring in the less experienced batch of rats (see *Figure 15A*). *Figure 15A* compares the pre-violation and violation sequences of the two batches of subjects. The difference seen in the batch of rats with less experience between the 3rd signals of pre-violation and violation sequences is significant, $t(5)= 4.00$, $p<.01$, with violation sequences resulting in faster reaction times on this signal than pre-violation sequences.

No main effects of trial type or signal were observed for the backward probe type. A trial type by signal interaction was observed for the backward probes, *F*(1, 8)= 10.01, *p*<.05, with subjects slowing down across signals of pre-violation sequences and speeding up across signals of violation sequences. No main effect of batch was found for this probe type. *Figure 15B*, comparing pre-violation and violation sequences for each batch of animals shows differences between trial types of both signals for less experienced animals. These differences were both found to be significant; signal 2 resulted in faster reaction times on pre-violation than violation sequences, t(5)= -2.81, *p*<.05, whereas for signal 3, the opposite was true, t(5)= 2.15, *p*<.10.

A main effect of trial type was found for mirror probes, *F*(1, 8)= 22.97, *p*<.01, with faster reaction times on pre-violation than violation sequences. A main effect of signal was also observed, *F*(1, 8)= 60.31, *p*<.01, with 2nd signals resulting in faster reaction times than 3rd signals. No main effect of batch was found for these probes either. As seen in *Figure 15C*, more experienced animals produced faster reaction times on signal 3 of pre-violation than violation sequences, t(5)= -6.05, *p*<.01, and less experienced animals produced faster reaction times on signal 2 of pre-violation than violation sequences, t(5)= -2.27, *p*<.10.

Proportions of Perfect Sequences Data:

Training. No within subjects effects were observed for mean proportions of sequences across training sessions. No group effect was observed for the proportion of perfect sequences. As illustrated in *Figure 16*, an effect for batch, however, was observed, *F*(1, 8)= 129.87, *p*<.01. More experienced subjects (*M*=.613, *SE*=.034) produced almost 10 times the mean proportions of perfect sequences than subjects with less experience (*M*=.065, *SE*=.034).

Probes. As shown in *Figure 17*, a main effect of trial type was observed, $F(1, 8) = 9.15$, $p < .05$, for mean proportions of correct sequences across probe sessions. Subjects produced higher mean proportions on pre-violation than violation sequences. No effect of session was observed. *Figure 17a* illustrates the trial type by group interaction that was not found to be significant, $F(1, 8) = 5.05$, $p = .055$, but was almost so with pre-violation sequences resulting in higher mean proportions than violation sequences for both groups. *Figure 17b* clearly illustrates the trial type by batch interaction observed, $F(1, 8) = 9.92$, $p < .05$. Subjects with more experience made higher mean proportions on pre-violation than violation sequences; whereas, subjects with less experience produced opposite results.

Errors:

Figure 18 compares the total number of errors made by each group (left side) and batch (right side) on pre-violation and violation sequences across probe sessions. A main effect of trial type was discovered, $F(1, 8) = 9.30$, $p < .05$, with more total errors being made on violation than pre-violation sequences. No effect for group was found. As seen in the right panel of Figure 18, however, and supported by a batch effect, $F(1, 8) = 39.31$, $p < .01$, the batch of less experienced rats made more errors than the batch of more experienced rats.

Figure 19 compares the total number of (A) repeat, (B) other, and (C) correct errors made on pre-violation vs. violation sequences by each group (left side) and batch (right side) of subjects. A error type by group interaction, $F(1, 8) = 7.29$, $p < .05$, occurred with Fixed ISI subjects making fewer repeat errors and more other errors than Varied ISI subjects. A error type by batch interaction, $F(1, 8) = 66.61$, $p < .01$, also occurred with the more experienced subjects making fewer repeat and other errors than subjects with less experience. A three-way interaction of trial type by error type by batch was also found, *F*(1, 8)= 9.76, *p*<.05. This interaction revealed that most of the errors made were other errors by less experienced subjects on violation sequences and the least were repeat errors by more experienced subjects on pre-violation sequences. Effects of both group, *F*(1, 8)= 9.78, *p*<.05, and batch, *F*(1, 8)= 62.75, *p*<.01, were found for correct errors. It was determined that fixed ISI subjects were making more correct errors than varied ISI subjects and that the batch of less experienced subjects were making more correct errors than the batch of subjects with more experience.

Discussion

In this experiment, I investigated the possible effects of varying inter-signal intervals on performance of 3-item sequences governed by an overall pattern. Subjects were divided into two groups, a Fixed ISI and a Varied ISI group. Violation sequences were used to test whether learning in both groups had occurred, and whether the ISIs within sequences effected how subjects respond. It was predicted that subjects in the Fixed ISI group would be more disrupted (i.e., increase reaction times, decrease mean proportion of perfect sequences and increase errors) than those in the Varied ISI group by these violation sequences. This prediction was based on the finding by Church and Lacourse (1998) that rats are capable of tracking sequences of fixed intervals, but not sequences of random intervals. Based on the results of this experiment, I have found that varied inter-signal interval sequences do not produce different performance than that in the fixed inter-signal interval sequences. However, I have also observed that differences in amount of experience with pattern learning and pattern violations *do* result in performance differences. As indicated by comparisons of pre-violation and violation sequence data, increased experience with pattern learning and testing leads to greater disruption by violations. Results from proportion of correct sequences data, though, suggest that increased experience also leads to increased learning of new patterns. Finally, the error data suggests that increased experience results in less disruption by violation sequences in respect that fewer errors are made.

The two groups did not show differences in reaction times for pre-violation and violation sequences between the two groups for any of the three probe types. In *Figure 16a* the two groups appear to show differences, however, this was not found to be significant. The two groups also did not appear to produce different mean proportions of perfect sequences for training or testing sequences. Varied ISI subjects did not produce different total number of errors on either pre-violation or violation sequences, but did produce more repeat errors and fewer other errors than fixed ISI subjects. It appears that when the intervals between signals within a sequence are not identical, subjects are more likely to repeat the previous response. I suggest two possible reasons for this. First, perhaps while waiting for the unpredictable interval to end, subjects forget where they are in a sequence, leading them to repeat a response already made. Second, perhaps subjects are frustrated by longer ISIs, and are simply repeating previous responses in an attempt to hurry the appearance of the next signal. The fixed ISI group did also appear to produce more correct errors than the varied ISI group. This could suggest that the subjects in this group were responding more automatically than the varied group, using the governing pattern without noticing the violation quickly enough. However, this could also just be the result of chance, as subjects have approximately a 1/3 probability of making a correct error on violations. These two findings are not sufficient to indicate that real differences between the groups are due to the fixed vs. varied quality of the ISIs.

According to reaction time data, the more experienced batch performed similarly on both pre-violation and violation sequences for forward and backward probe types but were disrupted on the mirror probes on the 3rd signal. These findings suggest that pattern learning experience reduces the disruptive effects of violation sequences (at least by forward and backward violation sequences) on subjects' performance. This disruption did not occur on forward probes, which was expected as the pattern is not violated there (e.g., pre-vio: “1**2**0” vs. forward vio: “1**2**3”). The less experienced batch of subjects showed differences between pre-violation and violation sequence performance for all three probe types. These subjects appeared to be disrupted on the 2nd signal by both backward and mirror probe violations. These increases in reaction times suggest that violation of direction upsets performance on less experienced animals. Subjects with less experience actually performed faster on signal 3 of violations than pre-violations for both forward and backward probe types. However, this may simply be the result of signals 2 and 3 being spatially closer on these violations than on pre-violation sequences. At this time, I would like to point out that the increased experience batch animals had included considerably more exposure to probe sessions on previous experiment as well. The less experienced animals did receive some exposure to patterns from the previous two experiments, but received no experience with violations of these patterns. Overall, reaction time data suggests that previous extensive pattern learning can reduce the disruptive effects of violations on performance. It is plausible that having dealt with violations before may make subjects more flexible in future probe situations.

The more experienced subjects also produced higher proportions of perfect sequences during training sessions than less experienced animals, indicating that the former may have better learned the pattern better than latter. This is also supported by batch of more experienced animals' higher mean proportion of perfect pre-violation sequences than the less experienced batch. The higher proportion of perfect violation sequences by the more experienced batch also supports my supposition above – that subjects who have experienced violations of patterns before are more flexible when facing them in the future.

The higher number of total errors on pre-violation sequences by the less experienced than the more experienced batch may indicate that the former did not learn the pattern as well as the latter batch. The fact that less experienced animals were also making significantly more correct errors than those subjects with more experience may at first seem to conflict with the previous indication but, again, can be explained by flexibility due to experience. More experienced animals may be able to use the presence of one violation sequence to predict that more violation sequences are going to occur. This prediction would allow them to more quickly identify a violation, and in those cases, respond accordingly. This idea is also supported by the fewer total errors resulting in more experienced animals.

The findings in this experiment suggest that varying the inter-signal intervals within pattern governed 3-item sequences does not affect performance on these sequences. However, I would like to point out that the pairs of ISIs used in the varied group (50/450ms, 450/50ms & 250/250ms) were not excessively different from one another and only three pairs were used. Perhaps these pairs were not *varied* enough to produce differences. Church and Lacourse (1998) used entirely random sequences of intervals, which were also much longer – in seconds as opposed to my tenths of seconds – than mine, for their random group. This may explain why they found differences between groups, whereas I did not. If I were to rerun this study, I would use completely random pairs of ISIs for the varied group. This experiment also suggests that previous learning of patterns may improve subjects’ ability to learn new patterns. The subjects from batch 1 not only had more exposure to pattern-governed sequences, but also had experience with testing on these patterns. Batch 2 animals had never experienced violations to the patterns they were given limited training with, previous to the start of this experiment. Experience with patterns being violated may give subjects flexibility in their response patterns.

Future studies should look more closely at the effects of pattern learning experience by systematically controlling the amount of experience with both patterns and violations of patterns. Another possible future direction would be an experiment in which the inter-signal interval not only provides information about *when* a response must occur, but also informs the subject *where* they are to respond (e.g., “if the ISI is short, move back 1. If the ISI is long, move forward 1”).

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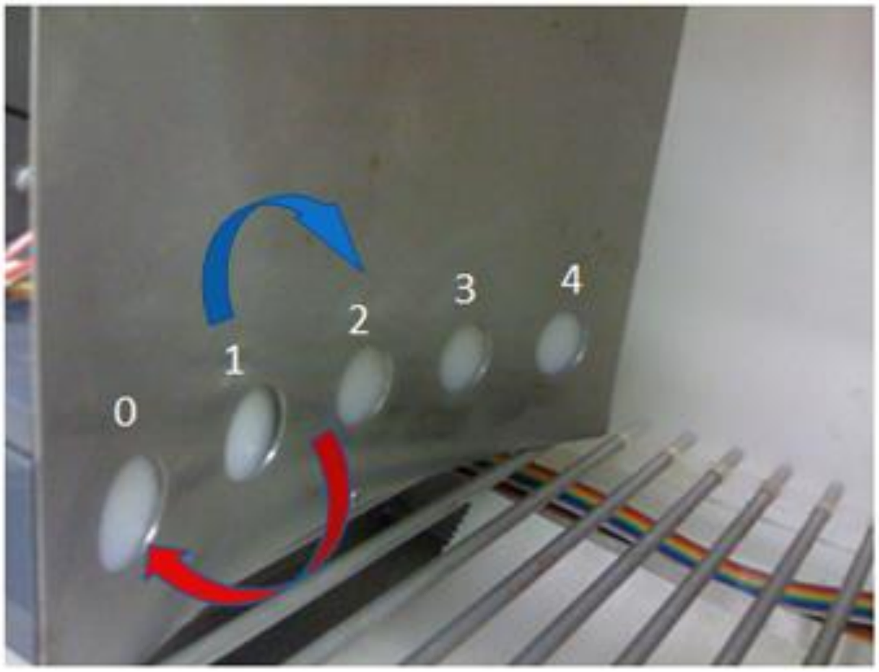

Figure 14. Linear signal panel illustrating example of governing pattern “F1B2” used in this experiment. The sample sequence shown is “1-2-0.”

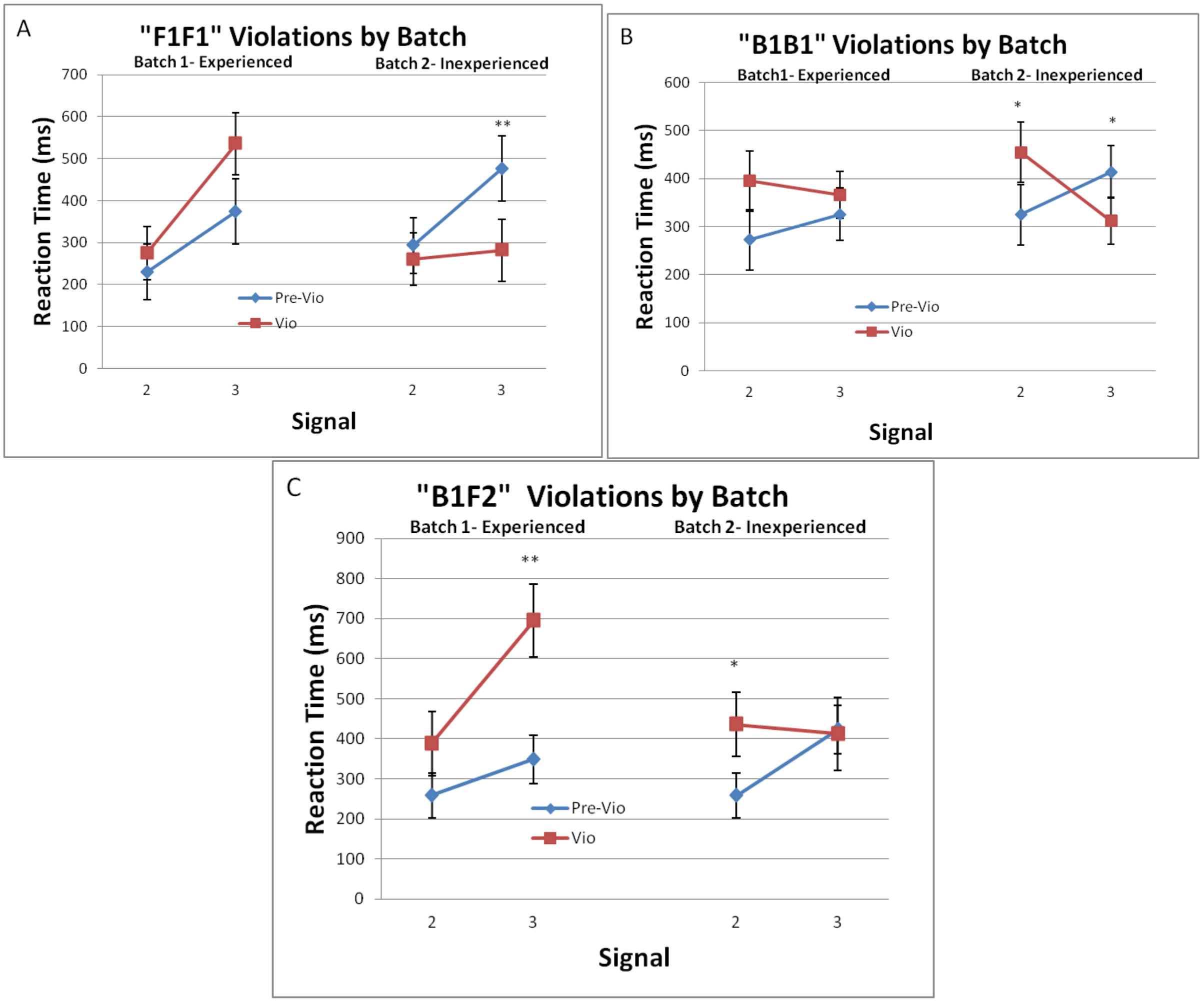

Figure 15. Comparison of reaction times to 2nd and 3rd signals of pre-violation and violation sequences of the (A) forward, (B), backward and, (C) Mirror type probes by batch.

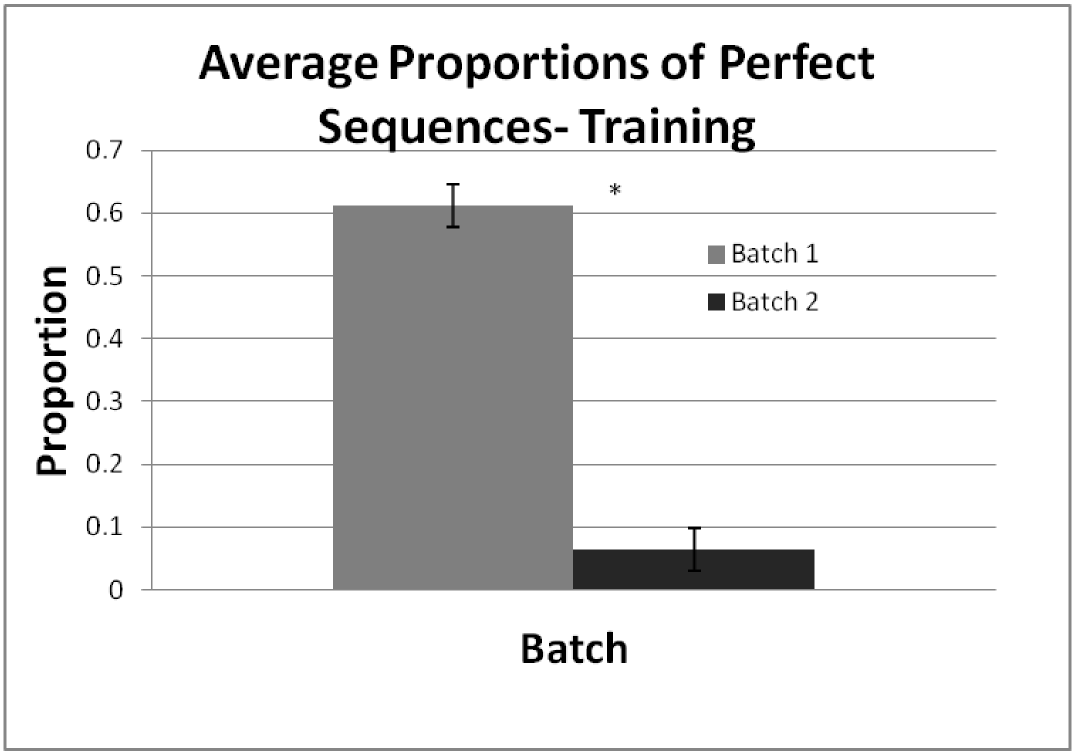

Figure 16. Average proportion of perfect training sequences collapsed across ISI groups.

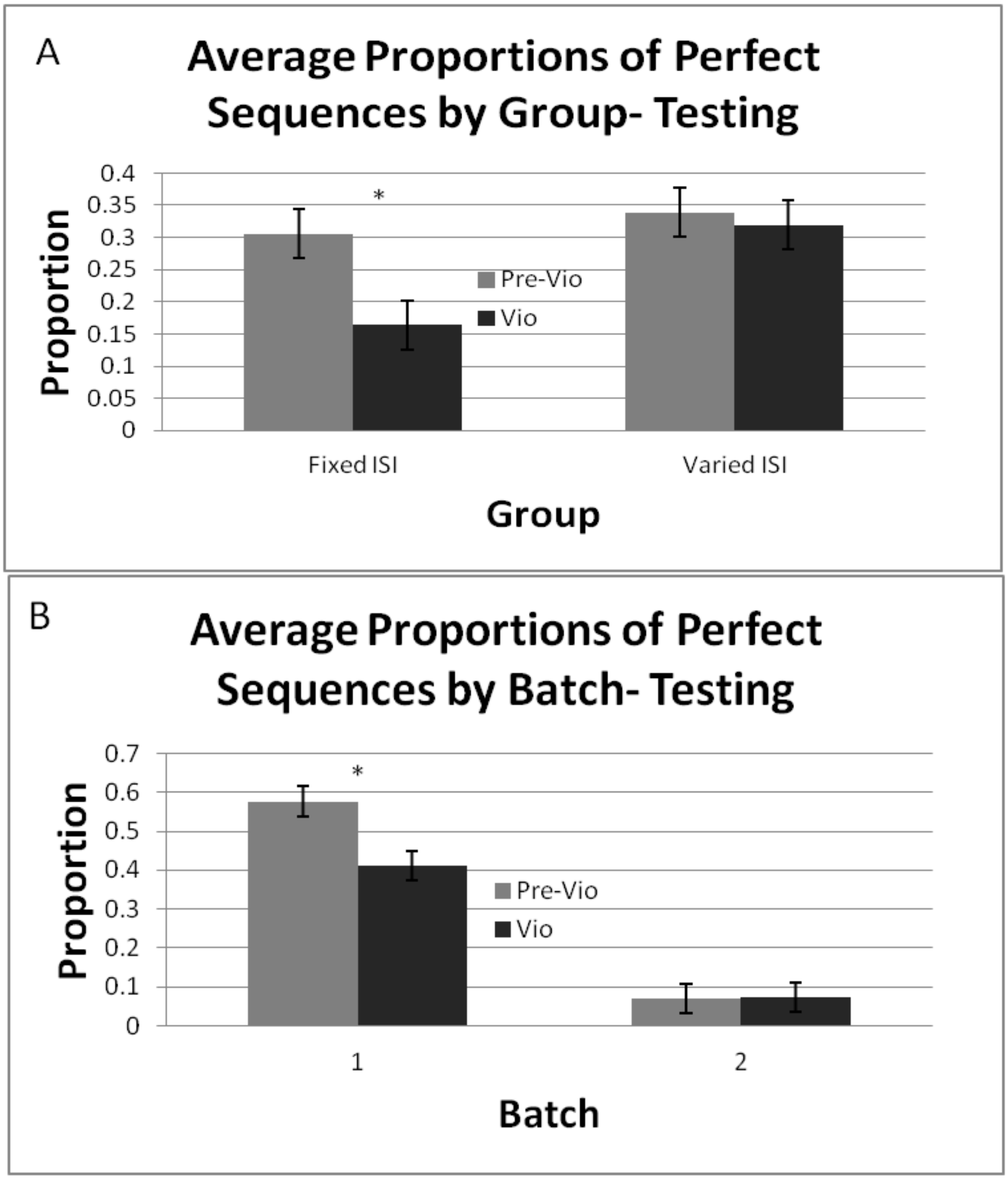

Figure 17. Average proportions of perfect pre-violation and violation sequences for A) Fixed ISI and Varied ISI groups and, B) Batches 1 and 2.

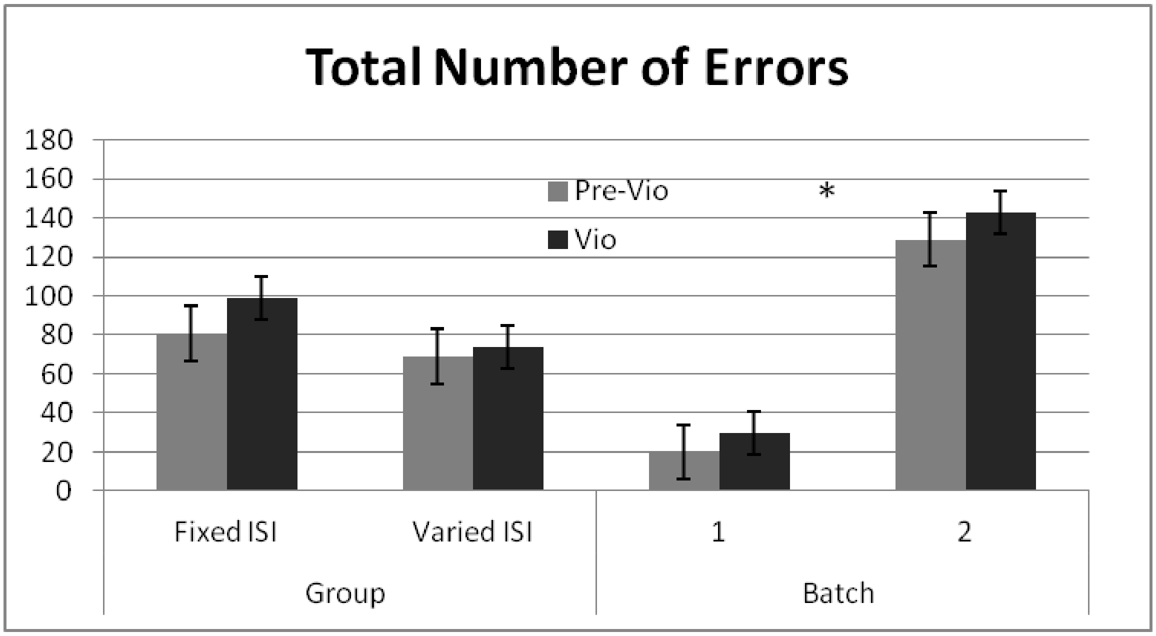

Figure 18. Comparison of total number of errors made on pre-violation and violation sequences across the two groups and across the two batches.

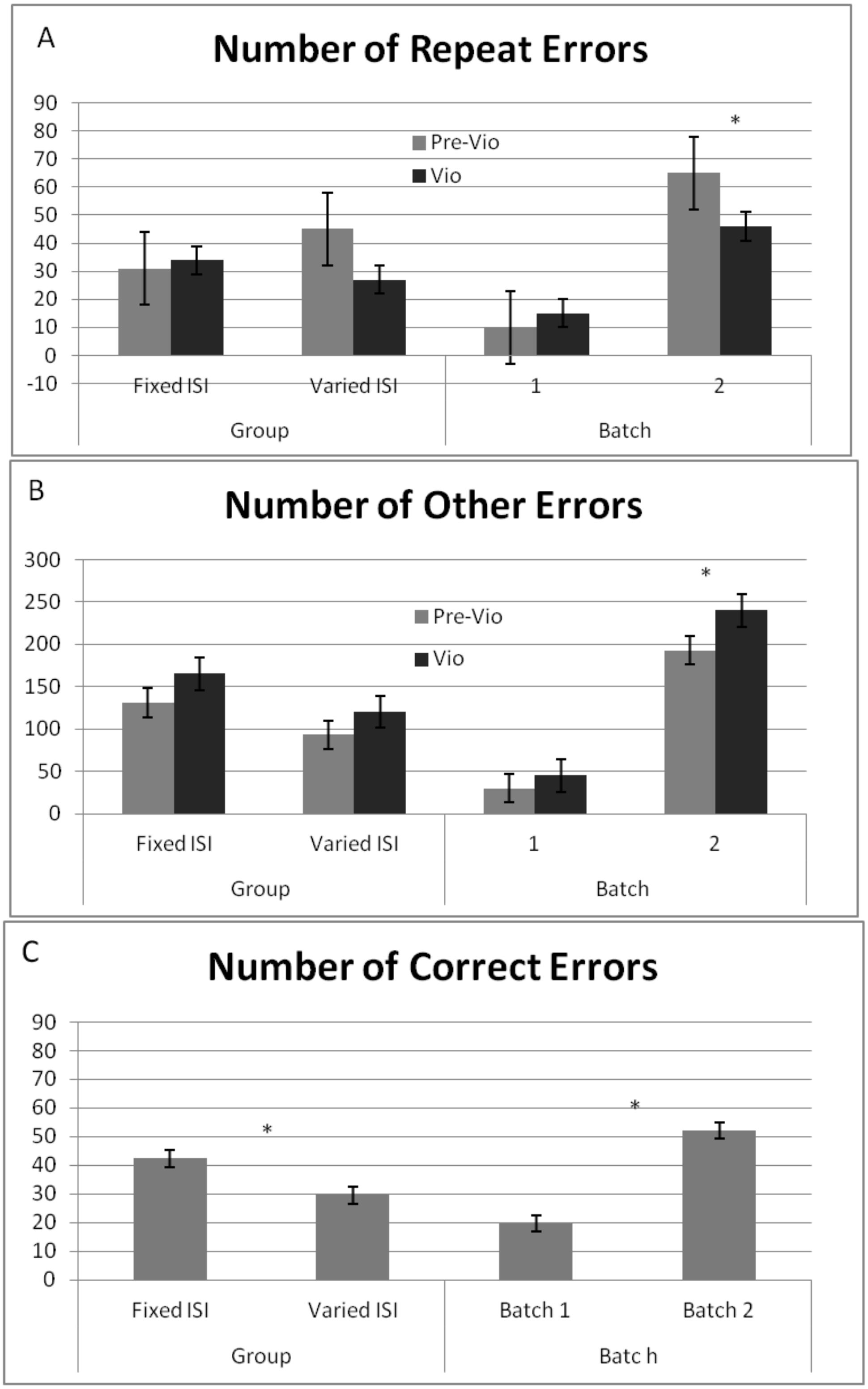

Figure 19. Total number of A) repeat, B) other and C) correct errors, across the two groups and two batches.

CHAPTER 5

GENERAL DISCUSSION

The findings of this project provide evidence that rats are capable of incidentally learning and using response patterns. My results also show that more complex patterns are more difficult for rats to learn as a rule. As shown by a high error rate on pre-violation sequences, pattern C ("F2B1") from my first experiment was more difficult for the rats to learn than patterns A ("F1B1") and B ("F1F1"). These findings are consistent with those of various studies by Fountain's Kent State and Kundey's Hood College research teams (Fountain, 2008; Fountain & Benson, 2006; Kundey & Fountain, 2014; Kundey et al., 2014). The results of the current project suggest that my simplified free operant, 3-item sequence procedure is sufficient for investigating pattern learning. Moreover, it is as effective in examining rats' serial pattern rule learning in rats as the more complex task used by Fountain and Kundey.

Results from the second experiment found that rats became poorer at switching from one response pattern within a session. This is inconsistent with findings from previous studies wherein rats were required to switch or use two patterns within a testing session (Fountain & Benson, 2006; Kundey et al., 2014). Discrepancies between my findings and those from the earlier studies may be due to different quantities of the patterns used and pattern-switches required. My experiment made use of three different governing patterns within each session. However, Kundey et al. (2014) and Fountain and Benson (2006) utilized only two patterns and sub-patterns, respectively, within sessions. This suggests that perhaps a limit exists as to the number of patterns rats can handle at one time. Also, my task required rats to make only two pattern switches throughout each session (i.e., from 1st to 2nd and from 2nd to 3rd pattern). The

Kundey et al. (2014) task required rats to switch back and forth between the two patterns up to nine times within a session (i.e., 10 sequences; 5 of each pattern). Fountain and Benson's (2006) task required rats to use two sub-patterns simultaneously to accomplish every sequence. If this switching experiment were to be attempted again, I believe that a task using only two response patterns should be used in order to test this hypothesis. Replications of this experiment using animals with no rule-violation experience may also produce different results. The previous training my animals had prior to this experiment may have influenced their responding behaviour.

Contrary to what I expected based on the literature (Roitblat et al., 1983; Church & Lacourse, 1998; Shin & Ivry, 2002), the findings of my third experiment show that varying inter-signal intervals did not result in performance differences than when these intervals were fixed. Roitblat et al. (1983) found that inter-run intervals (analogous to my ISIs) effects rats' ability to learn sequences. Findings from Church and Lacourse (1998) suggest that rats are capable of tracking sequences of fixed, but not random, intervals. Shin and Ivry (2002) have shown that rats can use temporal information to improve spatial sequence performance. It is a possibility that the varied ISIs were too short for the animals to perceive a difference in their temporal length. Future studies should utilize time periods in the unit of seconds when attempting to compare groups of animals using differing ISIs. For instance, 2s and 5s may be perceived as different more easily than 250ms and 450ms would be. It would also be interesting to see whether rats can be trained to detect the differences between 250ms and 450ms.

Finally, the third experiment provides evidence that subjects' experience with patterns and violations of those patterns affect their performance on a novel pattern. Rats with extensive pattern experience appear to only be disrupted by mirror type violations. However, those with less experience are disrupted by mirror, forward and backward violation types. I point out that not only did the younger, less experienced rats have less exposure to pattern learning in general, but they were also never previously exposed to pattern-violating sequences. This major difference may be a major contributor to the finding contrasts between the two batches of subjects. Perhaps previous experience with violations had made the older, more experienced rats more flexible during probe sessions with the novel pattern (i.e., they were not as "surprised" by a violation and understood that violation sequences did not necessarily make the governing pattern obsolete). This finding is an important one as it shows that previous learning with patterns cannot be assumed as forgotten when using subjects for successive experiments. Learning on a previous pattern influences how rats learn and use newer patterns. This information also suggests that the use of violations to test learning of novel patterns may not be viable after subjects have had experience with this type of testing. Subsequent research should seek to systematically control for the amount (i.e., number of trials and sessions) and type (i.e., how specific pattern types affect the learning of other pattern types) of pattern learning and/or testing (i.e., with/without violations) experience subjects are given in order to investigate more precisely how this affects future learning.

I believe that this project most strongly supports Hulse and Dorsky's (1977) idea of a *rule-encoding model* of serial learning. Hulse (1980) has argued that for rule learning to occur, there must be many instances in which one *class* of stimuli become associated with another class of stimuli in a consistent way. As the patterns used in my project governed multiple sets of stimuli (a.k.a. sequences), I have satisfied this rule-learning requirement. Each signal in a sequence (1st, 2nd, and 3rd) can be considered as its own *class* of stimuli. Under each governing rule, the relationship between signals 1 and 2 remains consistent in every sequence, as does the relationship between signals 2 and 3. Two other requirements for rule-learning that Hulse (1980) has listed are also met by my patterns. First, I provided my subjects with substantial amounts of training on the patterns – between 10 and 15 days, consisting of 64-66 trials each – before testing. Second, the inter-signal and inter-trial intervals used in all three experiments were not excessively long (i.e., ≤ 1s). It is fair to say then, according to Hulse, that my tasks provided ideal conditions for rule-learning.

My first experiment found that the more complex pattern was more difficult for the rats to learn than the simpler patterns; consistent with the rule-encoding model, which states that it is easier to learn and encode simple patterns than complex ones (Fountain & Hulse, 1981). This experiment also found that on violation sequences, rats were making significantly more *correct* (i.e., responses consistent with the governing pattern) than *other* errors, as predicted by the rule-encoding view (Fountain & Rowan, 1995). Fountain and Doyle (2011) have suggested that rule learners should be capable of using abstract rules to organize other, more complex pattern structures. The data from my third experiment, although not its original intention to do so, showed that rats may use their knowledge of previous pattern-learning to more quickly acquire a novel, complex pattern.

This project has also illustrated the suitability of my SRTT procedure for investigating serial pattern learning in relation to automated and deliberate processing. Although my third experiment did not produce differences between the fixed and varied ISI groups, it did demonstrate that rats are capable of reverting from automated to deliberate responding when necessary. This reversion was shown by the well experienced subjects during probe testing. These experienced animals were not significantly disrupted by violations, but did produce data showing that they had learned the pattern. These results combined suggest that the rats were using a follow-the-light (deliberate) responding pattern for probe sequences. The less experienced subjects, on the other hand, made many errors on violation sequences and their reaction times were significantly disrupted by violations; this is more representative of automated responding. The well-experienced subjects were rats that had been run on experiment 1. As experiment 1 had found that these animals' performance was indicative of automated responding, I can conclude that rats are capable of reverting from automated to deliberate responding when experience has shown this to be advantageous (e.g., in the case of violation sequences). The ability of my task to determine whether rats are using automated or deliberate responding makes it a useful tool for studying certain neurodegenerative diseases.

According to Carlson (2013), certain diseases cause neurodegeneration in structures and pathways of the brain in which automated responses are controlled. One such set of important structures is the basal ganglia, consisting of the caudate nucleus, putamen, and globus pallidus. There is evidence to suggest that once a learned behaviour has become automated, its processing is transferred to the basal ganglia. More specifically, while learning a new behaviour, the basal ganglia remain non-active, and observational in the processing. Over time, however, they take over, freeing up the more deliberate cortical circuits. I can conclude from this that when the basal ganglia are impaired (i.e., degenerated neurons, lesioned pathway, etc), the ability to perform automatically declines or even disappears. Two neurological diseases that are known to involve destruction/degeneration of the basal ganglia are Parkinson's (Deumens, Blokland, & Prickaerts, 2002; Carlson, 2013; Muthukumaran et al., 2014) and Huntington's diseases (Carlson, 2013).

One of the functions of the basal ganglia is to communicate with the substantia nigra, a structure of the brainstem (Carlson, 2013). Characteristic of Parkinson's disease is the substantial loss of dopaminergic neurons in the substantia nigra (Muthukumaran et al., 2014) as well as in the putamen and caudate nucleus of the basal ganglia (Deumens, Blokland, & Prickaerts, 2002). A study by Gobel et al. (2013) found that Parkinson’s disease patients had impaired sequence-specific learning abilities, due to the loss of circuitry between the basal ganglia and motor cortex regions of the brain. Gobel et al. (2013) concluded that this sequence-learning impairment was unlikely the result of the movement impairments characteristic of Parkinson’s disease. Huntington’s disease also affects the basal ganglia, specifically the putamen and caudate nucleus. It is characterized by jerky movements of the limbs due to the loss of neurons that secrete GABA - an inhibitory neurotransmitter (Carlson, 2013).

The ability to detect impairments of the basal ganglia as close to when they occur as possible, can allow for early detection of these neurological diseases. Early diagnosis can allow for early intervention, and those patients whose symptoms are detected as early as possible have a great advantage over those whose symptoms are not. A basic SRTT procedure, like the one I have used in this project, is a non-invasive way of detecting and monitoring the progress of basal ganglia diseases. In using this procedure with animal models of neurological diseases, I can test the effectiveness of possible drug treatments as well as develop a better understanding of the degenerative processes that occur in the brain.

A possible Parkinson’s disease (PD) experiment would be one in which I could test the effectiveness of a possible neuro-protectant, Ubisol Co-enzyme Q-10 (Muthukumaran et al., 2014). This experiment would use four groups: control/no Ubisol, control/with Ubisol, PD/no Ubisol, and PD/with Ubsiol. I would train all subjects on a basic 3-item pattern before disease-induction. After induction, I would then allow sufficient time to pass (~8weeks) for neurodegeneration to occur in the disease groups. Then I would begin pattern testing with violation sequences for one week. I would predict a few differences and similarities among the four groups. First, I would expect that the PD/ no Ubisol group to make the slowest reaction times during training and testing but produce fewer errors on violation sequences than the other three groups. Second, if Ubisol was a successful neuro-protectant, I would expect the PD/with Ubsiol group to perform on par, or almost on par, with the control/no Ubisol group. Finally, I would expect the control/with Ubisol group to perform as well as (i.e., quickly and with few errors), or possibly better than, the control/no Ubisol group.

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