EFFECTS OF THE NMDA RECEPTOR ANTAGONIST MK-801 ON THE TIMING AND
TEMPORAL PROCESSING OF SHORT-INTERVALS IN RATS

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ABSTRACT

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The present series of studies examined effects of MK-801, an NMDA antagonist, on short-interval timing in rats. The first study used two experiments to examine timing performance using the peak-interval (PI) and PI-gap procedures during chronic exposure of MK-801. The first of these experiments investigated the effects of three MK-801 doses (0.025 mg/kg, 0.05 mg/kg, & 0.2 mg/kg) administered for 10 sessions; the second experiment examined a single dose (0.2 mg/kg) administered for 15 sessions. MK-801 interfered with short interval timing by producing an over-estimation of time and a non-scalar increase in variability. Additionally, MK-801 increased response rate, suggesting a decrease in response inhibition. The influence of MK-801 on the formation of temporal memories was examined by switching the temporal criterion (Meck, 1988; Meck, Komeily-Zadeh, & Church, 1984). Spontaneous alternation and water maze tasks were determined whether the dose of MK-801 (0.05 mg/kg) also influenced spatial memory. Results suggested that MK-801 did not alter temporal learning and that this dose (0.05 mg/kg) had no effect on spontaneous alternation and water maze. MK-801 did increase the rate of responding, demonstrating that the drug had positive effects. However, the dose may have been insufficient to alter both spatial and temporal learning. Therefore, more work needs to be done with high concentrations of MK-801 before concluding that NMDA receptor antagonists have no influence on the learning of temporal durations. Finally, modeling simulations were used to qualitatively fit the MK-801 data using Scalar Expectancy Theory (SET) in order to better understand the effects of MK-801 on timing and
temporal processing. Modeling results suggest MK-801 has a wide ranging effect on the mechanisms of timing and temporal processing. Based on the simulations, the best fit to the data was obtained when five of the eight parameters were altered. Clock speed was slowed, while variability of clock speed, memory transformation, threshold, and base-rate of responding values were all increased. In summary, no definitive conclusions can be made about MK-801 effects on temporal learning. However there are two clear effects on timing and temporal processing, MK-801 causes an over-estimation of time and an increased rate of responding.
I dedicate this project to my friends and family (both living and deceased) who have all had a great impact in making me the person that I am today. Thank you for your understanding and tolerance during all my years of schooling.
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I would like to thank the members of my committee for their valuable feedback and help in conducting these experiments. I also thank Liz Hartmann for her help in constructing all of the operant boxes, Kelly Wright, Trisha Straight, Steve Borawski, and Leslie Gulvas for their help in conducting the experiments, and the members of the BGSU Timing Research group for providing a sounding board for many ideas. In addition, we would like to thank Dr. Peter Balsam for his suggestion to explore a state-dependent learning explanation for our data, which was used in the design of Experiment 2 in Chapter 2. This study is supported by PHS grant AG20560 and the generous donations of Mrs. Dorothy Price.
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CHAPTER 1. INTRODUCTION

The ability to accurately time and anticipate environmental events is an integral part of daily living. For example, routine tasks such as walking, dancing to a beat, or optimal forging in animals all require an ability to time and anticipate events. Because of its importance to daily living, timing and temporal processing has been a topic of study in both humans and non-human animals. Many different procedures have been developed to study timing behavior. Killeen and Fetterman (1988) proposed a taxonomy that is useful for organizing these different types of interval timing tasks. They identify three categories into which timing tasks falls: prospective, retrospective, and immediate timing. Prospective timing tasks gives information about what duration an animal anticipates will occur. Retrospective tasks give insight into the animals subjective experiences of a given duration, specifically how it relates to previously learned durations. An advantage of these types of tasks is that an animal need only give one response per trial. Although, these tasks only give information about how an animal perceives the duration of an event relative to other durations. In immediate timing tasks, the responses emitted by an animal give insight into the perceived duration of an interval being timed. In these types of timing tasks, the animal is typically free to respond at any point. However, responding is only maximally efficient around the time of the target duration, so the animal typically learns to withhold responded until the time at which the subjective end of the duration is near. Two immediate timing tasks were focused on in the following chapters: the peak-interval (PI) procedure and differential-reinforcement-of-low-rate of responding (DRL) schedule (see Appendix A).

Many models, both qualitative and quantitative, have been proposed in an attempt to explain the mechanisms of timing and temporal processing. These models have provided guides
for investigation into the biological mechanisms of timing and temporal processing. SET is a formal mathematical information processing (IP) model that has been proposed to address the sources of variance and the mechanisms involved in interval timing tasks (Church, 1984; Gibbon, Church, & Meck, 1984). SET attempts to characterize the processing involved in timing; including the different properties and sources of variability that produce timescale invariance (Gibbon, 1977). Three general stages are included in SET: clock, memory, and decision. Each of the stages has individual components with their own properties (see Appendix B for a thorough review of the workings of SET). The clock stage consists of three components: pacemaker, switch, and accumulator. The memory stage consists of two components: working memory and reference memory. The decision stage primarily consists of one major component, a comparator. SET has been useful in that it is relatively straightforward. It has been used to explain much behavioral data and it makes clear testable predictions. Of all the different timing models, SET is the dominant model used in animal timing research and other timing models have yet to be utilized to the extent of SET. Therefore, the SET framework was used to interpret the result in the following chapters.

The neuropharmacology of timing and temporal processing has been the focus of much empirical investigation aimed at understanding the biological underpinnings of the temporal processing mechanisms. In order to better understand the neurobiology of temporal processing, research has utilized techniques including behavioral manipulations, observations of clinical populations (i.e. Parkinson’s patients), and neurobiological manipulations, such as drugs and lesions (see Appendix C). Much of this work has focused on the roles of dopamine, acetylcholine, and 5-hydroxytryptamine (5-HT or serotonin) in temporal processing using a variety of different time production and estimation tasks. Another neurochemical that has been
investigated for its effects on temporal processing is glutamate (Sanger, 1992; Stephens & Cole, 1996; Tonkiss, Morris, & Rawlins, 1988; Welzl, Berz, & Battig, 1991).

Glutamate is a prominent excitatory transmitter found in the central nervous system (Cotman, Monaghan, Ottersen, & Stormmathisen, 1987). Glutamate acts on a number of receptor types, one type of which is involved in the gating of ion channels. Three primary subtypes of the ion gating glutamate receptors have been identified: N-methyl-D-aspartate (NMDA), α-amino-3-hydro-5-methyl-4-isoxazolpropionic acid (AMPA), and kainate receptors (Watkins, Krogsgaard-Larsen, & Honore, 1990). Although the AMPA receptor has received some interest for its role in timing and temporal processing (Stephens & Cole, 1996), the NMDA receptor was focused on for the following reasons. NMDA receptors have been found throughout the brain with high concentrations in telencephalic regions, the highest concentration of these receptors being located in the hippocampus (Monaghan & Cotman, 1985). Some evidence that NMDA receptor antagonists disrupt hippocampal function comes from previous studies of spatial learning and temporal processing. Administration of NMDA receptor antagonists results in impairment of spatial learning (Morris, Anderson, Lynch, & Baudry, 1986) and temporal processing (Tonkiss et al., 1988), similar to those observed with hippocampal lesions. Based on these findings, it has been assumed that the effects of NMDA receptor antagonists are primarily due to a disruption of hippocampal function (Morris et al., 1986; Tonkiss et al., 1988).

As compared to the research on dopamine, acetylcholine, and 5-HT systems in timing and temporal processing, glutamate has been the focus of much less investigation. Its effects have only been explored using a single timing related task; the DRL schedule (see Appendix A). This research on the DRL task found that NMDA receptor antagonists have three main effects: increased response rate, reduced efficiency for obtaining a reinforcer, and shortening of the
distribution of inter-response-times, IRTs (Sanger, 1992; Stephens & Cole, 1996; Tonkiss et al., 1988; Welzl et al., 1991). These studies concluded that NMDA receptors antagonists influence temporal processing by altering either clock or memory mechanisms via disruption of the hippocampus. The aim of this set of studies was to further explore the effect of NMDA receptor antagonists on timing and temporal processing.

Chapter 2 describes a study that investigated the effect of an NMDA receptor antagonist, MK-801, on a previously learned duration. Two experiments were conducted and are reported in Chapter 2. The first experiment examined the effects of MK-801 on timing performance using the PI and peak-interval gap (PI-gap) procedures (see Appendix A). Performance on the PI and PI-gap procedures was investigated following a chronic 2-week exposure of MK-801 at three doses (0.025 mg/kg, 0.05 mg/kg, & 0.2 mg/kg). The second experiment further examined the influence of MK-801 on performance in the PI procedure with only one dose (0.2 mg/kg) of the drug but with a 3-week treatment that did not include the PI-gap procedure.

Since some form of memory must be involved in the learning of temporal durations, studying of role of glutamate and the NMDA receptor in temporal learning may lead to greater understanding of the mechanisms used to learn and remember durations. Chapter 3 describes a study where the aim is to determine if the NMDA receptor is involved in formation of temporal memories. In sum, this study examined the effects of an NMDA antagonist, MK-801, on learning a new temporal memory using a PI procedure that switches the temporal criterion (Meck, 1988; Meck, Komeily-Zadeh et al., 1984). In addition, spontaneous alternation and Morris water maze tasks further examined the influence of MK-801 on working memory to ensure that the dose level of MK-801 used, 0.05 mg/kg, is sufficient to disrupt a task where NMDA receptor
antagonist are known to cause behavioral disruptions (Holter, Tzschentke, & Schmidt, 1996; Lennartz & Gold, 1995).

The aim of Chapter 4 is to gain further understanding into the mechanisms that are altered by NMDA receptor antagonists. This was accomplished by using Scalar Expectancy Theory (SET: see Appendix B). The data obtained from the previous two chapters was modeled using an implementation of SET (Church, 2003). Qualitative fits to the data were made by altering the parameters of the model in order to determine which theoretical mechanisms may be the target of the effects observed with MK-801.

Finally, Chapter 5 provides a general discussion of the finding from each of the studies. The discussion entails what the results from these series of studies tell us about the role of NMDA receptor antagonists is in timing and temporal processing. The findings are compared to the broader neuropharmacological context, with emphasis on comparing and contrasting the effects of NMDA receptor antagonist with those of other systems.
CHAPTER 2. EFFECTS OF THE NMDA RECEPTOR ANTAGONIST MK-801 ON SHORT-INTERVAL TIMING IN RATS

Glutamate is a prominent excitatory transmitter found in the central nervous system that interacts with several receptor subtypes (Cotman et al., 1987; Watkins et al., 1990). The N-methyl-D-aspartate (NMDA) receptor subtype has received much interest because of its role in learning, memory and synaptic plasticity (Morris, 2003; Morris et al., 1986; Shapiro & Caramanos, 1990; Shapiro & O'Connor, 1992). Because of the high concentration of NMDA receptors in the hippocampus (Monaghan & Cotman, 1985), NMDA receptor antagonists often have effects similar to hippocampal lesions (Morris et al., 1986; Tonkiss et al., 1988). Although the study of NMDA receptor involvement in memory has proven very fruitful (Butterfield & Pocernich, 2003; Kumar, 2004; Minkeviciene, Banerjee, & Tanila, 2004; Rogawski & Wenk, 2003), NMDA receptors may also be involved in non-mnemonic cognitive processes.

Evidence that NMDA receptors may be involved in timing and temporal processing comes chiefly from studies using a differential-reinforcement-of-low-rate of responding (DRL) schedule (Sanger, 1992; Stephens & Cole, 1996; Tonkiss et al., 1988; Welzl et al., 1991). In a DRL task (Kramer & Rilling, 1970; Zeiler, 1977), subjects are trained to emit an operant response (typically a lever press) after the passage of a target duration following the last response. If the response occurs after the target duration has passed, the subject receives reinforcement and a new interval begins. If the response occurs before the target duration elapses, then the duration resets and no reinforcement is given.

NMDA antagonists alter DRL performance. Chronic intra-ventricular infusions of a competitive antagonist of the NMDA receptor D,L-2-amino-5-phosphonopentanoic acid (AP5) increased response rate, decreased efficiency (the number of reinforcements delivered compared
to responses made), and shortened the distribution of inter-response-times (IRTs: Tonkiss et al., 1988). Upon termination of AP5 administration, the observed impairments disappeared and animals receiving AP5 returned to perform like control animals (Tonkiss et al., 1988). Acute systemic injections of MK-801 (dizocilpine), a noncompetitive NMDA receptor antagonist (Wong et al., 1986), produced impairments similar to those observed with chronic infusions of AP5 (Welzl et al., 1991). Single injections of MK-801 generally increased response rate, reduced efficiency, and caused a shortening, or leftward shift, of the distribution of IRTs. However, the highest dose of MK-801 (0.30 mg/kg) depressed response rate below the level of controls and eliminated any observable peak in the distribution of IRTs (Welzl et al., 1991).

Other competitive and noncompetitive NMDA antagonists, including phencyclidine, CGS 19755, eliprodil, memantine, and dextromethorphan, also disrupt DRL performance (Sanger, 1992). Because these NMDA antagonists bind to different sites on NMDA receptors, Sanger (1992) examined the effects of these compounds, in addition to MK-801, on the DRL task. All drugs produced a flattening of the distribution of IRTs, and all except eliprodil produced a leftward shift in the distribution similar to that observed with MK-801 and AP5. The effects of these different drugs on response rate varied, with some increasing response rate while others decreasing response rate depending on the drug and dosage level. These findings suggest that NMDA antagonists consistently disrupt timing behavior on DRL tasks (Sanger, 1992).

Because of the shortening of IRTs in DRL tasks, NMDA antagonists have been suggested to alter the short-interval timing system (Meck, 1996; Tonkiss et al., 1988; Welzl et al., 1991). According to this idea, NMDA antagonists may speed up the “clock” or alter the “memory” of the previously rewarded duration such that the subjective experience of the rewarded duration is an under-estimate of the actual duration. Alternatively, disruptions observed in DRL tasks with
NMDA antagonists could be due to the increased response rate or impairment in the ability to withhold lever responses for the length of the target duration. In order to dissociate an effect on temporal processing/memory from one on response inhibition, the peak-interval (PI) procedure may be useful.

In the PI procedure, animals must time the duration of a stimulus and develop a memory for this duration similar to the DRL task. However, unlike the DRL task, animals are free to emit any number of responses without affecting when reinforcement will be delivered. In the PI procedure, peak-time is a measure of timing accuracy, while peak-rate is a measure of response rate. In rats, these two measures of performance have been shown to be independent because they can be individually manipulated by changing parameters of the task (S. Roberts, 1981). If NMDA antagonists affect the ability to inhibit responding without altering timing ability, then peak-rate will be altered, but peak-time will not. On the other hand, if NMDA antagonists alter temporal processing without affecting response inhibition, then peak-time should shorten while peak-rate may or may not change, similar to hippocampal lesions (Buhusi, Mocanu, & Meck, 2004; Meck, Church, Wenk, & Olton, 1987; Olton, Wenk, Church, & Meck, 1988). It is also possible that both temporal processing and response inhibition could be altered by NMDA antagonists.

Effects of NMDA antagonists on DRL performance have generally been assumed to be due to disruption of hippocampal function for two main reasons (Tonkiss et al., 1988; Welzl et al., 1991). First, NMDA receptors have been found throughout the brain with high concentrations in telencephalic regions and the highest concentrations in the hippocampus (Monaghan & Cotman, 1985). Second, administration of NMDA receptor antagonists results in impairment of spatial leaning (Morris et al., 1986) and temporal processing (Tonkiss et al., 1988).
similar to those observed with hippocampal lesions. Based on these findings, it has been assumed that the effects of NMDA receptor antagonists are primarily due to a disruption of hippocampal function (Morris et al., 1986; Tonkiss et al., 1988).

A timing task related to the PI procedure that has shown effects of hippocampal lesions is the peak-interval gap (PI-gap) procedure (Meck, Church, & Olton, 1984; Meck et al., 1987; Olton et al., 1988; S. Roberts, 1981). In this task, a gap (or break in the stimulus) is added to the PI procedure as a test of working memory (S. Roberts, 1981). Four patterns of response have been previously observed: run, stop, partial reset and reset (Meck et al., 1987; Olton et al., 1988; S. Roberts, 1981). In the run pattern, the subject ignores the gap. For the stop pattern, the subject stops timing when the stimulus goes off, and continues timing from that point when the stimulus resumes. In the partial reset pattern, subjects stop timing when the stimulus goes off, and continues timing when the stimulus resumes. However, the remembered duration prior to the gap is not accurate resulting in a lengthened estimation of time. The reset pattern supposes that the subjects stop timing when the stimulus goes off, and begin timing as if a new trial has started when the stimulus resumes. Normal rats typically adhere to a stop, or partial reset (a response pattern between stop and reset) pattern, whereas animals with hippocampal lesions typically follow a reset pattern (Buhusi & Meck, 2000; Buhusi, Sasaki, & Meck, 2002; Meck et al., 1987; Olton et al., 1988; S. Roberts, 1981). If the effect of NMDA receptor antagonists is similar to the effects of hippocampal lesions on timing, administration of NMDA antagonists should result in animals using a reset pattern instead of a stop pattern in the PI-gap procedure (Meck et al., 1987; Olton et al., 1988).

Experiment 1 examined the effects of an NMDA antagonist, MK-801, on timing performance using the PI and PI-gap procedures. Performance on the PI and PI-gap procedures
was investigated following a 2-week series of daily injections of MK-801 at three doses. Experiment 2 further examined the influence of MK-801 on performance in the PI procedure with only one dose of the drug but with a 3-week treatment that did not include the PI-gap procedure.

Experiment 1

Method

Animals

Thirteen male, adult Fisher 344 rats (Harlan, Indianapolis, IN) were subjects for the study, were between two and four months of age at the start of the study, and weighed between 250-300 grams. Housing was in a room maintained near 22°C and on a 12-hour light-dark cycle with lights on at 7 a.m. local time. Rats were housed individually or in pairs. Four groups (n = 4) were treated (i.p. injections) with saline, 0.025, 0.05, or 0.2 mg/kg MK-801 (Three of the animals receiving 0.2 mg/kg had been given saline as part of the control group. All other animals were experimentally naïve at the start of testing). Prior to training, animals were food restricted until their body weight reached 85% of their ad lib weight. Supplemental food was given so that each rat gained approximately five grams per week in body weight. Testing sessions were conducted once each day, five days per week (Monday – Friday). All procedures used in this study followed NIH guidelines for handling and caring of animals and were approved by the Bowling Green State University Institutional Animal Care & Use Committee.

Apparatus

Testing was conducted in sixteen similar custom-made operant boxes (28 x 28 x 37 cm) constructed of clear acrylic. A hinged door was available on one side of the box, and a water bottle was located next to the door. Sucrose pellets (45mg, PJFSC-0045; Research Diets, Inc.,
New Brunswick, NJ) were delivered by a pellet dispenser (ENV-203; Med-Associates, St. Albans, VT) into a food cup on one wall. A response lever (11.0 cm above the floor) was to the right of the food cup, and a stimulus lamp (4.8 watts) was positioned directly above the lever (29.0 cm from the floor). Approximately 15 g of force was required to depress the lever. Additional experimental equipment (a sound generator, a stimulus lamp, a small cup for delivery of water, and a response lever) was located on another wall; however, this equipment was not used in the current study and was only present for use in other experiments that were being conducted in the lab. A house lamp (2.8 watts) located outside of the operant box provided indirect lighting. A solenoid valve (Z723A; Sirai Elettromeccanica, Bussero, Milano, Italy) was located outside the box to provide an audible click, and was activated along with the pellet dispenser to act as a secondary reinforcer.

Each operant box was housed in a larger chamber (61 x 61 x 61 cm) constructed from laminated particle board. This chamber acted to minimize light and sound originating from sources outside of the operant box. A fan provided ventilation to each chamber, and a small peep hole could be used to observe the behavior of the animal. A Dell (Optiplex GX240) computer with a Med-Associates SmartCtrl system (MED-PC IV; DIG-716; SG-716; Med-Associates, St. Albans, VT) controlled the presentation of stimuli and delivery of reinforcement, and recorded the times of lever responses.

Drug

MK-801 ((+)-5-methyl-16, 11-dihydro-5H-dibenzo[a,d] cyclohepten 5, 10-imine; M-107, Sigma, St. Louis, MO) was dissolved in physiological saline. Saline or MK-801 (0.025, 0.05, or 0.20 mg/kg, i.p.) was administered 30 minutes prior to testing sessions.
Procedure

Animals were first trained to press the lever to receive a sucrose pellet using a continuous reinforcement schedule (CRF, 3-5 sessions). Following the CRF sessions, animals received training on a variable reinforcement schedule (VR-3, 5 sessions), where, on average, every third lever press resulted in the delivery of a sucrose pellet. A minimum of one and a maximum of six lever presses were required to obtain a sucrose pellet.

Following the VR-3 sessions, rats were trained on a 12 s fixed-interval (FI) reinforcement schedule. FI trials started with the illumination of a light stimulus. After the 12 s target duration had elapsed, the first lever response resulted in delivery of a sucrose pellet and termination of the light stimulus. Any lever response prior to the passage of the FI duration did not have any effect. If an animal made no lever response after the FI duration, the stimulus light extinguished after 60 s. Following the completion of an FI trial, an inter-trial interval (ITI) began; ITIs were randomly selected from a uniform distribution of times between 30 and 60 s. Any lever response during the final 10 s of the ITI resulted in a 5 s timeout period during which the animal was placed into darkness by extinguishing the house light. Following the timeout period, the house light was re-illuminated and a minimum of 10 s without a lever press was required in order to begin the next FI trial. An individual FI session typically lasted 1 hour and 30 minutes during which an animal would complete approximately 70 trials.

After approximately ten sessions of FI training, rats began the peak-interval (PI) procedure. This procedure was the same as the FI procedure, except that half of the trials were probe trials, where a sucrose pellet was never delivered and the stimulus light remained on for the duration of the trial (60 s). During this time, the animal was free to respond. An individual PI
session typically lasted 1 hour and 30 minutes during which an animal would complete approximately 70 trials (≈ 35 FI trials and 35 probe trials). Random selection without replacement was used to select the trial type for an individual trial. The selection group consisted of four trial types (2 FI trials & 2 probe trials). Once each of the four trial types had been selected the selection group was reset. This procedure was used to ensure that no more four consecutive probe trials could occur between FI trials. Rats were trained on the PI procedure until the peak of the temporal response function for individual sessions was within two seconds of the target duration (12 s, approximately 30 sessions). At this point, rats were tested for five additional sessions to establish baseline performance.

Following the baseline sessions, rats received injections of either saline or MK-801 for 10 consecutive sessions. After the 10 sessions, injections continued as the rats performed 3 sessions of the PI-gap procedure. The PI-gap procedure is a modification of the PI-procedure in which a random half of probe trials (gap trials) have the light stimulus interrupted. Six seconds after the start of the trial, the stimulus light extinguished for a 6 s gap period and then illuminated again for the remainder of the trial. As with probe trials, no sucrose pellets were delivered during gap trials. After three sessions on the PI-gap procedure, rats were returned to the PI procedure for five additional sessions without injections.

Data Analysis

Peak-interval procedure: Data were analyzed in blocks of 5 sessions: Sessions 1-5 (Baseline), Sessions 6-10 (Drug 1), Sessions 11-15 (Drug 2), Sessions 16-18 (PI-gap), and Sessions 19-23 (Post-Drug). Only data from probe trials were analyzed. Lever responses were added for each successive 1-second interval (bin) throughout the 60 s signal duration. The total responses in each bin were divided by the total number of trials to determine the mean number of
lever responses per trial. The mean lever responses for each bin were plotted as a function of
time to create a temporal response function. Response rate was calculated by multiplying the
number of responses in each bin by 60 so that response rate could be expressed as responses per

A Gaussian + linear equation (Buhusi et al., 2002) that gave the best fit to the temporal
response function was determined by minimizing root mean-square error using the Solver add-in
package for Microsoft Excel 2002 (Version 10.65, Microsoft Corporation, Seattle, WA). The
following was the generalized Gaussian + linear model that was fit to the temporal response
function:

\[
R(t) = a \exp(-0.5\left(\frac{t-t_0}{b}\right)^2) + c(t-t_0) + d,
\]

where \( t \) is the current time bin and \( R(t) \) is the mean response rate at time \( t \). Model fitting
determined estimates for the parameters \( a, b, c, d, \) and \( t_0 \). Peak-time was estimated by \( t_0 \), peak-
rate was estimated by \( a + d \), and variability was estimated by \( b^2 \).

Parameter estimates were obtained for each animal in each session and then mean
parameter estimates were obtained over all sessions within a test block: Baseline, Drug 1, Drug 2
and Post-Drug. Data analyses for the PI-gap block are discussed below, and will be treated
separately. Separate mixed model ANOVAs were conducted in SPSS for Windows (Version
12.0, SPSS, Inc., Chicago, IL) to examine effects of drug group (saline, 0.025 mg/kg MK-801,
0.05 mg/kg MK-801, and 0.2 mg/kg MK-801) and test block (Baseline, Drug 1, Drug 2, and
Post-Drug) on peak-time, peak-rate, and variability. Greenhouse-Geisser corrections were used
in all cases where the assumption of sphericity was violated; for consistency, the degrees of
freedom reported in these instances are uncorrected. Post-hoc tests for the between subjects
factors were conducted, when necessary, using Tukey’s HSD with \( \alpha \) set at .05.
Peak-interval gap procedure: Because the testing procedures during the PI-gap sessions differed slightly from the PI sessions, data from PI-gap block were analyzed separately. Although probe trials were included in this test block as a part of the PI-gap procedure, the primary interest in this procedure is performance on gap trials. Therefore, only data from gap trials were analyzed. Peak-time, peak-rate and variability measures were calculated as in the PI sessions with one exception. Because of the small number of gap trials in each session, mean lever response data for each bin were calculated across all trials and sessions prior to parameter estimation using the Gaussian + linear equation.

Separate one-way ANOVAs were conducted in SPSS for Windows for peak-time, peak-rate and variability as dependent measures. Drug condition was a between subjects factor. Post hoc tests, when necessary, were conducted using Tukey’s HSD with \( \alpha \) set at 0.05.

Results

Peak-Interval Procedure

During Baseline sessions, temporal response functions for all animals peaked near the 12 s target duration (Figure 1). Peak-times were not altered by saline, 0.025 mg/kg, or 0.05 mg/kg MK-801 (Figure 2A). However, the 0.2 mg/kg dose of MK-801 produced a dramatic lengthening of peak-time during Drug 1 sessions. During Drug 2 sessions, peak-times began to return to the target time, although they remained longer than Baseline values. Peak-times for this group returned immediately to Baseline levels following cessation of the drug, Post-Drug: Mean Difference = 0.10 s, \( t(15) = 0.66, p > 0.05 \). These observations were confirmed by the ANOVA on peak-times, which showed a main effect of session block, \( F(3, 36) = 30.01, p < 0.001 \), a main effect of drug group, \( F(3, 12) = 5.71, p < 0.05 \), and a drug group by session block interaction, \( F(9, 36) = 22.30, p < 0.001 \).
Post-hoc tests on drug group revealed that the 0.2 mg/kg MK-801 group had significantly longer peak-times than the saline group ($p < 0.05$), the .025 mg/kg MK-801 group ($p < 0.05$), and the 0.05 mg/kg MK-801 group ($p < 0.05$). No other significant differences in peak-time were found between drug groups.

Peak-rate remained stable across all test session blocks for the saline groups (Figure 2B). However, MK-801 groups increased response rate upon receiving the drug injections (Figure 2B, Drug 1). This increased response rate was maintained throughout Drug 2 sessions. Upon the termination of drug injections, response rate for the 0.025 mg/kg, and 0.05 mg/kg MK-801 groups remained somewhat elevated above baseline levels, although these differences were not

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**Figure 1:** Temporal response profiles for each drug condition across testing blocks. Inset figures are response profiles normalized by both peak-rate and peak-time. (Panel A: Saline; Panel B: 0.025 mg/kg MK-801; Panel C: 0.05 mg/kg MK-801; Panel D: 0.2 mg/kg MK-801.)
Figure 2: Peak-time (Panel A), peak-rate (Panel B), and variability (Panel C) plotted by drug group (Saline, 0.025 mg/kg MK-801, 0.05 mg/kg MK-801, and 0.20 mg/kg MK-801) and by test block (Baseline, Drug 1, Drug 2, and Post-Drug). Error bars express standard error of the mean.
statistically significant, \( t(3) = -2.18, p > 0.05 \) & \( t(3) = -1.06, p > 0.05 \), respectively. The 0.2 mg/kg MK-801 group displayed a much sharper drop in peak-rate than the other two drug groups, returning to Baseline levels during the Post-Drug sessions, \( t(3) = 1.68, p > 0.05 \). To further analyze the peak-rate data, the results for each test block were normalized by animal relative to their Baseline block peak-rate (Normalization relative to baseline levels effectively eliminating the large individual differences in overall response rate typically observed between animals). An ANOVA was then performed on the normalized peak-rate data with the Baseline block omitted (for the Baseline Block all animals were necessarily at 100% of Baseline performance levels), which resulted in a change in degrees of freedom for the analysis of peak-rate. This analysis showed a main effect of session block, \( F(2, 24) = 9.08, p < 0.001 \) no main effect of drug group, \( F(3,12) = 0.881, p > 0.05 \), but a significant interaction between drug group and session block, \( F(6, 24) = 3.72, p < 0.01 \).

Variability remained stable across test blocks for the saline, 0.025 mg/kg, and 0.05 mg/kg MK-801 groups (Figure 2C). However, the 0.2 mg/kg MK-801 group displayed a dramatic increase in variability during Drug 1 sessions. Variability decreased during Drug 2 sessions, although variability was still larger than during Baseline sessions, \( t(3) = -3.55, p < 0.05 \) (Figure 2C). Variability for this group returned to Baseline levels upon termination of the drug, \( t(3) = -2.56, p > 0.05 \). These observations were confirmed by the ANOVA on variability, which revealed a significant main effect of session block, \( F(3, 36) = 12.76, p < 0.001 \), a main effect of drug group, \( F(3, 12) = 33.52, p < 0.001 \), and a significant drug group by session block interaction, \( F(9, 36) = 11.19, p < .001 \). Post hoc tests on the drug groups indicated that the 0.2 mg/kg MK-801 group had significantly greater variability than the saline group (\( p < 0.05 \), 0.025
mg/kg MK-801 group \( (p < 0.05) \), and 0.05 mg/kg MK-801 group \( (p < 0.05) \). No other significant differences in variability were found between drug groups.

Because the highest dose of MK-801 increased peak-time, a proportional increase in variability would be expected based on scalar timing (Gibbon, 1977; Gibbon et al., 1984). In order to determine whether increases in variability observed for the 0.2 mg/kg MK-801 group were consistent with scalar timing (i.e., Weber’s law), the data were plotted using a normalized time scale. Temporal response functions were also normalized with respect to peak-rate in order to facilitate comparisons between blocks. Results of this analysis are displayed in the inset graphs found in Figure 1A-D. Response functions of Drug 1 and Drug 2 sessions for the saline, 0.025 mg/kg, and 0.05 mg/kg groups (Figure 1A-C) superimposed, whereas functions for 0.2 mg/kg MK-801 group did not. These results demonstrate that the increase in variability observed in the 0.2 mg/kg MK-801 is greater than would be expected based on scalar timing.

**Peak-Interval Gap Procedure**

No differences between drug groups were observed in the PI-gap procedure for peak-time, \( F(3, 15) = 0.73, p > 0.05 \). All groups displayed a pattern of responding that was consistent with a *reset* pattern. In addition, no differences between drug groups were observed for either peak-rate, \( F(3, 15) = 1.40, p > 0.05 \), or variability, \( F(3, 15) = 0.90, p > 0.05 \). The findings for peak-rate and variability for the PI-gap procedure contrast with those observed for the standard PI procedure.

**Discussion**

Effects of the NMDA antagonist MK-801 on timing were examined using the peak-interval (PI) procedure and the peak-interval gap (PI-gap) procedure for three drug doses (0.025 mg/kg, 0.05 mg/kg and 0.2 mg/kg). Four main results were observed. First, injections of 0.2
mg/kg MK-801 produced an immediate lengthening of peak-time that attenuated with continued training. This result was not observed at lower doses. Second, injections of MK-801 increased peak-rate of responding. Third, injections of the highest dose (0.2 mg/kg) resulted in an increase in variability, beyond that expected from Weber’s law; this increase in variability was not observed at lower doses of MK-801. Finally, during the PI-gap procedure a reset pattern was observed for all rats (MK-801 and saline) and the findings for peak-rate and variability during PI-gap performance differed from those observed for the standard PI procedure.

Overall, the present results are only partly consistent with the results reported in previous studies using the DRL task. MK-801 increases responding in the DRL task, which is consistent with our finding of an increase in response (peak) rate in the PI procedure. Moreover, an increase in behavioral activity is common in studies using MK-801 (Ford, Sanberg, Norman, & Fogelson, 1989; Welzl et al., 1991; Whishaw & Auer, 1989). While this is not a new finding, the increase in peak-rate in the PI procedure for the two lowest doses of MK-801 (0.025 mg/kg & 0.05 mg/kg) indicate that the drug doses were of a sufficient level to alter some aspects of behavior but not other measures (peak-time and variability).

The effect of MK-801 on peak-time was different from that reported for DRL performance. In the current study, injections of MK-801 at the highest dose (0.2 mg/kg) produced an abrupt lengthening (rightward shift) of peak-time in the PI procedure, unlike the shortening (leftward shift) of the IRT distribution during DRL (Sanger, 1992; Stephens & Cole, 1996; Tonkiss et al., 1988; Welzl et al., 1991). The finding of a non-scalar increase in variability at the highest drug dose may partially explain some of the differences found in performances of the DRL and PI procedures. Examination of the temporal response profiles in Figure 1 revealed that animals given the 0.2 mg/kg dose of MK-801 started to respond sooner and continued to
respond longer than control animals. This type of early responding in the PI procedure would manifest itself as a shortening of the IRTs in DRL. Taken together, our findings with peak-time and variability suggest that the shortening of the IRTs in the DRL task is not due to a timing problem, but more likely due to the inability to inhibit responding.

The effect of MK-801 on peak-times was also different from previous reports of the effect of hippocampal lesions. Lesions of the hippocampal system have been observed to produce a gradual and permanent leftward shift in peak-times, whereas we observed an abrupt rightward shift that appeared to partially renormalize with continued training (Buhusi et al., 2004; Meck et al., 1987; Olton et al., 1988). According to Scalar Expectancy Theory (SET), the type of gradual and permanent shift in peak-time associated with hippocampal lesions has been attributed to alterations in “memory storage”, whereas an abrupt but transient shift in peak-time has been previously attributed to a change in “clock speed” (Church, 2003; Meck, 1994, 1996; Meck, Church et al., 1984; Meck et al., 1987).

The issue of hippocampal involvement is potentially addressed by the PI-gap procedure, as previous reports in the literature have found that control animals typically display a *stop* or *partial-reset pattern*, whereas animals with hippocampal lesions typically follow a *reset pattern* (Buhusi et al., 2002; Cabeza de Vaca, Brown, & Hemmes, 1994; Meck et al., 1987; Olton et al., 1988; S. Roberts, 1981). However, in the present study, saline control animals, as well as those receiving MK-801, displayed a *reset pattern*, limiting the value of the PI-gap procedure in terms of evaluating the effects of MK-801 on hippocampal function. The factors influencing whether animals *reset* or *stop* during a gap are difficult to tease apart. Animal species (i.e., rat vs pigeons) and strain (i.e., pigmented vs albino rats), stimulus modality and intensity have been shown to affect pattern of responding to gaps (Buhusi, Perera, & Meck, 2005; Buhusi et al., 2002). Buhusi
et al. (2005) concluded that albino rats (Sprague Dawley) usually demonstrate a *stop pattern* during a gap in a visual stimulus. However, the present study found that Fisher 344 rats, also an albino rat strain, followed a *reset pattern* during a gap in a visual stimulus. It is possible that other experimental design characteristics such as duration of target interval, gap location, gap duration, length of probe trials and duration of the inter-trial-interval may all be influencing the response strategy of our control animals (Buhusi et al., 2005; Buhusi et al., 2002; Cabeza de Vaca et al., 1994). Overall, it is clear that additional research is needed to disentangle the various factors responsible for response strategy shifts in the PI-gap procedure and the influence of NMDA receptor function in the PI-gap procedure.

Supporting the view that MK-801 mediates clock function, the present results are similar in some ways to distortions in timing reported with dopamine antagonists (Meck, 1983, 1986, 1996; Meck & Church, 1987b). Like the effects of MK-801, dopamine antagonists produce an immediate rightward shift in the peak-time that gradually returns towards its pre-drug value with continued training on the drug (Maricq, Roberts, & Church, 1981; Meck, 1996). In addition, continued training upon removal of the dopamine antagonist results in an immediate, but temporary, leftward shift in peak-time, which has been called a rebound effect (Maricq & Church, 1983; Meck, 1983, 1996).

Our results with MK-801 differ from those reported with dopamine antagonists in a few important respects. First, the shift we observed in peak-times with MK-801 did not completely return to pre-drug values with continued training, although the trend was in that direction. Second, no obvious rebound effect (an abrupt and transient shift in peak-times in the opposite direction) was observed following the cessation of MK-801 injections, as has been observed with dopamine antagonists (Meck, 1983, 1986, 1996; Meck & Church, 1987b).
It is possible that the lack of a rebound effect is due to state dependent learning (Beninger & Hahn, 1983; Ohyama et al., 2000; Siegel, 1988). In the present study, rats were first trained without any injection (saline or MK-801), which may be considered one state. A new state may have accompanied the initiation of injections, either from the sensation of the injections or from the sensations produced by the MK-801. Perhaps following the cessation of injections, animals returned immediately to the state formed without injections; thus no rebound in peak-time was observed. One method to address this possibility is to continue injections during the Post-Drug sessions, by substituting saline for MK-801.

Experiment 2

Experiment 2 addressed several issues raised in Experiment 1. First, a critical test of whether MK-801 mediates clock function is that changes in peak-time completely return to the criterion time with continued training. In Experiment 1, 2-weeks of training under the influence of MK-801 may have been insufficient for complete return of the peak-time to 12 s although the trend was in the right direction. Experiment 2 thus added a third week of training with MK-801 to determine whether further testing with drugs would produce a more complete renormalization of peak-times. Second, another characteristic of an alteration of the clock stage of SET is a rebound effect following cessation of the drug. However, a rebound effect was not observed in Experiment 1. To investigate the possibility that the lack of a rebound effect may have been due to state-dependent learning, saline injections were administered during Post-Drug sessions in Experiment 2. Third, all animals in Experiment 2 were experimentally naïve, unlike Experiment 1 in which some rats were tested in both saline and drug conditions. Fourth, only the highest dose of MK-801 (0.2 mg/kg) was examined in Experiment 2 because this dose produced the most dramatic effects on peak-time, response rate, and variability. Finally, PI-gap sessions were
removed from the testing procedures since the reset pattern observed for all rats (saline and MK-
801) in the preceding experiment precluded the use of the PI-gap procedure to test the role of
hippocampal NMDA receptors in timing behavior.

Method

Animals

Fifteen male, adult Fisher 344 rats similar to those used in Experiment 1 were subjects for
the study. Housing, food restriction, and training were the same as in Experiment 1. Rats were
divided into two groups: saline (n = 8) and 0.2 mg/kg MK-801 (n = 7). All animals were
experimentally naïve at the start of testing. Testing sessions were conducted once each day, five
days per week (Monday – Friday). All procedures followed NIH guidelines for handling and
caring of animals and were approved by the Bowling Green State University Institutional Animal
Care & Use Committee.

Apparatus

This experiment used the same operant boxes as used in Experiment 1.

Drug

Thirty minutes prior to the beginning of testing, rats were injected i.p. with saline or 0.20
mg/kg MK-801.

Procedure

The training procedures for Experiment 2 were identical to Experiment 1. After reaching
the training criterion (peak-time within 2 s of the target 12 s duration), rats were tested for 5
sessions to establish a baseline measure of performance. For the next 15 sessions, animals were
tested with either saline or 0.2 mg/kg MK-801. Following the 15 sessions, all rats were tested for
an additional 5 sessions with saline.
Data Analysis

For the purpose of analysis, data were divided into 5 blocks of sessions: Sessions 1-5 (Baseline), Sessions 6-10 (Drug 1), Sessions 11-15 (Drug 2), Sessions 16-20 (Drug 3), and Sessions 21-25 (Post-Drug). Data collection and the fitting procedure for estimating peak-time, peak-rate and variability were identical to Experiment 1.

Separate mixed model ANOVAs were conducted to examine effects of drug group (saline & 0.2 mg/kg MK-801) and test block (Baseline, Drug 1, Drug 2, Drug 3 & Post-Drug) on peak-time, normalized peak-rate, and variability. Greenhouse-Geisser corrections were used in any case where the assumption of sphericity was violated; for consistency, the degrees of freedom reported in these instances are uncorrected. Post-hoc tests for the between subjects factors were conducted, when necessary, using Tukey’s HSD with \( \alpha \) set at .05. As in Experiment 1, data for peak-rate were normalized relative to the Baseline performance prior to conducting statistical analyses, and the Baseline values were omitted from analysis.

Results

During Baseline sessions, temporal response functions for saline and 0.2 mg/kg MK-801 groups peaked near the FI target indicating that the animals learned to accurately produce responses that were centered on the target interval (Figure 3A & 3B). As in Experiment 1, peak-times remained stable across all test session blocks for the saline group, whereas the 0.2 mg/kg MK-801 group displayed a dramatic lengthening of peak-time upon initiation of drug injections (Drug 1; Figure 4A). With continued testing on MK-801 (Drug 2 & Drug 3), peak-times migrated toward the target time. Even with additional testing on MK-801, peak-times remained longer than those observed during baseline (see Drug 3 in Figure 4A). Peak-times for the final block of saline (Post-Drug) were slightly shorter than those observed during Baseline sessions,
Mean Difference = 0.38 s, \( t(17) = 2.20, p < 0.05 \). Greenhouse-Geisser corrections were used on the ANOVA on peak-time, which confirmed a significant main effect of session block, \( F(4, 52) = 13.61, p < 0.001 \), a main effect of drug group, \( F(1, 13) = 11.76, p < 0.01 \), and a significant session block by drug group interaction, \( F(4, 52) = 13.13, p < 0.001 \).

Similar to Experiment 1, MK-801 produced an overall increase in responding (compare Figure 4B with Figure 2B). For the saline group, peak-rate was stable across all test session blocks. In contrast, the MK-801 group displayed an immediate increase in response rate upon receiving the drug (Drug 1). Response rate continued to increase over the next two testing blocks

**Figure 3:** Temporal response profiles for each drug condition across testing blocks. Inset figures are response profiles normalized by both peak-rate and peak-time. Panel A: Saline; Panel B: 0.2 mg/kg MK-801.
Figure 4: Peak-time (Panel A), peak-rate (Panel B), and variability (Panel C) plotted by drug group (Saline and 0.2 mg/kg MK-801) and by test block (Baseline, Drug 1, Drug 2, Drug 3, and Post-Drug). Error bars express standard error of the mean.
Upon switching from MK-801 to saline injections, response rate returned back to levels similar to Baseline sessions (Post-Drug). Overall, the ANOVA on normalized peak-rate indicated a significant main effect of session block, $F(3, 39) = 20.52, p < 0.001$, a significant main effect of drug group, $F(1, 13) = 9.39, p < 0.01$, and a significant session block by drug group interaction, $F(3, 39) = 18.60, p < 0.001$.

Variability was increased by MK-801 (Figure 4C). Variability remained stable across test session blocks for the saline group. However, the MK-801 group displayed a dramatic and immediate increase in variability following drug injections (Drug 1). During Drug 2 and Drug 3 sessions, variability decreased although it remained larger than Baseline sessions. Variability for this group returned to pre-drug levels upon termination of the injections (Post-Drug). Consistent with these observations, the ANOVA on variability (with Greenhouse-Geisser corrections applied) revealed a significant main effect of session block, $F(4, 52) = 43.85, p < 0.001$, a main effect of drug group, $F(1, 13) = 42.14, p < 0.001$, and a significant session block by drug group interaction, $F(4, 52) = 33.59, p < 0.001$.

In order to determine whether the increase in variability observed in the MK-801 group was scalar, the data were plotted on a normalized time scale. For the saline group, the normalized response functions (Figure 3A) superimposed, supporting scalar timing. However, as in Experiment 1, the MK-801 group (Figure 3B) revealed a different scenario; the normalized temporal functions for Baseline and Post-Drug sessions superimposed and the normalized temporal functions for Drug 1, Drug 2, and Drug 3 sessions superimposed, but the three drug blocks did not superimpose with the two non-drug blocks, suggesting that, as in Experiment 1, the effect of MK-801 at the highest dose (0.2 mg/kg MK-801) was non-scalar.
Discussion

Overall, Experiment 2 replicated the three primary findings of Experiment 1. First, the 0.2 mg/kg dose of MK-801 produced an over-estimation of the criterion time that was reduced with continued training, as measured by peak-time. Second, MK-801 increased response rate, as measured by peak-rate. Third, MK-801 produced a non-scalar increase in variability. Additionally, Experiment 2 addressed two questions that tested the possibility that MK-801 mediates timing function in a manner similar to dopamine antagonists.

The first question addressed by Experiment 2 was whether peak-times would completely return to the criterion time with further training. However, even with the additional week of MK-801, we found that produced peak-times did not return to the criterion time. In terms of scalar expectancy theory (SET), rapid shifts in peak-times have typically been attributed to a change in the speed of an internal pacemaker or clock, with the renormalization of peak-times attributed to an updating of memory based on the new clock speed (Meck, 1983, 1996). One possible reason for the failure of peak-times to completely renormalize with additional training is that MK-801 may have also interfered with the updating of memory processes after a change in clock speed.

The second question addressed by Experiment 2 was whether the lack of a rebound of peak-time following the cessation of MK-801 might be due to animals learning different criterion times for two states, non-injected and injected. To test this possibility, saline injections were administered during the Post-Drug test block. Although no clear rebound was found, peak-times did reliably overshoot the criterion time following cessation of the drug, but only by a small degree. This result suggests that the absence of a rebound in Experiment 1 was not due solely to change in behavioral states caused by the experience of injections. We cannot discount the possibility, however, that state-dependent learning contingent to the subjective experiences of
MK-801 may be important. Matell et al. (2004) also failed to find an expected rebound in peak-times follow long-term daily cocaine injections. They hypothesized that the clock speed itself may have been readjusted instead of the temporal memories; a process that might change depending on subtle differences in administration schedules of drugs. It is possible that a similar phenomenon occurred in the present study. Taken as a whole, the results from Experiment 2 suggest that effects of MK-801 on PI performance are at least partially consistent with a change in clock speed.

General Discussion

Effects of the NMDA antagonist MK-801 on short-interval timing in Fisher 344 rats were examined in two experiments. There were four main findings. First, for the PI procedure, the highest tested dose of MK-801 (0.2 mg/kg) produced a rightward shift in the distribution of peak-times (over-estimation of time) that attenuated with continued testing, but did not completely return to the criterion time (Experiment 1 & 2). Second, MK-801 increased peak-rate of responding (Experiment 1 & 2). Third, the highest tested dose of MK-801 (0.2 mg/kg) produced a non-scalar increase in variability ( Experiment 1 & 2). Finally, a reset pattern was observed during the PI-gap procedure for all animals (Experiment 1).

The rightward shift in the distribution of peak-times (over-estimation of time) produced by MK-801 stands in contrast to the effects of MK-801 and other NMDA receptor antagonists on DRL performance. Both chronic and acute administration of NMDA antagonists result in leftward shifts of inter-response-times, or IRTs (Sanger, 1992; Stephens & Cole, 1996; Tonkiss et al., 1988; Welzl et al., 1991). The leftward shift in IRTs has been interpreted as an underestimation of time, possibly due to speeding up the clock used for short interval timing or shortening of the remembered time of reinforcement (Tonkiss et al., 1988; Welzl et al., 1991).
Alternatively, the leftward shift in IRTs could be due to a drug-induced increase in response rate or impairment in the ability to withhold lever responses for the length of the target duration. The present study used the PI procedure to try to distinguish between these two interpretations.

Our results demonstrate that MK-801 produces at least two effects, an impairment of short-interval timing and a disruption of the motor action system (lack of response inhibition). With regard to the effect of short-interval timing, MK-801 produced an over-estimation of time, rather than an under-estimation as suggested by the DRL studies. This pattern of responding on the peak-interval (PI) procedure under the influence of MK-801 is different from that observed after hippocampal lesions. In the PI procedure, rats with lesions of the hippocampus show a gradual and sustained under-estimation of time (Buhusi et al., 2004; Meck, 1988; Meck, Church et al., 1984; Meck et al., 1987; Olton et al., 1988). On the other hand, rats in the present study given systemic injections of MK-801 showed an immediate and partially transient over-estimation of time. Thus, neither direction nor pattern of change caused by systemic NMDA antagonist and hippocampal lesions was similar in the peak interval procedure. These results suggest that the predominate effects of MK-801 on short-interval timing is unlikely to be occurring in the hippocampus, even though the hippocampus itself can influence timing as demonstrated by the lesion studies.

With regard to the effects of MK-801 in disrupting motor inhibition, we found that MK-801 increased response rate. Further analysis of our data showed that animals under the influence of MK-801 had lever responses that started earlier, as well as a greater number, than those treated with saline. The earlier onset of responding is consistent with the pattern of results observed in DRL studies, and would lead to a leftward shift in IRTs. Based on the results of the present study, our interpretation is that the leftward shift in IRTs in DRL studies is not due to an
under-estimation of time, but rather to the enhanced responding. This effect on responding may involve the hippocampus as direct administration of MK-801 into the hippocampus has the same effect on DRL performance as systemic administration (Sanger, 1992; Sanger & Jackson, 1989; Tonkiss et al., 1988; Welzl et al., 1991). Previous studies have suggested a link between hippocampus and response inhibition (Jarrard, 1973; Tracy, Jarrard, & Davidson, 2001). In the present study, effects of MK-801 in the hippocampus may manifest as an increase in the rate of responding.

In regards to the MK-801 induced impairments on short interval timing, it is important to comment here that the shift in peak-time and increase in variability was observed only at the highest dose of MK-801 (0.2 mg/kg). It is possible that the initial very large right-ward shift in peak-times may have been partly due to the dramatic increase in response rate and variability produced by the highest drug dose in the first drug block. Note, however, that in the second drug block response rate and variability decreased, but peak-times remained significantly longer than the criterion time of 12 s, and the effects seen on this second drug block may be more representative of the initial magnitude of over-estimation than the first drug block. The fact that alterations in peak-time were only observed at 0.2 mg/kg suggests a cautious interpretation is necessary. This dose is in the range in which MK-801 has been reported to cause disruption of performance on select timing tasks (Berz, Battig, & Welzl, 1992; Sanger & Jackson, 1989), although other studies using DRL have been successful in testing animals at 0.2 mg/kg (Sanger, 1992; Welzl et al., 1991). In DRL studies, it is typical to see a high dose of MK-801 causing a rapid drop in responding and an associated reduction in reinforcement (Sanger & Jackson, 1989; Welzl et al., 1991). For the present study, we did not observe uncoordinated movements, disorientation or a drop in lever responding at 0.2 mg/kg of MK-801. In fact, our animals
responded more than controls and the response profiles showed peaks suggesting that the stimulus still had some control over the behavior of the animals at this dose. Therefore, although it would be more convincing if the effects on peak-time and variability were observed at more than one dose, preferably a lower dose, our animals did not display any signs of non-specific effects typically associated with high doses of MK-801. One reason that we may not have observed the non-specific effects of MK-801 at doses that others have is that the function of glutamatergic receptors can be dependent on strain (Manahan-Vaughan & Braunewell, 2005).

The observed effects of MK-801 are partly consistent with those observed with drugs that interfere with the dopamine system (Hinton & Meck, 1997; Maricq & Church, 1983; Maricq et al., 1981; Meck, 1983, 1986). Dopamine antagonists produce similar immediate over-estimates of time (rightward shifts in peak-time) that renormalize with continued training (Maricq et al., 1981; Meck, 1996). Upon removal of such antagonists, an immediate under-estimate of time (leftward shift in peak-time) occurs, which also renormalizes with additional training. Dopamine agonists have been shown to produce the opposite pattern (Maricq & Church, 1983; Meck, 1986, 1996). Based on these results, the dopamine system has been hypothesized to have an effect on the speed of the internal pacemaker, or clock (Meck, 1983, 1986, 1996; Meck & Church, 1987b).

The notion that MK-801 may be interacting with the dopamine system to produce the effects observed here is consistent with other reports of interactions between NMDA receptor antagonists and the dopamine systems on behavior (Jeziorski, White, & Wolf, 1994; Marek, Benelihah, Gold, & Liebeskind, 1991; Trujillo & Akil, 1994) and memory processes (Castellano, Cestari, Ciamei, & Pavone, 1999; Castellano, Pavone, & Allegra, 1984; Cestari & Castellano, 1997; Quevedo, Moretto, Colvero, Roesler, & Ferreira, 1997). In fact, Meck (1996) hypothesized a role for interactions between glutamate and dopamine in temporal processing;
however, he predicted that peak-time under the influence of NMDA antagonists would shift in the opposite direction of what was observed in the present study.

Our results differ from the effect of dopamine antagonists in a few respects. First, peak-times shifts under MK-801 did not fully renormalize to the criterion time even with the addition of an extra test block in Experiment 2. Second, there was no meaningful rebound (abrupt opposite shift in peak-times during Post-Drug blocks) following removal of MK-801 injections, as is typically the case with dopamine antagonists (Meck, 1983, 1986, 1996; Meck & Church, 1987b). However, if, in the present work, there was interference from the initial dramatic increase in response rate during the first drug block, then (1) the first drug block may not have accurately measured the magnitude of over-estimation and (2) the magnitude of the expected rebound effect should be smaller than what we anticipated. Moreover, there is some precedent that drug manipulations known to affect the dopamine system do not always produce rebounds in peak-times (Matell, King, & Meck, 2004).

With regard to interactions between glutamatergic and dopaminergic systems in timing, future studies should examine the effects of NMDA antagonists on multiple durations. It would be important to know whether shifts in peak-time caused by NMDA antagonists are scaled to the duration being timed, as with manipulations of the dopaminergic systems (Meck, 1996). A scalar shift in peak-time would be consistent with an interaction of NMDA antagonist with the clock component of scalar expectancy theory. Non-scalar changes to peak-time might indicate a drug effect on latency to start/stop timing or alterations in the memory for the criterion time, but unlike that proposed for hippocampal or frontal cortex lesions (Meck et al., 1987; Olton, 1989).

In summary, we conclude that MK-801 has at least two effects. First, MK-801 interferes with short interval timing by producing an over-estimation of time and a non-scalar increase in
variability. Overall, the lengthening observed in the present study is most consistent with a slowing in the speed of the “clock”, suggesting that the primary effects of MK-801, on timing in tasks like the PI procedure, lies in non-hippocampal brain systems, or at least include other brain systems in addition to hippocampus (Church, 2003; Meck, 1996; Meck et al., 1987). Second, MK-801 increases response rate, suggesting a decrease in response inhibition. This latter result is consistent with the pattern of results observed in DRL studies, and this effect on responding may involve the hippocampus (Jarrard, 1973; Tracy et al., 2001). These results extend our previous knowledge of the role of the NMDA receptor on learning, memory and synaptic plasticity to that of short-interval timing (Morris, 2003; Morris et al., 1986; Shapiro & Caramanos, 1990; Shapiro & O’Connor, 1992). Evidence from this study suggests that NMDA receptors may interact with the dopaminergic system to influence short interval timing.
CHAPTER 3. EFFECTS OF MK-801 ON LEARNING NEW TEMPORAL MEMORIES FOR SHORT-INTERVAL TIMING IN RATS

The previous chapter examined the effects of MK-801 on a previously learned duration. There were three main findings regarding MK-801: over-estimation of time at the highest dose (0.2 mg/kg) that attenuated with continued testing, increased peak-rate of responding at all doses, and a non-scalar increase in variability at the highest tested dose. The chapter concluded that the N-methyl-D-aspartate (NMDA) receptor plays a role in the timing of a previously learned duration and suggested that blockade of the NMDA receptors results in patterns of timing behavior that is somewhat similar to those observed under dopamine antagonists. An important point is that the majority of effects observed in Chapter 2 were from the highest dose, which is a relatively large dose of MK-801 (Sanger, 1992; Shapiro & Caramanos, 1990; Shapiro & O'Connor, 1992; Welzl et al., 1991; Whishaw & Auer, 1989; Willetts, Balster, & Leander, 1990).

The present chapter considers effects of MK-801 on temporal learning. The NMDA receptor has received much interest, for its role in learning, memory and synaptic plasticity (Morris, 2003; Morris et al., 1986; Shapiro & Caramanos, 1990; Shapiro & O'Connor, 1992). Antagonists of the NMDA receptors have been observed to have effects in both spatial (Morris, 2003; Morris et al., 1986; Shapiro & Caramanos, 1990; Shapiro & O'Connor, 1992) and nonspatial (Traverso, Ruiz, & De la Casa, 2003; Xu & Davis, 1992) learning and memory tasks. Although there is evidence that NMDA receptors are involved in nonspatial types of learning, at present there is no study that examines its effects on the learning of temporal information.

Several models of timing and temporal processing predict that NMDA receptor antagonists should also disrupt temporal learning. The Striatal Beat-Frequency model of timing
(SBF; see Appendix B) hypothesizes that the memory for any temporal duration is stored in the form of synaptic connections between cortical and striatal cells (Matell & Meck, 2000) suggesting that the NMDA receptor, with its role in spatial learning and memory and synaptic plasticity (Morris, 2003; Morris et al., 1986; Shapiro & Caramanos, 1990; Shapiro & O'Connor, 1992), may be important for the learning of temporal durations. The SBF model hypothesizes a cortico-striatal–thalamic-cortical loop underlying the processes of timing behavior. Synaptic plasticity is theorized to be important during training trials where the delivery of a reward causes a burst of dopamine in the striatum, originating from the substantia nigra pars compacta, that strengthens the connections (via synaptic plasticity) between the spiny neurons of the striatum and the cortical cells that are firing together at the passage of the criterion duration (Matell & Meck, 2000). Therefore, the present experiment was designed to separate the effects of MK-801 on temporal learning from its effects on steady-state behavior (i.e., a previously learned duration). This chapter continues to examine the role NMDA receptors on timing and temporal processing by conducting an examination the effects of MK-801 on the learning of a new temporal criterion.

In order to examine the effects of MK-801 on the formation of new temporal memories, rats were trained with the peak-interval (PI) procedure on one temporal criterion; then when that criterion was learned, the temporal criterion was switched to a new duration (Meck, 1988; Meck, Komeily-Zadeh et al., 1984). Normal rats tested on this type of procedure have been reported to quickly produce the new temporal criterion (Meck, 1988; Meck, Komeily-Zadeh et al., 1984). If the memory for a temporal duration is stored via NMDA receptor mediated plasticity in the cortico-striatal synaptic connections as hypothesized by the SBF model (Matell & Meck, 2000),
then blocking the NMDA receptor with MK-801 should result in impairments in learn the new temporal duration.

If MK-801 does not impair the learning of a new temporal duration, this might suggest that temporal learning and other types of learning, such as spatial learning, have different sensitivity to NMDA antagonists. Alternatively, the concentration of MK-801 administrated may not have been sufficient to disrupt memory. In order to be able to distinguish between these two possibilities, a subset of animals were also tested on two separate spatial memory tasks that are affected by MK-801: spontaneous alternation and Morris water maze tasks.

The spontaneous alternation task (Dember & Fowler, 1958, 1959) is a measure of exploratory behavior that is dependent on spatial working memory (Isseroff, 1979; Lalonde, 1986, 2002). Maximal efficient exploration uses working memory to record which maze arms were entered most recently so that the number of reentries are minimized. Prior reports on NMDA antagonists and spontaneous alternation indicate that administration of NMDA antagonists reduce alternations of arm entries, suggesting that NMDA receptor antagonists impair spatial working memory (Holter et al., 1996; Lennartz & Gold, 1995). In addition to spontaneous alternation, NMDA receptor antagonists have also been reported to affect spatial working memory on a variety of other behavioral tasks (Caramanos & Shapiro, 1994; Puma, Baudoin, & Bizot, 1998; Shapiro & O'Conner, 1992; White & Best, 1998; Yoshihara & Ichitani, 2004).

A method used to assess spatial learning and reference memory is the Morris water maze (Morris, 1984). The goal of the water maze is locate a hidden platform. Prior reports on NMDA antagonists and the water maze indicate that administration of NMDA antagonist increase the latency to find the hidden platform; suggesting that NMDA receptor antagonists impair spatial
learning and memory (Caramanos & Shapiro, 1994; Heale & Harley, 1990; McLamb, Williams, Nanry, Wilson, & Tilson, 1990; Riedel, Platt, & Micheau, 2003; Whishaw & Auer, 1989).

The present experiment determined the effects of NMDA receptor antagonists on the learning of a new temporal criterion using a variant of the PI procedure that switches the temporal criterion (Meck, 1988; Meck, Komeily-Zadeh et al., 1984). Spontaneous alternation and Morris water maze tasks further examined the influence of MK-801 on working memory to ensure that the dose level of MK-801 used, 0.05 mg/kg, in the PI task was sufficient to result in memory disruptions in a task where NMDA receptor antagonist are known to cause such disruptions (Caramanos & Shapiro, 1994; Heale & Harley, 1990; Holter et al., 1996; Lennartz & Gold, 1995; McLamb et al., 1990).

Method

Animals

Thirty-two male, adult Fisher 344 rats (Harlan, Indianapolis, IN) were subjects for the study. Rats were between two and four months of age at the start of the study, and weighed between 250-300 grams. Rats were housed individually or in pairs in an animal colony room maintained near 22°C and on a 12-hour light-dark cycle with lights on at 7 a.m. local time. Rats were divided into four groups for testing. First, animals were divided into two groups for training on either a 9 s or 18 s criterion. Each of these two groups were then assigned for injection of either saline, or 0.05 mg/kg MK-801, resulting in four groups: 9 s saline (n = 8), 9 s MK-801 (n = 7), 18 s saline (n = 8), and 18 s MK-801 (n = 9). All animals were experimentally naïve at the start of testing. Prior to training, animals were food restricted until their body weight reached 85% of their ad lib weight (All animals were given ad libitum food and water access on Saturdays). Rats were given supplemental food so that each rat gained approximately five grams
per week in body weight. Testing sessions were conducted five days per week (Monday – Friday). All procedures used in this study followed NIH guidelines for handling and caring of animals and were approved by the Bowling Green State University Institutional Animal Care & Use Committee.

**Drug**

MK-801 ((+)-5-methyl-16, 11-dihydro-5H-dibenzo[a,d] cyclohepten 5, 10-imine; M-107, Sigma, St. Louis, MO) was dissolved in physiological saline. The rats were injected (i.p.) with saline or 0.05 mg/kg MK-801 30 minutes prior to PI, spontaneous alternation, and water maze testing sessions. All injections were administered intraperitoneally. The dose level of MK-801 selected, 0.05 mg/kg was selected because it has been shown not affect temporal production, as measured by peak-time and variability (see Chapter 2), it is a relatively small dose of the drug (Sanger, 1992; Shapiro & Caramanos, 1990; Shapiro & O'Connor, 1992; Welzl et al., 1991; Whishaw & Auer, 1989; Willetts et al., 1990), and similar dose levels have been previously shown to effect spatial memory (Caramanos & Shapiro, 1994; Heale & Harley, 1990; McLamb et al., 1990).

**Peak-Interval Procedure**

**Apparatus**

Testing was conducted in sixteen similar custom-made operant boxes (28 x 28 x 37 cm) constructed of clear acrylic. Chapter 2 provides a detailed description of these boxes. Briefly, a hinged door provided access on one side of the box; a water bottle was located next to the door. For reinforcement, sucrose pellets (45mg, PJFSC-0045; Research Diets, Inc., New Brunswick, NJ) were delivered by a pellet dispenser into a food cup.
Procedure

Animals were pre-trained with the same continuous reinforcement, variable reinforcement (VR-3), and fixed interval (FI) procedures as described in Chapter 2. One difference in this study was that rats were trained on either a FI-9 or FI-18 schedule instead of a FI-12. After approximately ten sessions of FI training, rats began the PI procedure, where half of the trials were probe trials. Rats were trained on the PI procedure until the peaks of the temporal response function for individual sessions were within 10% of the target durations: either 9 s or 18 s. Animals typically reached this criterion after approximately 30 PI sessions.

Following completion of the training sessions test sessions began and rats were tested for 5 additional sessions to establish baseline performance. Table 1 presents a breakdown of the testing blocks, drug administration schedule, and temporal criterion shifts. After the 5 baseline sessions, drug administration began and continued for the remaining 20 test sessions. Rats received injections of either saline or MK-801. For the first 10 of these sessions, animals remained on the original training program, PI-9 or PI-18. Then, the target criterion was switched (animals trained on PI-9 were changed to PI-18, and animals trained on PI-18 were changed to PI-9) and animals were tested for 10 more sessions. This procedure resulted in a total of 25 test sessions per animal. In sum, the 25 testing sessions divided into 5 session blocks were:

Table 1: Design layout

<table>
<thead>
<tr>
<th>Control Group</th>
<th>Drug Group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Testing Block</strong></td>
<td><strong>Injection Administration</strong></td>
</tr>
<tr>
<td><strong>Testing Block</strong></td>
<td><strong>Injection Administration</strong></td>
</tr>
<tr>
<td>Baseline</td>
<td>None</td>
</tr>
<tr>
<td>Drug 1</td>
<td>Saline</td>
</tr>
<tr>
<td>Drug 2</td>
<td>Saline</td>
</tr>
<tr>
<td>Drug 3</td>
<td>Saline</td>
</tr>
<tr>
<td>Drug 4</td>
<td>Saline</td>
</tr>
<tr>
<td>Baseline</td>
<td>None</td>
</tr>
<tr>
<td>Drug 1</td>
<td>MK-801</td>
</tr>
<tr>
<td>Drug 2</td>
<td>MK-801</td>
</tr>
<tr>
<td>Drug 3</td>
<td>MK-801</td>
</tr>
<tr>
<td>Drug 4</td>
<td>MK-801</td>
</tr>
</tbody>
</table>
Baseline (test sessions 1-5), Drug 1 (test sessions 6-10), Drug 2 (test sessions 11-15), Drug 3 (test sessions 16-20), and Drug 4 (test sessions 21-25).

Data Analysis

Analysis by session: Data was collected from the 25 testing sessions. Only data from probe trials were analyzed. Lever responses, temporal response functions, and fitting of a Gaussian + linear equation that gave the best fit to the temporal response functions was conducted by methods identical to those used in Chapter 2. Model fitting determined estimates for the parameters \( a, b, c, d, \) and \( t_0 \); peak-time was estimated by \( t_0 \), peak-rate was estimated by \( a + d \), and coefficient of variation (CV) was estimated by \( b / t_0 \). CV was used to assess timing variability instead of variance (as in Chapter 2) since the differing criterion times used would be expected to have a proportional amount of variance (see Appendix B).

Parameter estimates were obtained for each animal in each session. Then the data for each animal was averaged into 5 unique session blocks, each consisting of 5 sessions. The 5 session blocks were: Baseline, Drug 1, Drug 2, Drug 3, and Drug 4. Separate mixed model ANOVAs were conducted in SPSS (Version 12.0, SPSS, Inc., Chicago, IL) to examine effects of drug group (saline & 0.05 mg/kg MK-801), condition (9 s – 18 s switch & 18 s – 9 s switch), and test block (Baseline, Drug 1, Drug 2, Drug 3, & Drug 4) on peak-time, peak-rate, and CV. Greenhouse-Geisser corrections were used in all cases where the assumption of sphericity was violated; for consistency, degrees of freedom reported in these instances are uncorrected.

Analysis by trial: In addition to the block analysis, single trial analyses were also performed. Although FI trials were mixed with probe trials, single trial analyses examined only the probe trials before and after the temporal criterion shift. Specifically, analyses were conducted using the 200 probe trials (approximately 6 sessions) immediately prior to and 200
Probe trials immediately after the temporal criterion switch. Peak-time were determined for each individual trial (Church, Meck, & Gibbon, 1994; Meck, 1988; Meck, Komeily-Zadeh et al., 1984). The method used to determine the individual trial peak-times has been described elsewhere (Church et al., 1994). Briefly, this method divides a trial into three periods: an initial period of low responding, a middle period of high responding, and an ending period of low responding (see Appendix B). The two transition points between these periods have been labeled $s_1$ and $s_2$. For each trial, the placement of these points was determined by an exhaustive search of all locations to maximize the difference between low and high responding periods. This was accomplished by selecting positions of $s_1$ and $s_2$ values that maximized the following equation:

$$t_1(r - r_1) + t_2(r_2 - r) + t_3(r - r_3),$$

where $t_1$ was the time from the start of the trial to $s_1$, $t_2$ was the time between $s_1$ and $s_2$, $t_3$ was the time from $s_2$ to the end of the trial, $r$ was the overall mean response rate, and $r_1$, $r_2$, and $r_3$ were the mean response rates during $t_1$, $t_2$, and $t_3$, respectively (Church et al., 1994). The location of these points was determined for each trial using a custom Matlab program (V6.5, Release 13.0.1, The Mathworks, Inc., Natick, MA). After the location of the transition points $s_1$ and $s_2$ were identified, peak-time and the width of responding could be determined. Peak-time was defined as the midpoint between $s_1$ and $s_2$, or $(s_1 + s_2)/2$. Width of responding was defined as the spread of time between $s_1$ and $s_2$, or simply $s_2 - s_1$, and normalized width was defined as width/peak-time. Normalized width was used as a measure for comparison to CV in the session analysis. A trial was excluded from analysis if an animal did not make a lever response during the course of the trial, or if the produced peak-time was greater than 48 seconds.

The peak-times for the 200 probe trials preceding the change in criterion time and the 200 probe trials following the change in criterion time were identified and arranged chronologically.
so the 201 trial was the first probe trial following the change in criterion time. This collection of
peak-times was then smoothed using a nonlinear digital filter with a width of five intervals (5R
filter: Meck, 1988; Meck, Komeily-Zadeh et al., 1984). The 5R filter is a repeating smoothing
method that uses a running median of five and repeats until smoothing results in no change in the
data (Tryon, 1983; Velleman & Hoaglin, 1981). In order to examine the gradual transition in
peak-time from one temporal criterion to the next, a sigmoid equation that gave the best fit to the
distribution of smoothed peak-times was determined by minimizing root mean-square error using
the Solver add-in package for Microsoft Excel 2002 (Version 10.65, Microsoft Corporation,
Seattle, WA). Separate forms of the equation were used for the 9 s – 18 s switch and for the 18 s
– 9 s switch because of the differences in the shape of each distribution. The following was the
generalized sigmoidal equation that was fit to the smoothed peak-time distribution for the 9 s –
18 s switch condition:
\[
R(t) = t_{\text{min}} + (t_{\text{max}} - t_{\text{min}}) \left( \frac{1}{1 + e^{-\gamma(t - \theta)}} \right),
\]  
(3)
The generalized sigmoidal equation for the 18 s – 9 s switch condition was defined as:
\[
R(t) = t_{\text{max}} - (t_{\text{max}} - t_{\text{min}}) \left( \frac{1}{1 + e^{-\gamma(t - \theta)}} \right),
\]  
(4)
where \( t \) is the current trial and \( R(t) \) is the peak-time at trial \( t \). Model fitting determined estimates
for the parameters \( t_{\text{max}}, t_{\text{min}}, \gamma, \) and \( \theta \). The maximum asymptotic value was estimated by \( t_{\text{max}} \), the
minimum asymptotic value was estimated by \( t_{\text{min}} \), the gain (abruptness of change) was estimated
by \( \gamma \), and the bias was estimated by \( \theta \). These two parameters can be used to determine how fast
the new interval is learned. The speed of transition between the two criteria was determined by
the gain parameter (\( \gamma \)). Small gain values indicated a slow transition while large gain values
indicated rapid change. The bias parameter (\( \theta \)) measures indicated the 50% point between the
two criterions. Two 2 x 2 between subjects ANOVAs were conducted in SPSS to examine
effects of drug group (saline & 0.05 mg/kg MK-801) and condition (9 s – 18 s switch & 18 s – 9 s switch) on the gain ($\gamma$) and bias ($\theta$) parameters obtained from the curve fitting procedures.

Finally, single-trial analyses were conducted using all probe trials from each of the testing sessions. Single-trial analyses were structured similar to the session analyses for comparison between the two analysis methods, single-trial parameter estimates included: peak-time (corresponding to peak-time), average response rate ($r_2$: corresponding to peak-rate), normalized width of responding (corresponding to variability), start-time ($s_1$) and stop-time ($s_2$). For each animal, single-trial parameters were averaged by session and then each session was averaged into the same 5 unique session blocks used above: Baseline (sessions 1-5), Drug 1 (sessions 6-10), Drug 2 (sessions 11-15), Drug 3 (sessions 16-20), and Drug 4 (sessions 21-25). Mixed model ANOVAs were conducted in SPSS to examine effects of drug group (saline & 0.05 mg/kg MK-801) and test block (Baseline, Drug 1, Drug 2, Drug 3, & Drug 4) on peak-time, average response rate, normalized width, start-time, and stop-time. Greenhouse-Geisser corrections were used in all cases where the assumption of sphericity was violated; for consistency, the degrees of freedom reported in these instances are uncorrected.

**Spontaneous Alternation**

**Apparatus**

Spontaneous alternation testing was conducted on an elevated eight arm radial maze (Model 8900B; Lafayette Instrument, Lafayette, IN). The maze was configured into a plus maze by closing the access door to four of the arms. The maze was placed in the center of a dedicated testing room. Three different posters were hung on the walls, in order to provide distal visual cues to aid spatial orientation. A camera was suspended from the ceiling directly above the maze to allow video recording of the sessions. A small radio was located in one of the room corners.
and was played during the sessions in order to mask noises originating from outside the testing room that may startle the animals.

Procedure

Following the completion of all testing on the PI procedures, a subset of animals (n = 9 saline and n = 9 MK-801) were tested on a spontaneous alternation task (Dember & Fowler, 1958, 1959) in order to determine whether the tested dose MK-801 affects spatial working memory. Rats were individually placed in the center the maze, remained in the maze for 20 minutes, and were able to move freely between any of the four unbaited maze arms. The spontaneous alternation session for each animal was video recorded for later data analysis.

Data Analysis

Video recordings of the spontaneous alternation sessions were scored by recording the order of all arm entries. An entry was scored only if all four paws of the rat entered fully into an individual arm. The arm location and sequence of arm entries was determined for each animal. Alternation scores were calculated in the following way: A sliding window of five consecutive arm entries (i.e. 1-5, 2-6, 3-7, etc.) was examined. If the animal entered four different arms during a particular window, then the animal was considered to have alternated, otherwise, it did not alternate. After scoring the session, a normalized alternation score was computed by taking the total number of alternations and dividing by the total number of possible alternations (total number of entries minus four). In addition to the normalized alternation score, total number of arm entries was also determined as a measure activity. Separate independent measures t-test were conducted on the alternation scores and total number of entries to compare the saline and 0.05 mg/kg MK-801 groups.
Morris Water Maze

Apparatus

The water maze was a plastic circular container (1.5m diameter) filled with water to a height of approximately 45 cm. The water was made opaque with nontoxic acrylic white paint. A clear Lucite platform (10 cm x 10 cm) was placed 18 cm from the wall of the west quadrant (target quadrant). The level of the water was adjusted so that the platform was hidden 1.5 cm below the surface of the water. A variety of extramaze cues were located around the room, including posters, a sink, and doors. A camera was suspended from the ceiling directly above the water maze to allow video recording of the sessions.

Procedure

Following completion of the spontaneous alternation task, a subset of animals (n = 7 saline and n = 7 MK-801) were tested on the water maze task. Behavioral testing occurred during the light phase of the light-dark cycle, one session per day, and five days per week (Monday – Friday). Rats were tested in a water maze for ten sessions. There were a total of ten sessions on the water maze: eight training sessions for learning the location of the hidden platform and two probes sessions for the testing memory of the platform location. Animals began testing with four consecutive training sessions; the fifth session was a probe session. The animals then received four additional training sessions, with a second probe session on the tenth day. Each of the training sessions consisted of three trials. On each trial, the rat was placed in one of the non-target quadrants with its head facing the outer wall of the maze. Each of the non-target quadrants was used as the start location once throughout a session. Each trial ended when the rat found the platform or was led to the platform by the experimenter after 60 seconds. After reaching the
platform, the rat was left there for 15 seconds and then moved to a plastic holding container for 30 seconds. The time to reach the platform was recorded as the escape latency.

On probe session days five and ten, rats were tested for their ability to remember the hidden platform location using a single probe trial on each day. The platform was removed from the maze, and rats were started in the quadrant opposite the target quadrant. The probe trial had a duration of 60 seconds and was recorded on videotape. Total time spent in the target quadrant and the number of times the rat crossed the platform location were determined from offline analysis of the videotape.

Data Analysis

The mean escape latency time was averaged across trials for each training day for each subject and analyzed using a mixed design ANOVA with days as a within-subject factor, and drug treatment as a between-subject factor (note: only data from training sessions were used in this analysis; data from probe sessions are analyzed separately). Performance on the probe trials was analyzed using a mixed design ANOVA with the two probe sessions as a within-subject factor and drug treatment as a between-subjects factor. Each behavioral measure (time in target quadrant and the number of platform crossings) was analyzed separately.

Results

Peak-Interval Procedure

Analysis by Session

Peak-time: During Baseline sessions, animals produced peak-times near the target duration (19.03 ± 0.44 & 9.17 ± 0.14). After the criterion time changes, peak-times for all animals in each switching condition migrated towards the new criterion time during session block Drug 3 (the first test block following the criterion switch), and had reached the new
criterion by Drug 4 (Figure 5A). Greenhouse-Geisser corrections were required for the peak-time ANOVA, which resulted in a main effect of condition, \( F(1, 28) = 97.88, p < 0.001 \), but no main effects of session block, \( F(4, 112) = 1.32, p > 0.05 \), or drug group, \( F(1, 28) = 0.38, p > 0.05 \). The interaction term for session block and condition, \( F(4, 112) = 602.92, p < 0.001 \), revealed a change in produced peak-times coinciding with the criterions shifts. The peak-times for all animals in the 9 s - 18 s switch condition started near 9 s and gradually moved near 18 s reflecting the new temporal criterion, while the peak-times for all animals in the 18 s - 9 s switch condition change started near 18 s and gradually moved near 9 s resulting in the observed interaction between session block and switch condition (Figure 5A). However, there was no interaction between either session block and drug group, \( F(4, 122) = 1.45, p > 0.05 \), or condition and drug group, \( F(1, 28) = 0.02, p > 0.05 \). Finally, the analysis indicated a three-way interaction of session block, drug group, and condition, \( F(4, 112) = 3.17, p < 0.05 \). The three-way interaction was the only effect of drug group that we observed in this analysis. Upon examining the data (Figure 5A), it appeared likely that this interaction was due to either an increase in peak-time observed in the Drug 1 block for the 18 s -9 s MK-801 group, but no the others, or a slow transition of the 9 s -18 s Saline group in the Drug 3 block, but not the any of the other groups.

To further investigate these possibilities, analyses where conducted separating session blocks Drug 1 and Drug 2 from session blocks Drug 3 and Drug 4. This separation places the two possible interaction points separate analyses, which permits isolation of the reason for the interaction (If the interaction was located in the early test block, then an interaction should remain in the first analysis). If the true interaction was located in the later test blocks, then an interaction should remain in the second analysis. The result was two new ANOVAs: a session
Figure 5: Peak-time (Panel A), peak-rate (Panel B), and coefficient of variation (Panel C) plotted by drug group (Saline and 0.05 mg/kg MK-180) and by session blocks (Baseline, Drug 1, Drug 2, Drug 3, and Drug 4). Error bars express standard error of the mean.
block (either Drug 1 and Drug 2, or Drug 3 and Drug 4) x drug group x condition mixed measure ANOVA.

The results from the ANOVA using session blocks Drug 1 and Drug 2 revealed a main effect of condition, $F(1, 28) = 873.22, p < 0.001$, and no main effects of either session block, $F(1, 28) = 0.12, p > 0.05$, or drug group, $F(1, 28) = 1.39, p > 0.05$. In addition, there were no session block x condition, $F(1, 28) = 0.41, p > 0.05$, drug group x condition, $F(1, 28) = 2.45, p > 0.05$, or session block x drug group x condition, $F(1, 28) = 0.00, p > 0.05$, interactions. However, there was interaction between session block and drug group, $F(1, 28) = 4.59, p < 0.05$, suggesting an effect of drug administration on peak-time during the Drug 1 block that was not observed during the Drug 2 block.

The results from the ANOVA on peak-time using session blocks Drug 3 and Drug 4 revealed a main effect of condition, $F(1, 28) = 206.53, p < 0.001$, and no main effects either session block, $F(1, 28) = 0.08, p > 0.05$, or drug group, $F(1, 28) = 0.09, p > 0.05$. An interaction between session block and condition was observed, $F(1, 28) = 97.88, p < 0.001$. However, no interactions were observed between session block and drug group, $F(1, 28) = 0.30, p > 0.05$, drug group and condition, $F(1, 28) = 2.074, p > 0.05$, or session block, drug group, and condition, $F(1, 28) = 1.63, p > 0.05$.

The session block x drug group interaction in the ANOVA using session blocks Drug 1 and Drug 2 suggests that the three-way interaction observed in the initial ANOVA on peak-time was due to an effect of drug group in the initial session blocks, rather than the later blocks. This is supported by the lack of effect of drug group in the analysis of session blocks Drug 3 and Drug 4. Upon initial examination of the data (Figure 5A), the original three-way interaction of session block, drug group, and condition appeared to be due to one of two sources: an increase in peak-
time observed in the Drug 1 block for the 18 s -9 s MK-801 group, but no the others, or a slow transition of the 9 s -18 s Saline group in the Drug 3 block, but not the any of the other groups. The results of the follow up analyses suggest the original interaction was due to a temporary increase in peak-time observed in Drug 1 for the 18 s -9 s MK-801 group, rather than slower transition of the 9 s -18 s Saline group (Figure 5A).

**Peak-rate:** Administration of MK-801 increased peak-rate of responding across session block for animals in both 9 s – 18 s and 18s – 9 s switch conditions (Figure 5B). This observation is confirmed by the ANOVA (with Greenhouse-Geisser corrections applied), which revealed a main effect of drug group, \(F(1, 28) = 6.51, p < 0.05\), and of session block, \(F(4, 112) = 6.05, p < 0.01\). No main effect of condition was observed, \(F(1, 28) = 2.21, p > 0.05\). The analysis also indicated an interaction between session block and drug group, \(F(4, 122) = 4.831, p < 0.01\), which reflects the increase in peak-rate across all drug blocks from the Baseline block (Figure 5B). This interpretation was confirmed by a follow-up analysis omitting the Baseline block, which eliminated the interaction between session block and drug group, \(F(3, 84) = 0.68, p > 0.05\), but not the main effect of drug group, \(F(1, 28) = 9.26, p < 0.01\). In addition, there was an interaction between session block and condition, \(F(4, 112) = 4.04, p < 0.05\). During the Baseline testing block, peak-rate for the 18 s – 9 s switch condition was lower in than the 9 s – 18 s switch condition, however by the Drug 4 testing block there was no difference in peak-rate between the two switch conditions. No interactions between drug group and condition, \(F(1, 28) = 0.27, p > 0.05\), or session block, drug group, and condition, \(F(4, 112) = 0.48, p > 0.05\) were observed.

**Coefficient of Variation:** CV remained fairly stable across the Baseline, Drug 1, and Drug 2 session blocks and increased after the criterion switch (Figure 5C). The ANOVA (with Greenhouse-Geisser corrections applied) verified this observation, revealing a main effect of
session block, $F(4, 112) = 46.30, p < 0.05$. There were no main effects for switch condition, $F(1, 28) = 0.10, p > 0.05$, or drug group, $F(1, 28) = 3.94, p > 0.05$; however, the main effect for drug group did approach significance ($p = .057$), suggesting a trend of higher CV values for animals injected with MK-801. There was an interaction between session block and condition, $F(4, 112) = 10.32, p < 0.05$, indicating that the 18 s - 9s condition had slightly higher CV values prior to the criterion switch and slightly lower values after the switch (Figure 5C). There were no interactions between session block and drug group, $F(4, 112) = 2.31, p > 0.05$, drug group and condition, $F(1, 28) = 0.35, p > 0.05$, or between session block, drug group and condition, $F(4, 112) = 0.26, p > 0.05$.

**Summary:** Session-level analysis revealed that administration of MK-801 did not affect the learning of a new temporal criterion, as measured by change in peak-time. MK-801 increased responding rate across test blocks for animals in both switching conditions. In addition, there were no significant effects of MK-801 on variability (as measured by CV), although, a non-significant increase with MK-801 administration was observed.

**Analysis by Trial**

**Gain and Bias:** All animals generated peak-times on individual trials that approximated the criterion times and peak-times quickly changed following the criterion switch so that animals were producing peak-times that once again approximated the criterions times 200 trials after the criterion switch (Figure 6). The ANOVA on the gain ($\gamma$) parameter did not reveal differences between condition, $F(1, 28) = 0.58, p > 0.05$, or drug group, $F(1, 28) = 2.77, p > 0.05$. Additionally, there was no interaction between condition and drug group, $F(1, 28) = 0.00, p > 0.05$. The ANOVA on the bias ($\theta$) parameter also failed to reveal any differences between condition and drug group. There were no main effects of either condition, $F(1, 28) = 0.91, p >
0.05, or drug group, $F(1, 28) = 3.15, p > 0.05$. Additionally, there was no interaction between condition and drug group, $F(1, 28) = 0.06, p > 0.05$. Taken together, these analyses on gain and bias indicated that all animals learned the new criterion time at essentially the same rate regardless of switch condition and drug group.

**Peak-time by block:** The peak-time data from the individual trial analysis appear nearly identical to results from the session analysis of peak-time when averaged across testing sessions and arranged into session blocks (Figure 5A & Figure 7A). In addition, the pattern of results from the ANOVA on the single-trial peak-times almost mirrors those observed in the session-level analysis. Greenhouse-Geisser corrections were used on the ANOVA for peak-time, which resulted in main effects of condition, $F(1, 28) = 49.65, p < 0.001$, and session block, $F(4, 112) = 10.03, p < 0.001$, but no main effect drug group, $F(1, 28) = 0.29, p > 0.05$. The interaction term for session block and condition, $F(4, 112) = 409.22, p < 0.001$, revealed a significant interaction

![Figure 6](image)

*Figure 6:* Peak-times for the 400 individual trials surrounding the criterions shift. Testing on the new temporal criterion began on the 201st trial.
Figure 7: Peak-time (Panel A), average responding rate (Panel B), and normalized width (Panel C) plotted by drug group (Saline and 0.05 mg/kg MK-801) and by session blocks (Baseline, Drug 1, Drug 2, Drug 3, and Drug 4). Error bars express standard error of the mean.
reflecting the change in produced peak-times with the criterions shifts. As with the session-level data, the peak-times for the 9 s - 18 s switch condition started near 9 s gradually moving towards 18 s, while the peak-times for all animals in the 18 s - 9 s switch condition change started near 18 s gradually moving towards 9 s, resulting in the observed interaction between session block and switch condition (Figure 7A). However, there were no interactions between either session block and drug group, \( F(4, 122) = 2.43, p > 0.05 \), or condition and drug group, \( F(1, 28) = 0.04, p > 0.05 \). Finally, the analysis indicated a three way interaction of session block, drug group, and condition, \( F(4, 112) = 4.05, p < 0.05 \). As with the session level analysis, the three-way interaction was the only effect of drug group that we observed. Again, it appeared likely that this interaction was due to an increase in peak-time observed in the Drug 1 block compared to the Drug 2 block for the 18 s - 9 s MK-801 group (Figure 7A). In order to confirm this observation, analyses where conducted separating session blocks Drug 1 and Drug 2 from session blocks Drug 3 and Drug 4. The result was two new ANOVAs: a session block (either Drug 1 and Drug 2, or Drug 3 and Drug 4) x drug group x condition mixed measure ANOVA.

Similar to the sessions analysis of peak-time, the results from the current ANOVA on peak-time using session blocks Drug 1 and Drug 2 revealed a main effect of condition, \( F(1, 28) = 411.68, p < 0.001 \), and no main effects of either session block, \( F(1, 28) = 0.02, p > 0.05 \), or drug group, \( F(1, 28) = 3.18, p > 0.05 \). In addition, there were no session block by condition, \( F(1, 28) = 0.45, p > 0.05 \), or drug group by condition, \( F(1, 28) = 1.48, p > 0.05 \), interactions. However, there was session block by drug group interaction, \( F(1, 28) = 5.47, p < 0.05 \), and a session block, drug group and condition interaction, \( F(1, 28) = 5.08, p < 0.05 \), suggesting an effect of drug injections during these two blocks.
The results from the ANOVA on peak-time using session blocks Drug 3 and Drug 4, were also similar to the session level analysis, and revealed a main effect of condition, $F(1, 28) = 96.54, p < 0.001$, but no main effects either session block, $F(1, 28) = 1.63, p > 0.05$, or drug group, $F(1, 28) = 0.14, p > 0.05$. An interaction between session block and condition was observed, $F(1, 28) = 15.61, p < 0.001$. However, no interactions were observed between session block and drug group, $F(1, 28) = 0.00, p > 0.05$, drug group and condition, $F(1, 28) = 1.39, p > 0.05$, or session block, drug group, and condition, $F(1, 28) = 0.99, p > 0.05$.

As with the session-level analysis, the drug-group interactions in the ANOVA using the Drug 1 block and the Drug 2 block, combined with the lack of any effect of drug group in the ANOVA using the Drug 3 block and the Drug 4 block, indicates that the three-way interaction observed in the initial ANOVA was due to an effect of drug on peak-time in the Drug 1 blocks only. These results suggest the original interaction was due to a temporary increase in peak-time observed in Drug 1 for the 18 s -9 s MK-801 group (Figure 7A).

*Average response rate by block:* The overall pattern of average response rate, or the average rate of responding during the run phase ($r_2$), was obtained from the single trial analysis procedure that yielded peak-time for individual trials. This measure was the closest measure from the single-trial analysis available for comparison to the session level variable peak-rate. As can be seen, the general pattern of average response rate is similar to the pattern observed with peak-rate (Figure 5B & Figure 7B). Likewise, the results for the ANOVAs were also similar; administration of MK-801 increased average response rate across session block for animals in both conditions. This observation is confirmed by the ANOVA (with Greenhouse-Geisser corrections applied), which revealed a main effect of drug group, $F(1, 28) = 5.46, p < 0.05$, and of session block, $F(4, 112) = 4.06, p < 0.05$. No main effect of condition was observed, $F(1, 28)$
= 0.52, \( p > 0.05 \). The analysis also indicated an interaction between session block and drug group, \( F(4, 122) = 7.83, p < 0.01 \), reflecting an increase in average response rate from the Baseline block (Figure 7B). In addition, there was an interaction between session block and condition, \( F(4, 112) = 5.42, p < 0.01 \). However, no interactions between drug group and condition, \( F(1, 28) = 0.83, p > 0.05 \), or session block, drug group, and condition, \( F(4, 112) = 1.80, p > 0.05 \) were observed.

**Normalized width of responding by block:** The overall pattern of normalized width of responding (normalized width), calculated by \((s_2-s_1)/\text{peak-time}\), was obtained from the single-trial analysis procedure that yielded peak-time and average response rate for individual trials. This single-trial analysis measure was the closest comparable to the session level variable CV. As can be seen in Figure 5C & Figure 7C, the general pattern of normalized width is somewhat similar to the pattern observed with CV, although not as pronounced. Normalized width remained fairly stable across all session blocks. The ANOVA (with Greenhouse-Geisser corrections applied) yielded results similar to the ANOVA on CV, indicating main effects of session block, \( F(4, 112) = 7.91, p < 0.05 \) and drug group, \( F(1, 28) = 5.04, p < 0.05 \). No main effect of condition was observed, \( F(1, 28) = 0.25, p > 0.05 \). There was a session block x condition interaction, \( F(4, 112) = 3.32, p < 0.05 \), indicating that as a group the 18 s - 9s condition finished with slightly smaller widths of responding after the switch than did the 9 s – 18 s condition (Figure 7C). There were no interactions between session block and drug group, \( F(4, 112) = 2.31, p > 0.05 \), drug group and condition, \( F(1, 28) = 0.25, p > 0.05 \), or between session block, drug group and condition, \( F(4, 112) = 0.263, p > 0.05 \).

**Start-time and Stop-time by block:** The single-trial analysis allows for the calculation of two additional measures that are unable to be computed with the procedures used in the session
level analysis. These two measures are start-time ($s_1$) and stop-time ($s_2$). It is important to note that since these measures are used directly in computing both peak-time and normalized width, they are not completely independent of the preceding analyses. However, individual analyses on start-time and stop-time may lead to insights into temporal processing not directly observable through the preceding measures. For instance, the changes observed for peak-time and width of responding could be the result of difference in when to start and stop responding to the stimulus.

There are at least three patterns that could explain the increase in both peak-time and width of responding: animals could start responding later and stop later, animals could start responding sooner and stop responding later, or animals stop time only may change. Therefore, start-time and stop-time for individual trials were also analyzed.

In both switching conditions, start-times for both drug groups appeared to be similar across session block (Figure 8A). Greenhouse-Geisser corrections were used on the ANOVA, which resulted in a main effect of condition, $F(1, 28) = 20.16, p < 0.001$, but no main effects of either session block, $F(4, 112) = 2.18, p > 0.05$, or drug group, $F(1, 28) = 0.96, p > 0.05$. The interaction term for session block and condition, $F(4, 112) = 89.54, p < 0.001$, revealed a interaction reflecting the change in start-times with the criterion time shifts. The start-times began later following the temporal criterion shift for 9 s – 18 s switch condition, while start-times began earlier following the temporal criterion shift for 18 s – 9 s switch condition. There were no interactions between session block and drug group, $F(4, 122) = 1.25, p > 0.05$, or condition and drug group, $F(1, 28) = 0.04, p > 0.05$. Finally, the analysis indicated a three way interaction of session block, drug group, and condition, $F(4, 112) = 3.21, p < 0.05$. This three-way interaction was due primarily to a slower change in start-times for the 18 s – 9 s saline
group, as compared to the MK-801 group, following the temporal criterion shift, this difference between drug groups was not observed for the 9 s -18 s switch condition (Figure 8A).

Stop-times appeared to differ slightly based on drug group, with animals receiving MK-801 stopping later in the trial compared to saline animals (Figure 8B). This observation was confirmed by the ANOVA (with Greenhouse-Geisser corrections applied), which revealed a main effect of session block, $F(4, 112) = 22.17, p < 0.001$, condition, $F(1, 28) = 66.19, p < 0.001$, and drug group, $F(1, 28) = 4.11, p < 0.05$. The interaction terms for session block and

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**Figure 8**: Start-time (Panel A) and stop-time (Panel B) plotted by drug group (Saline and 0.05 mg/kg MK-801) and session blocks (Baseline, Drug 1, Drug 2, Drug 3, and Drug 4). Error bars express standard error of the mean.
condition, \( F(4, 112) = 523.94, p < 0.001 \), revealed differences in the change in stop-times corresponding to the criterion shifts. The stop-times were later following the temporal criterion shift for 9 s – 18 s switch condition, while stop-times were earlier following the temporal criterion shift for 18 s – 9 s switch condition. In addition, there were interactions between session block and drug group, \( F(4, 122) = 4.63, p < 0.05 \), and session block, drug group, and condition, \( F(4, 112) = 3.24, p < 0.05 \), indicating that stop-times lengthened upon initiation of drug administration (especially during the Drug 1 testing block). Finally, there was no condition and drug group interaction, \( F(1, 28) = 0.03, p > 0.05 \).

**Summary:** As with the session-level analysis, single-trial analysis revealed that administration of MK-801 did not affect the learning of a new temporal criterion, as measured by change in gain, bias, and peak-time. Also consistent with session-level analysis, MK-801 increased responding rate across test blocks for animals in both switching conditions. In contrast to what was observed in the session-level analysis, MK-801 increased variability (as measured by normalized width of responding). Although, remember that there was non-significant increase in CV observed with MK-801 administration in the session-level analysis. Single-trial analysis of start-time and stop-time of responding reveal that increased stop-time while it did not affect start-time.

**Spontaneous Alternation**

In order to be included in the analysis for spontaneous alternation, animals must have performed a minimum of 10 alternations. Six of the original 18 animals (n = 9 saline and n = 9 MK-801) did not meet this criteria, resulting in 12 animals (n = 5 saline and n = 7 MK-801) being included in the final analysis. Contrary to what was expected, injections of MK-801 did not alter performance on the spontaneous alternation task. The means and standard deviations for
percentage of alternation and total number of entries are presented in Table 2. MK-801 injections did not change the percentage of alternation, $t(10) = -1.18, p > .05$, suggesting that there was no spatial working memory difference between drug groups. In addition, MK-801 injections did not change the total number of entries, $t(10) = .78, p > .05$, suggesting that there was no difference in general locomotion and exploratory behavior between the drug groups for this particular task.

**Table 2: Spontaneous alternation scores**

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>MK-801</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. Deviation</td>
</tr>
<tr>
<td>Percent Alteration</td>
<td>49.60</td>
<td>11.80</td>
</tr>
<tr>
<td>Total Number of Entries</td>
<td>19.20</td>
<td>3.27</td>
</tr>
</tbody>
</table>

**Water Maze**

Fourteen animals (n = 7 saline and n = 7 MK-801) began water maze testing. One animal died half way through the testing procedure and therefore did not complete all testing days. Data from this animal were excluded from analysis resulting in a 13 animals (n = 7 saline and n = 6 MK-801) run on the water maze.

**Escape Latency**

Escape latency to reach the platform decreased across the 4 initial training days and remained stable across the last 4 training days (Figure 9A). Greenhouse-Geisser corrections were used on the ANOVA, which resulted in a main effect of training day, $F(7, 77) = 19.20, p < 0.001$. However, there was no main effect of drug group, $F(1, 11) = 0.65, p > 0.05$, and there was no interaction between training day and drug group, $F(7, 77) = 0.77, p > 0.05$. Examination of
Figure 9: No effect of MK-801 (Saline vs. 0.05 mg/kg MK-801) on the water maze. Panel A: Escape latency; Panel B: Time in target quadrant during probe trials; Panel C: Number of platform crossing during probe trials. Error bars express standard error of the mean.
the escape latency data (Figure 9A) suggests a possible trend for longer latencies of animals receiving MK-801 during the first 4 training days that may have been masked by the later training days (although one might have expected a significant interaction term if this were the case). To test this possibility, a new ANOVA was run on the escape latency data excluding training days 6 - 9. The results of this new analysis were essentially unchanged. The ANOVA indicated a main effect of training day, $F(3, 33) = 9.16, p < 0.001$. However, there was no main effect of drug group, $F(1, 11) = 1.41, p > 0.05$, or interaction between training day and drug group, $F(3, 33) = 0.29, p > 0.05$, indicating no effect of MK-801 injections on escape latency.

**Time in Target Quadrant & Platform Crossings**

As with latency, no effect of MK-801 was observed for either of the measures from the probe trials (Figure 9B & C). Time spent in the target quadrant (Figure 9B) was not different across probe day, $F(1, 11) = 0.05, p > 0.05$, or between drug group, $F(1, 11) = 0.03, p > 0.05$. In addition, probe day and drug group did not interact, $F(1, 11) = 0.53, p > 0.05$. Likewise, the analysis of number of platform crossings (Figure 9C) revealed no main effects of probe day, $F(1, 11) = 2.45, p > 0.05$, or drug group, $F(1, 11) = 0.45, p > 0.05$. In addition, there was no interaction between probe day and drug group, $F(1, 11) = 0.02, p > 0.05$. Therefore as with spontaneous alternation, there was no effect of MK-801 injections on any of the water maze measures.

**Discussion**

Effects of the NMDA antagonist MK-801 (0.05 mg/kg) on temporal processing, learning, and memory were examined using the peak-interval (PI) procedure. Animals were trained on one temporal criterion and then switched to a new criterion. Two groups of animals were used: one group was switched to a longer criterion (9 s – 18 s) and the other group was switched to a
shorter criterion (18 s – 9 s). In addition, spatial memory tasks, spontaneous alternation and water maze, were conducted to determine whether 0.05 mg/kg MK-801 would disrupt spatial memory.

Administration of 0.05 mg/kg of MK-801 had no effect on the rate of learning a new temporal criterion, spontaneous alternation, or water maze. Although, no effects of MK-801 on temporal learning were observed, there were four effects of MK-801. First, MK-801 did not produce lasting changes in peak-time. The apparent lengthening of peak-time during the initial administration of MK-801, Drug 1 block, abated with training (see Drug 2 block); this lengthening was only observed in the 18 s – 9 s switch condition and not in the 9 s - 18 s switch condition. Second, MK-801 increased peak-rate of responding. Third, MK-801 also resulted in an increase in variability, beyond that expected from Weber’s law; this increase in variability was observed in the single-trial analyses (normalized width), although a non-significant trend in this direction with the session level analyses was also observed. Finally, start-times were essentially unchanged by MK-801 administration whereas stop-times were slightly lengthened.

The main focus of the current study was the effects of MK-801 on the learning of a new temporal criterion. The results from this study indicated that the 0.05 mg/kg dose of MK-801 had no effect on learning a new temporal duration. Results from the analysis centered on the 400 trials immediately surrounding the criterion switch suggested that animals receiving saline and those receiving MK-801 learned to produce the new temporal duration at similar rates.

Although the current study failed to find a difference in rate of learning between the saline and MK-801 animals, the question of whether MK-801 can influence the learning of a new temporal duration remains open because the MK-801 injections did not affect performance on either spontaneous alternation or the water maze as has been previously reported (Caramanos &
Shapiro, 1994; Heale & Harley, 1990; Holter et al., 1996; Lennartz & Gold, 1995; McLamb et al., 1990; Riedel et al., 2003; Whishaw & Auer, 1989). For spontaneous alternation, MK-801 (0.1 mg/kg) reduces the percentage of alternations (Holter et al., 1996; Lennartz & Gold, 1995). On the water maze, MK-801 (0.05-0.1 mg/kg) has been reported to impair acquisition and produce hyperactivity, but impair not recall of a well-learned place response (McLamb et al., 1990; Whishaw & Auer, 1989).

One possible reason that may explain the lack of effect of MK-801 in the current study is that the 0.05 mg/kg dose of MK-801 was simply not of a sufficient concentration to cause any impairment in learning, even though it was sufficient to increase response rate. The null results from spontaneous alternation and water maze tasks support this suggestion. Previous reports suggest that MK-801 such reduce alternation scores on spontaneous alternation, and increase latency times for the water maze (Caramanos & Shapiro, 1994; Heale & Harley, 1990; Lennartz & Gold, 1995; McLamb et al., 1990; Riedel et al., 2003; Whishaw & Auer, 1989). Although similar dose levels have been reported to produce spatial memory impairments in other rat strains (Caramanos & Shapiro, 1994; Shapiro & Caramanos, 1990; Shapiro & O'Connor, 1992), the lack of effect observed in this study may be due to difference in glutamate function between rat strains (Manahan-Vaughan & Braunewell, 2005), animals may have also developed a tolerance for the drug because of the repeated administrations. The 0.05 mg/kg dose level was selected because it produced behavioral effects on the PI procedure, increases in response rate, without affecting the production of the previously learned duration, as was the case with the 0.2 mg/kg dose. Such effects on peak-time would have complicated the evaluation of temporal learning since peak-time never stabilized with 0.2 mg/kg MK-801 administration. Future studies on the effects of NMDA receptor antagonists on temporal learning and memory could first find dose
levels sufficient to produce deficits on tasks such as spontaneous alternation and water maze prior to the investigation of temporal learning.

Three other possibilities may also explain the lack of a MK-801 effect on temporal learning. First, the NMDA receptors are not involved in the process of learning temporal durations. While this is indeed a possibility, it would be premature to jump to this conclusion based on the null finding with one drug dose; a dose that failed to produce deficits which have been previously reported with administration of MK-801 and other NMDA receptor. Second, the drug used was inactive. The positive results obtained for response rate and variability argue that the drug had a general effect, just not on the learning on the new duration. Third, it is possible that the task difficulty was not sufficient to cause impairments in learning. Evidence from studies on discrimination tasks in monkeys suggest that task difficulty is an important factor in the appearance of deficits related to NMDA antagonists (Harder, Aboobaker, Hodgetts, & Ridley, 1998). The ratio of the two intervals to be learned was a simple 2:1 ratio. The Striatal Beat-Frequency model of time (SBF) suggests that the temporal memory two such durations may have an overlapping neural representation (Matell & Meck, 2000). Thus it may not require much effort to learn the second duration with the current design. Perhaps a more challenging task would be to learn a new temporal criterion that has a more complex ratio relationship to the original criterion, and that may therefore have less share representation in memory. However, this explanation is not likely since there were null effects of MK-801 administration for the spontaneous alternation and water maze tasks.

The effects of MK-801 on peak-time, response rate, and variability, prior to the criterion switch, were mostly consistent with the effects reported in the previous chapter. Peak-time was not greatly affected by MK-801 administration. One interesting effect on peak-time was a
temporary lengthening during the first drug block for animals in the 18 s – 9 s switch condition, that was not observed in the 9 s – 18s condition, and the same dose of MK-801 the previous study did not produce any changes in peak-time. The temporary lengthening observed in the current study was similar in direction (but not magnitude) with the temporary lengthening observed at the 0.2 mg/kg dose in the previous study. MK-801 also increased rate of responding, which is consistent with the increase in peak-rate observed for all dose levels of MK-801 the previous study. The results for variability are also partially consistent with what was reported in the previous chapter. As in the previous study, the session level analysis on variability found no significant increase in variability with 0.05 mg/kg MK-801; although, trial level analyses revealed a difference in width of responding and stop-times after the administration of MK-801.

In summary, MK-801 has little effect on temporal learning in Fisher 344 rats. However, more work needs to be done before concluding that NMDA receptor antagonists have no influence on the learning of temporal durations because the currently study failed to find effect of MK-801 on the spontaneous alternation and water maze task, which have been previously reported (Caramanos & Shapiro, 1994; Heale & Harley, 1990; McLamb et al., 1990; Riedel et al., 2003; Whishaw & Auer, 1989). The best explanation of these null effects seems to be that the 0.05 mg/kg dose of MK-801 was simply not of a sufficient concentration to cause any impairment in learning. The present study reinforced the finding of the previous chapter: MK-801 interferes with short interval timing by producing and MK-801 increases response rate.
CHAPTER 4. QUALITATIVE MODELING OF MK-801 DATA USING SCALAR EXPECTANCY THEORY

The aim of the current chapter was to gain a greater understanding into the nature of the effects of NMDA receptor antagonists on timing and temporal processing. This was accomplished by using Scalar Expectancy Theory (SET) simulations to model the data obtained in the experiment reported in Chapter 2 (see Appendix B for a detailed discussion of SET and other models of timing). The goal was to investigate whether SET could account for the data and if so, which parameter, or combination of parameters produce the behavioral patterns observed with administration of MK-801. Specifically, emphasis was given to the effects of clock and memory transformation parameters. These parameters were chosen based on analysis of the data in the previous studies (Chapter 2) that suggest that the effects of MK-801 maybe a mixed clock and memory pattern of distortion.

SET is a formal mathematical information processing (IP) model that has been proposed to address the sources of variance and the mechanisms involved in interval timing tasks (Church, 1984; Gibbon et al., 1984). SET attempts to characterize the processing involved in timing; including the different properties and sources of variability that produce timescale invariance (Gibbon, 1977). Three general stages are included in SET: clock, memory, and decision. Each of the stages has individual components with their own properties. The clock stage consists of three components: pacemaker, switch, and accumulator. The pacemaker is a mechanism that emits a continuous stream of pulses at a mean rate (Church, 1984; Gibbon, 1992; Gibbon et al., 1984). The switch is responsible for the transference of the pulses from pacemaker to the accumulator (Church, 1984; Gibbon et al., 1984). When the switch is opened, pulses cannot reach the accumulator; however, when the switch is closed, the pulses are able to be flow freely into the
accumulator. The accumulator sums the number of pacemaker pulses during the closure of the switch (Church, 1984; Gibbon et al., 1984). The number of pulses summed in the accumulator at any given moment is the estimation passage of subjective time.

The memory stage consists of two components: working memory and reference memory. The working memory component is continually updated with information from the accumulator. Working memory is important for its role in temporarily storing the information held in the accumulator (Church, 1984; Gibbon et al., 1984). Reference memory stores information about previous trials (Church, 1984; Gibbon et al., 1984). This component consists of a distribution of remembered durations. Reference memory is updated only after reinforced trials. On these reinforced trials, the working memory count is transferred into reference memory and is transformed by a memory transformation constant, K*. Perfect transfer from working memory to reference memory is achieved with a K* value of 1.0. If K* is above 1.0, then the remembered duration is longer, and if K* is less than 1.0, then a shorter duration is stored.

The decision stage primarily consists of one major component, the comparator. The comparator’s function is to determine whether to respond based on a decision rule (Church, 1984; Gibbon et al., 1984). The comparator receives input from working memory, reference memory, and a distribution of threshold values. The working memory value is compared, via a decision rule to a value randomly selected from the distribution of remembered times held in the reference memory. A threshold value is randomly selected from a distribution of thresholds and is the criteria used in the response decision. When the value of the decision rule is above the threshold value, a ‘do not respond’ decision is the output of the comparator. When the value passes below the threshold value, the comparator output switches to ‘respond’ until value from
the decision rule will become greater than the threshold value and the output will again switch to do not respond.

Modeling the data within SET may lead us to a greater understanding of the mechanisms by NMDA receptor antagonists affect timing and temporal processing by clarifying the changes in the component temporal processing mechanisms (as proposed by SET) necessary to simulate the behavioral data. Previous research on the neurobiology of timing has also used SET to understand the patterns observed in the data (see Appendix C). For example, the patterns of data observed with manipulations of the dopamine systems can be simulated in SET by making alterations to the speed of the clock/pacemaker (Hinton & Meck, 1997; Maricq & Church, 1983; Maricq et al., 1981; Matell et al., 2004; Meck, 1983, 1986, 1996). Likewise the data patterns observed with pharmacological manipulations of the cholinergic system can be simulated by changes to the memory transformation parameter, \( K^* \) (Hinton & Meck, 1997; Meck, 1983, 1994, 1996, 2002; Meck & Church, 1987a, 1987b).

In sum, the goal of SET modeling was to discover if the patterns of behavioral data observed with MK-801 could be replicated by SET. These patterns include an immediate lengthening of peak-time that does not fully return to pre-drug levels with further training and increase in the variability of the temporal response distribution. Specific emphasis was given to the effects of changes in the clock and memory parameters to further investigate the conclusion of Chapter 2; that MK-801 data appear to follow a clock like pattern of distortion.

**Method**

**Simulations**

Model simulations were conducted using a modified implementation of SET modified from a previously published series of programs (Church, 2003) written for Matlab (The
Mathworks, Inc., Natick, MA). This implementation models performance on the fixed-interval (FI) procedure for a single duration. Alterable parameters of this implementation included clock speed ($\Lambda$), variability of clock speed, memory transformation ($K^*$), variability of memory transformation, threshold ($B$), and variability of threshold.

The program series used for the current study (see Appendix D) built on this previous coding. Alterations included the addition of peak-interval (PI) trials, a capacity to learn two unique temporal criterions, each with unique memory of reinforcement history, an alterable parameter to control the size of the memory vector, and an alterable parameter to control the base-rate of responding (the probability of a response independent of the results from the model decision stage). Although the current study only simulated data from one temporal criterion, the second was added for modeling data from other experiments being conducted in the lab. Since the memories of the two criterions were completely independent of each other, the addition of the second criterion did not fundamentally alter the simulations in any fashion.

The final program had eight alterable parameters: clock speed ($\Lambda$), variability of clock speed ($\sigma_\Lambda$), memory transformation ($K^*$), variability of memory transformation ($\sigma_{K^*}$), threshold ($B$), variability of threshold ($\sigma_B$), memory size ($M$), and base-rate of responding ($R$). Clock speed was the mean rate that the clock generated pulses, and the variability of clock speed set the consistency with which the clock generated those pulses. Memory transformation was the value used to scale the information passing into reference memory on reinforced trials, and again, its variability described the consistency of this scaling. The threshold values described the mean of the distribution of thresholds which are randomly selected for the decision process; the variability of this distribution was described with the variability of threshold parameter. Memory size specified dimensions of the memory vector which determined how much the training history
effects future performance. Finally, base-rate of responding determined the probability of a
response independent of the stimulus and timing processes. Simulations were run in Matlab
(V6.5, Release 13.0.1) on a Dell (Optiplex GX260) computer with a 2.66 MHz processor and
512 MB of RAM.

Procedure

The data modeled in this chapter were the data from experiment two from Chapter 2.
Data from Chapter 3 was not modeled since there was no effect of drug on the learning of a new
temporal criterion. Saline and MK-801 groups were modeled separately. Start values for the
saline group for clock speed (5.0), variability of clock speed (0.0), memory transformation (1.0),
variability of memory transformation (0.2), threshold (0.1), and variability of threshold (0.0)
were obtained from values of previous model fits (Church, 2003; Meck, 1983). The start value
for base-rate of responding was set to zero. The value for memory size was fixed at 53
throughout all simulations; this value was selected based on the data from Chapter 3, which
indicated that on average animals learned the new temporal criterion after about 53 FI trials.

The simulations were structured to match the training procedures used in Experiment 2 of
Chapter 2. The training procedures for the simulations matched both the number of training
sessions and number of trials within individual sessions. Specifically, the modeling procedure
started with 10 sessions of FI training (∼70 FI trials per session) followed by 30 sessions of
peak-interval (PI) training (∼35 FI and ∼35 probe trials per session). Following the completion
of these training sessions, 5 test blocks of PI test sessions were run: Day 1-5 (Baseline), Day 6-
10 (Drug 1), Day 11-15 (Drug 2), Day 16-20 (Drug 3), and Day 21-25 (Post-Drug). In order to
simulate the same number of animals tested (Experiment 2 in Chapter 2), 8 unique simulations
were conducted for each of the saline and drug groups. Temporal response function were then
averaged across animals (unique simulations) for each test block and normalized by maximum rate of responding.

For simulations, the parameters that best fit the Baseline block were used for initial training. The five test sessions in the Baseline block were simulated with the same parameters used in training. Changes to the parameters to model the effects of MK-801 administration (i.e., to simulate the effects of drug injections) were made prior to simulation of test session in the Drug 1 block. No further changes were made to the parameters through the Drug 2 and Drug 3 test blocks. Following the completion of simulations for the Drug 3 block and prior to the Post-Drug test block simulation, parameters were reset to the original parameters used to train the model based on an assumption was made that the state of the system would return to normal upon cessation of drug injections.

Results

A qualitative fit was first made to the Baseline data (Days 1-5) for the saline group by altering the parameters of the model until the output of the simulation visually matched the shape and mean of the temporal response distribution. When the modeled Baseline results for the saline group visual matched the actual data, no further changes were made to the parameters to ensure that the parameters derived from Baseline would result in fits to the remaining test blocks. Reasonable visual fits to the saline data that matched shape and mean of the simulated temporal response functions were able to be obtained through the SET modeling simulations (Figure 10A & C). The final values for the SET parameters were 5.206 pulses per second for clock speed with 0.0 variability, the memory transformation value was 0.985 with a 0.150 variability value, the threshold value was 0.420 with a 0.280 variability value, and the final value for base-rate of responding was 0.001.
Qualitative fits for the drug group Baseline condition were conducted using the parameters obtained from the saline group fits for start parameters. Parameters of the model were then altered until the output of the simulation visually matched the shape and mean of the temporal response distribution of Baseline data. The parameters that provide the fits for the saline data also allowed for a good fit of the Baseline data for the drug group, with one exception: the memory transformation value was set to 0.975 (Baseline: Figure 10B & D).

**Single Parameter Manipulations**

When the parameters that gave the best visual fit for the Baseline data were identified, the focus shifted to fitting the data from the Drug 1 (Day 6-10) test block. To examine the effect of individual parameters, each parameter was changed, while the other parameters were held at

![Graphs showing temporal response functions for different conditions](image)

*Figure 10:* Scalar Expectancy Theory (SET) accounts for MK-801 data. Temporal response functions are plotted by time (seconds) and by proportion of maximal responding. Panel A: Saline data; Panel B: 0.2 mg/kg MK-801 data; Panel C: SET simulations for Saline; Panel D: SET simulations for MK-801.
baseline values. Initial attempts to model the Drug 1 test block began with the Baseline parameters and then each parameter was altered individually.

*Base-rate of Responding*

In an attempt to match the increase observed in the tails of the temporal response distribution (Drug 1 – 3; Figure 10B), the base-rate of responding parameter was increased. Increasing the base-rate of responding parameter (to 0.250) raised the left and right tails of the temporal response function without changing the shape or temporal placement of the function. When base-rate of responding was returned to the baseline value, the Post-Drug block temporal response function match the Baseline block, indicating that there was no lasting effects of the parameter change.

*Clock Speed*

The immediate rightward shift of the response distribution observed upon initiation of MK-801 administration (Drug 1; Figure 10B) could indicate a slowed clock speed. With the clock running at a slower speed, a greater amount of time needs to pass before the working memory count accumulates to matches the counts stored in reference memory. This effect is transient because with continued training the references memory will be filled with counts reflecting the new clock speed. The slowing of clock speed to 1.250 pulses per second resulted in a leftward shift for the Drug 1 test block; however by Drug 2 and Drug 3 testing blocks, the change in clock speed had been compensated for and the temporal response function matched the Baseline functions (Figure 11A). Upon the return to baseline parameters for the Post-Drug block, a leftward shift was observed that began to normalize during that testing block, as evidenced by a bi-modal response function.
Figure 11: Effects of single parameter changes. Temporal response functions are plotted by time (seconds) and by proportion of maximal responding. Panel A: Changing clock speed; Panel B: Changing variation of clock speed; Panel C: Changing memory transformation; Panel D: Changing variation of memory transformation; Panel E: Changing threshold; Panel F: Changing variation of threshold.
Variability of Clock Speed

An increase in variability of the temporal response distributions was observed for all three MK-801 test blocks. To model this increased variability, clock speed variability was increased. Increasing clock variability to 3.000 resulted in slight leftward shift and an increase in the right tail of the response functions for all drug blocks (Figure 11B). These changes disappeared and the response functions for the Post-Drug block matched the Baseline block when clock variability to reset to its baseline value, suggesting no lasting effects of this manipulation.

Memory Transformation

Increases in the memory transformation parameter resulted in a gradual rightward shift in the response distribution. Initially there was no effect because the reference memory representation was accurate; however, with the increased memory transformation value the new counts entering memory were larger than the actual counts. As reference memory was filled with these enlarged counts, the subject overestimates time because it tasks longer for the clock to reach the newly stored values. The result was a permanent rightward shift in response distributions, as long as the transformation parameter remained increased. MK-801 administration resulted in a permanently rightward shifted response distribution. Perhaps this shift was due in part to an increase in the memory transformation. As expected, an increase in the memory transformation parameter (to 1.500) caused a rightward shift in the temporal response function that gradually become greater across Drug 1, Drug 2 and Drug 3 test blocks (Figure 11C). When this parameter was returned to its baseline value for the Post-Drug block, the response function shifted back to the left; however, it had a greater spread than the Baseline respond function.
Variability of Memory Transformation

Variability of memory transformation is another parameter that might explain the increase in variability observed in the temporal response distributions. Increasing the values of this parameter should result in response distribution that gradually becomes more variable as reference memory is filled with new temporal representation formed under these new conditions. Increasing the value for variability of memory transformation to 0.750 resulted in changes in the temporal response functions that included a rightward shift, an increase in variability, and an increase in the right tail (Figure 11D). These changes increased in magnitude from Drug 1 to Drug 2 and Drug 3. During the Post-Drug block, the resetting of the variability of memory transformation allow the response functions to nearly return to baseline; although a slight increase in the right tail of the distribution remained.

Threshold

Another way to model the increased variability of the response distributions would be to increase the threshold value. Increase in the threshold values results in a wider window of responding because the ‘respond’ decision from the comparator would reached soon, and consequently the return to a ‘do not respond’ decision would take longer (see Appendix B; Figure 15). Any changes in threshold should not effect the temporal placement of the response distribution, just its width. As expected, an increase in the width of the response function without a change of its temporal placement threshold was observed when threshold was increased to 0.900 (Figure 11E). This result was consistent across all drug test blocks. Upon return to baseline conditions, the temporal response function for the Post-Drug block matched the Baseline block indicating no lasting effect of the threshold change.
Variability of Threshold

The last single parameter that could model the increases in variability observed in the MK-801 data is threshold variability. As with increases to the threshold value, increases in variability of threshold should increase the width of the response distribution without changing its temporal placement. Increases in the variability of the threshold (to 0.400) cause a widening of the base of the response functions for all drug blocks (Figure 11F). As with changes to the threshold parameters, return to baseline conditions resulted in a return of the temporal response function for the Post-Drug block to a form identical to the Baseline block, indicating no lasting effect.

Summary

None of the single parameter changes were sufficient to reproduce the pattern in the temporal response observed under MK-801 (compare Figure 10B to Figure 11). Next, select combinations of parameter changes were made, motivated by the hypothesis from the previous chapters and the patterns observed with the single parameter manipulations. These combinations of parameter changes were conducted based on observation from the MK-801 data. Specifically, data fits were first attempted by both changing the clock speed and memory transformation parameters, then both the threshold and base rate of responding parameters, and finally all four of these parameters together.

Multiple Parameter Manipulations

Clock Speed & Memory Transformation

The first combination examined the effects of changing clock speed and memory transformation parameters together. This combination was selected because the MK-801 data contained features that partially fit the pattern of disruptions observed with changes of either
parameter only. Specifically, an immediate rightward shift was observed in the data, consistent with a slowing of the clock speed. In addition, the rightward shift appeared to be permanent, consistent with an increase in the memory transformation parameter. The slowing of clock speed to 3.250 pulses per second combine with an increase in memory transformation parameter, to 1.100, resulted in a leftward shift for the temporal response function for Drug 1 test block. The temporal response functions for Drug 2 and Drug 3 testing blocks were found to be placed to the left of the Drug 1 function, indicating a renormalization of the temporal placement with training. However they were both still located to the right of the Baseline block (Figure 12A), a pattern similar to the actual data (Figure 10B). However, the tails of the distributions were much lower than those observed in the actual data for all drug blocks. Returning the parameters to baseline values resulted in temporal response function that was similar in shape to the Baseline block. In addition, the response function was shifted slight left of the Baseline block, which was also similar to the actual data.

*Threshold & Base-rate of Responding*

In an attempt to account for the increased variability and increased tails observed in the MK-801 data (which remained unaccounted for with the clock speed and memory transformation changes), the next combination of changes examined the effects of changing threshold and base-rate of responding parameters together. This combination of change should result in a more variable response distribution that has elevated tails. As expected, increasing threshold (to 0.900) and base-rate of responding to (0.250) resulted in an increase in the variability of the response function and an increase in the right tail of the response function, without altering its temporal placement (Figure 12B). Returning the parameters to baseline values resulted in temporal response function that was identical in both shape and temporal placement to the Baseline block.
Figure 12: Effects of combinations of parameter change. Temporal response functions are plotted by time (seconds) and by proportion of maximal responding. Panel A: Changing clock speed and memory transformation; Panel B: Changing threshold and base-rate of responding; Panel C: Changing clock speed, memory transformation, threshold and base-rate of responding.
Although this combination of change to the parameters resulted in the variability and shape of the distributions tails similar to the actual data, the temporal placement of the drug blocks differed.

*Clock Speed, Memory Transformation, Threshold, & Base-rate of Responding*

The clock and memory combination and the threshold and base-rate combination each provided only partial replication of the actual data, with each combination replicating a different aspect of the data. Each combination seemed to provide only components of the response pattern; the clock and memory combination provided temporal placement lacking in the threshold base-rate combination, while the threshold base-rate combination provided increases in variability and the tail absent in the clock and memory combination. So, these four parameters were changed. Simulations were run systematically changing one of the four parameters values until the parameter values were set give the best visual fit for the data. The simulations resulted in the following changes: clock speed was decreased to 3.250 beats per second, memory transformation was increased to 1.150, threshold was changed to 0.800, and the base-rate of responding was set to 0.175. As can be seen (Figure 12C), this combination of parameter change lead to temporal response functions closer to the actual data than any single change or combination of changes previously examined. However, the tails of the distribution were lower than those observed in the actual data (Figure 10B).

*Clock Speed, Memory Transformation, Threshold, Base-rate of Responding, & Variability of Clock Speed*

The tails of the simulated temporal response functions needed to be increased to better match the actual data. Since similar increases were observed with changes in variability of clock speed (Figure 11B), the effect of changing this parameter along with clock speed, memory
transformation, threshold, and base-rate of responding were investigated. Increasing variability of clock speed did increase the tail of the response function in the manner expected; however, no pattern of changes in these parameters before the Drug 1 block allowed for the separation observed in the tails of Drug 1, Drug 2, and Drug 3. Therefore variability of clock speed was allowed to vary between these drug blocks. By allowing this one parameter to vary, good fits were obtained between the actual and simulated data (Figure 10B & D). The parameter values that gave this fit were a clock speed of 2.950 pulses per second, a memory transformation of 1.300, a threshold of 0.700, and a base-rate of responding of 0.200. As with all previous simulations these four parameters were changed prior to Drug 1 and held constant until the return to baseline parameters prior to Post-Drug; variability of clock speed, however, was allowed to change between drug blocks. Variability of clock speed was 1.450 for Drug 1, 0.600 for Drug 2, and 0.200 for Drug 3.

Discussion

The current chapter modeled the data from Chapter 2 using SET in order to obtain a greater understanding of the mechanisms by which NMDA receptor antagonists affect timing and temporal processing. This modeling approach to understanding the mechanisms affected by drug administration has been utilized in previous research on the neurobiology of timing (see Appendix C). The goal of the modeling was to discover which patterns of MK-801 behavioral data could be replicated by SET, and which combination of parameter changes might yield behavioral patterns observed in the previous chapters.

Results indicate that the effects of MK-801 on timing and temporal processing can be reasonably reproduced using SET. Furthermore, the simulations suggest that MK-801 has wide ranging effects on the mechanisms involved in timing and temporal processing. In order to fully
account for the patterns observed in the behavioral data, five of the eight SET parameters were altered: clock speed was slowed, and the memory transformation, threshold, and base-rate of responding were all increased. Variability of clock speed was increased for all drug testing blocks; however, in order to fit the data, this parameter needed to change across drug blocks: the value for variability of clock speed was highest for Drug 1, lower for Drug 2, and further decreased in value for Drug 3.

The combination of SET parameter changes needed to simulate the data is consistent with the interpretation in Chapter 2 that suggested that either clock, memory, or a combination of the processes were being effect by injection of MK-801. The slowing of the clock speed parameter is consistent with the effect of dopamine antagonists (Hinton & Meck, 1997; Maricq & Church, 1983; Maricq et al., 1981; Matell et al., 2004; Meck, 1983, 1986, 1996), and the increase in the memory transformation parameter is consistent with the effects of cholinergic antagonists (Meck, 1983, 1996; Meck & Angell, 1992; Meck & Church, 1987a). Therefore a reasonable conclusion would be that MK-801 interacts with the dopamine system (Jeziorski et al., 1994; Marek et al., 1991; Trujillo & Akil, 1994) and the cholinergic system (Arias, McCardy, & Blanton, 2001; Covasa, Ritter, & Burns, 2003; Fenu, Acquas, & Di Chiara, 2001; Lydie & Baghdoyan, 2002) to produce the effects observed in the temporal response distribution. Moreover, the change in base-rate of responding and change in threshold would be expected because of the observed increase in rate of responding and the extra responding observed across probe trials for drugged animals relative to control (Chapters 2 & 3). In addition, increases in behavioral activity is common in studies using MK-801 (Ford et al., 1989; Welzl et al., 1991; Whishaw & Auer, 1989).
One unanticipated finding was the need to alter variability of clock speed. It is unclear exactly what this parameter change means. One possibility is that MK-801 interferes with the clock processes in a way that cause the clock speed to fluctuate. With continued exposure to MK-801, the clock system adjusts to the presence of the drug allowing for increased stability in clock function over time. A related possibility is that the instability of clock speed is due more to the dramatic slowing of the speed caused by MK-801 rather than a direct influence of the drug on the mechanisms controlling variability. Again, over time the clock mechanism is able to reduce variability as it adjusts to the slowed clock. This second explanation less likely because if the variability increase was due solely to changes in clock speed, one might expect an increase in variability as the clock resumes it normal speed once the drug is removed. However, the actual data does not have an increased variability in the Post-Drug block; rather it appears that variability of clock speed is returned to baseline levels.

The variability of clock speed was altered to account for the increase in the right tail of the temporal response distribution. In this implementation of SET, the change to variability of clock speed was the only parameter that could adequately produce the changes needed to simulate the MK-801 data. However, another variation of the SET implementation may have also helped simulate the increase tails. The implementation of SET used in the current experiment utilized a single threshold value that was used in the comparison stage. This single threshold value was used to decide when to begin responding as well as when to stop responding. However, at least one study has suggested that a model with two independent thresholds better describes that single trial data (Church et al., 1994). In this implementation, the first threshold value is used for the decision of when to begin responding and the second threshold value is used to determine when to stop responding. Based on the start-times and stop-
times observed during MK-801 administration, the first threshold value might be unchanged from Baseline values since start-times were essentially unchanged and the second threshold value might increase from Baseline values since stop-times increased. This pattern of change could account for the increase in right tail of the temporal response distribution. However, adding a second threshold would result in the addition of two parameters (the threshold and its variability) to a model already full of free parameters.

Another surprising finding from the clock speed and memory transformation combination was that a rebound in temporal placement of the response distribution does not always follow the return to the training parameters; a rebound is almost always observed when clock speed is changed by itself. This effect is dependent on the relationship of change between the clock speed and memory transformation parameters. If clock speed is changed a greater degree more than the memory transformation, a rebound may be observed. However, the correct relationship of change in both clock speed and memory transformation parameters can result in an immediate return to baseline performance when the parameters are reset to training values (i.e., no rebound in the temporal placement). This finding could explain the lack of a rebound follow cessation of MK-801 administration, reported in Chapter 2. Another study has also failed to find an expected rebound in peak-times (Matell et al., 2004). Matell et al. (2004) found a clock shift pattern following long-term daily cocaine injections. However, following the cessation of cocaine injection, no rebound in peak-times was observed. They hypothesized that the clock speed itself may have been readjusted instead of the temporal memories; this is a process that might change depending on subtle differences in administration schedules of drugs. It is difficult to discount the possibility that a similar phenomenon occurred in the present study.
The simulations reported in this chapter may resolve the conflicting findings using the PI procedure and those reported using differential-reinforcement-of-low-rate of responding (DRL) schedule (Sanger, 1992; Stephens & Cole, 1996; Tonkiss et al., 1988; Welzl et al., 1991). The results from the PI procedure indicate that with the administration of MK-801, there is a lengthening of temporal estimation (rightward shift), and for the DRL schedule there is a shortening of temporal estimation (leftward shift). Chapter 2 concluded that this difference was not due to timing per se but rather to a failure to inhibit responding that would necessarily result in a leftward shift in the inter-response-times (IRTs) for DRL based on the task constraints. The change in base-rate of responding is consistent with this interpretation. Interestingly, the change observed in the threshold values also can illuminate reasons for the conflicting findings between PI and DRL. As the threshold values increases, animals begin to respond much sooner in the trial regardless of changes in clock speed (See Appendix B & Figure 15). Since the animal can make only one response per trial in the DRL task, an increased threshold value would result in a leftward shift in the IRTs for DRL. Since increases in both threshold and base-rate of responding would cause the leftward shift in IRTs in the DRL task, it is difficult to determine from this study whether the one or both of these are actually responsible for this shift.

Three SET parameters were not altered during the simulations, these include: memory size, variability of memory transformation and variability of threshold. Memory size could have had an effect during the transitions stages between drug states (i.e., Baseline to Drug 1 and Drug 3 to Drug 4 testing blocks); however, a decision was made to fix this parameter prior to any simulations. The memory size value was set based on the data from the temporal criterion switch in Chapter 3. Increases in the value for variability of memory transformation result in a rightward shift, an increase in variability, and an increase in the right tail of the temporal response.
functions (Figure 11D). However, the increase observed in the right tail became more pronounced as testing proceeded, rather than the reduction in the tail observed in the data. So, changes to this parameter would not improve the simulation fits to the data. Finally, increases in variability of threshold result in greater variability in the temporal response function; however, these changes were not in a manner that improved the fits to the data.

In summary, SET can reproduce that the effects of MK-801 on timing and temporal processing, and furthermore these effects of MK-801 on the mechanisms of timing and temporal processing are wide ranging. To account of the effects of MK-801, five of the eight SET parameters needed to be altered including, clock speed, variability of clock speed, memory transformation, threshold, and base-rate of responding. Memory size, variability of memory transformation and variability of threshold were not altered to simulate the MK-801 data. These findings are at least partially consistent with the conclusions of Chapter 2 that the lengthening observed in the present study is most consistent with a slowing in the speed of the “clock” and that MK-801 increases response rate, suggesting a decrease in response inhibition. However, based on the results of the SET modeling simulations, a conclusion must be drawn that MK-801 affects more timing mechanisms than just clock speed alone. Instead, administration of MK-801 appears to affect a wide range of mechanisms used in short-interval timing. In addition, these finding may explain the difference between the effects on MK-801 on the PI and DRL procedures.
CHAPTER 5. GENERAL DISCUSSION

The neuropharmacology of timing and temporal processing has been the focus of much empirical investigation aimed at understanding the biological basis of time perception in humans and other animals. In order to better understand the neurobiology of temporal processing in animals, research has utilized techniques including behavioral manipulations, observations of clinical populations, and neurobiological manipulations, such as drugs and lesions. Much of this work has focused on the roles of dopamine, acetylcholine, and 5-hydroxytryptamine (5-HT or serotonin) in temporal processing using a variety of different time production and estimation tasks. Another neurochemical that has been investigated for its effects on temporal processing is glutamate (Sanger, 1992; Stephens & Cole, 1996; Tonkiss et al., 1988; Welzl et al., 1991). Glutamate is a prominent excitatory transmitter found in the central nervous system (Cotman et al., 1987), and the N-methyl-D-aspartate (NMDA) glutamate receptors have been implicated in both spatial learning (Morris et al., 1986) and temporal processing (Tonkiss et al., 1988).

The aim of the current set of studies was to explore the effect of the NMDA receptor antagonist, MK-801, on timing and temporal processing using the peak-interval (PI) procedure. Specifically, the effects of MK-801 on timing of a previously acquired temporal criterion and on the learning of a new temporal criterion were examined. The results from the timing of a previously acquired criterion indicated that administration of MK-801 caused an over-estimation of time that attenuated with continued testing, increased peak-rate of responding, and increased variability in a non-scalar manner. Modeling these effects using Scalar Expectancy Theory (SET) simulation suggested that MK-801 has a wide range of effects on the mechanisms of timing, including clock speed, variability of clock speed, memory transformation, base-rate of responding, and threshold. With regard to the effects of MK-801 on learning a new temporal
criterion, the MK-801 did not have noticeable effects on the rate of learning a new temporal criterion; however, the same dose did not impair two tests of spatial memory.

Previous research on the effect of NMDA receptor antagonists on timing had been limited to a single timing task, the differential-reinforcement-of-low-rate of responding (DRL) schedule (Sanger, 1992; Stephens & Cole, 1996; Tonkiss et al., 1988; Welzl et al., 1991). This research on the DRL task found that NMDA receptor antagonists have three main effects: increased response rate, reduced efficiency for obtaining a reinforcer, and a shortening of the distribution of inter-response-times (IRTs: Sanger, 1992; Stephens & Cole, 1996; IRTs: Tonkiss et al., 1988; Welzl et al., 1991). The leftward shift in IRTs was interpreted as an underestimation of time, possibly due to speeding up the clock used for short interval timing or shortening of the remembered time of reinforcement (Tonkiss et al., 1988; Welzl et al., 1991).

The major findings from the present study using the PI procedure were effects on timing: an over-estimation of time and a disruption of the motor action system (lack of response inhibition). The rightward shift in the distribution of peak-times (over-estimation of time) found in the present study using the PI procedure stands in contrast to the underestimation of time observed in DRL tasks. Based on the findings from the PI procedure, an alternative explanation of the shift in IRTs for DRL could be a drug-induced increase in response rate or impairment in the ability to withhold lever responses for the length of the target duration. The SET simulations found that the disruption of the motor system can be attributed to two factors: an increase in base-rate of responding and a change in the threshold of responding. With regard to the effects of MK-801 in disrupting motor inhibition, we found that MK-801 increased the rate of responding. In the PI procedure animals are free to emit any number of responses per trial; however, on the DRL task animals may make only one response per trial. The increase in base-rate of responding
results in an animal being more likely to make a response independent of its estimation of time. So, failure to inhibit this tendency to respond would result in a leftward shift IRTs on the DRL independent of time estimation. The effect on the PI procedure is much different; an increased base-rate of responding would result in an overall increase in the tails of the response distribution, without a change in the peak-time. Threshold changes may also contribute to the differ results observed between the DRL and PI procedures. By increasing threshold, the decision to ‘respond’ is made much earlier than normal (Figure 15 in Appendix B). Again, this change would effect the IRT distribution from DRL quite differently because of the procedural differences between DRL and PI (e.g., DRL allows only one response per trial where the PI procedure allows multiple responses); the end result would be a leftward shift in the IRTs that does not truly reflect a change in the accuracy of timing.

SET simulations also suggested that the over-estimation of time observed in the PI is consistent with a combination change including a slowing of clock speed and an increase in the value of the memory transformation parameter. Changes to either of these parameters alone results in over-estimations of time; in combination, however, these changes help explain the effects of MK-801 on timing. Specifically, slowing of clock speed with an increase in memory transformation constant resulted in an immediate over-estimation of time that did not renormalize, as was observed with administration of MK-801. Furthermore, when the clock speed and memory transformation parameters were reset to baseline values (e.g., the cessation of MK-801 administration), temporal estimation returns to baseline level without any effect of the previous parameter changes.

The effects of MK-801 administration can be compared to the effects of other neurobiological manipulations. Previous studies on DRL have generally assumed that the effects
of NMDA antagonists are due to disruption of hippocampal function for two main reasons (Tonkiss et al., 1988; Welzl et al., 1991). First, NMDA receptors have been found throughout the brain with high concentrations in telencephalic regions and the highest concentrations in the hippocampus (Monaghan & Cotman, 1985). Second, administration of NMDA receptor antagonists results in impairment of spatial learning (Morris et al., 1986) and temporal processing (Tonkiss et al., 1988) similar to those observed with hippocampal lesions. However for the PI procedure, the effect of MK-801 on peak-times was different from previous reports of the effect of hippocampal lesions. Lesions of the hippocampal system have been observed to produce a gradual and permanent leftward shift in peak-times, whereas we observed an abrupt rightward shift that appeared to partially renormalize with continued training (Buhusi et al., 2004; Meck et al., 1987; Olton et al., 1988). Although disruption of the hippocampus does not seem to explain the effects on MK-801 on timing, the effects of MK-801 on rate of responding may involve the hippocampus. Previous studies have suggested a link between hippocampus and response inhibition (Jarrard, 1973; Tracy et al., 2001), which would be consistent with the increase in responding rate observed with MK-801 administration.

Since the over-estimation of time observed with MK-801 are inconsistent with the effects of hippocampal lesions on peak-time in the PI procedure, the MK-801 data can be compared to the effects of neuropharmacological manipulations. The effects of MK-801 on timing and temporal processing are similar in some ways to distortions observed with drugs that antagonize the dopamine system (Hinton & Meck, 1997; Maricq & Church, 1983; Maricq et al., 1981; Meck, 1983, 1986; Meck & Church, 1987b). Dopamine antagonists produce immediate over-estimates of time (rightward shifts in peak-time) that renormalize with continued training (Maricq et al., 1981; Meck, 1996). Upon removal of such antagonists, an immediate under-
estimate of time (leftward shift in peak-time) occurs, which also renormalizes with additional training; a pattern that has been called a rebound (Maricq & Church, 1983; Meck, 1983, 1996). Based on these results, the dopamine system has been hypothesized to have an effect on the speed of the internal pacemaker, or clock (Meck, 1983, 1986, 1996; Meck & Church, 1987b).

The notion that MK-801 may be interacting with the dopamine system to produce the clock speed shifts is supported by reports of interactions between NMDA receptor antagonists and the dopamine systems on behavior (Jeziorski et al., 1994; Marek et al., 1991; Trujillo & Akil, 1994) and memory processes (Castellano et al., 1999; Castellano et al., 1984; Cestari & Castellano, 1997; Quevedo et al., 1997). However, these effects appear to rise from interactions between the NMDA receptor and the dopamine systems rather than from any direct effects.

The temporal patterns of change with MK-801 differ from the effects of dopamine antagonists in a few respects, however. First, peak-times shifts under MK-801 did not fully renormalize to the criterion time even after fifteen testing sessions. However, peak-times did continue to drift back toward the criterion time. A possibility remains that peak-time may have renormalized given a sufficient amount of further testing. Second, there was no meaningful rebound (an abrupt and transient shift in peak-times in the opposite direction) following cessation of MK-801 administration, as is typically the case with dopamine antagonists (Meck, 1983, 1986, 1996; Meck & Church, 1987b). These differences suggest that MK-801 may influence other timing mechanisms in addition to clock speed. One such possibility is an alteration in the scaling of the memory representation ($K^*$: see Chapter 4 & Appendix B).

Manipulations that cause changes in the scaling of memory are characterized by gradual shifts in peak-time that eventually stabilize, and that do not renormalized with continued (Hinton & Meck, 1997; Meck, 1996). This pattern of disruption is observed with systemic
pharmacological manipulations of the cholinergic system (Hinton & Meck, 1997; Meck, 1983, 1994, 1996, 2002; Meck & Church, 1987a, 1987b) as well as lesions to the frontal cortex, nucleus basalis magnocellularis (NBM), medial septal area, and hippocampus (Buhusi et al., 2004; Meck et al., 1987; Olton et al., 1988). Systemic administrations of cholinergic agonists, as well as lesions of the medial septal area, and hippocampus, have been shown to cause gradual and sustained leftward shift in peak-time (Buhusi et al., 2004; Meck, 1996; Olton et al., 1988). Systemic administrations of cholinergic antagonists, as well as lesions of the frontal cortex and NBM, have been shown to cause gradual and sustained rightward shift in peak-time (Meck, 1996; Olton et al., 1988). The sustained rightward shifts with MK-801 appear similar to the sustained changes observed with cholinergic antagonists. The gradual and sustained rightward shift in peak-times has been attributed to an increase in the value of K* (Meck, 1983, 1996; Meck & Angell, 1992; Meck & Church, 1987a). In fact, MK-801 has been reported to interact with the cholinergic system reducing acetylcholine function (Arias et al., 2001; Covasa et al., 2003; Cui, Urich, & Hess, 2004; Fenu et al., 2001; Lydic & Baghdoyan, 2002; Pessoa, Castro, & Noel, 2005). Indeed, results from SET simulations indicate that administration of MK-801 increases that values of memory transformation (K*), in a fashion similar to choline antagonists.

Based on the SET simulations, simultaneous alterations of clock speed and memory transformation can cause immediate shifts in peak-time that do not fully renormalize with additional training, which is similar to pattern observed under administration of MK-801 (see Chapter 4). Interestingly, when these two parameters are returned to their normal states, there is no observable rebound in peak-time as was observed with administration MK-801. This finding suggests that the effect of MK-801 on timing may be an indirect effect expressed through a combined interaction between MK-801 and the dopaminergic (Jeziorski et al., 1994; Marek et
al., 1991; Trujillo & Akil, 1994) and cholinergic systems (Arias et al., 2001; Covasa et al., 2003; Cui et al., 2004; Fenu et al., 2001; Lydic & Baghdoyan, 2002; Pessoa et al., 2005).

The final aim of these series of studies was to examine the effects of MK-801 on the learning of a new temporal criterion. The NMDA receptor has received much interest because of its role in learning, memory and synaptic plasticity (Morris, 2003; Morris et al., 1986; Shapiro & Caramanos, 1990; Shapiro & O'Connor, 1992). Antagonists of the NMDA receptors have been observed to have effects in both spatial (Morris, 2003; Morris et al., 1986; Shapiro & Caramanos, 1990; Shapiro & O'Connor, 1992) and nonspatial (Traverso et al., 2003; Xu & Davis, 1992) learning and memory tasks. Further, some models of timing and temporal processing predict that NMDA receptor antagonists should disrupt temporal learning. The Striatal Beat-Frequency model of timing (SBF; see Appendix B) hypothesizes that the memory for any temporal duration is stored in the form of synaptic connections between cortical and striatal cells (Matell & Meck, 2000) suggesting that the NMDA receptor, with its role in spatial learning and memory and synaptic plasticity (Morris, 2003; Morris et al., 1986; Shapiro & Caramanos, 1990; Shapiro & O'Connor, 1992), may be important for the learning of temporal durations. The SBF model hypothesizes a cortico-striatal–thalamic-cortical loop underlying the processes of timing behavior. Synaptic plasticity is theorized to be play an important role during training trials where the delivery of a reward causes a burst of dopamine in the striatum, originating from the substantia nigra pars compacta, that strengthens the connections (via synaptic plasticity) between the spiny neurons of the striatum and the cortical cells that are firing together at the passage of the criterion duration (Matell & Meck, 2000). Prior to this work, however, there have been no studies examining the effects of NMDA receptor antagonists on the learning of temporal information.
The results from this study indicated that the 0.05 mg/kg dose of MK-801 has no effect on the speed or pattern of learning a new temporal duration. The 0.05 mg/kg dose level was selected because it produced behavioral effects on the PI procedure, increases in response rate, without affecting the production of the previously learned duration, as was the case with the 0.2 mg/kg dose. A dose that did not affect production of a previously learned duration was important because the measure of learning used was the pattern of change in peak-time across trials. If the drug dose affected peak-time, it would be difficult to determine which effects were due to temporal learning and which were due to the drug administration. Although no difference was observed in rate of learning, the question of whether MK-801 can influence the learning of a new temporal duration remains unanswered because the MK-801 administration did not affect performance on either spontaneous alternation or the water maze as has been previously reported (Caramanos & Shapiro, 1994; Heale & Harley, 1990; McLamb et al., 1990; Riedel et al., 2003; Whishaw & Auer, 1989).

At least three possible reasons may explain the lack of effect of MK-801 on learning new temporal memories. One possibility is that the NMDA receptors are not involved in the process of learning temporal durations. This is still an open question because of the null effects obtained in the spontaneous alternation and water maze tasks, two task which MK-801 have been previously reported to affect (Caramanos & Shapiro, 1994; Heale & Harley, 1990; McLamb et al., 1990; Riedel et al., 2003; Whishaw & Auer, 1989). Second, the drug administered in the study was ineffective; although, this is not a strong argument because the drug administration did have positive results for both response rate and variability. If the drug was ineffective, there would be no reason why its administration would results in any behavioral effects.
A third reason may be that the task difficulty was not sufficient to cause an impairment in learning (Harder et al., 1998). Evidence from studies on discrimination tasks in monkeys suggest that task difficulty is an important factor in the appearance of deficits related to NMDA antagonists (Harder et al., 1998). The ratio of the two intervals to be learned was a simple ratio. The SBF model of time suggests that the temporal memory of two durations which a simple ratio relationship many have an overlapping neural representation (Matell & Meck, 2000). SBF proposes that coincidence detectors are the spiny neuron of the striatum and that the oscillating units are neurons of the cortex, which project to the striatum. The SBF model hypothesizes that the stimulus that signal the start of a trial resets the oscillation of the cortical neurons so that there is a synchronous beginning to the oscillations. The neurons in the cortex then continue oscillating independent of one another each with its own period of oscillation. When the cortical neurons fire synchronously in the pattern learn during training, the spiny neurons of the striatum send an output signal, that it is time to respond, to the thalamus, which in turn passes the signal onto the cortex so that the animal can initiate a response (Matell & Meck, 2000). Since 9 second and 18 second durations have a 2:1 ratio, the neural representation for 9 seconds is repeated within the 18 second duration. Therefore, little effort may have been required to learn the new temporal criterion.

A final explanation for the lack of effect of MK-801 on learning new temporal memories may be that although the NMDA receptor is important for the formation of temporal memories, the administered dose of MK-801 (0.05mg/kg) was not sufficient to cause learning impairments. The null results from spontaneous alternation and water maze tasks support this suggestion. Although similar dose levels have been reported to produce spatial memory impairments in other rat strains (Caramanos & Shapiro, 1994; Shapiro & Caramanos, 1990; Shapiro & O'Connor,
1992), the lack of effect observed in this study may be due to differences in glutamate function between rat strains (Manahan-Vaughan & Brauneewell, 2005), or animals may have also developed a tolerance for the drug because of the repeated administrations.

Future studies using higher concentrations of MK-801 are needed before a conclusion can be made about the effects of NMDA receptor antagonists on temporal learning and memory. The greatest pressing issue remaining is the further study of the effect of MK-801 on temporal learning. Future studies could first find dose levels sufficient to produce deficits on tasks such as spontaneous alternation and water maze prior to the investigation of temporal learning. In addition to exploring the effect of MK-801 on temporal learning, more research needs to be conducted to confirm the effects of NMDA receptor antagonist and timing and temporal processing. Future work could investigate other timing tasks (see Appendix A) to ensure that the results are consistent across tasks. Based on the SET simulations, strong predictions can be made about how MK-801 will affect each new task. Additionally, other NMDA receptor antagonists should be used to investigate whether the findings reported here are specific to MK-801, or if the findings are general a wider range of NMDA receptor antagonists. It remains possible that the wide range of effects on the mechanisms of time observed in the present study is specific to MK-801. Administering other NMDA receptor antagonists and modeling their effect should lead to a clear picture of which change are general effects NMDA receptor antagonists and which may be specific to MK-801.

In conclusion, the most significant results from the present set of studies are that the NMDA receptor antagonist MK-801 effects timing and temporal processing by causing over-estimation of time. This over-estimation of time can be attributed to changes in two timing mechanisms: a slowing of clock and change in scaling of memory as it passes into reference
memory. In addition to these effects, MK-801 causes an increased base-rate of responding and threshold of responding, which results in an increase in responding rate throughout the trial. The changes in base-rate of responding and threshold of responding help account for the difference observed between the DRL and PI procedures. In regard to learning new temporal memories however, the role of MK-801 plays remains unclear.
APPENDIX A: AN OVERVIEW OF ANIMAL INTERVAL TIMING TASKS

The ability to time events is an important part of daily living. Routine tasks such as walking, dancing to a beat, or optimal forging in animals all require an ability to time or anticipate the occurrence of an event in time. Timing and temporal processing has been a topic of study in both humans and non-human animals. The study of timing processes can be divided into at least four ranges: millisecond, interval, circadian, and circannual timing (Hinton & Meck, 1997). Millisecond timing deals with intervals with duration of less that a second, interval timing considers timing on the order of seconds to minutes, circadian timing examines rhythms that oscillate around 24 hours, and circannual timing takes into account timing of behavior coordinated with changes in season.

The focus of this review is on interval timing and the methods used to study it in non-human animals. Many different procedures have been used to study interval timing behavior in animals. Killeen and Fetterman (1988) proposed a taxonomy that is useful for organizing these different types of interval timing tasks. They identify three categories into which timing tasks falls: prospective, retrospective, and immediate timing.

Prospective Timing

Prospective timing tasks are tasks in which the animal emits discriminating responses based on knowledge of the intervals that will follow (Killeen & Fetterman, 1988). For example, the subject many have to choose between responses that result in either a short delay and a small reward or longer delays with a larger reward. Normal and control animals typically will select the short a delay to reward unless the amount of reward available is substantially greater for the longer deal. In such a case, animals will delay getting an immediate reward to obtain a greater reward to be delivered later (Mazur, 1984, 1986; Mazur, Snyderman, & Coe, 1985). Timing is
related to these types of tasks because they typically require the subject to choose between two behaviors that will determine the length of delay until a reward will be delivered.

Retrospective Timing

Retrospective timing tasks are tasks in which the animal emits different responses based on the duration of an elapsed interval (Killeen & Fetterman, 1988). For example, an animal may subjectively classify the duration of a stimulus as either short or long based on the type of response emitted following the termination of the stimulus. In these types of timing tasks, there is typically only one response made per trial. Two common tasks used in studying animal timing behavior that fall into this category are temporal bisection and temporal generalization.

Temporal Bisection

The temporal bisection procedure (Church & Deluty, 1977) requires animals to discriminate two target signal durations by making a response on one of two levers. This procedure can be divided into two phases: training and testing. During the training phase, trials begin with the onset of a signal, after an intertrial interval. The signal is maintained for one of two target durations: short (e.g. 2 sec) or long (e.g. 8 sec). The animal is trained to press a particular lever (e.g. the right lever) for the short duration and the other lever (e.g. the left lever) for the long duration (the pairing of levers and durations is typically counterbalanced across animal to account for response basis). A correct response leads to the delivery of a reward. No reward is given for an incorrect response. The animal is given a window of time to respond, after this time has passed or after a lever response, an intertrial interval begins. For each trial, only one response is allowed after the cessation of the signal and the start of the intertrial interval. When performance reaches a predetermined criterion (e.g. 90% correct responses) the animal is moved onto the testing phase.
During the testing phase of the temporal bisection procedure, animals continue to receive reward for correct responses to the target durations. However, a new type of trial is introduced, the probe trial. Probe trials are unrewarded trials with signal durations lasting somewhere between the two target durations. The data of interest are the lever presses in response to the stimulus duration on the probe trials (whether the right or left lever is pressed). These data plotted are the probability of a long response by the signal durations. The result is an ogive, or s-shaped, curve that shows an increase probability of a long response with increased signal duration. The point at which the probability of a long response is .50 is labeled the point of subjective equality, PSE (Church & Deluty, 1977). Conceptually, the PSE is the point at which the animal cannot consistently classify a duration as being either short or long. In normal and control animals the PSE has typically been reported as being at the geometric mean of the two target durations (Church & Deluty, 1977; Maricq & Church, 1983; Maricq et al., 1981; Meck, 1983). Other important measures from temporal bisection are the difference limen (DL) and Weber fraction. The DL is essentially a measure of spread or reliability of the PSE. It is calculated by taking half the difference of the duration associated with a long response 25% and the duration associated with a long response 75%. The Weber fraction is a measure of proportionality of the data. It is used to normalize data that were presented with differing durations because the longer durations tend to have larger DL values. The Weber fraction is calculated by divided the DL by the PSE (Church & Deluty, 1977; Meck, 1983). If the data is proportional, Weber fractions will be equal.

**Temporal Generalization**

The temporal generalization procedure (Church & Gibbon, 1982) is similar to temporal bisection in that the subject animal makes a response decision based on a presented stimulus.
However in this task, there is only one target duration, as opposed to the two target durations used in temporal bisection. A single trial begins, after an intertrial interval, with the initiation of the trial stimulus. The stimulus stays on for some period of time, and then is turned off. If the signal duration was equal to the target duration, a lever press response leads to the administration of a reward. If the signal duration was either longer or shorter than the target duration, no reward is administered. The trial ends and an intertrial interval begins after a response is made or after a response window has elapsed (i.e. 5 seconds), whichever occurs first.

The data of interest from the temporal generalization procedure are lever presses in response to the different signal durations, specifically, whether or not a response was made for a given stimulus duration. The data can be graphed by plotting the probability of response by duration (Church & Gibbon, 1982). When plotted, these data look quite different than the data from temporal bisection. Instead of an s-shaped curve, the plotted data from temporal generalization appear to approximate a normal distribution with a peak near the time of the target duration. Measures of interest are the peak-time, which is determined by locating the bin at which the distribution is at its maximum value, and the spread, or variability, of the distribution.

Immediate Timing

Immediate timing tasks are tasks in which the animal can emit responses during the ongoing presentation of an interval to be timed (Killeen & Fetterman, 1988). In these types of timing tasks, the subject is typically free to respond at any point during the interval; however, responding is only maximally efficient around the time of the target duration. Differential-reinforcement-of-low-rate of responding schedule, the fixed-interval procedure, and the peak-interval procedure and its variations all fall into the immediate timing category.
Differential-Reinforcement-of-Low-Rate of Responding Schedule

On the differential-reinforcement-of-low-rate of responding (DRL) schedule (Kramer & Rilling, 1970; Zeiler, 1977), subjects are trained to emit an operant response (typically a lever press) after the passage of a target duration following the last response. If the next response falls after the target duration has passed, then the subject receives a reward and a new interval begins. If the next response occurs before the target duration elapses, then the duration resets and no reward is given.

Subject performance under DRL schedules is typically characterized by low responding rates. Data are plotted with an inter-response-time (IRT) frequency histogram. This distribution has a primary peak approximately at the target duration. Additionally, the distribution may have a secondary peak around short IRTs (Harzem, 1969; Platt, 1979; Wearden, 1990).

Fixed-Interval Procedure

In the fixed-interval (FI) procedure (Ferster & Skinner, 1957), a reward becomes available to a subject animal after an operant response (typically a lever press) has been performed following the passage of a target duration. For an FI procedure with discrete trials, the FI trial begins with the onset of a stimulus. The subject is free to make any number lever responses during the presentation of the stimulus, however, no reward is presented until the target duration has elapsed and a lever response is made.

When the data is examined over the course of several trials, a subject tends to respond at lower rates at the beginning of the trial, and increasingly high rates toward the end. This low-high pattern of responding has been called a “break-run” pattern (Schneider, 1969). When data are collapsed across trials, there appears to be an increase in responding from the start of the trial that increase faster as the time of the target duration approaches; responding drops off.
dramatically once the target duration has passed. This pattern of responding has been labeled the FI-scallop (Boren, 1960; Ferster & Skinner, 1957; Hanson, Campbell, & Witoslawski, 1962; Trapold, Carlson, & Myers, 1965).

**Peak-Interval Procedure**

The peak-interval (PI) procedure (Catania, 1970; S. Roberts, 1981) is a combination of reinforced FI trials and probe trials. No reward is given on probe trials, and the trial typically lasts at least twice that of the target duration from the FI trials. Probe trials are added in order to see how responding changes after the expected time of reward has passed. This information is not present in FI trials because lever pressing rates drop off dramatically after the target time because of the presentation of the reward.

The data of interest from the PI procedure are obtained by summing and binning lever responses across trials; when this is done the data appear to have an approximately normal distribution with a slight positive skew; this distribution is referred to as a peak function (Hinton & Meck, 1997; S. Roberts, 1981). Three variables can be obtained from the peak function: peak-rate, peak-time, and spread, or variability. Peak-rate is the maximum rate of responding, and generally occurs near the FI target duration. Peak-time is the mode of the peak function, and is what is used to infer the subject’s remembered time of reinforcement. While the peak-time and peak-rate are located at the same point of the peak function (peak-time is location on the x-axis while peak-rate is location on the y-axis), these two variable can be manipulated independent of each other by changes in the experimental procedure (S. Roberts, 1981). Spread is the width of the peak function at a specified percentage (typically 50%) of the maximal responding rate and is taken to be a measure of the precision of temporal discrimination (Hinton & Meck, 1997). A Weber fraction can be obtained by dividing the spread by the peak-time.
Peak-Interval Gap Procedure

The PI gap procedure (Meck, Church et al., 1984; Meck et al., 1987; Olton et al., 1988; S. Roberts, 1981) is a modification of the PI procedure that included a “gap”, or “break”, in the presentation of the stimulus during probe trials. The gap trial starts just like an FI or standard prove trial, with the onset of the stimulus. After some time the stimulus is turned off for the duration of the gap, and then is turn back on. The trial continues as a probe trial until the onset of the intertrial interval. This gap was added to probe trials to test working memory (S. Roberts, 1981). A testing session on the PI gap procedure includes FI trials, probe trials, and probe trials with the gap.

Three patterns, of how a subject might respond to the gap, have been described: run, stop, and reset (Meck et al., 1987; Olton et al., 1988; S. Roberts, 1981). In the run pattern, the subject ignores the gap, estimating time from the initial onset of the stimulus. In this case, the peak-time would be identical to that of the probes trials without gaps. For the stop pattern, the subject stops timing when the stimulus goes off. The amount of time that had passed prior to the gap is held in working memory through the gap, and time begins from that point when the stimulus resumes. Peak-time for a stop pattern would be shifted longer by the amount of the gap duration. The reset pattern supposes that the subject stops timing when the stimulus goes off, and when the stimulus resumes, the subject begins timing as if a new trial has started. No part of the interval prior to the gap is retained in the reset pattern. Peak-times for a reset pattern would be shifted by the duration of the gap plus the duration of the pre-gap interval.

Studies suggest that different species may respond to the gap differently. Rat appear to have the stop pattern (Meck, Church et al., 1984; Meck et al., 1987; Olton et al., 1988; S. Roberts, 1981) and pigeons appear to display a reset pattern (Cabeza de Vaca et al., 1994; W. A.
Roberts, Cheng, & Cohen, 1989). However, there are recent indications that rats and pigeons, under the right conditions, can either stop or reset. In addition, they may at times have a pattern of responding that falls somewhere between stop and reset, which has been labeled a partial reset pattern (Buhusi & Meck, 2000; Buhusi et al., 2002; Cabeza de Vaca et al., 1994; W. A. Roberts et al., 1989).

Prior-Entry Method

The prior-entry (PE) method (Meck, 1984; Penney, Holder, & Meck, 1996) is an alteration of the PI procedure designed to test attention. In the PE method, subjects are trained on two FI intervals that are cue by stimuli of different modalities (e.g. light vs. tone). Then probe trials are added so that trials may either be an FI light, FI tone, probe light, or probe tone. In the next phase, warning cues are added so that light trials are preceded by a light cue and tone trials are preceded by a tone cue. The cues are believed to direct attention to the upcoming trials and give a cue as to which modality trial to expect (Meck, 1984; Penney et al., 1996). In the final testing phase, the cues were sometimes mismatched, for example a light cue would be follow by a tone stimulus. The mismatched condition has been labeled the prior-entry-reversal (PER) method (Meck, 1984). Data from the PE method has shown a slight shortening (leftward shift) of the peak-time, while data from the PER method has shown a lengthening (rightward shift) of the peak-time.

Simultaneous Temporal Processing

During simultaneous temporal processing (STP), a subject may be required to time two or more intervals concurrently (Leak & Gibbon, 1995; Meck, 1987; Meck & Church, 1984; Meck & Williams, 1997; Olton et al., 1988; K. C. Pang, Yoder, & Olton, 2001; K. C. H. Pang & McAuley, 2003). This procedure is used to test a subject’s ability to divided attention between
multiple stimuli. In one STP method (Olton et al., 1988), the subject is first trained on an FI procedure followed by a PI procedure each with two or more distinct stimulus each associated with different target durations. During an STP session, the different stimuli are presented concurrently with differing onsets. STP trials may consist of concurrent FI trials, concurrent peak trials, or a mixture of FI and peak trials. Another STP method, also begins with training on FI and PI procedures, however the STP session follows a different pattern. The trial begins with the onset of the one of the stimuli, then after some delay, the second stimulus is initiated. Both stimuli remain on together for a while before one of the two is turned off. The subject just then finishes timing the remaining stimuli (Meck & Williams, 1997). The STP trials can be either rewarded or probe trials. Both rat (Meck, 1987; Meck & Church, 1984; Meck & Williams, 1997; Olton et al., 1988) and pigeon (Leak & Gibbon, 1995) normal/control animals can be trained to accurately time up to three different durations that are presented in different modalities.

Conclusion

In sum, prospective, retrospective, and immediate timing tasks each have differing strengths and weaknesses. Prospective timing tasks gives information about what duration an animal anticipates will occur. However, the response is also very much dependent on the expected magnitude of reinforcement at the end of the delay. It is difficult to separate the influences of the duration and reward in these types of tasks and therefore prospective timing task are not frequently used to assess temporal perception and processing in animals.

Retrospective and immediate timing tasks are much more commonly employed in the study of timing and temporal processing. Retrospective tasks give insight into the animals subjective experiences of a given duration, specifically how it relates to previously learned durations. An advantage of these types of tasks is that an animal need only give one response per
trial. So if a biological manipulation affects speed, consistency, inhibition or ability of responding, these types of tasks may still be useful to gain insight into the time perception of the subject. However, these tasks only give information about how an animal perceives the duration of an event relative to other durations. In order to determine an animal subjective estimate of a duration, or how long something should last, then immediate timing tasks are necessary.

In immediate timing tasks, the responses emitted by an animal give insight into the perceived duration of an interval being timed. In these types of timing tasks, the animal is typically free to respond at any point. However, responding is only maximally efficient around the time of the target duration, so the animal typically learns to withhold responded until the time at which the subjective end of the duration is near. A major advantage of these types of tasks is that the animal itself marks the perceived passage of time, so the subjective experience of the animal is more obvious. However, these tasks are more sensitive to biological manipulations that affect speed, consistency, inhibition or ability of responding, and such manipulations may make data difficult to interpret. Another disadvantage of these tasks is that no information is given about how the animal perceives the duration of an event relative to a different duration; retrospective timing tasks are better suited for this type of information.
APPENDIX B: THEORIES OF ANIMAL TIMING

Examination of the data from the various timing tasks has lead to the discovery of properties that are similar across the various tasks. When the data are plotted on a normalized time scale, the distributions appear to overlap, superposition (Gibbon, 1977). Quantitative support of this superposition has been found in that the coefficient of variation (standard deviation / mean) is approximately the same across intervals. Based on these findings, the standard deviation and mean of a timing distribution has a scalar relationship and holds to a generalized form of Weber’s law (Gibbon, 1977). This property is commonly referred to as timescale invariance (Church, 2003). Scalar Expectancy Theory (SET) isn’t simply a description of how time is accuracy estimated, but it is an attempt to characterize the nature of variability in timing; including the different properties and sources of variability that result in the observed timescale invariance (Gibbon, 1977).

Scalar Expectancy Theory

An information processing (IP) model of SET has been proposed to address the sources of variance and the mechanisms involved in interval timing tasks (Church, 1984; Gibbon et al., 1984). A diagrammatic representation of the IP model of SET (for the remainder of this discussion the IP model will be simply referred to as SET) is shown in Figure 13. SET is composed of three general stages: clock, memory, and decision. Each of the stages has individual components with their own properties, which will be examined individually later.

Although SET has been utilized to explain data from most of the timing tasks previously described, the following description will be discussed as the model applies to the PI procedure. In general, the pacemaker emits a continuous stream of pulses. The onset of stimulus initiates the closing of a switch, which allows the pulses of the pacemaker to flow into the accumulator. In
Figure 13: An information processing model of Scalar Expectancy Theory. The model is divided into three stages: clock, memory, & decision. The parameters of the model include: Λ – speed of the pacemaker, \( D_T = T - T_0 \) – duration of switch closure, \( A_T \) – number of pulses accumulated, \( M_T \) – value stored in working memory, \( K^* \) - memory storage constant, \( M_{S+}' \) – value stored in references memory, & \( B \) – threshold value.
the accumulator, the pulses are summed over the duration of the stimulus. The count from the accumulator gets passed along to working memory. The comparator then compares the information in working memory with a value pulled from reference memory. If the ratio of working memory to reference memory is less than some selected threshold value, then the animal responds; if not, the animal does nothing. If upon responding the animal receives a reward, then the value currently held in working memory gets stored in reference memory.

Components of Scalar Expectancy Theory

The previous section illustrated the flow of information through SET from the onset of a stimulus to the animal’s response decision. This section will describe some of these properties and discuss how each part adds variance to the output. The discussion of each component will be organized according to stage: clock, memory, or decision.

Components of the Clock Stage

The clock stage consists of three components: pacemaker, switch, and accumulator. As mentioned above, the pacemaker is a mechanism that emits a continuous stream of pulses that conforms to a Poisson distribution with a mean rate of \( \Lambda \) (Church, 1984; Gibbon, 1992; Gibbon et al., 1984). While this mean rate is generally consistent, it can be altered by drug, dietary, or environmental changes (Church, 1984; Hinton & Meck, 1997; Maricq et al., 1981; Meck, 1983, 1996).

The switch is responsible for the transference of the pulses from pacemaker to the accumulator (Church, 1984; Gibbon et al., 1984). When the switch is opened, pulses cannot reach the accumulator, however, when the switch is closed, the pulses are able to be flow freely into the accumulator. The switch’s default setting is open, and it remains open until the onset of a stimulus, which is believed to be the signal for closing the switch. Likewise, when a stimulus is
terminated, the switch is reopened. Independent random delays in the closing ($t_1$) and opening ($t_2$) of the switch add to the total variability of timing (Figure 14A). The length of time that the switch remains closed ($D_7$) differs from the total length of the stimulus ($T$) by the difference between $t_1$ and $t_2$ (Gibbon et al., 1984). The delays in closing and opening the switch can be altered by changes in attention such that increases in attention reduce latency to closure (Meck, 1984; Penney et al., 1996).

The accumulator sums the number of pacemaker pulses during the closure of the switch (Church, 1984; Gibbon et al., 1984). The number of pulses summed in the accumulator at any given moment is the estimation passage of subjective time (Figure 14B). The expected value for the accumulator count is the product of mean rate of the pacemaker and the duration of the switch closure.

Components of the Memory Stage

The memory stage consists of two components: working memory and reference memory. The working memory component is continually updated with information from the accumulator. Working memory is important for its role in temporarily storing the information held in the accumulator (Church, 1984; Gibbon et al., 1984). This memory component is important for retaining pre-gap durations on PI-gap trials and for retaining the duration of a stimulus on trials consisting of retention interval prior to the opportunity for response.

Reference memory stores information about previous trials (Church, 1984; Gibbon et al., 1984). This component consists of a distribution of remembered durations that is randomly sampled from in the decision stage. Reference memory is updated after each reinforced trial, and on probe trials, the information from working memory is not passed into the reference memory. On reinforced trials, the count that is being held in working memory is transferred into reference
Figure 14: SET representation of the switch (Panel A) and working memory (Panel B).
Parameters include: T – time duration of stimulus, t₁ – delay for switch to close, t₂ – delay for switch to open, T₀ – net delay for switch, Dₜ – duration of switch closure, Aₜ – number of pulses in accumulator, Λ – speed of the pacemaker, & Mₜ – working memory representation of time.
memory and is transformed by a memory storage constant, K* (Figure 13). Perfect transfer from working memory to reference memory is achieved with a K* value of 1.0. If K* is above 1.0, then the remembered duration is longer, and if K* is less than 1.0, then a shorter duration is stored. This memory transformation during the storage does not have an inverse (1/K*) transformation during retrieval (Church, 1984), so once a memory is transformed it remains in that state. While this constant many vary from animal to animal, the memory storage constant any one animal generally remains reliable, however, it can be altered by factors, such as drugs and diet (Hinton & Meck, 1997; Meck, 1994, 1996, 2002; Meck & Church, 1987a, 1987b).

Components of the Decision Stage

The decision stage primarily consists of one major component, the comparator. The comparator’s function is to determine whether to respond, or not to respond based on a decision rule (Church, 1984; Gibbon et al., 1984). The comparator receives input from three main sources: working memory, reference memory, and a distribution of threshold values (Figure 13). The count stored in working memory is imported directly into the comparator. The working memory value is compared, via a decision rule (Figure 13) to a value randomly selected from the distribution of remembered times held in the reference memory.

A threshold value, B, is randomly selected from a distribution of values and is the criteria used in the decision of whether or not to respond (Figure 15A). When the value of the decision rule is above the threshold value, then the comparator outputs do not respond. When the value passes below the threshold value, the output switches to respond. If the stimulus remains on, eventually the value from the decision rule will become greater than the threshold value and the output will again switch to do not respond.
**States of Responding on Individual Trials**

The decision rule used in SET has been employed to account for data from probe trials in the PI procedure that indicate animals switch between states of responding during individual trials, and has been called the *break-run-break* pattern (Cheng & Westwood, 1993; Church et al., 1994; Gibbon & Church, 1990). The length of the run state depends on the threshold and the duration of the interval being timed (Figure 15A). The length of a duration, S, has a narrower run state as compared to a duration twice that length, 2S. However, when scaled on a relative time scale (objective time divided by the target duration), the two responding windows overlap (Figure 15B).

**Other Timing Theories**

SET is not the only theory of timing used to explain results from animal research. Other theories of timing, some similar and some quite different that SET, also exist. The next section will give a brief overview of some of these other theories and compare them to what is proposed by SET.

**Behavioral Theory of Timing**

The Behavioral Theory of Timing (BeT) is a theory that emphasizes observed stimuli and behavior in the tradition of quantitative behavioral theories (Killeen & Fetterman, 1988). BeT assumes that an animal tracks time by traversing through a series of behavioral states. These behaviors might be overt and visible to an observer (e.g. grooming, sniffing in the corner, or turning in a circle), or they may be covert behaviors that are not observable (e.g. tensing of different muscle groups). The animal learns the duration of an interval by associating the delivery of a reward with a particular behavior during the behavior chain. Changes in behavioral states are triggered by pulses from a pacemaker. BeT assumes a pacemaker that generates pulses...
Figure 15: The decision stage of SET. When the subjective discrepancy becomes less than the threshold (B) the subject enters a run/respond state (shaded area); responding continues until the subjective discrepancy becomes larger than the threshold at which time the subject returns to a break/no responding state. Timing longer durations result in larger variability (shaded area: Panel A), however when time is normalized to a relative time scale, variability is approximately equal (shaded area: Panel B).
according to a Poisson process. The rate of the pacemaker is not fixed, but varies with the rate of reinforcement (Bizo & White, 1994; Killeen & Fetterman, 1988).

BeT is a behaviorally oriented approach to explaining data obtained from timing tasks in response to the more cognitively oriented SET model. One feature that is similar between the two is a pacemaker with a Poisson process that emits a steady stream of pulses. However, in SET, the rate of the pacemaker is generally fixed with a rate assumed to be around 5 pulses per second, and in BeT, the rate of the pacemaker varies with the rate of reinforcement and is assumed to generate about 3 or 4 pulses per reinforcement (Church, 1997; Killeen & Fetterman, 1988; Meck, Church, & Gibbon, 1985). SET describes the representation of time as a number of pulses and BeT represents time as a behavioral chain. Response decisions are assumed to be made by a decision rule in SET, however, the decision to response in BeT is made from strength of association of reinforcement with a particular behavior in the sequence of the behavioral chain.

**Connectionist Timing Model**

The Connectionist Timing (CT) model (Church & Broadbent, 1990a, 1990b) has been developed as an alternative to the IP model of SET. The CT model is designed to only require standard assumptions of neural network operation, in contrast with some of the components of SET, which require activities that are difficult to implement biologically (Church & Broadbent, 1990a, 1990b). The clock of the CT model is constructed from a bank of oscillators, each having a unique period. Church and Broadbent (1990a) proposed a bank of 11 coupled oscillators, with the fastest oscillating near 200 milliseconds, and with each consecutive oscillator double that period of the previous one (e.g. 200ms, 400ms, 800ms, etc.). However, if this bank contained 30 oscillators, then the slowest oscillator would not complete a cycle in the lifetime of a rat. Thus,
any point in time has a unique representation in the bank of oscillators. The bank of oscillators is reset by stimuli, such as the start of a trial.

Each oscillator has an accompanying status indicator that detects the half-phase (+1 or -1) of the corresponding oscillator. The status indicators output a vector of values recording the half-phase of each oscillator, which is the representation of time (Church, 1997; Church & Broadbent, 1990a, 1990b). In working memory, information from this output vector is transformed into an autoassociation matrix, which retains information about the relationship between adjacent status indicators. Reference memory is composed of an autoassociation matrix, with the same dimensions as working memory, which stores the time of reinforcement from all previous trials. Upon reinforcement, information from working memory is combined with reference memory by a linear rule that specifies the new content of reference memory being 99% the value of reference memory and 1% the value of working memory (Church, 1997; Church & Broadbent, 1990a, 1990b; Hinton & Meck, 1997). A decision rule compares weighted average vector from reference memory to the current content of working memory, and a decision is made based on the relationship of these two vectors to a threshold, similar to SET.

The CT and SET models have a similar organization and flow of information. The pacemaker in SET is comparable to the bank of oscillators in the CT model. The accumulator is replaced in CT by the status indicators. The information then flows into working memory and, upon reinforcement, into reference memory, however the content of these memory differ between SET and CT models. Finally, both models propose a comparator, but each model has different decision rules based off the nature of the content in memory.
Other Neural Network Based Models of Timing

The CT model is just one model that has attempted to mimic biological mechanisms when constructing a model of timing behavior. Other models use mathematically based neural networks to account for timing behavior. These models can look quite different than IP models, such as SET. The Spectral Timing model (Grossberg & Schmajuk, 1989) and a Beat-Frequency model (Miall, 1989) are two such neural network models.

Spectral Timing Model

The Spectral Timing model is designed to simulate trace conditioning experiments, a classical conditioning design where the animal anticipates the presence of an unconditioned stimulus (US) sometime after the presentation and removal of a stimulus (Grossberg & Schmajuk, 1989). However, it also can simulate data consistent with operant timing task, such as the PI procedure. The model is able to learn an association in the absence of a conditioned stimulus (CS), because an internal representation of the CS (ICS) is activated during the presentation of the CS. The ICS then remains active until the end of the trial. The signal from the ICS outputs to an array of units, or activation spectrum, with different activation functions that peak at different points in time when activated. The signals from activation spectrum are gated by another array of units, the gated signal spectrum, which are in turn differentially associated with the US based on the duration of the delay between the presentation of the CS and the US. With slight modifications, the Spectral Timing model can also simulate data from the PI gap procedure (Hopson, 1999). To accomplish this, the ICS is removed from the model, and the activation spectrum responds directly to the CS. In addition, a decay process is added to the activation spectrum, such that, the value of the activation function is gradually reduced when the CS is removed. Interestingly, the modification that enables this model to simulate the data from
the PI gap procedure also renders it incapable of simulating the trace conditioning data, which it was originally designed to simulate.

The Spectral Timing model differs from SET in many aspects. There are no pacemaker or pacemaking units that present in SET. Although the accumulator in the SET might be considered analogous to the activation function of the Spectral Timing model, with an increased count in the accumulator corresponding to an increase in the value of the activation functions. However, in SET there is one accumulator used to time a single signal, and there are multiple activation units and functions in the Spectral Timing model. Memory in the Spectral timing model is stored within the weights of the connections between the gated signal spectrum and output unit, whereas in SET, memory is a distribution of counts stored from previous trials.

A Beat-Frequency Model

Another neural network based model designed to simulate timing data is the Beat-Frequency model (Miall, 1989, 1992, 1996). The Beat-Frequency model is intended to learn time intervals using a population of oscillating neurons, which project to a single output neuron. The population of oscillating neurons consists of around 500 pacemaking units that oscillate with frequencies between 5-15 Hz, with an average frequency near 10 Hz and a standard deviation of 1.6 Hz (Miall, 1989, 1992, 1996). Units have output values of either 0 or 1. Unit threshold are set so that the unit has a 0 value for 85-90% of its cycle and a value of 1 for the remaining 10-15% of the cycle. In this model, a time interval can be stored by a group of pacemaker units that are all active, having activation value of 1, at the same time. The duration of time between repeated simultaneous activations of all units in a given group is called the beat-frequency. The shortest beat-frequency that can be stored is limited by the unit with the shortest period. The longest beat-
frequency is difficult to determine, however, test have shown the model capable of learning at least 20 second durations (Miall, 1989, 1992, 1996).

At the start of a trial, all pacemaker units are reset and begin oscillating together. Each pacemaking unit is connected to an output unit; the weights of these connects are modified during training via a Hebbian learning rule (Miall, 1989, 1992, 1996). During training trials, the weights of the connections from pacemaker units to the output unit are modified; the units active after the passage of the target interval duration are strengthened. For test trials, the output unit responds when the pattern of activation in the pacemaker units matches the pattern that was activated during training. In other words, the task of output unit is to detection the coincidence of activation of the pacemaker units, for this reason the output unit can be called a coincidence detector (Miall, 1989, 1992, 1996).

The Beat-Frequency model of timing is quite different than SET. Instead of a constant running pacemaker, the Beat-Frequency model proposes a population of oscillatory units which have a distribution of oscillatory periods and all of which can be reset at the start of a trial. The use of a bank of oscillatory units is similar to the CT model; however, the units CT model have a strictly defined relationship in regards to their oscillatory period, which was previously described (e.g. 200ms, 400ms, 800ms, etc.). The Beat-Frequency model does not impose such strict control of the individual units. As described above, the population consists of around 500 units oscillating between 5-15 Hz, with a 10Hz mean and a 1.6 Hz standard deviation. Memory in the Beat-Frequency model is in the strength of the weights connecting the pacemaker units to the coincidence detector. The decision stage for the Beat-Frequency model is based on a pattern of activation in the pacemaker units rather than a direct comparison between a working memory value and a reference memory value.
Striatal Beat-Frequency Theory

The Striatal Beat-Frequency (SBF) model of timing is a revision and extension of the neural network based Beat-Frequency model of timing that attempts to explain the neurobiological mechanisms of timing behavior (Matell & Meck, 2000). SBF proposes that coincidence detectors are the spiny neuron of the striatum and that the oscillating units are neurons of the cortex, which project to the striatum. The SBF model hypothesizes that the stimulus that signal the start of a trial resets the oscillation of the cortical neurons so that there is a synchronous beginning to the oscillations. The neurons in the cortex then continue oscillating independent of one another each with its own period of oscillation. On training trials, the delivery of a reward causes a burst of dopamine in the striatum, originating from the substantia nigra pars compacta, strengthens the connections between the spiny neurons of the striatum and the cortical cells that are firing together at the passage of the criterion duration. On testing trials, the oscillations of the cortical neuron are reset as before, and the interval is timed by wait until the set of cortical neurons fire simultaneously in the pattern that was learned during training trials. When the cortical neurons fire synchronously in the pattern learn during training, the spiny neurons of the striatum send an output signal, that it is time to respond, to the thalamus, which in turn passes the signal onto the cortex so that the animal can initiate a response. Thus, the SFB model hypothesis a cortico-striatal –thalamic-cortical loop underlying the processes of timing behavior (Matell & Meck, 2000).

This SBF model makes specific predictions regarding neural activity during a timing task. However, little research has been conducted directly testing this model. One main prediction, which has received some empirical support, is that activity in the striatum should peak near the criterion time (Hinton, Meck, & MacFall, 1996; Matell, Meck, & Nicolelis, 2003;
Meck, Hinton, & Matell, 1998). Although, this same study failed to locate cortical or striatal neurons the fired in oscillatory patterns (Matell et al., 2003).

Conclusion

Of all the models discussed, SET is the predominant model used to explain timing data in animal research. SET has been useful in that it is relatively straightforward. It has been used to explain much behavioral data and it makes clear testable predictions. However, a major shortcoming of this model is its biological plausibility. It is difficult to conceive of biological mechanisms that function quite like the components of the model. Many of the other models discussed in the section have been proposed in response to this shortcoming of SET. However, at least until this point, SET is still the dominant model used in animal timing research and the other models have yet to be utilized to the extent of SET. Therefore SET remains an important and influential model of timing behavior.
APPENDIX C: TEMPORAL PATTERNS OF INTERVAL TIMING REVEALED BY BEHAVIORAL AND NEUROBIOLOGICAL RESEARCH

Research on timing and time perception has utilized many different techniques including behavioral manipulations and neurobiological manipulations, such as drugs and lesions, to better understand the biological underpinnings of the processing of temporal information (for reviews see: Al-Ruwaitea, Al-Zahrani, Ho, Bradshaw, & Szabadi, 1997; for reviews see: Hinton & Meck, 1997; Ho, Velazquez-Martinez, Bradshaw, & Szabadi, 2002; Meck, 1996). Several different patterns of distortion of timing have been observed as a result of these studies, and are frequently interpreted within the framework and processes proposed in Scalar Expectancy Theory (SET). These patterns of distortion in the data identify specific types of changes in timing, including temporal shifts and non-scalar changes in the variability of the distribution of the data. There are two types of temporal shifts: leftward and rightward. The two shifts derive their names from the direction of displacement when time is plotted on the x-axis of a graph. A shortening in the estimation of time has been classified as leftward shift because the distribution moves towards the zero point. Likewise, a lengthening in time estimation has been classified as a rightward shift because the distribution is moving away from zero. Many of these patterns will be briefly discussed in the following section.

Clock Pattern

The clock pattern of distortion is assumed to be linked to changes in the speed of the pacemaker or clock, \( A \). Changes in the speed of the clock are typified by abrupt shifts in data that renormalize (a return to the reinforced time) with continued training at the new clock speed. The renormalization of the data is related to the updating of reference memory with continued training, so that the remembered counts now reflect the new clock speed. Another abrupt shift,
this time in the opposite direction is seen when the clock speed is returned to normal, and with continued training, the data will renormalize once again.

The clock pattern of disruption is observed with pharmacological manipulations of the dopamine system (Hinton & Meck, 1997; Maricq & Church, 1983; Maricq et al., 1981; Matell et al., 2004; Meck, 1983, 1986, 1996). Dopamine agonists, such as methamphetamine, speed the clock and produce an immediate leftward shift in the peak-time (Maricq et al., 1981; Meck, 1996) and point of subjective equality (PSE) in temporal bisection tasks (Maricq & Church, 1983; Maricq et al., 1981; Meck, 1983, 1986). With continued training with dopamine agonists, the subject learns to time accurately. Upon removal of the drugs, an immediate rightward shift, which gradually renormalizes, is observed in the data. Dopamine antagonists, such as haloperidol, have an effect opposite of that observed with dopamine agonists (Maricq et al., 1981; Meck, 1996). Testing under dopamine antagonists results in an abrupt rightward shift, which disappears with continues training with the antagonist. Removal of the antagonist causes an immediate leftward shift, which gradually renormalizes with continued training. The effects noted above are only seen if there is training without drugs prior to the testing on the drugs. If training and test are carried out under stable drug conditions, either dopamine agonists or antagonists, then no difference will be observed because the same clock speed will be utilized during the initial learning and testing phases (Hinton & Meck, 1997).

Reference Memory Pattern

In contrast to the abrupt changes observed in clock pattern disruptions, manipulations that cause disruptions of the reference memory are characterized by gradual shifts in peak-time and PSE that eventually stabilize. These shifts do not renormalized when training is continued under the conditions that created the shift. The manipulations that cause reference memory
disturbances are thought to change the value of the $K^*$ parameter to values greater or less than 1 (Hinton & Meck, 1997; Meck, 1996).

The reference memory pattern of disruption is observed with pharmacological manipulations of the cholinergic system (Hinton & Meck, 1997; Meck, 1983, 1994, 1996, 2002; Meck & Church, 1987a, 1987b). Cholinergic agonists, such as physostigmine which increases effective levels of acetylcholine (ACh), have been shown to cause a gradual leftward shift in the data; a decrease in the value of $K^*$ (Meck, 1994, 1996; Meck & Church, 1987a, 1987b). Cholinergic antagonists, such as atropine which is an ACh receptor blocker, have been shown to cause a gradual rightward shift in the data; an increase in the value of $K^*$ (Meck, 1983, 1996; Meck & Angell, 1992; Meck & Church, 1987a). In addition to pharmacological manipulations, reference memory patterns have also been observed with lesions (Meck, Church et al., 1984; Meck et al., 1987; Olton, 1989; Olton et al., 1988). Lesions of the frontal cortex (FC) and nucleus basalis magnocellularis (NBM) produce rightward shifts in peak-time, with approximately a 20% rightward shift for FC lesions and a 10% rightward shift for NBM lesions (Meck et al., 1987; Olton et al., 1988). Lesions to the medial septal area (MSA) and the fimbria-fornix (FF) result in leftward displacement in peak-time, with approximately a 20% leftward shift for FF lesions and a 10% leftward shift for MSA lesions (Meck et al., 1987; Olton et al., 1988).

Working Memory Pattern

The working memory pattern is observed with data from the peak-interval gap (PI-gap) procedure (Meck, Church et al., 1984; Meck et al., 1987; Olton et al., 1988; S. Roberts, 1981). As described earlier (see Appendix A), the PI-gap procedure begins like a typical probe trial, with the initiation of a stimulus. However, at some fixed point into the trial, the stimulus is turn
off for a brief duration prior to it turning back on. Under these conditions, normal and control
rats seem to stop timing during the duration of the gap and restart timing at that point following
the return of the stimulus (Meck, Church et al., 1984; Meck et al., 1987; Olton et al., 1988; S.
Roberts, 1981). These data show a rightward shift in peak-time that equals the duration of the
gap, and is consistent with what as being described as a stop pattern of responding (See PI-gap
section in Appendix A). Rats with lesions to the FC and NMB appear to follow the stop pattern
like control animals and do not appear to have any working memory disruptions (Meck et al.,
1987; Olton et al., 1988). However, rats with lesions to the MSA and the FF display a different
response to the presences of a gap. These animals restart timing as if new trial begins following
the gap (Meck et al., 1987; Olton et al., 1988). These data, which have been interpreted as an in
ability to hold the accumulator count in working memory for the duration of the gap, show a
rightward shift in peak-time that equals the duration of the gap plus the length of the pre-break
duration; consistent with what has been described as a reset pattern (See PI-gap section in
Appendix A).

Selective Attention Pattern

The influence of selective attention, the ability to attend to a relevant stimulus while
ignoring irrelevant distracter stimuli, on timing can be observed using the prior-entry (PE) and
prior-entry-reversal (PER) tasks (Hinton & Meck, 1997; Meck, 1984; Penney et al., 1996). As
discussed above (see Appendix A), the PE and the PER tasks contains two target intervals cued
by stimuli from different modalities (e.g. light and tone). In the PE task, a brief cue, designed to
direct attention to the following trial, is presented prior to the beginning of some of the randomly
selected trials; in this instance, the modality of the cue matches the modality of the stimulus
presented in the following trial. In the PER task, the brief cue, when presented, mismatches the
stimulus presented in the following trial (Meck, 1984; Penney et al., 1996). The effects of the cue is interpreted as effecting the latency to begin timing (i.e. the latency to close the switch), with the PE task reducing the latency causing a leftward shift in peak-time and the PER task increasing the latency causing a rightward shift.

Administration of clonidine, a noradrenergic agonist that acts on the $\alpha_2$ receptor, results in rightward shifts in the peak-time in the PI procedure, the PER task and even in the PE task, which normally would show a leftward shift (Penney et al., 1996). In addition, administration of idazoxan, a noradrenergic antagonist which also acts on the $\alpha_2$ receptor, results in leftward shifts in peak-time on the PI procedure (Penney et al., 1996). These data suggest levels of norepinephrine influence the latency to begin timing, or the latency to close the switch, and suggest that the locus ceruleus, the primary source of norepinephrine in the brain, may have a role in selective attention during timing task (Hinton & Meck, 1997; Penney et al., 1996).

Divided Attention Pattern

Simultaneous temporal processing (STP) tasks (see Appendix A) are used for testing the subject’s ability to divided attention (Leak & Gibbon, 1995; Lustig & Meck, 2002; Meck, 1987; Meck & Church, 1984; Meck & Williams, 1997; Olton et al., 1988; K. C. Pang et al., 2001; K. C. H. Pang & McAuley, 2003). In order to properly perform an STP task, in contrast to selective attention tasks where the subject must selectively attend or ignore a stimulus, the animal must attend to two or more stimuli and time them concurrently. As mentioned above, both rat (Meck, 1987; Meck & Church, 1984; Meck & Williams, 1997; Olton et al., 1988) and pigeon (Leak & Gibbon, 1995) normal/control animals can accurately time up to three different durations.

Rats with lesions of the FC and NBM appear to be unable to time two stimuli at the same time (Olton et al., 1988). Instead, these animals seem to time the first stimulus until the second
begins. Upon initiation of the second stimulus, the animals switch to timing the second exclusively until that duration has passed, then the first stimulus is timed and timing appears to start where it left off. In this case, the peak-time for the second stimulus is accurate, however, the peak-time for the first stimulus is shifted to the right by an amount equal to the duration of the second stimulus (see Olton et al., 1988 Figure 14). Recording of cellular activity in the lateral agranular frontal cortex indicate that a large percentage (approximately 60%) of cells in the area increase responding only to compound trials, or trials that have both stimuli present, with no increase in responding on simple trials, or trials with only one stimulus (K. C. Pang et al., 2001). These findings further support the role of the FC, specifically the lateral agranular cortex, in divided attention tasks. Other studies on the STP task show that performance can be improved with the administration of arginine vasopressin (Meck, 1987) and that age-related declines in performance can be eliminated with prenatal choline supplementation (Meck & Williams, 1997).

Other Neurobiological Data

Timing and temporal processing has also been examined with other neurobiological manipulations. These results do not clearly fall into the disruption patterns discussed above, so they will be discussed separately below. The two major data that will be examined include manipulations to central 5-hydroxytryptamine (5-HT) and to the N-methyl-D-aspartate (NMDA) glutamate receptor.

5-Hydroxytryptamine

Central 5-HT depletion results in different types of disruptions depending on the type of task (prospective, immediate, or retrospective timing tasks: see Appendix A) being used (Al-Ruwaitea et al., 1997; Ho et al., 2002). In addition, differing results may be obtained, in some cases, depending on the method used to deplete 5-HT: lesions to the 5-HTergic pathways (dorsal
and median raphe nuclei) or acute drug treatment. For prospective timing tasks, which require an animal to make a choice between two responses which result in differing delays till delivery of reinforcement, lesions to the 5-HTergic pathways results in response choices that yield smaller amounts reinforcement which are delivered earlier when choosing between two delayed reinforce (Al-Ruwaitea et al., 1999; Mobini et al., 2000; Wogar, Bradshaw, & Szabadi, 1993). 5-HT$_2$ receptor antagonists seem to produce the same results as 5-HT pathway lesions, however selective antagonists of the 5-HT$_{1A}$ and 5-HT$_3$ receptors do not appear to have an effect on choice (Evenden & Ryan, 1999). There are mixed results with the effect 5-HT agonists on choice. Some data indicate that choice preference is shifted toward rewards that are larger and delivered at longer delays (Bizot, Thiebot, Lebihan, Soubrie, & Simon, 1988; Poulos, Parker, & Le, 1996), however, other data indicate no effect of 5-HT agonists on choice (Charrier & Thiebot, 1996; Evenden & Ryan, 1996).

At least two types of immediate timing tasks have been used to examine the effects of lesions to the 5-HTergic pathways: differential-reinforcement-of-low-rate of responding (DRL) schedules and the PI procedure. Lesions impair acquisition, shorten interresponse times (IRTs), and increase the Weber fraction on DRL schedules (Fletcher, 1995; Jolly, Richards, & Seiden, 1999; Wogar, Bradshaw, & Szabadi, 1992). On the PI procedure, animals with 5-HT pathways lesion produced peak-times similar to that of control animals, however, as in the DRL schedules, lesioned animals display impaired acquisition and an increase in the Weber fraction, indicative of a non-scalar increase in variability (Morrissey, Ho, Wogar, Bradshaw, & Szabadi, 1994). The shortening of the IRTs on the DRL schedules may be due to a greater difficulty, for subjects with lesions, to withhold responding. In the DRL task, this would cause the interval-to-be-timed to
reset (Ho et al., 2002; Soubrie, 1986). In the PI procedure, there is no such penalty for early responses.

The temporal bisection task (see Appendix A) is a common retrospective timing task which has been used to test the effects of 5-HT pathway lesions on timing and temporal processing. Early research examining the effects of 5-HT lesions on performance in the temporal bisection task found a leftward shift in the point of subjective equality (PSE) with no change to the Weber fraction (Morrissey, Wogar, Bradshaw, & Szabadi, 1993). This leftward shift in PSE has been attributed to a lesion induced facilitation in switch between behavioral states (Fletcher, 1993; Graham, Ho, Bradshaw, & Szabadi, 1994; Ho et al., 1995; Morrissey et al., 1994; Soubrie, 1986). In this case, the subjects with a 5-HT lesion switch from one behavioral state (attending to the “short” lever) to another state (attending to the “long” lever) more quickly than control subjects. An alternative explanation of the effects of 5-HT lesions is that clock speed has changed. However, other studies examining 5-HT lesions find that neither the PSE nor the Weber fraction are effected by the lesion when the rat is required to make a nose poke response on a panel placed between the two levers prior to making a lever response (Ho et al., 1995), or when intervals to be discriminated are too short too allow this movement to the new lever to occur during the presentation of the stimulus (Graham et al., 1994) which argue against the clock explanation of the effects of 5-HT lesions.

In sum, different manipulations of 5-HT result in a variety of effects on timing behavior. These effects seem to be dependent on the type of task being used to assess its effects. However, the most coherent explanation of the effects of 5-HT appear to be that 5-HT does not effect timing directly, but decisions of how to respond based upon the information from timing and temporal processing.
The effects of antagonists to the NMDA glutamate receptor on timing and temporal processing has been chiefly examined with DRL tasks (Sanger, 1992; Stephens & Cole, 1996; Tonkiss et al., 1988; Welzl et al., 1991). Chronic intra-ventricular infusions a competitive antagonist of the NMDA receptor D,L-2-amino-5-phosphonopentanoic acid (AP5), which acts at the glutamate binding site (Davies, Francis, Jones, & Watkins, 1981), have resulted in impairments on DRL performance (Tonkiss et al., 1988). These impairments include an increase in response rate, a decrease in efficiency (the number of reinforcer deliveries compared to responses), and a leftward shift in the distribution of IRTs. Upon completion of the AP5 infusion, the observed impairments disappeared and the animals, which had been receiving AP5 injection, began to perform like control animals (Tonkiss et al., 1988).

Acute systemic injections of MK-801, or dizocilpine, a noncompetitive NMDA receptor antagonist that acts as a blocker of the receptor’s Ca\(^{2+}\) ion channel (Wong et al., 1986), has been shown to produced impairments similar to those observed with chronic infusions of AP5 (Welzl et al., 1991). One time injections of MK-801 generally increased response rate, reduced efficiency, and cause a leftward shift in the distribution of IRTs. However, the highest does of MK-801 (.30 mg/kg) depressed response rate below the level of controls and eliminated any observable peak in the distribution of IRTs (Welzl et al., 1991). The above results were from an unsignaled DRL task. However, in a signaled DRL task, where a stimulus light was used to signal the end of the target interval (10 sec), acute injections of MK-801 did not results in a shift in the distribution of IRTs (Although, a valid argument can be made that timing ability is not needed the signaled DRL task, since an animal simply needs to wait for the cue to respond, removing any anticipatory aspect from the task). Based on these two finding from signaled and
unsignaled DRL performance under the influence of MK-801, the authors conclude that since the
drug injected animals were able to produce accurate IRTs in the present of the external cue, the
animals could inhibit the early responding observed in the unsignaled DRL task suggesting that
the observed leftward shift was not simply due to a response inhibition failure. Further, they
conclude the drug injections interfere with animals’ ability to use internal cues to solve the DRL
task as a result of either a memory or timing dysfunction (Welzl et al., 1991).

Other NMDA antagonists, aside from AP5 and MK-801, also disrupt timing behavior on
DRL tasks (Sanger, 1992). Sanger (1992) examined the effects of six different NMDA
antagonists (phencyclidine, MK-801, CGS 19755, eliprodil, memantine, and dextromethorphan),
with differing sites of action on the NMDA receptor, in order to determine whether injection of
the different compounds would lead to qualitatively different effects on the DRL task. The
results suggest that each drug produced a flattening of the distribution of IRTs, and all drugs,
except eliprodil, produced a leftward shift in the distribution similar to what had been previously
observed with MK-801 and AP5. The effects of these different drugs on response rate varied,
with some observed increase in responses rate and some decreases dependent on the drug and
dosage level. The finding suggest that NMDA antagonists generally produce consistent
disruptions of timing behavior on DRL tasks, specifically shortening of the distribution of IRTs
(Sanger, 1992).

Conclusion

This review has covered many of the behavioral patterns observed in timing as a result of
several different biological manipulations. The major patterns discussed include: clock, reference
memory, working memory, selective attention, and divided attention patterns. The clock pattern
involves temporary shifts in peak-time and appears related the dopamine system. Permanent
shifts in peak-time characterize the reference memory pattern, which seems to be linked to the cholinergic system. The working memory pattern involves impairment in holding temporal information through a stimulus gap and is affected by lesions the hippocampal system. Selective attention patterns relate to changes in time estimation dependent on cue that precede the to-be-timed stimulus and are related to norepinephrine levels. The divided attention pattern involves an ability to time to stimuli simultaneously; this ability appears to be affected by the function of the frontal cortex and the NBM. In addition to these data, other timing data from manipulation of the 5-HTergic and glutamatergic systems were discussed. However, these data do not fall into consistent and coherent patterns.
APPENDIX D: MATLAB PROGRAMS USED IN SCALAR EXPECTANCY THEORY MODELING

function m = Run_Sim();

global SET
global DATA
Initialize_SET;

%Start values for the SET parameters
SET.Speed_mean = 5.2059;
SET.Speed_sd = 0.0;
SET.k_mean = 0.975;
SET.k_sd = 0.15;
SET.Mem_Size = 53;
SET.Threshold_mean = 0.42;
SET.Threshold_sd = 0.28;
SET.Base_rate = 0.000998;

%Criterion times
Light_FI = 12;
Tone_FI = 0;

%FI training 10 sessions w/ 70 FI Light trials per session
sessions = 10;
trials = 280;
Run_SET('FI_training',Light_FI,Tone_FI,sessions,trials);

%PI training 30 sessions w/ 35 FI & 35 PI Light trials per session
sessions = 30;
trials = 140;
Run_SET('PI_training',Light_FI,Tone_FI,sessions,trials);

%Baseline 5 sessions w/ 35 FI & 35 PI Light trials per session
sessions = 5;
trials = 140;
Run_SET('Baseline',Light_FI,Tone_FI,sessions,trials);

%Parameter changes for drug blocks
SET.Speed_mean = 5.2059;
SET.Speed_sd = 0.0;
SET.k_mean = 0.975;
SET.k_sd = 0.15;
SET.Mem_Size = 53;
SET.Threshold_mean = 0.42;
SET.Threshold_sd = 0.28;
SET.Base_rate = 0.000998;

%Drug 1 5 sessions w/ 35 FI & 35 PI Light trials per session
sessions = 5;
trials = 140;
Run_SET('Drug1',Light_FI,Tone_FI,sessions,trials);

%Drug 2 5 sessions w/ 35 FI & 35 PI Light trials per session
sessions = 5;
trials = 140;
Run_SET('Drug2',Light_FI,Tone_FI,sessions,trials);

%Drug 3 5 sessions w/ 35 FI & 35 PI Light trials per session
SET.Speed_sd = .2;
sessions = 5;
trials = 140;
Run_SET('Drug3',Light_FI,Tone_FI,sessions,trials);

%Parameters return to Baseline following drug blocks
SET.Speed_mean = 5.2059;
SET.Speed_sd = 0.0;
SET.k_mean = 0.975;
SET.k_sd = 0.15;
SET.Mem_Size = 53;
SET.Threshold_mean = 0.42;
SET.Threshold_sd = 0.28;
SET.Base_rate = 0.000998;

%Washout 5 sessions w/ 35 FI & 35 PI Light trials per session
sessions = 5;
trials = 140;
Run_SET('Washout',Light_FI,Tone_FI,sessions,trials);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%Initialize_SET
global SET

%Model name
SET.Model = 'SET';

%Clock stage
SET.Speed_mean = 20.0;
SET.Speed_sd = 2;
SET.Speed = (SET.Speed sd)*randn + SET.Speed_mean;
SET.Accumulator = 0;

%Memory stage
SET.k_mean = 1.0;
SET.k_sd = 0.0;
SET.Light_Memory = [(SET.Speed)*120];
SET.Light_Memory_sample = [(SET.Speed)*120];
SET.Tone_Memory = [(SET.Speed)*120];
SET.Tone_Memory_sample = [(SET.Speed)*120];
SET.Mem_Size = 100;

%Decision stage
SET.Threshold_mean = 0.2;
SET.Threshold_sd = 0;
SET.Threshold = (SET.Threshold_sd) * randn + SET.Threshold_mean;

%Base rate of responding
SET.Base_rate = 0.04;

function m = Run_SET(sim_id,FI_light,FI_tone,nsessions,ntrials,block)

global DATA
dt = 0.5;
all_fi_light = [];
all_fi_tone = [];
all_probe_light = [];
all_probe_tone = [];

for i = 1:nsessions
    Initialize_PI(FI_light,FI_tone);
    for j = 1:ntrials
        Model_start;
        % Trial type (FI Light = 1; FI Tone = 2; Probe Light = 3; Probe Tone = 4)
        Trial_type = Get_Trial_type;
        switch Trial_type
            case {1}
                FI = FI_light;
                DATA.sfi_light_cnt = DATA.sfi_light_cnt + 1;
            case {2}
                FI = FI_tone;
                DATA.sfi_tone_cnt = DATA.sfi_tone_cnt + 1;
            case (Wozniak, Olney, Kettinger, Price, & Miller)
                FI = FI_light;
                DATA.sprobe_light_cnt = DATA.sprobe_light_cnt + 1;
            end
        end
    end
end
```matlab
% Model decides whether to respond
Response = Model_decide(Trial_type, Cycle_clock);

bin_index = ceil(Cycle_clock);

% Record response
if (Response)
    switch Trial_type
    case {1}
        DATA.sfi_light_rf(bin_index) = DATA.sfi_light_rf(bin_index) + 1;
    case {2}
        DATA.sfi_tone_rf(bin_index) = DATA.sfi_tone_rf(bin_index) + 1;
        DATA.sprobe_light_rf(bin_index) = DATA.sprobe_light_rf(bin_index) + 1;
    case {4}
        DATA.sprobe_tone_rf(bin_index) = DATA.sprobe_tone_rf(bin_index) + 1;
    end
end
end

% If response after FI on FI trial, give reward and update model
if (Prime == 1 & Response == 1)
    Model_update(Trial_type);
    Prime = 0;
    break;
end

% Update real time
Cycle_clock = Cycle_clock + dt;
end
```

```
DATA.sfi_light_rf = DATA.sfi_light_rf*60/DATA.sfi_light_cnt;
DATA.sfi_tone_rf = DATA.sfi_tone_rf*60/DATA.sfi_tone_cnt;
DATA.sprobe_light_rf = DATA.sprobe_light_rf*60/DATA.sprobe_light_cnt;
```
DATA.sprobe_tone_rf = DATA.sprobe_tone_rf*60/DATA.sprobe_tone_cnt;

all = transpose(vertcat(1:DATA.range, DATA.sfi_light_rf, DATA.sfi_tone_rf, DATA.sprobe_light_rf, DATA.sprobe_tone_rf));

ofile = strcat(sim_id,'-L',int2str(FI_light),'-T',int2str(FI_tone),'-',int2str(i),'.xls');
oloc = strcat('C:\SET\',ofile);
dlmwrite(oloc,all,'	');

res = [DATA.sfi_light_cnt, DATA.sfi_tone_cnt, DATA.sprobe_light_cnt, DATA.sprobe_tone_cnt];

all_fi_light = [all_fi_light; DATA.sfi_light_rf];
all_fi_tone = [all_fi_tone; DATA.sfi_tone_rf];
all_probe_light = [all_probe_light; DATA.sprobe_light_rf];
all_probe_tone = [all_probe_tone; DATA.sprobe_tone_rf];
end

all_fi_light = [1:DATA.range;all_fi_light];
all_fi_tone = [1:DATA.range;all_fi_tone];
all_probe_light = [1:DATA.range;all_probe_light];
all_probe_tone = [1:DATA.range;all_probe_tone];

all_fi_light = transpose(all_fi_light);
all_fi_tone = transpose(all_fi_tone);
all_probe_light = transpose(all_probe_light);
all_probe_tone = transpose(all_probe_tone);

DATA.all_sfi_light_rpm = all_fi_light;
DATA.all_sfi_tone_rpm = all_fi_tone;
DATA.all_sprobe_light_rpm = all_probe_light;
DATA.all_sprobe_tone_rpm = all_probe_tone;

ofile = strcat(sim_id,'FI-Light-',int2str(nsessions),'.xls');
oloc = strcat('C:\SET\',ofile);
dlmwrite(oloc,all_fi_light,'	');

ofile = strcat(sim_id,'FI-Tone-',int2str(nsessions),'.xls');
oloc = strcat('C:\SET\',ofile);
dlmwrite(oloc,all_fi_tone,'	');

ofile = strcat(sim_id,'Probe-Light-',int2str(nsessions),'.xls');
oloc = strcat('C:\SET\',ofile);
dlmwrite(oloc,all_probe_light,'	');

ofile = strcat(sim_id,'Probe_Tone-',int2str(nsessions),'.xls');
oloc = strcat('C:\SET\',ofile);
dlmwrite(oloc,all_probe_tone,'	');
oloc = strcat('C:\SET\',ofile);
dlmwrite(o loc, all_probe_tone,'\t');

m = res;

***************************************************************************
function m = Initialize_PI(FI_light,FI_tone)

global PROCEDURE
global DATA

PROCEDURE = 'PI';

rand('state',sum(100*clock)); %Initialize Random Number Generators
rand('state',sum(100*clock));

DATA.sfi_light_cnt = 0;
DATA.sfi_tone_cnt = 0;
DATA.sprobe_light_cnt = 0;
DATA.sprobe_tone_cnt = 0;

if (FI_light > FI_tone)
    %DATA.range  = 2*FI_light;
    DATA.range  = 60;
else
    %DATA.range  = 2*FI_tone;
    DATA.range  = 60;
end

DATA.sfi_light_rf = zeros(1,DATA.range);
DATA.sfi_tone_rf = zeros(1,DATA.range);
DATA.sprobe_light_rf = zeros(1,DATA.range);
DATA.sprobe_tone_rf = zeros(1,DATA.range);

***************************************************************************
%Model_start
global SET

% Get new sample of clock speed
SET.Speed  = (SET.Speed_sd) * randn + SET.Speed_mean;

% Get new sample from memory
j1 = ceil(rand * length(SET.Light_Memory));
SET.Light_Memory_sample = SET.Light_Memory(j1);

j2 = ceil(rand * length(SET.Tone_Memory));
SET.Tone_Memory_sample = SET.Tone_Memory(j2);

% Get new sample of threshold
SET.Threshold = (SET.Threshold_sd) * randn + SET.Threshold_mean;

function m = Get_Trial_type()

%Determine trial type (FI Light = 1; FI Tone = 2; Probe Light = 3; Probe Tone = 4)
Trial_type = round(rand*4);
if (Trial_type == 0) Trial_type = 4; end
m = Trial_type;

function m = Model_decide(Trial_type,Cycle_clock)

%No response as default
Response = 0;

%Update accumulator
SET.Accumulator = SET.Speed*Cycle_clock;

%Make decision based on trial type
switch Trial_type
    case {1,3}
        Discrepancy = abs(SET.Light_Memory_sample - SET.Accumulator) /
                    SET.Light_Memory_sample;
        if (Discrepancy < SET.Threshold) Response = 1; end
    case {2,4}
        Discrepancy = abs(SET.Tone_Memory_sample - SET.Accumulator) /
                    SET.Tone_Memory_sample;
        if (Discrepancy < SET.Threshold) Response = 1; end
end

%Mean operant rate of 1 response in 100 seconds, baseline responding
if (rand < SET.Base_rate) Response = 1; end
m = Response;

function m = Model_update(Trial_type);

global SET
\[ \text{SET.k} = (\text{SET.k}_{sd}) \times \text{randn} + \text{SET.k}_{\text{mean}}; \]

\[ \text{lmem}_{\text{size}} = \text{length(SET.Light\_Memory)}; \]

\[ \text{tmem}_{\text{size}} = \text{length(SET.Tone\_Memory)}; \]

\[
\begin{align*}
\text{switch} & \ \text{Trial\_type} \\
& \quad \text{case} \ {1} \\
& \quad \quad \text{if} \ (\text{lmem}_{\text{size}} \geq \text{SET.Mem\_Size}) \\
& \quad \quad \quad \text{SET.Light\_Memory} = [\text{SET.Light\_Memory}(2:\text{lmem}_{\text{size}}), (\text{SET.k}) \times \text{SET.Accumulator}]; \\
& \quad \quad \quad \text{else} \\
& \quad \quad \quad \quad \text{SET.Light\_Memory} = [\text{SET.Light\_Memory}, (\text{SET.k}) \times \text{SET.Accumulator}]; \\
& \quad \quad \quad \text{end} \\
& \quad \quad \text{case} \ {2} \\
& \quad \quad \quad \text{if} \ (\text{tmem}_{\text{size}} \geq \text{SET.Mem\_Size}) \\
& \quad \quad \quad \quad \text{SET.Tone\_Memory} = [\text{SET.Tone\_Memory}(2:\text{tmem}_{\text{size}}), (\text{SET.k}) \times \text{SET.Accumulator}]; \\
& \quad \quad \quad \quad \text{else} \\
& \quad \quad \quad \quad \quad \text{SET.Tone\_Memory} = [\text{SET.Tone\_Memory}, (\text{SET.k}) \times \text{SET.Accumulator}]; \\
& \quad \quad \quad \text{end} \\
& \text{end}
\end{align*}
\]
APPENDIX E: REFERENCES


Fletcher, P. J. (1995). Effects of combined or separate 5,7-dihydroxytryptamine lesions of the dorsal and median raphe nuclei on responding maintained by a DRL 20s schedule of food reinforcement. *Brain Research, 675*(1-2), 45-54.


Manahan-Vaughan, D., & Braunewell, K. H. (2005). The metabotropic glutamate receptor, mGluR5, is a key determinant of good and bad spatial learning performance and hippocampal synaptic plasticity. *Cerebral Cortex, advance access, 2/9/05*.


*Psychopharmacology, 111*(2), 239-243.


