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Specific dopaminergic manipulation of the internal clock mechanism

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Abstract

Using non-specific dopaminergic agents, Meck (1983, 1986) and his colleagues have repeatedly demonstrated manipulations of the internal clock mechanism, while showing no other changes to the psychophysical function (DL & WF). The current study used analyses and procedures similar to those of Meck (1983) and investigated the internal clock mechanism with the specific D2 agonist, quinpirole. Two groups were trained with saline and tested with quinpirole (0.08 mg/kg). One group was naive to the drug prior to testing (DN), while the other had previous drug exposure (DE). A third group (DT) was trained with quinpirole, and tested with saline. The DN and DE groups revealed no differences in acquisition, no predicted shift in the point of subjective equality (PSE), and changes in the DL and WF, when compared over phase. The DT group acquired the task more slowly and to a lower criterion, and no differences were found in the PSE, DL, or WF, over phase. The data presented here do not support dopaminergic control over the clock component as represented in the Internal Clock Model (Church, 1989). Results are consistent with other research (Lejeune et al., 1995; Rapp & Robbins, 1976; Santi et al., 1995; Stubbs & Thomas, 1974) in suggesting that dopaminergic control of the internal clock is more complex than was once thought.

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> Please sir, my mind cries for knowledge. I wish to cup knowledge in my hand and drink it as one drinks water by the side of stream. I am naked without knowledge, I am nothing without learning. Please sir, give me this knowledge, give me this learning, so that I too can be a man. [Gideon Mandoma, in *The Power of One*. (p.465)].

Aici este ceva din ce am învăţat multe.

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Specific Dopaminergic Manipulation of the Internal Clock Mechanism

There are many evolutionary pressures that would positively select for organisms that could determine the duration of events. Mastery of timing such events is important in behaviours as diverse as the mating rituals in ring-tailed doves, the development of feeding schedules, and circadian rhythms (Morell, 1996). Likely, there are a number of different internal timing mechanisms controlling or monitoring a variety of different behaviours, such as migration, sleep, temperature regulation, and the estrous cycle. The concept of an internal clock mechanism responsible for measuring durations of immediately relevant events and a cognitive model describing its function has been developed over the last twenty-five years.

In a review article, Church (1989) outlined a cognitive model of the internal clock and attempted to explain timing of brief temporal durations. He also discussed the biological and pharmacological implications of this cognitive model. The pharmacological aspect of the internal clock has been studied extensively by Meck (1983, 1986). He manipulated the dopamine and acetylcholine systems to differentially affect the internal clock. The internal clock model will be introduced with literature that provides support for its concepts. Santi, Weise, and Kuiper (1995), Rapp and Robbins (1976), and Stubbs and Thomas (1974) have also investigated the role of dopamine in the operation of the internal clock. All of their results were inconsistent with one particular aspect of the model, namely the notion that the pacemaker is affected by dopamine, because no shift in the perception of the duration was observed. This discrepancy could either be due to procedural differences or due to the data analysis methods.

Church (1989) provided an outline for the concept of the internal clock model based on behavioural, biological and psychological perspectives. He concluded that animals are sensitive to stimulus durations and to the time between responses to stimuli and outcomes. Based on this, he suggested that temporal characteristics could serve as discriminative stimuli in operant conditioning tasks. Historically, this type of research has been conducted with pigeons or rats, but evidence supporting the internal clock model has also used a number of other species, including humans (Rammsayer, 1993).

There are two methods commonly used to study timing of short intervals. The first utilizes time production, using procedures which are essentially variations on a Fixed-Interval (FI) schedule. An animal is trained on a Fixed-Interval (FI) schedule (e.g., FI-10 s), whereupon the first response immediately after the time is reinforced. Various measures are used to define time production, namely peak response rate (the maximal rate of responding) and peak time, the time at which the greatest amount of responding occurs. After the FI has been learned, test trials are interspersed within the session. During the test trials, the reward at the fixed time is removed, so that responding on these trials is never rewarded. The test trial is much longer than the FI duration (e.g., 120 s), and is independent of the animal's response. Peak time on these empty trials is usually very close to the training FI value, and is indicative of the ability of the animal to produce the desired duration.

The second method involves time perception, or temporal discrimination. In this procedure, animals are typically trained in a forced choice task to respond to a series of sample durations. Response to one lever will be correct for the longer sample, while

responses to the other lever will be correct for the shorter sample. After this task is acquired, durations intermediate to both durations are added for testing. Over the session, the number of responses associated with the long response is recorded and a proportion is generated with the total number for each sample duration presented. The proportion of long response choices versus the sample duration produces an ogival psychophysical function. Once the psychophysical functions are determined, points of subjective equality (PSE) are calculated. The PSE is defined as the signal duration associated with the 'long' response 50% of the time, and is an indicator of time perception for that particular range of stimuli. Under normal conditions, the PSE is close to the geometric mean of the endpoint samples. This method is interpreted as a perception of temporal signals, unlike the time production procedure discussed above.

One advantage of time perception studies is the requirement for different responses, which limits interpretive problems compared to the time production procedures. In a time production procedure, the use of drugs may have confounded results with respect to the peak rate and peak time. As only one response is required, any increase (or decrease) in responding or the peak rate could be attributed to altering the time production mechanism, or to some other physiological action of the drug. In the time perception paradigm, it is assumed that any of these other physiological measures would be disrupted equally for responses to each lever, so that more subtle alterations could be observed.

A model has been proposed which combines processes of perception, attention, memory, motivation and decision making to define the operation of the internal clock. As

illustrated in Figure 1, the internal clock model includes three basic components; a clock, memory, and a comparator. The clock, which is the basis for the perception or production of time, further consists of three elements; a pacemaker, a switch and an accumulator. The pacemaker produces regular pulses. At the onset of a timed event, the switch closes and allows for the transfer of pulses from the pacemaker to the accumulator. The accumulator integrates the pulse count and passes this information onto memory. The memory consists of reference and working memory elements. Working memory is the storage location for temporarily relevant information such as the particular pulse count associated with the event duration of the current trial. On the other hand, a reference memory for the duration develops through repeated exposure to trials with similar durations. The comparator receives inputs from the pulse accumulation in working memory and compares it to the stored representation in reference memory. On the basis of this comparison, a response decision is made and as an output, behaviour is initiated. Based on the trial outcomes, a representation of reinforced durations is developed in reference memory. Church (1989) used this model as the psychological explanation of timing intervals from seconds to minutes, as demonstrated either through time production or perception. If this model accurately represents the processing of temporal events, it was proposed that the clock or reference memory components could be manipulated independently of each other.

A biological perspective was used to form the basis for the separation of the clock and memory components and was based on research utilizing pharmacological manipulations. Drugs that affect the dopamine system were shown to modify time

perception and production, presumably by altering the pacemaker speed. These drugs were argued to produce alterations in transient neural processes initiated by temporal stimuli. The pacemaker speed, and therefore the clock speed, was temporarily changed, thus altering the perception of time. General dopamine agonists increased clock speed and general dopamine antagonists decreased clock speed when compared to saline treated animals. This temporary disruption of temporal perception was defined by Church (1989) as a phasic shift in the function of the perceived temporal duration compared to the physical temporal duration. The phasic shift occurs immediately upon application of a dopamine altering treatment. After repeated or chronic exposure to the dopamine treatment, the shift is no longer present, as the reference memory is readjusted to the new pulse count. The shift also immediately reappears, but in the opposite direction, when the chronic dopamine treatment is removed. An observed change in the PSE is the described shift in the temporal discrimination functions previously discussed.

Maricq, Roberts and Church (1981) investigated whether methamphetamine would alter the clock. In their first two experiments, they tried to generate the shift. In the third experiment, they tried to discern if it was the rate of the clock that was affected. In Experiment 1, rats were trained with two types of trials with equal probability of presentation. The first trial type, 'food' trials, established the responding time. Here the first response after 40 s was rewarded. The second type of trial was 'empty', wherein no food was delivered, and these trials lasted a minimum of 80 s, plus a random period of time ranging from 10 to 80 s. These trials ended independently of responding. After 8 sessions, injections of either saline or methamphetamine began. The major result was that

methamphetamine reduced the peak-time. This suggests that methamphetamine was affecting the internal clock, either by reducing latency of the clock to start or by increasing the speed of the clock. In Experiment 2, the procedure was the same, except for two manipulations. First, there were two groups of rats trained on the FI schedule, one on FI-20 s and the other FI-40 s. This was done in an attempt to determine if the clock was altered by an absolute amount or in a proportional manner. The second difference was that the animals were run for many more sessions, so the days during which drug was administered could be spread out to eliminate the possibility of developing tolerance to the drug. It was found that methamphetamine reduced the peak time, and also increased the response rates. The authors reported there was too much individual variability to determine if the shift was constant or proportional in nature. Experiment 3 used psychophysical functions, where the rats were trained to distinguish between long and short periods of darkness by responding to the correct one of two levers. They used this time perception task in an attempt to generalize from the time production procedure used in Experiments 1 and 2, and to try once again to determine if there was a constant or proportional change in the internal clock. During training, rats learned to distinguish between either 1 and 4 s, or 2 and 8 s, or 4 and 16 s. After this was done, five intermediate times were introduced to the set, and psychophysical functions were generated. Three sessions of saline injections were followed by three sessions of methamphetamine injections. Results were reported with two modifications made to the data. First, they removed all trials that had a response latency of greater than the mean of all response latencies, and second, they made a correction for responses not controlled by

duration. The saline generated functions were similar to those found in previous literature, and the response latency and the correction for inattention to the sample 'cleaned' up the functions. The point of indifference, or the point of subjective equality is the point at which the subject will choose one of the two samples 50 % of the time. It is independent of the slope and other characteristics of the curves. The difference limen (DL) is the slope of the function between two points. As the DL increases, the animal's ability to identify changes in stimuli decreases. The discriminability of the sample durations increases as the DL decreases. A third characteristic of psychophysical functions is the Weber fraction, which can be interpreted as the proportion change on some dimension of the stimulus required to detect the change. The WF is similar to the DL, as they are both measures of discrimination, but the DL is for a specific range of stimuli, whereas the WF is for any range of stimuli.

The PSE was near the geometric mean for the two endpoints. The temporal discrimination, as represented by the difference limen (DL), was lower when the correction for inattention and response latency was considered. Also, after these manipulations, the DL, and thus temporal discrimination, was not affected under the influence of methamphetamine. This provides for unconfounded interpretation of the point of indifference, as any change in the PSE could not be due to a change in the temporal discrimination of the stimuli. They reported less disruption due to the drug than was reported elsewhere. They attributed this stability in the DL to the method used to derive the psychophysical functions. They also found that the shift in temporal perception was proportional, as relative shifts were the same for each of the groups (1:4 s, 2:8 s, 4:16

s). Their main conclusion was that methamphetamine changed the internal clock used to measure duration signals in the magnitude of seconds. This change was due to a proportional increase in the clock, and not simply a change by a constant amount.

Meck and Church (1983) studied the concepts of timing and counting. They conducted a series of experiments to determine if number and duration could serve as discriminative stimuli. Initially, rats were trained on compound signals with varying number of sound segments and signal durations of 2 or 8 s. Using psychophysical functions, they found the accuracy for the duration and number classifications to be equivalent. This led to two conclusions, either there was one mechanism for analysis of the duration and number samples, or there were two mechanisms which had coincidently the same sensitivity. The PSE was near the geometric mean of the two reinforced durations. They also found that rats under the influence of methamphetamine had the PSE shifted in both the counting and duration psychophysical functions. It was hypothesized that, if there were two mechanisms, then differences in performance would be found in either the timing PSE or the counting PSE. If there was only one mechanism, then the PSE for both stimulus modalities would be affected equally. The same decrease in PSE was found for duration and counting behaviours. This suggests that there is a fundamental similarity between counting and timing processes.

Further, they tested for cross-modal transfer of counting, where foot shock or auditory signals served as the cue for either counting or timing. Meck and Church (1983) found that there was substantial simultaneous cross-modal transfer from auditory to cutaneous segments. The fact that there was translation of number and duration for

auditory to cutaneous stimulation adds additional support to the hypothesis that the same internal mechanism is used for timing and counting. From these experiments, it was concluded that a single mechanism with a specialized clock that could time and count was preferred, as it was the most parsimonious model that met the needs of the data.

Not only pharmacological manipulations, but also specific methods used to assess coding of temporal durations, have been found to affect the perception of time. Fetterman (1995) reported two experiments designed to explore the psychophysical functions of temporal memory. In the first, pigeons were rewarded for correctly responding to the keys assigned to either 2 or 10 s samples. After acquisition of this task, tests were conducted where the offset of the sample and the onset of the comparison was separated by 2 to 15 s. For the first two tests, no other sample stimuli were used. After this, durations intermediate to the endpoints were also used. Over the delay, responses to the long sample (10 s) decreased in accuracy more than the accuracy for the responses to the short (2 s) duration. Results indicated that the response bias was consistent with subjective shortening and was also consistent with the internal clock model. Subjective shortening has been attributed to the loss of pulses in either the working memory or accumulator component of the model. This loss in pulses leads to an increased probability that the animal will choose the short comparison stimulus. Depending upon the procedure, the interpretation of the internal clock shows either a shift to the left in the psychophysical function or a choose 'long' bias. Likewise, a shift to the right in psychophysical function analyses shows the same effect on the internal clock model as a choose 'short' bias.

In the second experiment, the procedure was modified slightly. Subjects were trained with a set of six signal durations. Responses to one comparison stimulus were reinforced for the three shortest durations, while responses to a second comparison stimulus were reinforced for the three longest durations. Training was provided until the pigeons responded reliably on a partial reinforcement schedule. Testing again used delays ranging from 2 to 15 s. The delay functions decreased equally for short and long durations. With this altered procedure, there was unbiased responding as the delay increased and therefore no evidence for subjective shortening.

From this, the specificity of the procedure used in psychophysical function studies is emphasized. The differences between Experiments 1 and 2 can only be accounted for by the introduction of the modified method in Experiment 2, namely one response was now correct for a group of sample stimuli and another response was correct for a second group of sample stimuli. As a result of this modification, the Experiment 1 procedure had intermediate sample stimuli that were never rewarded, whereas the Experiment 2 procedure rewarded all sample stimuli. It can be seen from Fetterman (1995) that there is a difference in coding of temporal durations depending upon the methodology utilized. Future studies using the psychophysical timing function must be aware of the effects of methodological differences such as this. In the current research, it is desirable that the time is retained by the animals as a precise value as in the first experiment, instead of having the durations converted to concepts of 'short' or 'long', as in the second experiment.

Using psychophysical functions with endpoints of 2 and 8 s, Meck (1983) reported

that when tested with the sympathomimetic adrenergic stimulant methamphetamine, rats who had been trained with saline but had previously received the drug outside of the experimental paradigm demonstrated a left shift in the temporal discrimination function, indicating a lengthening of the perception of the temporal duration. This agonist is known to increase the level of dopamine in the synaptic cleft by increasing the release of dopamine and inhibiting its synaptic reuptake. This results in more dopamine in the synaptic cleft making longer the period of time that the post-synaptic neuron could be activated by available dopamine. It was proposed that the leftward phasic shift produced by the methamphetamine was due to the increased speed of the clock. Reference memory for reinforced durations on short and long signal trials had been established under drug-free conditions. The pulse count for each of the durations was stored in the reference memory component. When methamphetamine was used during testing, the clock speeded up, and the pulse counts for each duration were greater than the counts associated with the sample stimulus in reference memory. This would cause the animal to choose the lever associated with the long duration more frequently. Thus, the resulting function would be to the left of the training curve.

Similarly, rats who were experienced outside of the training paradigm with the general dopamine antagonist haloperidol, and tested under the influence of haloperidol, produced a rightward shift in the temporal discrimination function. This shift was subsequently eliminated with continued training in the drugged state, demonstrating the phasic, temporary nature of the dopamine manipulation. Termination of the chronic haloperidol treatment produced an immediate shift to the left in the chronic haloperidol

temporal function, further illustrating its phasic nature. Church (1989) in his review, concluded that manipulations affecting the dopaminergic system produce phasic shifts in timing functions while drugs or other manipulations affecting the cholinergic system produce chronic shifts in timing functions. The phasic shifts observed were characteristic of changes to the clock speed and also to changes in the dopaminergic system. With dopamine seemingly controlling the pacemaker component of the clock, the independent manipulation of the acetylcholine system was found to control the memory aspect of timing.

Church (1989) reviewed the use of the cholinergic agents physostigmine and atropine to test the reference memory component of the clock model. Physostigmine, an acetylcholinesterase inhibitor and indirect agonist, when administered repeatedly resulted in chronic shifts in the psychophysical functions to the left. The antagonist and muscarinic receptor blocker atropine also resulted in chronic shifts in the functions to the right when compared to curves generated under saline injections. A chronic shift in the temporal discrimination occurred gradually after training under application of the treatment. This shift remained as long as the training under treatment continued and was gradually eliminated when training without drug treatment was given. It was also noted that, compared to the saline treated rats, physostigmine increased the speed of task acquisition and reduced performance variability. The atropine treated group had a slower speed of acquisition and increased performance variability when compared to saline treated controls.

Most of the support for the chronic manipulation of the memory component of the

internal clock model is derived from Meck (1983, Experiment 4) who proposed that changes in the memory representation of the stimulus duration could be shown without affecting the speed of the clock. The purpose of these experiments was to determine if operating characteristics of the clock and memory stages were independent and thus could be selectively adjusted. He proposed that there were different pulse counts associated with the durations for animals having acquired a temporal discrimination while under the influence of a drug which affected the internal clock. This was compared to animals that had acquired the same temporal discrimination under similar conditions following saline injections.

It was hypothesized that an animal administered a drug that changes the clock component should display a different temporal discrimination pattern than an animal injected with a drug that affects the memory component. In the first experiment, methamphetamine and haloperidol were proposed to have differential effects on the clock, but not on the memory or decision components of the internal clock model. Rats trained with methamphetamine showed a proportionate increase in the speed of the pacemaker, and produced an increased clock reading compared to the saline rats. Methamphetamine trained rats then accurately stored this elevated clock reading in reference memory. Haloperidol trained rats learned the task with a decreased clock speed, and this reduced count was stored accurately in reference memory. From this experiment, Meck (1983) observed that the psychophysical functions developed in training were the same for the chronic dopaminergic drug conditions and the saline condition. The explanation was that the rats trained with the drug had learned to correctly associate a chronic

misrepresentation of time with a reinforced event. As long as the rats were tested in the drugged state, they performed at levels similar to those of the saline control group. Meck (1983) concluded that methamphetamine and haloperidol selectively affected the clock stage by altering the clock reading (number of pulses) associated with each stimulus duration. Methamphetamine increased the clock rate, while the haloperidol decreased the clock rate. There was no evidence that methamphetamine or haloperidol changed memory for the event. It appeared that levels of dopamine set the rate of the pacemaker in the clock (Meck, 1983).

Although dopamine appears to alter the rate of the pacemaker, it does not appear to modify the memory mechanism. Acetylcholine, however, appears to modify mechanisms of memory. Therefore, Meck (1983) determined if the process of storing clock readings in reference memory could be modulated by the active level of acetylcholine in the CNS. Rats were trained in a temporal task under either chronic atropine, an acetylcholine antagonist or chronic physostigmine, an indirect acetylcholine agonist. When compared to the saline trained rats, the physostigmine group had a permanent left shift in the psychophysical function. The rats trained with atropine (an acetylcholine antagonist), showed a permanent rightward shift in the psychophysical function relating the probability of a 'long' response to signal duration. Meck (1983) proposed that when trained with chronic physostigmine, the rats were unable to adjust their temporal criterion and when tested with saline, the reinforced temporal discrimination was consistently underestimated, resulting an a leftward shift. In the opposite direction, treatment with atropine caused a permanent rightward shift in the psychophysical function.

He argued that this shift was due to the pulse counts in reference memory being artificially established under the drugged state and the inability of the rats to quickly adjust the pulse count in reference memory. The rats trained under cholinergic drug conditions were then tested with saline injections. As the reference memory was previously established, no immediate shift in the psychophysical function was observed for the groups trained with either physostigmine or atropine.

Meck (1983) proposed that the number of pulses in the accumulator were defined as the perceived time. He argued that the transfer of the perceived time into reference memory could be biased in some way, represented by a memory storage constant, K*. If the time in reference memory differed from the perceived time, as was the case in the Meck (1983) studies, then the animal would reliably expect the event to occur at another time. If K* (the bias) was greater than 1.0, the event would be expected to occur later than scheduled. Conversely, if K* was less than 1.0, the event would be expected to occur earlier than scheduled. When K* was equal to 1.0, the event occurred without bias exactly as expected. For example, physostigmine, decreased K* and the predicted leftward shift (a shortening of the event) was observed. Atropine was argued to increase K* and the predicted rightward shift in the psychophysical function was found (a lengthening of the event).

To further study the role of dopamine on the internal clock, Meck (1986) performed an experiment that related the binding affinity of various neuroleptics or dopamine antagonists to their efficacy at altering the internal clock. He argued that the evidence for the internal clock model based on general dopamine agonists and antagonists

was compromised by two factors. First, there were at least three different types of dopamine receptors (D_1, D_2, and D_3), and each has different neurological effects (Beninger, Hoffman, & Mazurski, 1989). Second, neuroleptics and amphetamine interact with other neural receptor sites in the norandrenergic and seratonergic pathways.

In this study (Meck, 1986), the dose of neuroleptic required to induce a 15% to 20% increase in the point of subjective equality over the performance of a temporal task was determined. *In vitro* studies examining the affinity of radiolabelled neuroleptics to bind to D_2 receptor sites were also completed. Using five dopamine antagonists (chlorpromazine, haloperidol, pimozide, promazine and spiroperidol), Meck found that D_2, but not D_1, receptor binding affinity was highly correlated with the effectiveness of the drug to alter temporal discrimination functions. Therefore, Meck postulated the D_2 system could be responsible for control of the clock component in the internal clock.

Not only has the dopamine system been shown to modify timing behaviour in rats, it has also been shown to modify timing in humans. Rammsayer (1993) used human participants and two neuroleptics, haloperidol and remoxipride, to study the internal clock in two separate experiments. In one experiment, the standard stimulus was 50 milliseconds and in the other, it was 1000 ms. He suggested internal timing mechanisms were more likely to depend on specific changes in D_2 receptor activity than on changes in the rate of synthesis or release of dopamine. Haloperidol blocks both D_1 and D_2 receptors, but has a higher affinity for D_2 receptors. Remoxipride, a substituted benzamide, selectively blocks D_2 receptors. Rammsayer (1993) tested the hypothesis that changes in pacemaker speed of the internal clock were mediated by D_2 receptor activity

and that this change would alter time estimation. A reaction time test, included as a motor impairment control, indicated that motor control was not affected at the doses used. Therefore, performance on time estimation tasks could not be considered a function of generalized central nervous system depressant effects. Time perception performance under the influence of either haloperidol or remoxipride suggested that the pharmacological agents modulated the internal clock mechanism in a specific manner. Perception for the small durations was found to be altered by haloperidol, but not by remoxipride, while perception for the longer durations was affected by both remoxipride and haloperidol. Remoxipride has more pronounced action on D_2 receptors in the mesolimbic and mesocortical areas. Haloperidol more selectively affects the D_2 receptors in the striatum. Rammsayer (1993) concluded that temporal information processing was affected by the reduction of D_2 receptor activity. He also suggested that time perception was related to the potency of the drugs to block striatal D_2 receptors. Time estimation for longer durations is more dependent upon cognitive processing and both of the D_2 neuroleptics produced deficits in processing of times in this range.

Also, Spetch and Treit (1984) trained pigeons to respond to samples of 1 or 5 s of houselight. After this task was acquired, they ran delay tests to monitor the memory for time. They delayed presentation of the comparison stimuli by 0, 5, or 20 s and ran birds with either saline or d-amphetamine injections. Data showed that the dose of the drug was critical to demonstrating the effect, and they chose 2.0 mg/kg. When birds were run with saline injections, there was no difference in accuracy for the short and long samples at the 0 s delay. At 20 s delay, the accuracy for the 1 s sample was higher than that for the 5 s

sample. This demonstrates the 'choose-short' effect, and is consistent with the internal clock model. The choose short effect has been explained by subjective shortening of the pulse count stored in working memory. After a timed event occurs, the pulse count is stored in working memory. If a delay is imposed between the end of the sample stimuli and the beginning of the comparison stimuli, the count in working memory degrades and looses counts. So as the delay increases, there is an increased likelihood that the animal will choose the comparison associated with the short sample, was found in this case.

The results achieved while the birds were tested with d-amphetamine were the exact opposite. Here the difference was in accuracy at the 0 s delay, as the long sample was chosen as correct more often. At the 20 s delay, there was no difference between short and long samples, with accuracy at chance. Spetch and Treit (1984) concluded that the effect of d-amphetamine on pigeons' ability to perceive sample durations was compatible with reports that amphetamine produces an overestimation of real time intervals in rats and humans. Also, monitoring the memory under d-amphetamine showed there was a reduction in the tendency to display a 'choose-short' bias at the 20 s delay. Overall, they concluded that, in addition to lengthening the perceived duration of the samples, d-amphetamine produced a general short-term memory deficit. Such a deficit eliminated sample duration control at long delays and thus overrode the perceptual changes produced by the drugs.

Contrary to previous reports, Santi et al. (1995) found that amphetamine did not modify the internal clock of rats and pigeons. For the rat study, sample stimuli were 2 and 12 s of either tone or house light. The procedure assessed time perception (at zero

delay), as well as memory for time durations at delays of 1, 3, and 9 s. If methamphetamine increased the speed of the rats' internal clock, it was hypothesized that there would be a choose-long bias at the 0 s delay. The temporal task was acquired free of drug. Half of the rats were tested in the order of saline-drug, while the other half were tested in the reverse order. There were 15 days of testing in each condition.

No 'order of treatment' effect was found, and overall accuracy for the methamphetamine phase was lower than that for the saline phase. There was also a choose-short bias as the retention interval increased. However, none of the groups demonstrated the choose-long bias at the 0 s delay, which would be predicted if methamphetamine had selectively affected the internal clock.

There have been other studies similar to Santi et al. (1995) that investigated pharmacological effects on temporal perception and memory. Rapp and Robbins (1976) studied the effect of amphetamine on temporal discrimination in rats. They used 3 and 7 s tone stimuli. At the largest dose (0.8 mg/kg), accuracy was differentially disrupted as evidenced by a higher error rate for the 7 s sample. This shows a bias for the short durations. The internal clock model would predict a bias to select the 'long' lever when trained under saline and tested with amphetamine.

Using pigeons, Stubbs and Thomas (1974) did not find the choose-long bias as reported by Meck (1983). They used various temporal sample stimuli mapped onto coloured key comparison stimuli. They assigned one response to be correct if the sample was between 1 and 5 s, and another comparison stimulus to be correct if the sample was between 6 and 10 s. After this was learned, the pigeons were tested under the influence of

d-amphetamine. They discovered that, regardless of the absolute lengths of the two durations used, d-amphetamine increased errors more with the longer sample duration than with the short sample duration. The bias against the long samples was the largest at the highest dose (2.5 mg/kg i.m.). d-Amphetamine was found to produce a choose-short effect, as the drug dose increased. This is of interest as this procedure is similar to the second experiment by Fetterman (1995). Fetterman also did not find support for the internal clock model using this procedure. Another interesting finding in Stubbs and Thomas (1974) was that, as the drug dose increased, the response latencies increased, and the psychophysical functions were more devastated. By the time 2.5 mg/kg d-amphetamine was used, the psychophysical function was nearly flat for the one bird that was reported.

The methodologies of Santi et al. (1995) and Meck (1983) differed in three major aspects. First, Santi et al. (1995) used two rewarded sample durations, 2 and 12 s. Meck (1983) used two rewarded durations, 2 and 8 s, as well as 5 intermediate unrewarded durations. This could be an important factor because if psychophysical functions shift only a small amount (10 to 20%), then rats may still be able to distinguish between 2 and 12 s. If the samples, as in Meck (1983), were 2.0, 2.6, 3.2, 4.0, 5.0, 6.4 and 8.0 s, these temporal durations would be easier to confuse, and slight shifts in the psychophysical functions would be more easily detected. For example, if a rat was trained with saline and during test, was injected with amphetamine, the sample duration of 6.4 s may be perceived as 8.0 s, one of the samples in the Meck (1983) procedure. It is not likely that a 2 s sample would be perceived as the 12 s sample in the Santi et al. (1995) procedure because

much larger effects on the internal clock would be required. Similarly, it was found that after amphetamine training, rats tested with saline in the Meck (1983) study would choose the 2.0 s lever when the 2.6 s stimulus had been presented. Thus, the shift can be detected at both ends of the temporal discrimination function. The Meck (1983) procedure, using psychophysical functions, may be more sensitive to the perception of sample duration as it provided detection of slight shifts in temporal perception, while the Santi et al. (1995) procedure would need more obvious perceptual differences. However, if there was a predicted choose-long bias at the 0 s delay, accuracy for the long duration would be higher than was observed. If there was a perceived lengthening of the 12 s sample, the accuracy should increase when compared to saline treated sessions. This was not found to be the case, because correct choices for both durations dropped in accuracy. It could very well be that the sensitivity of the procedure could make a difference, but here it seems unlikely, because predictions made not supported. The theory of the internal clock should be open to investigation from a variety of procedures. The Santi et al. (1995) procedure was an attempt to support the internal clock theory from a different angle, and the results could not fully support the model.

Second, Santi et al. (1995) used all data collected in their analyses. Meck (1983) excluded from the data any response that had a reaction time greater than 3.0 s. He argued that these excluded data points (18% of trials when using haloperidol, 23% for the methamphetamine group and 20% for the saline control group) were no longer under temporal control. From the remaining data, psychophysical functions were calculated and a point of subjective equality (PSE) was determined. While both of these methods were

sensitive to the changes in the perception of time, they are different, and failure of Santi et al. (1995), Stubbs and Thomas (1974), and Rapp and Robbins (1976) to find the internal clock effect may be in part due to the alternative data analysis presented by Meck (1983). It is not possible to further analyse the Santi et al. (1995) data to take response latency into consideration because the pertinent data were not collected.

Third, Meck (1983) used a specialized procedure to ensure that the rats had previous exposure to the drugs outside of the training and testing paradigms. He argued that preliminary exposure to the drugs could become a confound in studying the perception of temporal durations. To eliminate the drug exposure confound, the naive rats were not trained each day and received drug injections on non-training days. This ensured that the drug naive group was experienced with the drug outside the training paradigm. In fact, Meck and Church (1983), used saline and methamphetamine, but the attention to providing the rats with exposure to the drug was only applied in one time production study. Apparently, this aspect is not as important as first thought. Santi et al. (1995), Rapp and Robbins (1976), and Stubbs and Thomas (1974) did not use the non-training drug exposure paradigm in their studies. In fact, Santi et al. (1995) offered an explanation for the lack of a choose-long effect at the zero delay in terms of a generalized attention disruption induced by the amphetamine.

A biological basis for the cognitive internal clock mechanism has been presented. Dopamine has been proposed to be responsible for manipulations in the clock component of the internal clock model. The dopamine manipulation produces a temporary, phasic shift in the psychophysical function for the perception of temporal events (Church, 1989).

Dopamine agonists increase the speed of the clock, and produce a phasic leftward shift in the psychophysical functions but do not affect the memory of the event (Meck, 1983). Dopamine antagonists decrease the speed of the clock, and cause decreases in temporal estimations (Meck, 1983; Rammsayer, 1993). The D_2 receptor subsystem is proposed to control the clock component of the internal clock. There are correlational studies suggesting a relationship between the D_2 receptor affinity of various neuroleptics and the dose required to alter the internal clock by 15% to 20% (Meck, 1986). The work by Rammsayer (1993) suggested that the D_2 receptor antagonists haloperidol and remoxipride show evidence for dopaminergic control of the internal clock in humans.

The present experiment was designed to increase the knowledge of the dopaminergic control over the internal clock. Unlike previous studies, this experiment used a specific D_2 receptor agonist (quinpirole) manipulation. The methodology attempted to provide a bridge for the discrepancies observed between Santi et al. (1995) and Meck (1983).

This experiment tested the hypothesis that rats treated with the D_2 agonist quinpirole will have discrimination functions shifted in a nonpermanent, phasic manner. It was further hypothesized that previous exposure to the drug outside of training would reduce the generalized disruption sometimes found with dopamine agonist use, and allow for measurable manipulations of the internal clock. It was predicted that drug naive rats, when tested with quinpirole, would not display a shift in the psychophysical function, but would display a general disruption in the overall accuracy of the two endpoint sample stimuli in a manner similar to the Santi et al. (1995) procedure. It was further predicted

that when rats with drug experience outside of the training paradigm were tested with quinpirole, a shift in the PSE towards the left would occur. It was also predicted that animals that received quinpirole during training, when tested with saline, would show a right phasic shift in the psychophysical function compared to the training phase.

Method

Subjects

Twenty-four adult male, Sprague-Dawley rats (Charles River, Canada), naive in drug and temporal discrimination experiments started as subjects. They were individually housed in clear Plexiglas shoebox cages in a vivarium with 12 h light : 12 h dark cycles, with fluorescent lights on at 8:00 a.m. and ad libitum access to water. During testing they were food deprived and maintained at approximately 85% of their normal body weight with supplemental feeding of LabDiet for Rodents (PMI Feeds). At the beginning of the experiment, the rats were approximately 90 days old.

Apparatus

Four Colbourn operant chambers (Model #E10-10) were used, each individually housed in isolation chambers (Model #E10-20) and equipped with baffled exhaust fans. On the front wall of each chamber, two retractable levers (Model #E23-07 in two of the boxes and Model #E23-17 in the other two) were positioned on either side of a pellet feeder (Model #E14-06) approximately 3 cm from the grid floor and 14 cm apart. The pellet feeder was placed in the centre of the front wall with the opening approximately 3 cm from the floor of the chamber and provided access to 45 mg pellets (Bioserve Universal Research Test Diets grain-based rodent pellets). A house light (Model #E11-01, bulb #SL1819x) positioned 6.5 cm directly above the pellet feeder and reflecting toward the ceiling of the chamber remained on throughout each trial. The absence of the houselight was the carrier of the temporal signal. All events and responses were arranged and recorded by a microcomputer system located in the same room.

Experimental Design

The 24 rats were randomly assigned to one of three groups, so that each group consisted of 8 animals. One group of animals had drug injections during training (DT), while another experienced the drug outside of the training paradigm (DE). The last group had no experience with the drug prior to testing (DN).

It was predicted that these three groups would behave in the following manner. The drug trained group (DT) would show a phasic shift in the PSE to the right when tested with saline injections. It was also proposed that there would be a leftward phasic shift in the PSE when the drug experienced (DE) group was tested with quinpirole. It was also hypothesized that drug naive animals (DN) would experience disruption and this would be indicated by a reduction in accuracy at the endpoints. Performance could be so disrupted that the selective clock shift would not be detected.

Procedure

Training and testing of the animals consisted of four stages; Pre-training, Two-signal training, Seven-signal training and Seven-signal testing. Pre-training consisted of magazine and lever training. This occurred over a period of seven sessions and ensured that all rats reliably pressed either lever for a continuous schedule of 45 mg pellets. This pretraining was achieved by exposing each rat to a computerized autoshaping procedure. During this procedure, either the right or left lever was presented in the chamber and after 60 s, if the rats had not pressed the lever, it was retracted and a pellet was delivered to the food hopper. After four 60 min sessions, only two of the rats had adequately acquired the bar pressing response, as defined by 60 bar presses in 5 min or less. As a result, all rats

had one session in an auxiliary operant chamber so that successive approximations could be used if needed to achieve adequate acquisition of the bar press response. All rats were run for two more automated sessions (60 trials each) to ensure a high rate of lever pressing was achieved.

Two-signal training developed the discrimination between the two temporal stimuli (2 and 8 s) by reinforcing responding to the left lever after 2 s of darkness, and to the right lever after 8 s of darkness. The right and left levers were counterbalanced for temporal durations across and within groups. Throughout this experiment, rats received two types of sessions which will be labelled as 'Operant' sessions or 'Exposure' sessions. The 'Operant' days consisted of either the training or testing procedure. For 'Exposure' days, the rat was placed in the non-functional, but illuminated operant chamber with the levers retracted for 20 minutes. The order of these was assigned in a pseudo-random manner so that there was a maximum of two consecutive days of one type of session. The running sequences for each group is included as Appendix A. The overall probability of either an 'Operant' or 'Exposure' day was approximately 0.5.

Injections began during Two-signal training and remained throughout Two- and Seven-signal training. All injections were administered 30 min prior to the beginning of the session. During 'Operant' training days, the DT group received quinpirole, and the DN and DE groups were administered saline. During the 'Exposure' days, the DE group received quinpirole, and the DT and DN rats received saline. This allowed for one group (DE) to have experience with the drug in the same environment as during training. It also limited the possibility of sensitization to the drug in the DT group, as the drug was

received on an unpredictable schedule, so that the injection procedure and even placement in the operant chamber only predicted the drug 50% of the time. This design paradigm is outlined in Table 1, and provides a summary of training and testing drug manipulations. In addition to this, half of the rats in each group were given 'Operant' sessions on each day, so that one calendar day consisted of three 'Exposure' runs (20 min each), and three 'Operant' runs (1 hour each).

In each session, there were 50 presentations of each temporal sample, resulting in 100 trials. There was a time limit of 60 min to complete the 100 trials established in the computer programme. If all of the trials were not completed in time, the programme was halted, and the rat lost the opportunity to gain the remaining reinforcers. This was done to ensure efficient use of time, and accurate timing of injections. There was a variable intertrial interval (ITI) of 5, 10, 15, 20, or 25 s with the average being 15 s. Each trial consisted of a period of darkness (the sample stimulus), presentation of the levers, possible presentation of the reward, and the ITI. If the rat performed correctly by choosing the lever associated with the duration, a food pellet was delivered and the next trial began. If the rat performed incorrectly by choosing the lever not associated with the duration, no food pellet was received and the correction procedure was initiated. The correction trial presented the same temporal sample after a delay of 5 s. If during a correction trial, the rat chose the lever not associated with that duration, the correction trial was re-presented. Choice of the correct lever for the associated duration during a correction trial resulted in the delivery of a pellet and the next trial beginning. Only the first lever choice was included in the recorded data. Two-Signal training continued until the average accuracy in

each group for short and long samples was 85% over four consecutive sessions, with no session having either the short or long response accuracy less than 80%. Once this response criterion was achieved, the probability of being rewarded for a correct response was reduced to 0.5. Once performance was again stabilized to the aforementioned criterion, Seven-signal training began. After 84 training sessions, two rats from the quinpirole trained group could not meet the criterion for 2-signal training, so they were dropped from the study.

Seven-signal training included the original 2 and 8 s stimuli endpoints and five intermediate sample durations, so the sample stimuli set was 2.0, 2.6, 3.2, 4.0, 5.0, 6.4, and 8.0 s of darkness. The rats were still rewarded with a probability of .5 for responding correctly to the 2 and 8 s samples, but responses to either lever in response to the intermediate stimuli never resulted in food reward. The intermediate stimuli were introduced so that choices associated with the 'long' lever could be monitored and psychophysical analysis could be performed. The drug injection schedule remained the same as Two-signal training. Each endpoint sample was presented with equal probability on half of the trials in each session. The remaining half of the trials were equally distributed among the five intermediate samples. This resulted in 100 trials per session, 25 trials of each endpoint (2 and 8 s), and 10 trials for each intermediate duration. The correction procedure was used for the endpoint duration trials (2 or 8 s) if an incorrect response occurred. The correction procedure was not used for the intermediate samples as there was never an incorrect response. All rats remained at Seven-signal training for eight sessions.

Seven-Signal testing had the drug injections reversed. During Operant days, the DT group received saline while the DN and DE groups received quinpirole. During the Exposure days, the DT group got quinpirole, and the DN and DE groups received saline. Seven-Signal testing also lasted for eight sessions.

Drug

The highly effective D_2 agonist, quinpirole, was used (Research Biochemicals International, Natick, MA). The dose of 0.08 mg/kg for quinpirole was administered intraperitonally using a distilled water vehicle and dissolved so that injection volumes were 1 ml/kg. This dose was used for all rats during the first 45 sessions. At this point, the DN and DE groups had completed the study, and the dose was cut to 0.04 mg/kg for the DT group. Bushnell and Levin (1993) trained rats in an appetitive operant task that permitted quantification of working memory, reference memory and motor function. They studied doses of d-amphetamine from 0.3 to 1.0 mg/kg, as well as doses of quinpirole from 0.01 to 0.056 mg/kg. Bushnell and Levin (1993) found similar disruption of spatial delayed nonmatching-to-position over these doses of d-amphetamine and quinpirole, with the exception that response latency was increased more with quinpirole. Maricq et al. (1981) used 1.5 mg/kg methamphetamine in a timing perception task and found a shift in the clock. By extrapolating the drug doses used in Maricq et al. (1981), which were required to achieve a clock shift, to the Bushnell and Levin (1993) paradigm, the estimated dose of quinpirole was 0.08 mg/kg. It was postulated from these data, and other behavioural quinpirole studies, that although motoric interference would occur under quinpirole, the rats would have adequate time to complete the task (Garrett & Holtzman, 1993; Hoffman

& Donovan, 1994; White, Packard & Seamans, 1993).

Analyses

A data file was generated that included the following information for each trial; date, rat, session, trial number, total number of trials presented in that session, sample presented, choice of long lever, and response latency. No data were recorded for correction trials, and is not included in the calculations presented here. The number of responses to the lever associated with the long duration and the total number of presentations of each sample were used to derive the proportions required for the psychophysical functions. This was done, because not all rats completed all trials in each session, and the proportions were corrected for this failure to complete trials, by generating these two pieces of data. Calculation of the psychophysical functions and PSE shifts were made for each rat, using means from the eight days of Seven-signal training and Seven-signal testing.

Results

Acquisition

Acquisition data include the number of sessions required to complete 2-signal training with a probability of reinforcement of 1.0. The DE and DN groups, trained with saline, acquired this discrimination after 23 training sessions. The acquisition criterion used the average of the group (n=8) being 85% correct for both endpoints over 4 consecutive days, with no score for either endpoint alone being less than 80%. The two groups then took 6 sessions to complete the 0.5 probability reinforcement Two-signal training and advance to Seven-signal training.

The DT group, trained with quinpirole, exhibited great difficulty in learning this discrimination. After 45 training sessions, the quinpirole dose was reduced to 0.04 mg/kg. At this point, the criterion required to continue with Two-signal training (0.5 probability of reinforcement) was changed to an 80% four-day average (n=2) for both endpoints, for pairs of rats. With these reduced criteria, 4 rats acquired the discrimination after 12 additional training sessions. Two more rats achieved the 80% criterion after 16 training sessions on the reduced drug dose. The remaining 2 rats in the DT group did not acquire the task in the next 37 sessions and were, as a result, dropped from the study. Acquisition functions are shown in Figure 2, where the DN group is represented by 'x', and DE group is represented by '+'. The DT (n=6) group, represented by '■', shows impaired training.

These data are interesting for two reasons. First, there was no difference between the acquisition curves for the DN and DE groups. This shows that, despite experience with quinpirole, rats in the DE group were able to learn the discrimination as fast and to

the same level as the DN group. Second, acquisition for the DT group was devastated. The slope of the acquisition function appears to be zero from training days 25 and 45, and as a result of this, the drug dose was cut in half. From Figure 2, it was not apparent that this step improved the rate of acquisition. Clearly, this dopamine agonist affected the learning of this task.

In subsequent sections of the results, the data for the quinpirole trained (DT) group were analysed separately as this arrangement of data facilitates the presentation of the results.

DN and DE Groups; Seven-Signal Analyses

Each psychophysical function was generated with the mean proportion of 'long' responses as the dependent variable. This is represented in Figure 3, which depicts the mean proportion of long responses for the DN and DE groups. Training is represented by solid lines and testing by dashed lines. The DE group is symbolized by triangles while the DN group is symbolized by crossed lines. A 2 x 2 x 7 (group by phase by sample duration) mixed analysis of variance was conducted with group as a between factor and phase and sample duration as within factors. Group was defined as either drug naive (DN) or drug experienced (DE). Phase was defined as either training or testing. Sample duration was defined as the seven sample stimuli used in Seven-signal training and testing.

The analysis for DE and DN groups resulted in no significant main effects of group or phase (F's (1,14) <1) but there was a main effect of sample duration ($F(6,84) = 331.85$, $MSE = 0.008$, $p < .001$). The phase by group interaction was not significant (F (1,14) < 1). The sample duration by group, and the sample duration by phase interactions were

both significant (F's (6,84) = 2.77 and 24.69, MSE's = 0.008 and 0.006, p's < .02 and < .001). The three way interaction of group by phase by sample duration was not significant (F(6,84) < 1). The sample duration by group interaction is depicted in the top panel of Figure 4. Further examination of this interaction showed group differences only at sample durations 2.0 and 2.6 s (F's (1,14) = 5.03, 5.88, MSE's = 0.009, 0.009, p's < .04, .03).

The sample duration by phase interaction is illustrated in the bottom panel of Figure 4. The testing curve, illustrated as a dashed line with large squares, showed less accuracy at the two endpoints compared to the training curve, shown as a solid line with small dots. Actually, the testing curve showed less discrimination for the task at each sample duration when compared to training, because the points were closer to random choice. Significant differences were found at all sample durations (F's (1,14) = 37.09, 28.23, 16.77, 5.11, 10.45, 10.89, and 28.47, MSE's = 0.007, 0.008, 0.004, 0.011, 0.010, 0.007, 0.004, p's < .001, .001, .001, .04, .01, .01, .001). For the three shortest sample durations, the testing curve had a higher proportion of 'long' responses compared to the training curve, while for the four longest sample durations, the testing curve had a higher proportion of 'short' response choices when compared to training.

In addition to these ANOVAs, for each rat a set of five regressions was also generated from the proportion of 'long' responses for each of three consecutive sample durations. From these regressions, the sample duration combination that created the linear equation with the greatest slope was determined for each rat. From this equation, points of subjective equality (PSE) and points at which the sample duration was associated with the long response 25% and 75% of the time were calculated. The difference limen (DL)

was calculated as the average of the difference between the sample duration associated with the long lever 75% of the time and the sample duration associated with the long lever 25% of the time. The Weber Fraction (WF) was calculated as the DL/PSE. The PSE was calculated to determine whether changes in the pacemaker pulse rate had occurred. The DL was calculated to give an indication of the rats' ability to discriminate the range of samples used here; the lower the DL value the better the rats were in discriminating the samples. The WF, a corollary of the DL, was used to determine the animals' ability to disciminate differences over any sample range in that modality. It was expected that there would be a decrease in the PSE showing a shift to the left, and low DL and WF scores, as this shows good discrimination of the samples.

Three analyses, one for each of PSE, DL, and WF, were conducted as a 2 x 2 mixed ANOVA (phase by group) with phase as a repeated measure variable and group as a between variable. For the PSE analysis, neither the main effects nor the interaction were significant (p's < 0.13). The grand mean was 4.0 s. The PSE mean for the DN group was 3.9 s, and for the DE group was 4.0 s. During training the mean PSE was 3.9 s, and during testing it was 4.1 s. The analysis of the difference limen (DL) revealed significant main effects of group and phase (F's (1,14) = 4.83 and 16.19, MSE's =0.249, 0.244, p's < .05 and .001) although the interaction of group by phase was not reliable. These data are presented in the top panel of Figure 5, where it can be seen that the DN group had less temporal discrimination than the DE group during both training and testing, as represented by a higher DL. The mean DL during training was 0.79 s and this increased during testing to 1.4 s. For the DN group, the DL increase from training to testing was significant (F

(1,14) = 12.92, *MSE* = 0.244, $p < .001$), as was the DL increase in the DE group (F (1,14) = 4.39, *MSE* = 0.244, $p < .05$). The analysis of Weber Fractions resulted in significant main effects of phase and group (F (1,14) = 11.28 and 6.07, *MSE's* = 0.021, 0.016, $p < .001$ and .03), but again the interaction of phase by group was not significant. The bottom panel of Figure 5 shows these main effects, where the WF was greater in both groups during testing, and overall increased from 0.20 in training to 0.37 in testing.

DT Group

As illustrated in Figure 6, the DT group analysis resulted in a main effect of sample duration (F (6,30) = 42.11, *MSE* = 0.016, $p < .001$). Neither the main effect of phase (F(1,5) <1), nor the phase by sample duration interaction (F(6,30) = 2.08, *MSE* = 0.004, $p < .09$) were significant. The quinpirole trained (DT) group did not show any difference between training and testing for the PSE, DL, or WF. The PSE overall was 4.1 s, DL was 1.4 s and the WF was 0.36. Overall, there was no difference found between training and testing on any measure calculated. The main effect of sample duration shows this group was able to perceive the samples, but performance did not change from training to testing.

The testing phases of the DN and DE groups were compared with the training phase of the DT group. The analysis, comparing sessions with 0.08 mg/kg quinpirole and those trained with 0.04 mg/kg quinpirole, showed no differences among any of the groups ($F < 1$). There was no apparent drug dose effect, as was assumed, because the dose was different from the DN and DE group training (0.08 mg/kg) and DT (0.04 mg/kg). Because there were no differences, the observed disruption was not related to the drug dose as would be predicted.

Summary

Analyses determined that, for the DN and DE groups, there were significant effects of sample duration, and the interactions of sample duration by group and sample duration by phase. The three-way interaction was not significant. No differences were found in the PSE between group, phase, or their interaction. The DL and WF differed over both phase and group alone, but neither interaction was significant.

The DT analysis showed that only the main effect of sample duration was significant. There were no phase differences found in the psychophysical function, PSE, DL, or WF.

Additional Analyses

A secondary analysis was performed with a modified data set, similar to procedures outlined by Meck (1986). The modified data set was obtained by removing any trial with a response latency greater than 3.0 s. The proportion of trials with latencies less than and including 3.0 s was used as the dependent variable in a 2 x 2 x 7 mixed ANOVA [group (between) by phase (within) by sample duration (within)]. For the DN and DE groups, which were again treated separately, the Small data set included 93.65% of the Total data set. The main effect of phase ($F(1,14) = 31.49, MSE = 0.017, p < .001$) and the interaction of phase by sample duration ($F(6,84) = 2.69, MSE = 0.0006, p < .02$) were significant. The DN group included 97.33% of the Total data set for the training phase and 86.18% of the Total data set in the testing phase. The DE group included 99.74% of the Total data set in the training phase and 91.36% of the Total data set in the testing phase. The phase by sample duration interaction is represented in Figure

7, which illustrates significant but small differences at each sample duration (*F's* (1,14) = 39.74, 29.83, 19.76, 35.24, 30.94, 15.74, and 31.76, *MSE's* = 0.001, 0.002, 0.004, 0.003, 0.004, 0.005, 0.002, all *p's* < .001). Thus, quinpirole increased response latencies, as predicted. For the DT group, the Small data set was reduced from the Total data set by 5.78%. The analysis conducted for the DT group revealed no significant differences between the two data sets. This is particularly interesting because the DT group had fewer trials removed than the DE and DN groups.

All of the analyses conducted on the original data set were repeated with the smaller data set. To quickly highlight, all of the results were consistent with the main analyses with two exceptions. The first was the difference between the DN and DE groups at sample codes 2.0 and 2.6 s was eliminated. The second exception was the PSE during testing increased compared to training and this became significant.

The analysis for the DE and DN groups resulted in a significant main effect of sample duration ($F(6,84) = 343.35$, $MSE = 0.008$, $p < .001$). The mean proportion of 'long' responses for each sample duration at each phase are presented in the top panel of Figure 8. Training is represented by solid lines and testing is represented by dashed lines. The interaction of phase by sample duration ($F(6,84) = 19.64$, $MSE = 0.006$, $p < .001$) was significant. The three-way interaction of phase, sample duration and group was not significant. The phase by sample duration interaction is represented in the bottom panel of Figure 8. The testing curve when collapsed over group was less accurate at both endpoints compared to the function generated during training. Significant differences were found at all sample durations (*F's* (1,14) = 29.73, 22.40, 7.76, 4.77, 9.02, 8.89, and

18.94, *MSE's* = 0.006, 0.008, 0.004, 0.015, 0.012, 0.009, 0.004, *p's* < .001, .001, .01, .05, .01, .01, .001). For the first three sample durations, testing had higher proportions of long responses compared to training. For the last four sample durations, the testing curve had lower proportions than the training curve.

For the DN and DE groups, three ANOVAs were run with either PSE, DL or WF as the dependent variable. For the PSE, the main effect of phase was significant ($F(1,14) = 4.79$, $MSE = 0.167$, $p < .05$). The training PSE was 3.9 s, and the testing PSE was 4.2 s. This suggests that the clock speed was slowed down and that quinpirole acted more like an antagonist, causing the observed rightward shift. The difference limen ANOVA resulted in a significant main effect of phase ($F(1,14) = 14.95$, $MSE = 0.177$, $p < .001$). The DL during training was 0.78 s and during testing was 1.35 s. The Weber Fraction ANOVA also showed a phase effect ($F(1,14) = 11.87$, $MSE = 0.01$, $p < .001$) increasing from 0.19 during training to 0.32 during testing.

The data for the DT group psychophysical function showed significance for sample duration ($F(6,30) = 44.30$, $MSE = 0.016$, $p < .001$). The phase by sample duration interaction approached significance ($F(6,30) = 2.28$, $MSE = 0.004$, $p < .06$), and this is seen in Figure 9. These data suggest that there was less accuracy for the temporal task when the group was tested with saline. The DT group did not show an effect of phase for either the PSE, DL or WF ANOVA. The overall PSE was 3.9 s, the DL was 1.44 s and the WF was .46.

Summary for Additional Analyses

Analysis with the DE and DN groups determined significance for the main effect of

sample duration and the interaction of sample duration by phase. The three-way interaction of group, phase and sample duration was not significant. The PSE, DL and WF were all found to differ only over phase, and all increased from training to testing. The DT group only showed differences as a function of sample duration. For the curve characteristics, there were no differences found in the PSE, DL or WF.

Discussion

There are a number of specific conclusions that can be drawn from this experiment regarding acquisition, the entire data set, the secondary analyses run with the modified data set, and the comparison of the two data sets.

During acquisition, the DN and DE groups were indistinguishable in their performance on Two-signal training. This was despite the DE group having experienced the drug outside of the training paradigm. After equating for the number of trials received in each session, the DN and DE acquisition curves were very close to the saline acquisition curves reported by Meck (1983). When quinpirole was paired with the temporal task in the DT group, learning of the task was severely hindered, even when the drug dose was halved after 45 training sessions (90 days in total). Meck (1983) argued that dopaminergic manipulations act on the internal clock component, while cholinergic manipulations affect reference memory. In his study, training under the influence of haloperidol or amphetamine produced slightly lower acquisition curves compared to animals trained under saline. By contrast, rats in the DT group in the present study were very impaired in the learning of the temporal task. It is interesting to note that animals in Meck's study, despite having a lower rate of, and lower accuracy during, acquisition, show Seven-signal training where accuracy on the endpoints actually improved, so that all animals were exhibiting very good discrimination of the temporal samples. This did not occur here, as the rats remained, at best, at the same level of accuracy during the Seven-signal phases.

Using all the data collected, DE and DN groups showed the sample duration by

group interaction. The DE group had finer discrimination compared to the DN group regardless of the phase. Further examination showed that this effect was localized at the two shortest durations. There were also group effects for the DL and WF.

The sample duration by phase interaction was also significant for the combined DE and DN psychophysical function. Further examination showed no significant shift in the PSE. The PSE moved to the right, and although this was not significant, it was in the direction opposite to that predicted by the internal clock model. The main effect of phase was present in both the DL and WF, resulting in higher values during testing sessions compared to training sessions. Quinpirole decreased the rats' ability to discriminate the sample durations, regardless of whether the rats had previous exposure to quinpirole (DE group) or not (DN group).

Taken together, the main effects of phase and group show two things. First, the use of 0.08 mg/kg of quinpirole during test was not successful in speeding the clock and causing a shift to the left in the PSE, as demonstrated in other studies using non-specific dopaminergic agonists. Meck (1983) reported no phase effect for the DL or WF, showing a stable perception of the samples. His phase effect was found only for the PSE. This was exactly opposite of what was found here, as there was no leftward shift in the PSE and disruption was found in the perception of the samples. Despite this, during training under saline, both groups (DE + DN) combined had values for the PSE, DL, and WF (3.9 s, 0.79 s, and 0.20) that were very close to those reported by Meck in 1983 (4.03 s, 0.85 s, and 0.21). The saline curves in the present experiment successfully reproduced the curves reported by Meck (1983), with discriminability in the present experiment as good as

previously reported. The DT group psychophysical function characteristics (4.1 s, 1.4 s, 0.36) also highlighted that the PSE was close to the PSE of the saline treated animals, in both the Meck (1983) and the current procedure, but discriminability was affected as indicated by the higher values for the DL and WF. Second, experience with quinpirole aided in the discrimination of the shorter signal durations because the DN group had poor discrimination when compared to either the training or testing phases of the DE group.

The secondary analysis using the modified data set found there were differences only with phase and any group differences were eliminated. Both DN and DE groups lost accuracy for the endpoints in testing compared to training. The entire function showed decreased discrimination when compared to training. The PSE also shifted over phase with this modified data set, but it *increased* from training to testing. Meck argued that the phasic shift in the curve was due to influence over the dopamine system, and that when tested with agonists, the shift should be to the left of the saline curve. The reduction in the data set caused the slight rightward shift in the PSE to become significant. This increase was approximately 7% as the PSE increased from 3.9 to 4.2 s, contrary to the internal clock model. Similar to the main data set, both the DL and WF, for DN and DE groups combined, increased significantly from training to testing. Once again, the curve generated with saline injections (3.9 s, 0.78 s, and 0.19) reproduced the saline curve results reported by Meck (1983). The modified data set curve for the DT group (3.9 ,1.4, 0.46) was similar to the DT group total data set curve (4.1 s, 1.4 s, and 0.36), with the exception of the increase in the WF.

The analyses of the two data sets highlight a number of things. The differences

between the DN and DE groups were eliminated by removing trials with response latencies greater than 3.0 s. The trials that were most confusing, as measured by longer response latency, were helped by pre-exposure to the drug. If there was little confusion, as indicated by shorter response latency, the prior drug experience did not influence the lever choice.

The saline curve for the DN and DE groups combined was very similar to the saline training data curves reported by Meck (1983), regardless of the data set that was used. The DT group in either analysis showed a similar PSE, but the DL and WF were higher than those reported by Meck (1983). This decrease in discrimination could be due to the disrupting effect demonstrated by the drug.

The testing curves with quinpirole were very different from the testing curves reported by Meck (1983) for the general dopamine agonists. Meck found no change in the DL or the WF from training to testing. He reported a lower PSE for the testing curve. In the current analyses, the testing curve for the DN and DE groups combined showed higher values for both the DL and WF compared to the training sessions. The PSE shifted to the right in both analyses, and reached significance in the smaller data set, but the shift was opposite to that found by Meck (1983). The DT testing curve, using saline injections, showed no difference in the PSE, DL or the WF, compared to the DT training curve but compared to Meck, the testing curve was very different. Meck (1983) found that rats trained with amphetamine and tested with saline injections had no change in the DL or the WF, but the PSE shifted to the right of the training PSE. Here, there were no changes in the DL, WF, or the PSE.

Lejeune et al. (1995) used a number of measures to study time discrimination and response timing in rats. Using the dopamine re-uptake inhibitor and tricyclic antidepressant amineptine, at doses between 1 and 20 mg/kg, they looked at the discrimination accuracy for 2 and 8 s durations of auditory stimuli. They reported that the drug did not induce intrastimulus shifts in the proportion of correct responses, and accuracy was close to 90% correct for both short and long samples. They argued that amineptine altered neither the representation of the duration, nor decision rules used by subjects to choose a lever. Lejeune et al. (1995) presented this as evidence against the concept of speeding the internal clock. Amineptine is a dopamine agonist, and no bias in accuracy to the lever associated with the long response was found after the first injection. Two of the three time production studies included in Lejeune et al. (1995) yielded a dose-related increase in response rates correlating with a dose-related impairment in the temporal regulation of performance. They also found decreases in response latency, indicating that amineptine improved vigilance, and reactivity to specific stimuli.

Lejeune et al. (1995) used a general dopamine re-uptake blocker, while the current research used a specific dopamine receptor agonist. Like the current research, Lejeune et al. (1995) also found no evidence for the speeding of an internal clock, even though they used a slightly different temporal perception procedure. The saline results reported by Lejeune et al. (1995) were consistent with the Meck (1983) data, but the drug manipulation was ineffective.

Frederick and Allen (1996) used a time production study (FI 140 s) to look at the effect of specific D_1 and D_2 dopaminergic agonists and antagonists, including 0.01 mg/kg

quinpirole. They found a decrease in both peak time and peak rate with quinpirole, compared to the saline controls. Neither the D_1 nor the D_2 antagonist was found to cause significant increases in the peak time. Frederick and Allen (1996) concluded that a temporal schedule can be differentially affected by agents specific for dopamine receptors, and therefore indirectly suggest that the dopamine system may also play a role in the expression of timing behaviours. This corroborates the data for the time production studies in Lejeune et al. (1995). The use of dopamine specific agonists in this time production paradigm was able to produce results that were consistent with the internal clock model. In the Frederick and Allen (1996) time production study, the D_1 and D_2 antagonists did not cause shifts, even though this result has been widely reproduced in the literature for the time perception studies (e.g., Meck, 1983; Rammsayer, 1993). This aspect is inconsistent with the internal clock model, and the authors attributed the inconsistency to a small sample size. They also suggested that the effect of the D_2 drugs on the peak time occurred despite the overall depressive effects found on the peak rate. Unfortunately, in both Lejuene et al. (1996) and Frederick and Allen (1996), the subjects were trained only with saline, and tested with the drug, so the reverse manipulations of the timing, and effects on learning can not be compared.

Santi et al. (1995) found that using both rats and pigeons, the methamphetamine treated animals did not produce results consistent with the internal clock model. They found that under the influence of methamphetamine, there was reduced accuracy for both signal durations at the 0 s delay. Comparing the Santi et al. (1995) 0 s delay data to the data generated for each endpoint in the current research, there was a decrease in accuracy

for the endpoint samples while under the influence of the drug when trained under saline and tested with the drug.

Rapp and Robbins (1976) found also that with rats, there was a marked disruption of temporal discrimination (between 3 and 7 s). They also found that in general, d-amphetamine increased response latencies. This is similar to the current study which reported an increase in response latencies while in the drugged state. Rapp and Robbins (1976) found a greater effect on accuracy for the long duration while under the influence of d-amphetamine. In the present study, differences were found at both endpoints.

Stubbs and Thomas (1974) also found that their pigeons did not demonstrate the predicted 'choose long' effect. Unlike the current study, the psychophysical functions in Stubbs and Thomas (1974) showed a greater decrease in accuracy for the long durations compared to the short durations. Here, a decrease in accuracy was found at both endpoints.

Research by Meck and his colleagues has provided considerable support for the involvement of dopamine in altering the speed of the internal clock (Maricq et al., 1981; Meck, 1983; Meck, 1986; Meck & Church, 1983). However, other studies of dopaminergic effects on temporal discriminations in both rats and pigeons have failed to produce a choose-long response bias which would be consistent with speeding of the internal clock (Lejeune et al., 1995; Rapp & Robbins, 1976; Santi et al., 1995; Stubbs & Thomas, 1974). It is widely recognised that dopaminergic agents may not exclusively affect processes related to time discrimination. For example, there is considerable evidence that dopaminergic drugs like amphetamine affect nontemporal working and

reference memory. Nevertheless, it is important to know precisely what experimental conditions are necessary in order to produce selective effects such as those reported by Meck. Maricq et al. (1981) noted that "in order to determine if clock rate is affected, it is essential to observe the effect of a drug that does not lead to a marked attenuation of temporal control" (pg. 29). Most studies that report results inconsistent with the internal clock have demonstrated an attenuation of temporal control. However, this is not true of Lejeune et al. (1995). Their drug failed to attenuate temporal control, but it did not affect clock rate.

Can either procedural details and/or methods of analysis of the data account for differences between studies that show effects on clock rate and those that don't? Drug dosage and type of drug can probably be ruled out as Meck (1983) and Santi et al. (1995) were identical in these parameters. However, previous exposure to the drug, the use of a psychophysical procedure and discarding of trials with latencies greater than a criterion value could all have been important. The role of previous exposure to the drug seems not to be important for two reasons. First, Meck and Church (1983) were able to get selective effects on clock rate and no attenuation of temporal control in rats that had no previous exposure to methamphetamine. Second, in the present experiment, the exposure to the drug was a manipulation, and no differences were found in the secondary analyses, and only minor differences were found in the original analyses between the groups (DE and DN). In this study, there was no effect on the internal clock, but there was attenuation of the temporal function. Also, the acquisition functions were similar in both the DE and DN groups, as would be expected if prior drug exposure was not an important

factor in a manipulation of the internal clock.

In addition, the use of the psychophysical function procedure has also been shown not to be important in providing evidence for the internal clock mechanism. The current parameters matched those used by Meck (1983). Use of the procedure was not helpful in obtaining support for the internal clock model in this case, while it was in the Meck procedures. Also, Frederick and Allen (1996) were able to find support for the internal clock model, while using a time production procedure. It seems that the use of a psychophysical function is not a key measure in demonstrating the functioning of the internal clock.

It has also been suggested that by adding a response latency criterion, the demonstration of the internal clock model will be made clear, as was illustrated by Meck (1983). In the current experiment, analyses were conducted with and without responses greater than 3.0 s. Even with the response latency criterion, there was no predicted manipulation of the internal clock, just attenuation of temporal control. Clearly, it has been argued that demonstration of the internal clock mechanism is not due to prior exposure to the drug, the use of a psychophysical procedure, or to the use of a specific drug.

The role of dopamine in timing is more complex than once thought, as demonstrated by the D_2 agonist quinpirole showing evidence of disruption in learning, while showing no effect on the PSE. The hypotheses that dopamine controls temporal perception, while acetylcholine controls temporal memory appears to be too simplistic. Fine tuning of the internal clock model can be obtained using pharmacological

manipulations. Time production studies using dopamine agonists (D_1 or D_2) and time perception studies using dopamine antagonists (D_1 or D_2) have provided evidence for the internal clock model. Conversely, time perception studies using D_1 or D_2 agonists and time production studies using D_1 or D_2 antagonists have not consistently provided support for the internal clock model. It could be that some receptors activate the switch and others deactivate it. Or there could be differential notions regarding the working memory or reference memory components. Perhaps this information is transferred using a specific receptor type, and thus, this particular stage was affected. In theory, if the dopamine component of the internal clock model is going to stand up to scrutiny, then a complete analysis of antagonists and agonists may be necessary to separate the role of receptors on the internal clock. Until this is done, work such as this will only begin to provide insight into the exact role of the dopamine system in timing.

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Table 1: Drug injection regime for Seven-signal training and testing for all rats.

Training

Group	Day '*A*' (Operant)	Day '*B*' (Exposure)
Drug Naive (DN)	Saline	Saline
Drug Trained (DT)	Quinpirole*,†	Saline
Drug Experienced (DE)	Saline	Quinpirole*

Testing

Group	Day '*A*' (Operant)	Day '*B*' (Exposure)
Drug Naive (DN)	Quinpirole*	Saline
Drug Trained (DT)	Saline	Quinpirole†
Drug Experienced (DE)	Quinpirole*	Saline

* 0.08 mg/kg quinpirole

† 0.04 mg/kg quinpirole

*,† 0.08 mg/kg (45 Training Sessions), 0.04 mg/kg (remainder of Training Sessions)

On any session half of the rats were on day 'A' and half of the rats were on a 'B' day.

Figure Captions

Figure 1: Internal Clock Model as modified from Church (1989).

Figure 2: Acquisition functions for the three groups (DN, DE and DT). The horizontal dashed lines represent the criterion used for DN and DE groups, and the DT group.

Figure 3: Psychophysical functions for the DN and DE groups as a function of the mean proportion 'long' responses and sample duration.

Figure 4: Sample duration by group (DN and DE) interaction (top) and the sample duration by phase interaction (bottom).

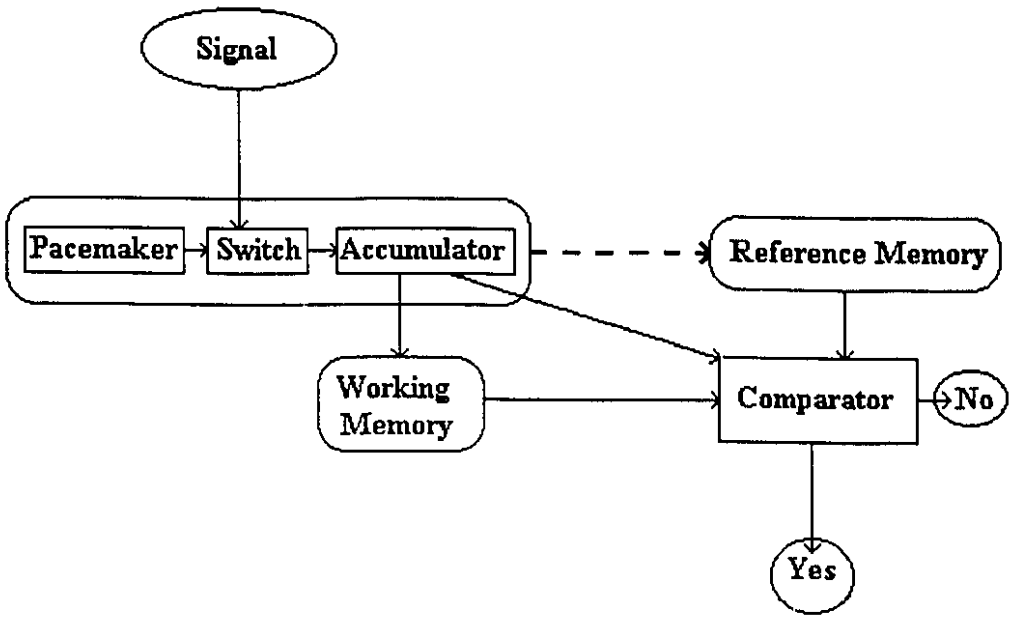
Figure 5: Mean Difference Limens as a function of group and phase (top) and mean Weber Fractions as a function of group and phase (bottom).

Figure 6: Psychophysical functions for the DT group as a function of the mean proportion long responses and sample duration.

Figure 7: Mean proportion of trials with response latency less than or equal to 3.0 s during training and testing.

Figure 8: Psychophysical functions for DN and DE groups generated from data with response latencies less than and including 3.0 s (top), and the main effect of phase (bottom) for the same data set. Training is represented by solid lines, testing by dashed lines.

Figure 9: Psychophysical functions generated from data with response latencies less than and including 3.0 s for the DT group. Training is represented by solid lines and testing is represented by dashed lines.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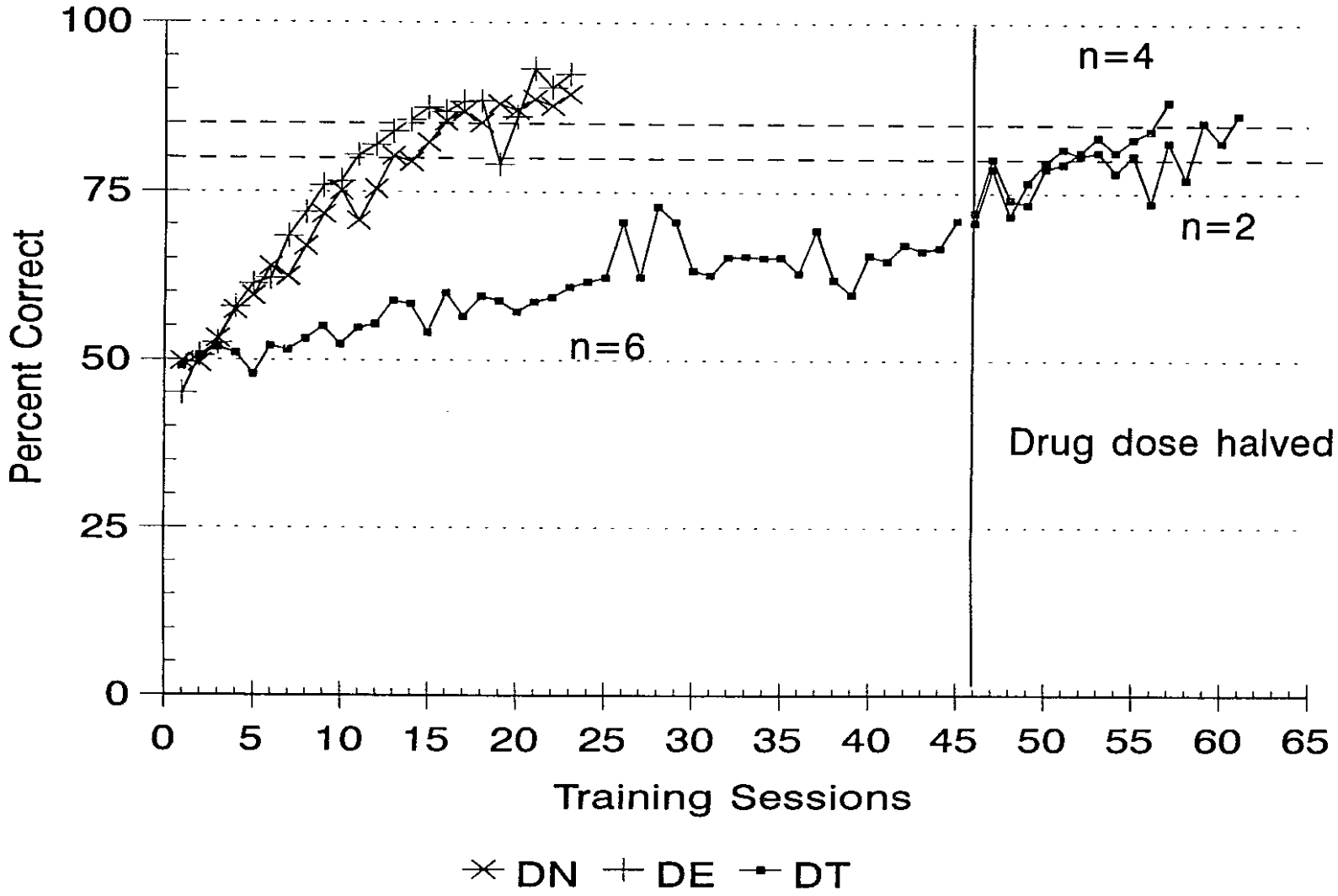
Reference Memory
Working Memory
Comparator
No
Yes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75
50
25
0
Percent Correct
n=4
n=2
n=6
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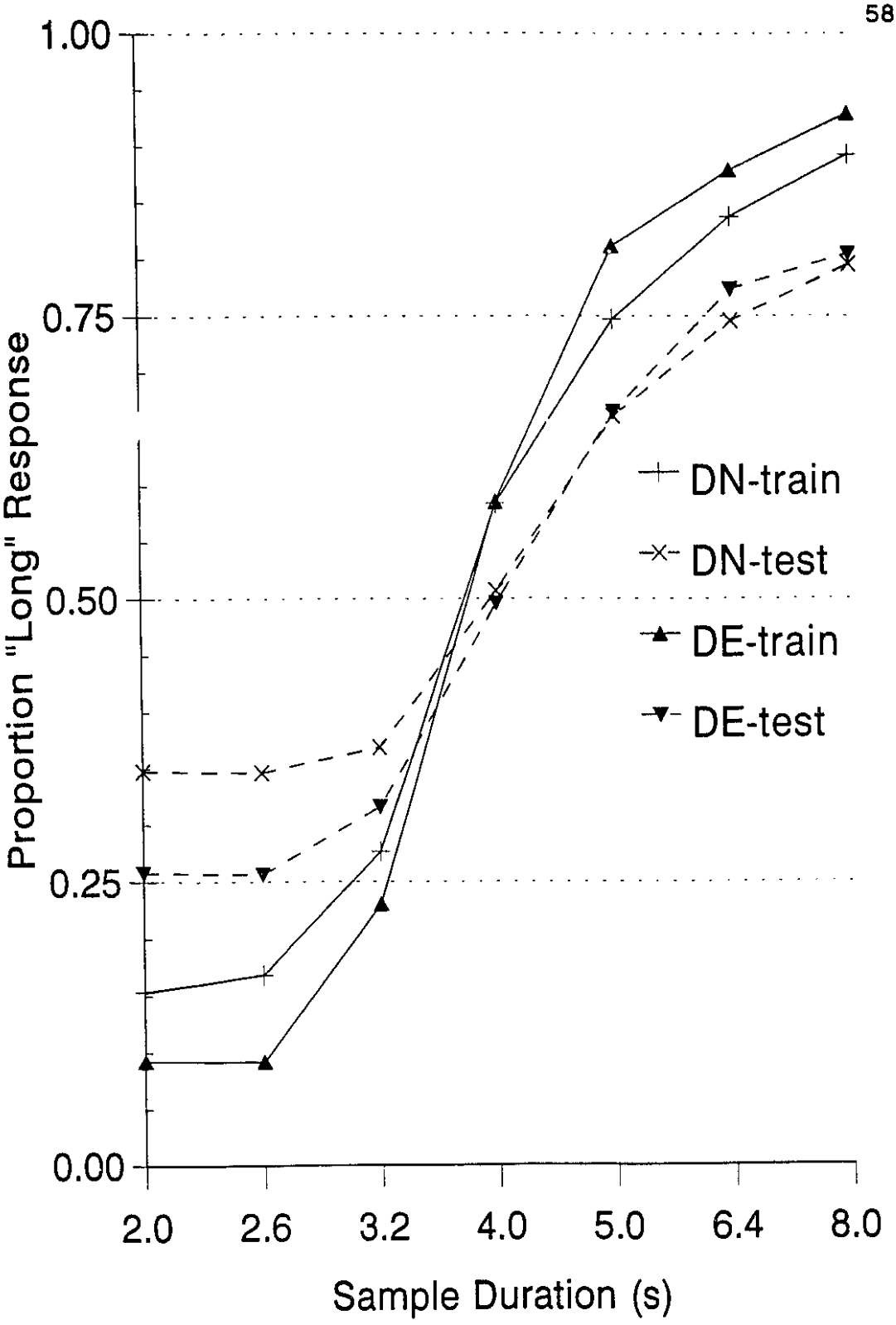
Training Sessions
DN
DE
DT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Proportion "Long" Response
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DN-test
DE-train
DE-test
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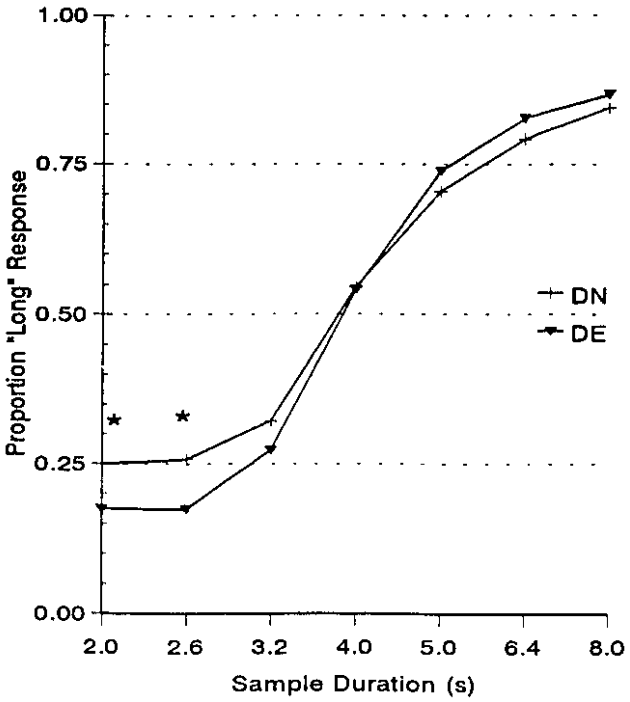
6.4
8.0
Sample Duration (s)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Proportion "Long" Response
DN
DE
2.0
2.6
3.2
4.0
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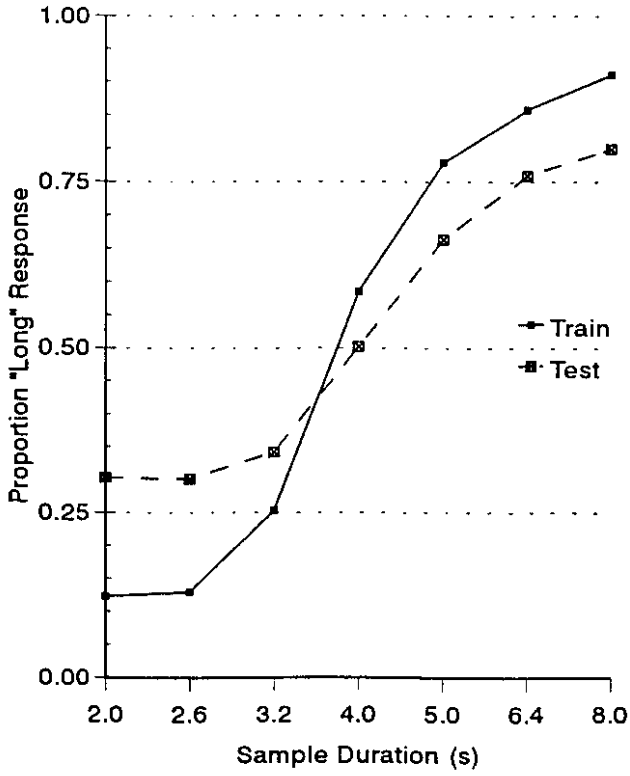
Sample Duration (s)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Proportion "Long" Response
Train
Test
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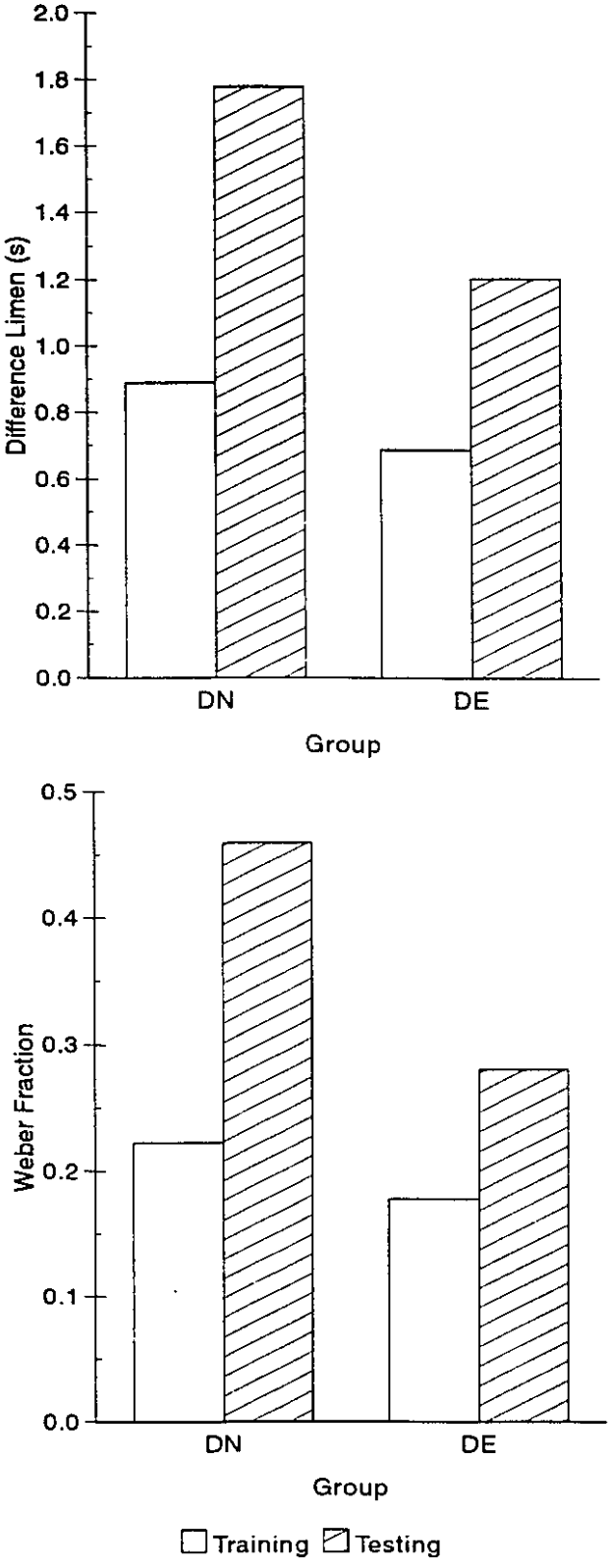
5.0
6.4
8.0
Sample Duration (s)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1.4
1.2
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0.2
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Difference Limen (s)
DN
DE
Group
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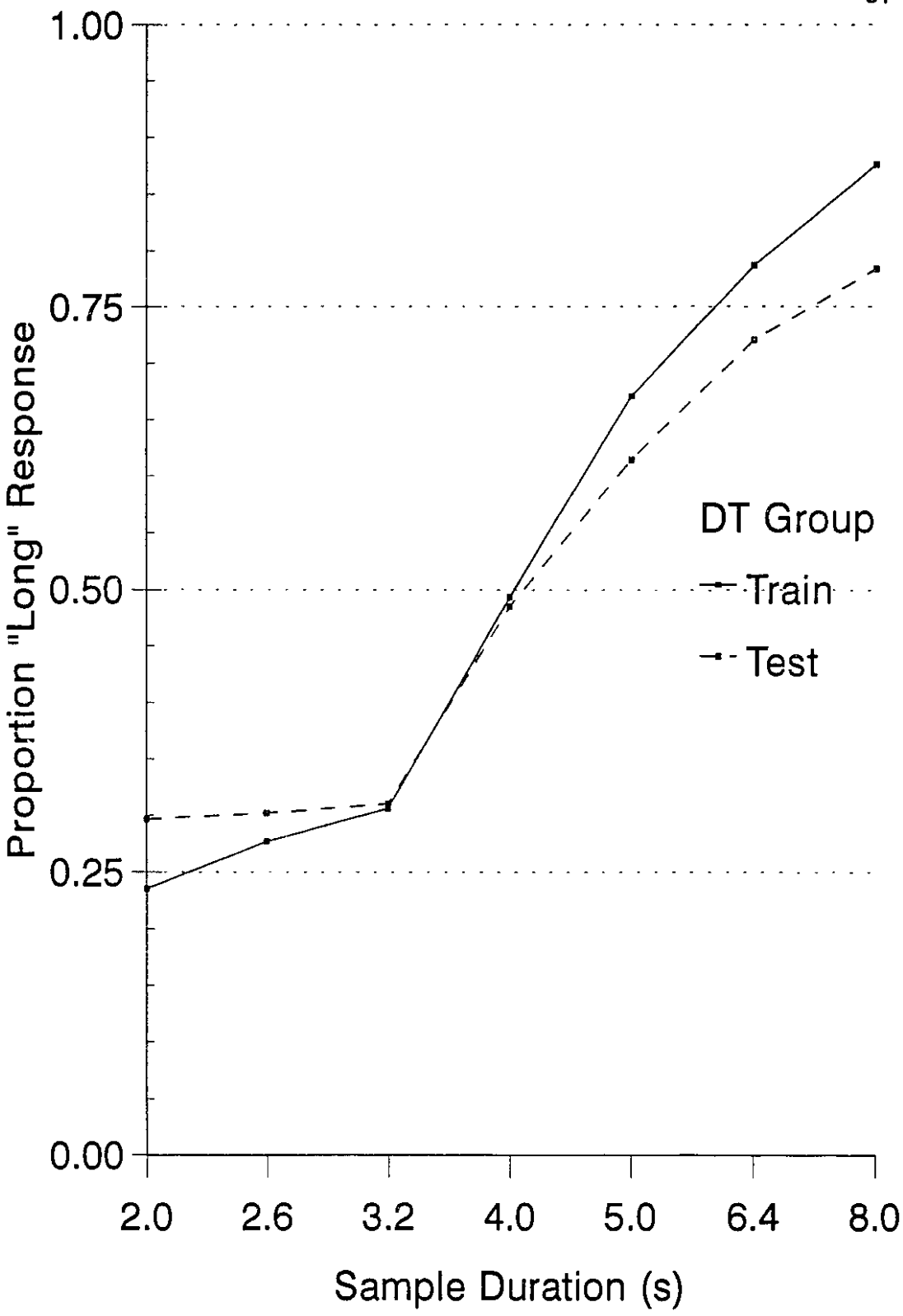
0.3
0.2
0.1
0.0
Weber Fraction
DN
DE
Group
Training
Testing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Proportion "Long" Response
DT Group
Train
Test
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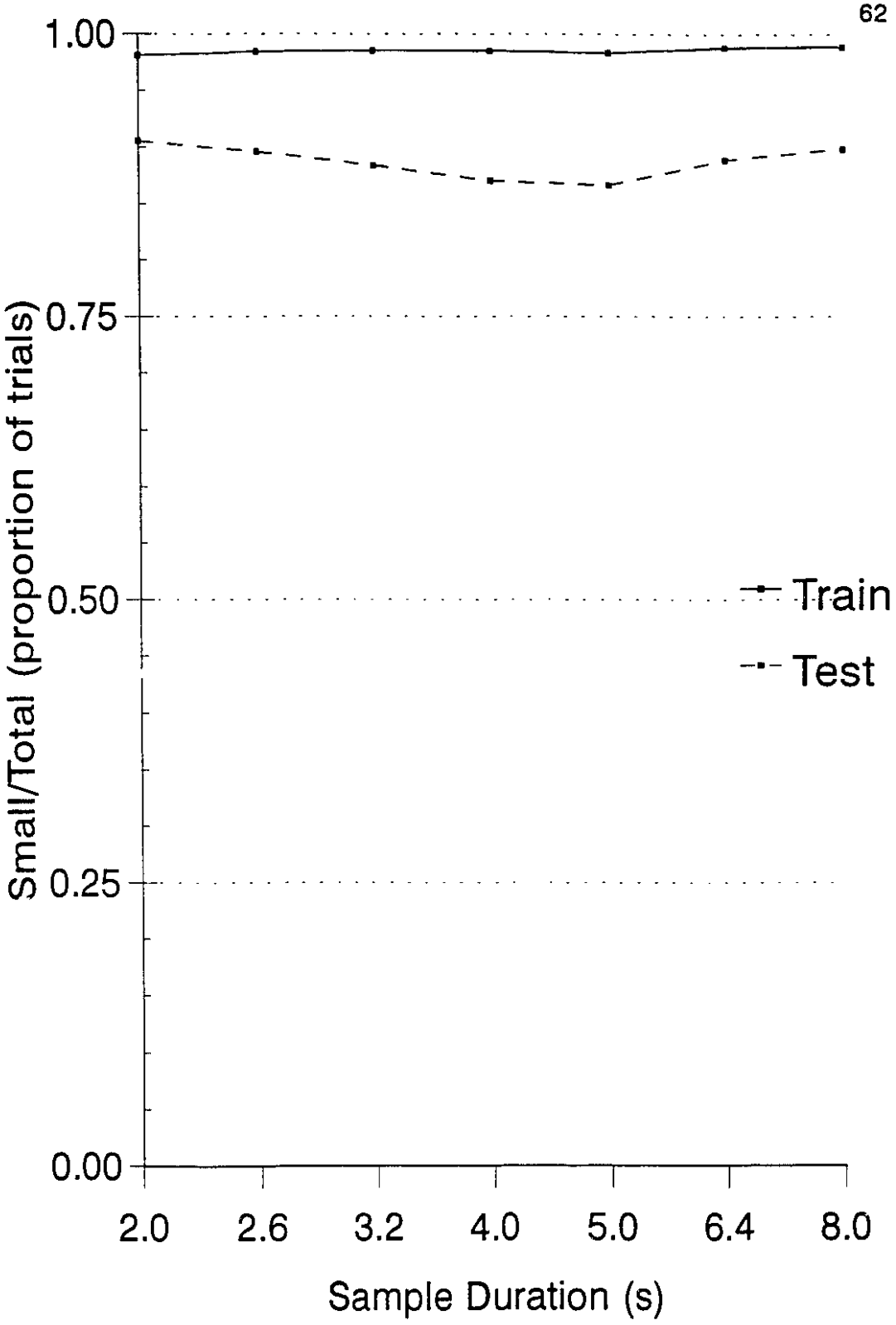
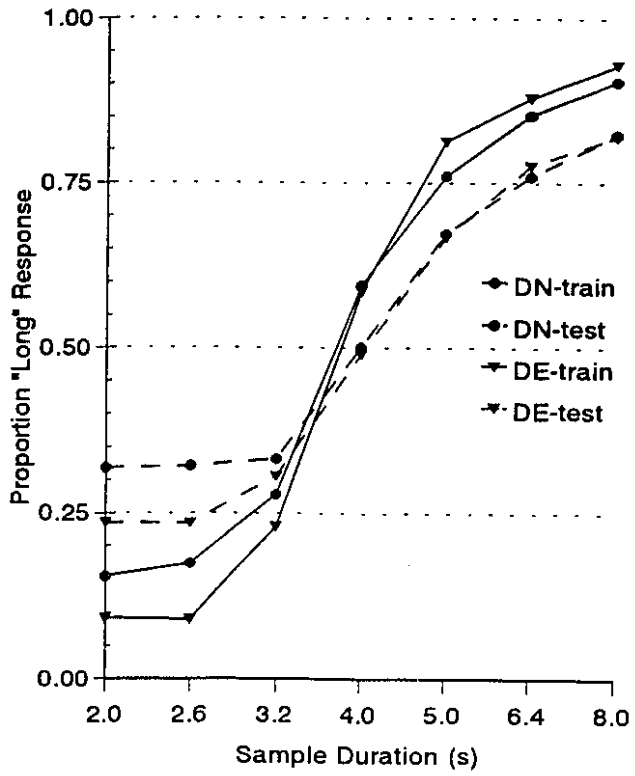
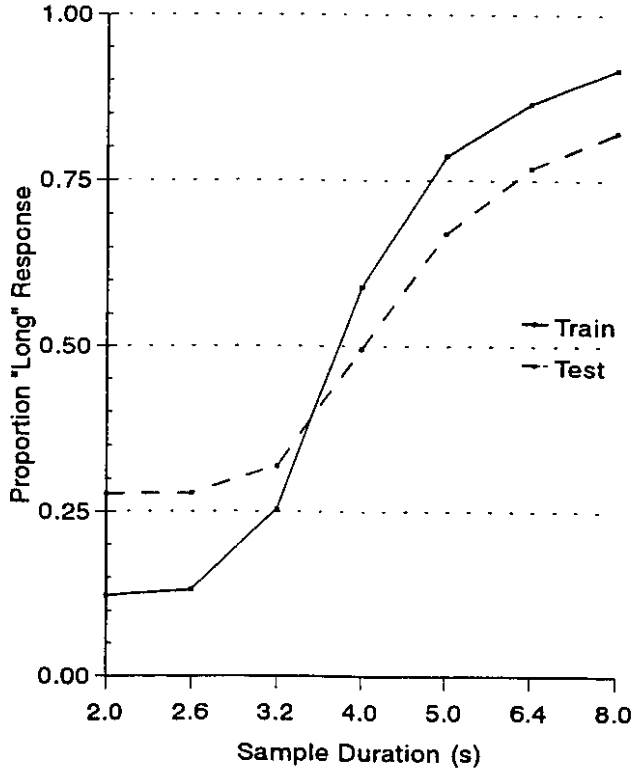
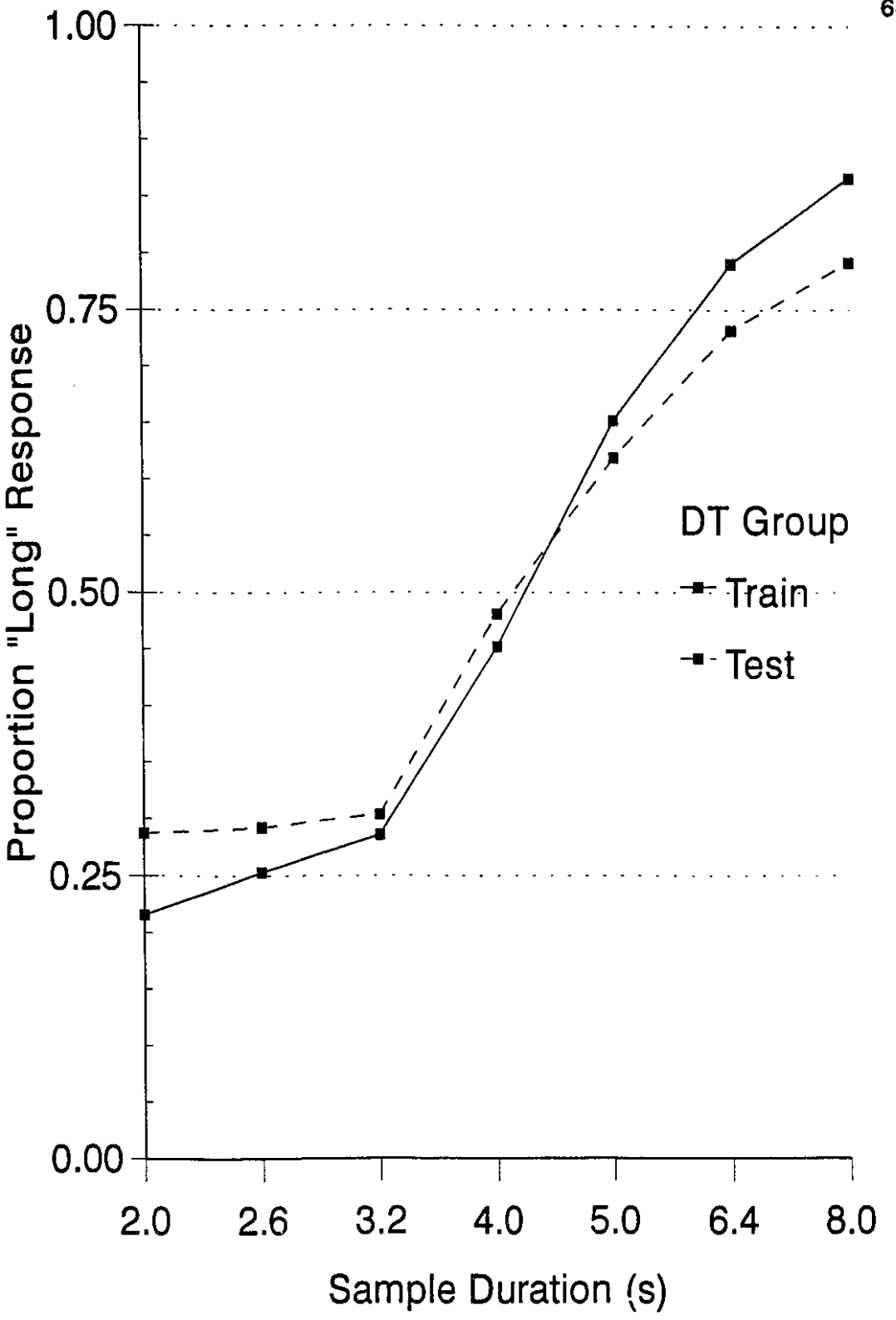
6.4
8.0
Sample Duration (s)


1.00
0.75
0.50
0.25
0.00
Small/Total (proportion of trials)
Train
Test
2.0
2.6
3.2
4.0
5.0
6.4
8.0
Sample Duration (s)


1.00
0.75
0.50
0.25
0.00
Proportion "Long" Response
DN-train
DN-test
DE-train
DE-test
2.0
2.6
3.2
4.0
5.0
6.4
8.0
Sample Duration (s)


1.00
0.75
0.50
0.25
0.00
Proportion "Long" Response
Train
Test
2.0
2.6
3.2
4.0
5.0
6.4
8.0
Sample Duration (s)


1.00
0.75
0.50
0.25
0.00
Proportion "Long" Response
DT Group
Train
Test
2.0
2.6
3.2
4.0
5.0
6.4
8.0
Sample Duration (s)

Appendix A

The following outlines the order of running for half of the rats. Four rats from each group were run on this schedule. The remainder of the rats (Four rats each in the DE and DN groups, and two rats in the DT group) were run with the mirror image pattern, so that the Type of day was exactly reversed. 'O'(operant)days consisted of the rats performing the operant task. 'E' (exposure) days consisted of 20 min of exposure to the non-operational and illuminated operant box. 'Day' was the number of consecutive days the rats were run.

Day	Type	Day	Type	Day	Type	Day	Type	Day	Type
1	O	25	O	49	O	73	O	97	E
2	O	26	E	50	E	74	E	98	E
3	E	27	E	51	E	75	E	99	O
4	O	28	O	52	O	76	O	100	O
5	E	29	E	53	O	77	E	101	E
6	O	30	E	54	E	78	O	102	O
7	E	31	O	55	O	79	E	103	E
8	O	32	O	56	E	80	O	104	O
9	O	33	E	57	O	81	O	105	E
10	E	34	E	58	E	82	E	106	E
11	E	35	O	59	O	83	E	107	O
12	O	36	O	60	E	84	O	108	O
13	E	37	E	61	O	85	O	109	E
14	O	38	E	62	E	86	E	110	E
15	E	39	O	63	O	87	O	111	O
16	O	40	O	64	E	88	E	112	E
17	O	41	E	65	O	89	O	113	E
18	E	42	O	66	E	90	E	114	O
19	E	43	E	67	O	91	E	115	O
20	O	44	O	68	E	92	O	116	E
21	E	45	E	69	O	93	E	117	E
22	O	46	E	70	E	94	E	118	O
23	O	47	O	71	O	95	O	119	E
24	E	48	E	72	E	96	O	120	O

Day	Type	Day	Type
121	E	145	O
122	E	146	O
123	O	147	E
124	E	148	O
125	O	149	E
126	O	150	O
127	E	151	E
128	E	152	O
129	O	153	O
130	E	154	E
131	O	155	O
132	E	156	E
133	E	157	O
134	O	158	O
135	O	159	E
136	E	160	O
137	E	161	O
138	O	162	E
139	O	163	O
140	E	164	O
141	O		
142	E		
143	O		
144	E		