To Donald and Myrna
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Emerging evidence suggests that nicotine administration may enhance short-term remembering. Much of this evidence comes from animal models using a procedure called delayed matching-to-sample, wherein the animal is trained to select a comparison stimulus that matches some physical property of a previously presented sample stimulus. Delays between sample stimulus offset and comparison stimuli onset are manipulated, accuracy is measured, and a forgetting function (i.e., the relation between accuracy and delay) is derived. The present research first examined nicotine’s effects on delayed matching-to-sample performance in pigeons (a commonly used species in behavioral pharmacology). In a second experiment, the effects of nicotine administration were assessed under a second, more dynamic remembering task called titrating-delay matching-to-sample. In this procedure, the delay between sample offset and comparison onset adjusts as a function of the subject’s performance. Specifically, correct matches increase the delay and incorrect matches decrease the delay, and steady-state titrated delays serve as the chief dependent measure. This procedure allows for both tests of short-term remembering (i.e., how long a delay a subject can tolerate and still perform accurately), and also drug effects on short-term remembering and the time course of these effects. Both studies examined nicotine’s effects under acute (i.e., drug administrations separated by several days) and
chronic (i.e., daily injections) administration to determine if any effects on short-term remembering would persist over multiple sessions, or if tolerance to nicotine’s effects would be observed. Neither experiment provided much evidence of enhancement of remembering following nicotine administration despite reliable and systematic dose-related changes in other measures of the remembering task. A modest dose-related effect on accuracy of a very circumscribed subset of trial types, however, was found, but both the magnitude and importance of the effect appears to be directly related to tactics of data analysis leaving the effect dubious at best.
CHAPTER 1
GENERAL INTRODUCTION

There is some emerging evidence that nicotine administration may have an enhancing-effect on short-term remembering. Although many dimensions of this effect currently remain unclear, experimental nicotinic therapies for those suffering from dementias, including Alzheimer's disease, continue (e.g., Jones, Sahakian, Levy, & Warburton, 1992; Knott, Engeland, Mohr, Mahoney, & Ilivisky, 2000; Newhouse, 1986; White & Levin, 1999). The study of memory and remembering is ubiquitous in several branches of psychology and neuroscience. Although the present studies focus only on two procedural measures of remembering, many others exist including those detailed in handbooks on various cognitive approaches (e.g., Honig & James, 1971), neuroscience approaches (e.g., Eichenbaum, 2002), and behavior analytic approaches (e.g., Kendrick, Rilling, & Denny, 1986).

One commonly employed recognition task that is believed to test remembering is delayed matching-to-sample (e.g., Berryman, Cumming, & Nevin, 1963; Blough, 1959). In a delayed matching-to-sample (DMTS) task, a subject is presented with a sample stimulus. Completion of an observing response to the sample stimulus terminates sample presentation and initiates a delay (usually called the retention interval) between sample offset and the onset of comparison stimuli. A response to the comparison stimulus that matches some physical property (e.g., hue) of the previously presented sample stimulus results in the delivery of reinforcement. A response to a comparison stimulus that does not match the sample results in a timeout. The chief dependent measure in the DMTS procedure is usually accuracy (i.e., percent correct). Accuracy values can be plotted across different delay values to determine a forgetting function for a given subject under this procedure. Fitting a simple negative exponential function to the forgetting function can also reveal more nuanced characteristics of remembering – the slope of the function is
related to the rate of forgetting and can be modulated by variables in the experimental
preparation, and the intercept is related to the initial discriminability of the preparation; that is,
the accuracy of remembering at no delay. These parameters have been empirically demonstrated
to be reliable and sometimes independent. For example, White (1985) controlled whether the
houselight was on during the retention interval and discovered that the forgetting functions had a
steeper slope when the houselight was on during the delay relative to when it was off, but little
change in intercept. In another experiment he found that increasing the response requirement on
the sample before initiating the delay increased the intercept without changing the slope. These
parameters, therefore, not only quantify values to different components of the DMTS memory
task but also help illuminate experimental variables to be manipulated.

The DMTS procedure has been widely used in studies with several species. Recent
investigations using DMTS as a procedure to assess short-term remembering include studies with
rats (e.g., Seif, Clements, & Wainwright, 2004), pigeons (e.g., Urcuioli, DeMarse, & Lionello,
1999), nonhuman primates (e.g., Sawaguchi & Yamane, 1999), humans with developmental
disabilities (e.g., Williams, Johnston, & Saunders, 2006), and typically developing humans (e.g.,
Critchfield & Perone, 1990). In addition, the DMTS procedure has been repeatedly employed to
assess the pharmacological effects on short-term remembering of several drugs including cocaine
(e.g., Branch & Dearing, 1982), amphetamine (e.g., Baron & Wenger, 2001), ethanol (e.g.,
Girard, Xing, Ward, & Wainwright, 2000), MDMA (e.g., Harper, Hunt, & Schenk, 2006), and
nicotine (see below).

There is emerging evidence that nicotine can enhance short-term remembering. Much of
the working hypothesis involves the fact that among the many neurochemical abnormalities
demonstrated to occur in the postmortem brains of Alzheimer’s patients is a loss of cholinergic
nicotinic receptor density (Julien, 2008). E. D. Levin and colleagues have promulgated much of the supporting early evidence of this claim by observing rat performance in the radial arm maze, an alternative task to the DMTS procedure that is thought to assess remembering. Several articles on nicotine’s enhancement of remembering in this procedure have been published and although some procedural details vary, the basic task involves baiting each of 8 arms with food and placing the rat in the center of the maze. Because under this preparation the food is not replaced during a session, only the first entry in each arm is reinforced, with subsequent entries scored as errors. Daily sessions continue until the rat enters all 8 arms or 5 min has elapsed and the chief dependent variable is the number of entries until a repeat is made (Entries to Repeat [ETR]). Because optimal performance involves entering and obtaining each reinforcer without repeating, that is, remembering which arms you have already collected reinforcement from, memory is thought to be central to optimal performance. And indeed, rats are able to engage in this task with fairly high levels on ETR measures with baseline ETR measures usually between 6.5-7.0 (8.0 indicating an errorless session). After a baseline is collected, nicotine at various doses is administered typically via implanted osmotic minipumps which provide a constant release over several days. Levin and colleagues’ usual effect is that moderate doses of nicotine (e.g., 5-10 mg/kg per day) are related to improvements in the memory task, that is, fewer session-wide repeats, usually approximating an ETR of 7.5 (e.g., Levin et al., 1990; Levin and Rose, 1990; Levin et al., 1993a, 1993b; Levin and Rose, 1995; Levin and Torry, 1996a; Levin et al., 1996b; and see Levin, 2002 for a recent review). This effect appears to be modest in nature with baseline measures approaching the ceiling leaving little room for improvement in performance; however, the enhancement appears reliable across empirical demonstrations.
A second demonstration of nicotine’s purported enhancement of remembering that is procedurally more similar to the present investigations is provided by several studies that have come out of J. J. Buccafusco’s nonhuman primate laboratory. In the first study of a series, Elrod, Buccafusco, and Jackson (1988) trained 5 adult rhesus monkeys on a DMTS procedure using visual stimuli (i.e., illuminated colored keys). After establishing a stable baseline of accuracies under a range of delay values, nicotine administration was studied both with and without pretreatment by nicotine antagonists (e.g., mecamylamine or hexamethonium). Comparisons of baseline performance to nicotine conditions (i.e., assessment of drug effect) were evaluated by percent change in accuracy. The results indicated that nicotine administration increased accuracy and showed the most effectiveness with moderate doses (e.g., 5.0 µg/kg); that is, an inverted U-shaped dose response function relating accuracy to dose was observed, and drug effects were especially notable at the longer delay values (e.g., 30 s and 60 s). Mecamylamine pretreatment abolished the enhancing effects of nicotine described above suggesting that central nicotinic receptors may be involved in short-term remembering. These findings of nicotine’s remembering-enhancement were later replicated (Buccafusco & Jackson, 1991) using 4 young (10 years old) and 2 aged (34 and 35 years old) rhesus monkeys. In addition to the replication of nicotine’s enhancing effects, it was discovered that the enhancement remained during DMTS tests administered 24 hours after the dose of nicotine. In another replication of nicotine’s effects on behavior in DMTS tasks, Buccafusco, Jackson, Jonnala, and Terry (1999) tested for sex differences in the rhesus monkey. Six male and 7 female rhesus monkeys received a series of nicotine doses over 5 weeks. Results indicated that the males demonstrated improvement across the entire dose range, but improvements in female accuracy were only observed following administration of the two highest doses in the range. The review above is not intended to be
exhaustive but merely a sample of Buccafusco and colleagues’ reliable published effect –
moderate doses of nicotine are related to modest increases of accuracy under the larger tested
retention intervals in nonhuman primates. Although the effect appears reliable, it should be
noted that the magnitude as well as the validity of the effect has yet to be conclusively
demonstrated. For example, in all three of the studies described above as well as others by
Buccafusco and colleagues, enhancement effects were usually between 5 and (rarely over) 10%
increase in accuracy, and importantly, those increases were circumscribed to accuracies under
only particular drug doses (usually the mid-level doses) and only under particular retention
intervals (usually but not always the largest tested values). Moreover, the retention intervals and
drug doses included in the analyses both varied across subjects, and no details of individual-
subject values were provided. The reported accuracy increases of approximately 5-10% are
shown as group data (bar graphs), and although error bars suggest fairly low between-subject
variability, individual data on which doses and which retention intervals went into the analysis
are not reported. This sort of selective inclusion into their chief dependent measures of memory
enhancement provides a clear demonstration of changes in performance, but leaves the question
of validity of the purported effects unclear.

Regardless of the extent of reliability and validity of the findings on nicotine and memory
described above, when considering that a vast majority of the results were obtained in essentially
only two laboratories, the reliability and generality of nicotine’s effects on remembering remain
worthy of further examination. In addition, the extent to which this effect can be demonstrated
in other species and under other memory tasks remain unclear. The present experiments attempt
to systematically replicate the memory-enhancing effects of nicotine under different but related
conditions.
CHAPTER 2
EXPERIMENT 1

Nicotine’s Effects on Delayed Matching-to-Sample

Although a majority of the findings on nicotine’s effect on delayed matching-to-sample (DMTS) have come out of the same laboratory, their careful replications of the effect are fairly compelling. To extend these findings further, Experiment 1 assessed nicotine’s effects on DMTS performance using pigeons as subjects. The pigeon is a commonly employed nonhuman animal subject in the study of behavioral pharmacology as well as DMTS investigations. Moreover, as a recent detailed review (Wright, 2007) has noted, published pigeon and monkey forgetting functions under the DMTS procedure often overlap (see Overman & Doty, 1980; Etkin & D’Amato, 1969; Moise, 1976 for individual monkey studies, and Berryman, Cumming, & Nevin, 1963; Blough, 1959; Roberts & Grant, 1976 for individual pigeon studies). Furthermore, as McCarthy and White (1985) and White (1985) have noted, pigeon forgetting functions can be well-described by simple negative exponential functions, and these functions have also been successfully employed in studies with monkeys (e.g., White & Harper, 1996), rats (e.g., Harper, McLean, Dalrymple-Alford, 1994), human children (e.g., Pipe, Gee, Wilson, & Egerton, 1999; MacDonald & Hayne, 1996), typically-developing adult humans (e.g., Rubin & Baddeley, 1989) as well as adults with mental retardation (e.g., Williams, Johnston, & Saunders, 2006). Therefore, in addition to assessing the generality of the findings by Buccafusco and colleagues using nonhuman primates, these findings can be integrated into an existing body of work on both drug effects and pigeon DMTS performance.
Method

Subjects

Six experimentally-naïve White Carneau pigeons (*Columba livia*) served as experimental subjects. They were maintained at approximately 85% of their ad libitum weight. Subjects had vitamin (Agrilabs™ Vitamins and Electolyte “Plus”®, St. Joseph, MO) enriched water available at all times in the home cage. Their experimental weight was maintained by post-session feeding of Purina Pro-11 Grains for Pigeons™ and Purina™ Pigeon Chow® Checkers® in a 50:50 mixture. Subjects were housed individually in a windowless colony room that was maintained between 19°C to 22°C, with ambient levels of humidity. Colony room lights were illuminated daily at 7:00 a.m. and the animals were subject to a 16/8 hr light/dark cycle.

Apparatus

The experiment was conducted in a sound- and light-attenuating BRS/LVE pigeon chamber with inside dimensions measuring 35 cm high, 30 cm long, and 35 cm deep. One side wall (the intelligence panel) contained a houselight, three horizontally arrayed response keys (2.5 cm in diameter) and a 6-cm by 5-cm opening for access to a solenoid-operated hopper filled with mixed grain. The opening was located 10 cm above the floor and centered below the center key. During each feeder operation, the aperture was illuminated, and all other lights in the chamber were extinguished. The center key was horizontally centered on the intelligence panel 25 cm above the floor. The two side keys were located 8 cm to the left and right of the center key. Each key could be transilluminated red, green, or white, and a peck with a force of at least 0.15 N counted as a response and was accompanied by a 30-ms feedback tone (2900 Hz) via the operation of a Mallory Sonalert™. To mask extraneous sounds, white noise at approximately 95 dB was present in the room in which sessions were conducted. Scheduling of experimental
events and data collection were controlled via a dedicated computer system (Palya & Walter, 1993) operating with a resolution of 1 ms.

**Initial Training**

Establishing DMTS performance requires a several-part training regimen. Prior to the experiment proper, each pigeon was first trained to eat food from the hopper and then trained by shaping (Catania, 1998) to peck the center key (illuminated white). After the pigeon pecked the center key reliably when lit, shaping was employed to induce it to peck the right and left key (illuminated white). After the pigeon was pecking all three keys reliably when lit, one of the three keys was illuminated red or green and pecks to the illuminated key resulted in access to grain. Additional shaping was used if necessary, and training trials continued until the pigeon reliably pecked each of the three keys when they were illuminated either red or green.

**Acquisition Training**

All six pigeons were then trained using a simultaneous matching-to-sample (MTS) procedure (e.g., Weinstein, 1941; Carter & Eckerman, 1975; Cumming & Berryman, 1961). Specifically, discrete trials began with the illumination of the houselight and the center (sample) key with either a red or green hue. A single peck to the sample key illuminated the two side (comparison) keys with matching and non-matching hues (i.e., sample and comparison keys were illuminated simultaneously). A single peck to the side key illuminated with the same color as the sample key (i.e., the correct match) turned off the houselight, the sample key, both comparison keys, and raised the food hopper for 3 s followed by a 10-s intertrial-interval (ITI). An intertrial interval was employed because previous research has shown that ITIs improve accuracy of pigeon MTS performance (e.g., Thomas, 1979; White, 1985). A single peck to the non-matching comparison key (i.e., the incorrect response) turned off all lights in the chamber and initiated a 13-s ITI. The 10-s ITI (plus 3-s hopper access) following a correct match, and 13-
s ITI following an incorrect match ensured equivalent times between trial onsets following a correct or incorrect response.

A two-color (red [R] and green [G]), two-comparison MTS procedure yields four possible trial configurations (left/center/right key = RRG, GRR, RGG, GGR). The computer arranged the presentation of these configurations on each trial in a quasi-random order. Specifically, each of the four configurations was presented before any configuration could be repeated (i.e., random selection without replacement). This procedure guarantees that the maximum number of consecutive identical trials is two, the maximum number of consecutive trials on which the same comparison color is correct is four, and the maximum number of consecutive trials on which the same side key is correct is also four. All subjects were exposed to daily sessions consisting of 60 trials.

After 10 consecutive sessions with 85% or greater accuracy under the simultaneous MTS procedure, each pigeon was then exposed to the DMTS procedure. One problematic issue that often occurs during early simultaneous MTS training is the development of stimulus or position biases. A recent investigation in our laboratory addressed this and empirically validated a correction procedure (i.e., repeating a trial if an error was made) designed to eliminate these biases (Kangas & Branch, 2008). This rapidly effective correction procedure was employed when trial configuration analyses suggested developing biases.

**Delayed Matching-to-Sample Training**

The general structure and consequences of the DMTS procedure was the same as that of the simultaneous MTS procedure described above with three exceptions. First, five responses to the sample stimulus were required, second, the sample stimulus was terminated after completion of the response requirement, and third, a variable delay was programmed between the offset of the sample stimulus and the onset of the comparison stimuli. The retention intervals were
alternated in a random-selection-without-replacement fashion, and were 0, 2, 4, 8, and 16 s with 60 trials per session. Thus, each delay occurred exactly 12 trials per session. All 6 subjects were exposed to a fixed time-interval stability criterion (Perone, 1991; Sidman, 1960) of 300 daily sessions of DMTS to serve as an extended baseline before determination of acute nicotine effects began.

Drug Procedure

Nicotine ([–]-Nicotine Hydrogen Tartrate Salt; Sigma, St. Louis, MO) dissolved in a potassium phosphate buffered saline solution (KPO₄), which served as both vehicle and buffer against nicotine’s acidity with a 0.1 molar potassium phosphate buffer prepped with K⁺phos and NaCl to obtain a pH of 7.4. Dose-concentrations were based on previous research involving nicotine administration to pigeons (e.g., Chadman & Woods, 2004). On drug administration sessions, doses were administered by an intra-muscular injection in the pigeon’s breast. During chronic administration the site of successive injections was alternated between the two sides of the breast.

Acute Administration

Following 300 sessions (3600 trials with each delay) of DMTS training, pigeons were administered one of several doses of nicotine prior to a session once every 4 days. The doses were the vehicle - (KPO₄ [0.0]), 1.0, 0.3, 0.1 mg/kg nicotine (from base) examined in that order. Two acute determinations (4-dose series) were administered to examine the stability in the dose response function. A fixed order of doses was be used to permit easy observation of any systematic changes (i.e., lessening or increasing) in effects as a result of previous drug exposure. Accuracies under the different delays during acute administration sessions were analyzed and compared to accuracies observed during pre-administration (control) sessions to determine the
extent to which nicotine had any enhancing or diminishing effects on remembering under the DMTS task.

**Chronic Administration**

After an acute dose-response function was derived, a dose for chronic administration was chosen. The chronic dose was that which had the greatest change (increase or decrease) relative to control without eliminating responding. The chronic dose was then administered for 30 consecutive sessions. Accuracy was the chief dependent variable and was analyzed against control (pre-chronic values). In addition, changes across the 30-session condition were assessed.

**During-Chronic Assessment**

Following the initial 30 sessions of chronic administration, we re-determined the dose-response function with a similar protocol as the acute determination described above. That is, a determination was conducted with a different dose administered once every 4 days with the chronic dose continuing to be delivered on intervening sessions. Two determinations were conducted and this dose-response function was compared to that obtained prior to chronic administration. This assessment identified any potential effects the chronic regimen had on the subject’s dose-response function (e.g., tolerance).

**Post-Chronic Assessment**

After completing the dose-response function during chronic exposure, subjects were exposed to a withdrawal phase in which chronic nicotine administration ceased and was replaced with daily injection of the vehicle (KPO₄) alone. Following 30 sessions of daily KPO₄ administration, a final dose-response function was conducted in the manner described above to determine if any changes to the effects of nicotine remained.
Results and Discussion

Acquisition Training

Figure 2-1 presents session accuracy by trial configuration for subjects during the first 30 days of simultaneous MTS training. Although all subjects initially experienced position or stimulus biases, four of the 6 subjects (268, 800, 808, and 939) engaged in the simultaneous MTS task with greater than 85% accuracy and no observed bias following extended exposure (upwards of 20 sessions) to the contingencies. Two of the subjects (876, 930), however, exhibited a prolonged bias (930) or highly variable and inaccurate performance (876) both resulting in low daily session accuracy. These two subjects were then exposed to a trials-repeat correction procedure that resulted in highly accurate performance (see Kangas & Branch, 2008, for more details of the correction procedure and initial baseline for these subjects). After 10 consecutive daily sessions of above 85% accuracy, all subjects were exposed to the DMTS procedure described above.

Delayed Matching-to-Sample Training

Figure 2-2 presents data from the 300 session baseline of DMTS performance. Specifically, the figure shows the development of DMTS performance (accuracy) under each delay value - 0, 2, 4, 8, and 16 s. In this figure, the delay value is related to the width of the data series line with the thinnest representing 0 s and the thickest representing 16 s. Each measure was assessed using a ten-session moving-window average across the 300 sessions of DMTS exposure. For example, for each measure the values obtained for sessions 1-10 are averaged to produce the leftmost point; the average of the values for sessions 2-11 result in the second point, and so on. Therefore, the x-axis is scaled from windows 1-291, where the points for window 291 are the averages over the last 10 sessions (i.e., the mean of sessions 291-300). As the figure shows, the specific rate of development under each delay value varied among the subjects, but
the general function is consistent. High accuracies under the 0 s delay emerged relatively quickly, within a few sessions for 876 and 930, and by 25 sessions for the other 4 subjects. In addition, there was a highly consistent inverse relationship between the delay value and accuracy for all subjects during baseline exposure. The extended baseline exposure (i.e., 300 sessions) provided generally steady-state performance upon visual inspection, but it should be noted that even after 300 sessions (i.e., 18,000 total trials of exposure [3,600 of each delay value]) there was some hint of upward trends in accuracy (e.g., 2 s data series of Subject 800 and 876). Despite imperfect baseline stability across delay value accuracies, the extended fixed time-interval criterion appeared to provide a reasonably stable baseline for drug administration.

**Acute Administration**

Figure 2-3 shows the functional relationship of accuracy across the delay values as a function of dose of nicotine. In this figure, the width of the data series line is related to the dose with the thinnest line representing vehicle (KPO₄) and the thickest line representing 1.0 mg/kg nicotine. The gray shaded area represents the range of control data; that is, the maximum and minimum accuracies observed during the sessions immediately before those preceded by drug administration. As Figure 2-3 shows, a vast majority of observed accuracies across delays and subjects are inside the shaded area suggesting that accuracy under all doses of nicotine was not affected in any significant fashion. If nicotine administration was related to improved remembering, the effective doses would be signified by the dose’s data path above control (i.e., the shaded area). No significant or reliable departure from control in either direction, however, was observed.

These acute dosing data on DMTS performance alone might suggest that the choice of dose of nicotine was not active in these subjects; however, there was a significant and very reliable dose-related effect on another recorded measure of performance. It was observed that
under the larger doses of nicotine (i.e., 1.0 mg/kg for all subjects and 0.3 mg/kg for some subjects) overall session lengths were substantially increased. Upon closer inspection it was discovered that there was a dose-related increase in latency to respond during the first trial of DMTS. That is, after administration of large doses of nicotine, the subject would not engage in the DMTS task for many minutes; after they completed the first trial, however, there was then the typical near-zero latency to engage in subsequent trials (i.e., a non-graded, step-function).

Figure 2-4 shows trial latency data from the session on which each subject received its first dose of nicotine (1.0 mg/kg [filled diamonds]) and the previous session (control [open squares]). As the figure indicates, there was a very long latency to complete the first trial – sometimes as long as 30-40 min, and all other trial latencies were well within control values. Although these data are only from the first dose of nicotine administered, the first-trial-only effect is highly representative of all large doses in all 6 subjects; furthermore the pattern did not change in any significant fashion across exposure. Interestingly, Dallery and Locey (2005) found a very similar dose-related nicotine effect on first trial latencies but with rats and under a very different procedure (i.e., a self-control task).

The filled circles in Figure 2-5 show the dose response relationship of latency on the first trial as a function of dose. A curvilinear dose-related increase was observed in all 6 subjects. The error bars represent the range across the two acute determinations indicating relatively high replication of this effect across determinations. Furthermore, a count of the two determinations (descending 4-dose series) across the 6 subjects revealed exactly 12 instances in which the first determination yielded a higher latency value and exactly 12 instances in which the second determination yielded a higher latency value providing evidence that there were no systematic changes across the two acute determinations.
In addition to these data suggesting that there was indeed a systematic drug effect, although not on our chief dependent measure of interest (DMTS accuracy), they also provided at least two reasons against testing higher doses. First, toxicologically speaking, nicotine has been shown to have a steep lethal dose (LD) function in rats (e.g., Aceto et al., 1979); however, that function has not been investigated for pigeon subjects. Second, higher doses of nicotine would likely induce even longer latencies to complete the first trial which would push the session length outside of the plasma half-life of nicotine, although we cannot be sure as that information is also unknown in pigeons; it is, however, approximately 45 min for rats (Matta et al., 2007).

**Chronic Administration**

Because there was no significant change in accuracy resulting from acute nicotine administration, the dose chosen for chronic administration was that which had the largest effect on latency to complete the first trial – 1.0 mg/kg for all subjects. Just as accuracies were largely unchanged during acute administration, they were also similar to pre-chronic values during the 30 days of chronic administration. The latency to complete the first trial, however, changed in a systematic fashion. Figure 2-6 shows the latency to complete the first trial across the 30 consecutive sessions of chronic administration. Although the magnitude of the effect varied across subject, as the figure shows, all latencies decreased over the chronic administration condition. That is, four of the 6 subjects approached near control levels of latencies, and all six subjects displayed tolerance (i.e., a reduced effect) to the effects of 1.0 mg/kg nicotine on this measure of performance.

**During Chronic Assessment**

To capture more fully the extent of tolerance to the latency effects of nicotine, the open circles in Figure 2-5 show the during-chronic dose response relationship of latency to complete the first trial as a function of dose. As the figure suggests, a rightward shift of the dose response
function indicates tolerance; indeed a dose larger than the highest given during the acute

determination (1.3 mg/kg nicotine) was needed to approximate the effects of 1.0 mg/kg nicotine
during the acute phase.

Figure 2-7 presents the dose-response relation of percent correct as a function of dose of

nicotine across the delay values. As with the acute determination, no significant or reliable
departures from control (shaded area) were observed. In addition, the control area did not
change in any appreciable way between acute and chronic phases (i.e., compare the shaded areas
of Figure 2-3 and Figure 2-7). An analysis of the shaded control accuracies in both the acute and
chronic phases are presented in Table 2-1. The values in Table 2-1 represent the summed
differential of the maximum and minimum accuracy of each delay value during control sessions.
The summed differentials are larger in the during-chronic determination relative to the acute
determination in 5 of 6 subjects suggesting some increased variability during the chronic phase;
however, the differences are small, as visual inspection confirms, providing evidence for the
statement above on nicotine’s minor effect on accuracy during chronic exposure relative to pre-
chronic administration sessions.

Post-Chronic Assessment

To determine the permanency of the changes in latencies to complete the first trial
observed during chronic administration, following the during-chronic determinations, each
subject was exposed to 30 consecutive sessions of daily administration of the KPO4 vehicle (i.e.,
the withdrawal phase) after which the dose-response function was re-assessed. The open squares
in Figure 2-5 show the post-chronic dose-response relationship between latency to complete the
first trial and dose. For most doses in all subjects, the post-chronic function is between those for
the acute and during-chronic determinations suggesting a partial loss of tolerance following the
30 sessions of withdrawal.
The Limits of Memory

The essentially null effect of nicotine on remembering, might suggest that memorial enhancement may have been impossible under these conditions due to the subject’s inability to respond better under the studied retention intervals. For example, perhaps the lengthy extended baseline exposure (i.e., 300 sessions) resulted in delay accuracies at levels at the limit of the animal’s ability. To test this hypothesis we attempted to engender higher accuracies by manipulating a nonpharmacological variable. One such variable that has been shown to increase accuracies is increased response requirements on the sample stimulus prior to initiation of the retention interval (e.g., Sacks, Kamil, and Mack, 1972; Roberts, 1972).

For example, Sacks, Kamil, and Mack (1972) studied the effects of four different FR response requirements on the sample key. Four groups of two pigeons experienced sample-observing response requirements of 1, 10, 20, or 40. The subjects in each group performed under a zero-delay MTS procedure until a criterion of 85% correct matching or better was maintained for three consecutive sessions and were then exposed to a DMTS task with the retention intervals increasing in each subsequent condition. Results indicated that increased FR response requirements increased speed of acquisition under the zero-delay baseline condition as well as accuracy in the conditions in the DMTS condition; for example, the two birds that experienced the FR 1 requirement were performing at chance after 2 s delays, and both birds that experienced the FR 40 requirement were performing at levels above 75% after 8 s delays.

Roberts (1972) also assessed the effects of sample-observing response requirements on performance in a DMTS task. Ten pigeons were first trained using a simultaneous MTS procedure with an FR 3 sample-observing response requirement. After performances exceeded 85% correct for 5 consecutive sessions, the conditions changed to a DMTS procedure in which delays of 0, 1, 3, or 6 s were initiated after the FR 3 observing response requirement was met.
After 15 days of delayed MTS training with the FR 3 response requirement, FR response requirements of 1, 5, or 15 were programmed across sessions. Each subject cycled through the series of FR values six times, each time in a different order. Results from the condition in which FR response requirements were manipulated showed significant increases in accuracy across all delays for all subjects as the FR value increased.

After completion of the post-chronic determination, we stopped administering KPO₄ and reestablished a baseline of accuracy during 15 consecutive daily sessions. On Session 16 we increased the response requirement on the sample from 5 (the value during the experiments examining nicotine’s effects) to 10 and then on Session 17 increased the response requirement again to 20. All subjects were exposed to 30 sessions under the FR 20 sample response requirement. All other details of the daily sessions remained the same. Following the 30 sessions we conducted a return to baseline reversal and reduced the response requirement back to FR 5 for 15 daily sessions. Figure 2-8 shows accuracy measures for the 8 s delay trials (16 s for subject 808) during each session. We decided to display accuracy for a single value instead of the composite because accuracies for the 0 and 2 s delay values were near 100% and thus would contribute to masking any possible effects of increased accuracies at other delay values; 8 and 16 s were chosen because they displayed the largest effect. As the figure shows, although most subjects displayed an initial disruption in accuracy, session-wide accuracy under the chosen delay value increased across subsequent sessions under the FR 20 sample response requirement condition in 5 out of 6 subjects and resulted in a higher condition-wide mean daily accuracies (solid horizontal line) than those observed in the FR 5 condition. Accuracies displayed a return to baseline when the response requirement was reversed in the same 5 of 6 subjects. Although this effect was by no means large, it was fairly consistent and mere extended exposure to the
DMTS contingencies alone (i.e., a practice effect) is unlikely to account for this effect as this manipulation occurred after the experiment proper, with over 400 sessions of prior exposure to the DMTS task. This effect, therefore, suggests that the observed accuracies in Experiment 1 were not at the highest levels possible in these pigeons, and if nicotine has enhancing effects on remembering, there was room for upward movement in that measure.
Table 2-1. Sum of accuracy differentials for each subject under control sessions during acute and chronic administration.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Acute</th>
<th>Chronic</th>
</tr>
</thead>
<tbody>
<tr>
<td>268</td>
<td>0.83</td>
<td>1.83</td>
</tr>
<tr>
<td>800</td>
<td>1.50</td>
<td>1.33</td>
</tr>
<tr>
<td>808</td>
<td>1.08</td>
<td>2.08</td>
</tr>
<tr>
<td>876</td>
<td>1.08</td>
<td>1.42</td>
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<tr>
<td>930</td>
<td>0.92</td>
<td>1.50</td>
</tr>
<tr>
<td>939</td>
<td>1.08</td>
<td>1.17</td>
</tr>
</tbody>
</table>
Figure 2-1. Accuracy by trial configuration during the first 30 sessions of exposure to the simultaneous MTS task. Each graph shows data for an individual pigeon.
Figure 2-2. Moving-window average (10-session window) of accuracy under each of the 5 delay values during the extended 300-session DMTS baseline. Each graph is for an individual subject. The thickness of the line is related to the value of the retention interval with the thinnest representing 0 s and the thickest representing 16 s.
Figure 2-3. Forgetting functions (proportion correct as a function of delay) under each dose of nicotine during the acute determination. The thickness of the line is related to the value of the dose of nicotine with the thinnest representing vehicle (i.e., KPO₄) and the thickest representing 1.0 mg/kg nicotine. Lines represent an average of the two determinations of each dose, and the gray shaded area indicates range of accuracies under control sessions.
Figure 2-4. Latencies to complete each trial during the session on which the first dose of nicotine (1.0 mg/kg) was administered (closed diamonds) and the session before (open squares). Ordinate is logged to normalize proportional change across subjects and to accommodate the long pauses on the first trial under drug.
Figure 2-5. Relationships between dose of nicotine and latency to complete the first trial of the session during the acute (filled circles), chronic (open circles), and post-chronic (open squares) conditions. Error bars indicate range.
Figure 2-6. Latency to complete the first trial during each of the first 30 sessions of chronic exposure.
Figure 2-7. Forgetting functions under each dose of nicotine during the chronic determination. The thickness of the line is related to the value of the dose of nicotine with the thinnest representing vehicle (i.e., KPO4) and the thickest representing 1.0 mg/kg nicotine. Lines represent an average of the two determinations of each dose and the gray shaded area indicates range of accuracies under control sessions.
Figure 2-8. Session accuracy of one delay value under 15 sessions with a sample response requirement of FR5, 30 sessions under FR20, and a return to 15 sessions under FR5. Vertical dashed lines indicate condition change and horizontal solid lines indicate condition-wide accuracy means.
Nicotine’s Effects on Titrating Delay Matching-to-Sample (TDMTS)

Experiment 1, unlike some experiments with monkeys, did not reveal memory enhancement by nicotine. Although there was suggestive evidence that the pigeons were not at some memory limit that precluded observation of memory improvement, it remains possible that the pigeons were too accurate to reveal any enhancement. The selection of another short-term remembering task could rectify two problematic features of the delayed matching-to-sample (DMTS) procedure. The DMTS procedure has proven to be useful for the study of short-term remembering in a whole host of experimental manipulations and investigations, but it does however, have two less than ideal features. First is its susceptibility to ceiling effects. Especially under smaller delay values between sample offset and comparison onset, the subject is likely to reach and maintain near perfect accuracy, thus potentially masking an enhancing effect of a programmed independent variable (e.g., a drug effect). This is an especially important limitation when the goal is to assess enhancement of performance. A second problematic feature of the DMTS procedure is that the arrangement of conditions (i.e., delay values) is fairly arbitrary. That is, the selection of tested delay values could have a direct consequence on the shape of the remembering function. For example, testing too few delay values may fail to capture certain parametric dynamics, and not testing values along a wide enough spectrum may fail to elucidate the full remembering capabilities of the experimental subject.

A procedure that employs the recognition component of DMTS but avoids the two problematic features described above is a titrating delay matching-to-sample (TDMTS) procedure. In a TDMTS procedure, the delay between sample offset and comparison onset adjusts as a function of the subject’s performance. Specifically, correct matches increase the
delay on the subsequent trial and incorrect matches decrease the delay. The chief dependent variable in the TDMTS procedure is titrated delay. This procedure was introduced by Cumming and Berryman (1965) in their seminal book chapter on delayed conditional discriminations. In their study, pigeons were presented with either red or green sample key lights and every two consecutive correct matches increased the delay between sample offset and comparison onset by 1 s and every incorrect match decreased that delay by 1 s. This contingency led to roughly 67% accuracy under steady-state performance. The virtues of the dynamic features of the TDMTS procedure were noted early on. As Cumming and Berryman (1965) pointed out, “This titrating schedule has the obvious advantages for work with psychopharmacological agents… since it provides an immediate and continuous record of the bird’s capability for sustaining delay (pp. 308-309).”

Subsequent researchers have employed this procedure to investigate a variety of variables that may affect remembering. For example, Poling, Temple, and Foster (1996) examined the differential outcomes effect in chickens using a TDMTS procedure. A differential outcomes effect refers to the increase in accuracy that occurs in discrimination training when each of two discriminative stimuli is correlated with a different outcome (e.g., type of reinforcer). This effect has been demonstrated in pigeons, rats, dogs, and humans (see Goeters, Blakely, and Poling, 1992 for a review) using the conventional DMTS procedure; that is, the subjects performed with higher accuracy under longer delays when there were differential outcomes relative to when there were not. In the Poling et al. (1996) study, results under a TDMTS procedure indicated that when differential reinforcer magnitudes were programmed, the birds were able to adjust the delay to significantly longer values when differential outcomes were programmed. In another study, Dayer, Baron, Light, and Wenger (2000) used the TDMTS procedure to examine the
effect of ethanol on “working memory and attention” in the pigeon. Several doses of ethanol were administered and dose-related decreases in accuracy were observed in the three highest doses (1.0, 1.8, and 3 g/kg) which decreased the mean titrated delay value. In another pharmacological study, Nordholm, Moore, and Wenger (1995) analyzed the effects of the proposed cognitive-enhancing agent Linopirdine on six pigeons and four squirrel monkeys. In this study, no dose-related effects on accuracy or titrated delays were observed, even under doses that affected response rate, leading the authors to conclude that if Linopirdine had any cognitive enhancing effects, they were not related to the enhancement of remembering.

One parameter of the TDMTS procedure that has been shown to be important in determining average titrated delay values is the number of responses required on the sample stimulus at the beginning of each trial. To assess its role on remembering in the pigeon, we employed the TDMTS procedure to conduct a parametric analysis of the role of multiple observing response requirements on the sample key (Kangas, 2005). That is, the number of pecks required on the sample key prior to sample offset and initiation of the delay was varied across conditions. Response requirements of 1, 2, 4, 8, and 16 were programmed and performance under these schedules was assessed. The results indicated that increasing the sample observing response requirement increased the amount of time between sample offset and comparison onset the pigeon could tolerate and still match accurately. This procedural manipulation was said to test the role of attention in remembering because by requiring the pigeon to engage in multiple responses on the sample key, the time the pigeon spent in the presence of (and presumably looking at) the sample stimulus was increased, and, perhaps more importantly, showed that the procedure is sensitive to variables known to improve remembering.
Although the TDMTS procedure has been used much less frequently than the DMTS procedure, as a paradigm it has proven to be amenable to both behavioral and pharmacological tests. Therefore, the purpose of Experiment 2 was to assess any memory-enhancing effects of nicotine under this second memory task.

**Method**

**Subjects**

Five experimentally-naïve male White Carneau pigeons (*Columba livia*) served as experimental subjects. They were maintained at approximately 85% of their free-feeding weights. All feeding and housing conditions were identical to those in Experiment 1.

**Apparatus**

This experiment was conducted in a different operant conditioning chamber than Experiment 1. Its construction, components, and measurements, however were the same as for the apparatus used in Experiment 1. See Experiment 1 for details and dimensions.

**Initial Training and Acquisition Training**

Initial key peck training was conducted as described in Experiment 1 above. Because it has been our experience that the development of position or stimulus biases is commonplace during initial MTS training and was also observed in Experiment 1, we exposed all subjects in Experiment 2 to the trials-repeat correction procedure for 30 sessions directly after key-peck training (see Kangas & Branch, 2008 for more details). Each subject was then exposed to a zero-delay MTS procedure. In this condition, a single peck to the center key turned off the sample and illuminated both side comparison keys with a zero-delay. The consequences for pecking the matching or non-matching key remained the same as before. After 10 consecutive sessions (48 trials/session) with 85% or greater accuracy, each pigeon was then exposed to the TDMTS procedure.
**Titrating Delay Matching-to-Sample Training**

The TDMTS procedure was identical to the zero-delay MTS procedure described above with the exception that the delay between sample-stimulus offset and comparison-stimuli onset was adjusted as a function of the pigeon’s accuracy on immediately preceding trials (see Fig. 3-1). Specifically, every 2 consecutive correct matches increased the delay by 1 s, and every incorrect match decreased the delay by 1 s (unless the delay was zero). The programmed contingencies of this titrating procedure eventually, during steady-state performance, held accuracy around 67% as the delay between sample offset and comparison onset titrated (Cumming & Berryman, 1965; Kangas, 2005). The first trial of the first session in this condition began with a zero-delay; thereafter, each daily session began with the delay value from the last trial of the previous session.

Each subject initially had a sample observing response requirement of 1 for the first 15 sessions and then was exposed to an ascending series of sample-observing response requirements (2, 4, 8, and 16) across conditions, each 15 sessions per condition. This sequence was designed to increase titrated delay values to a level sufficiently large in order to see any potential drug effects in either direction. The terminal response requirement of 16 was chosen because previous research (Kangas, 2005) indicated that 16 responses on the sample key engendered stable delay values between 10-15 seconds in all subjects, which allowed the opportunity to clearly observe any increasing or decreasing titrated delays as a function of exposure to nicotine.

Each subject remained on the TDMTS procedure with a sample observing response requirement of 16 for a minimum of 100 sessions. After 100 sessions, daily mean titrated delay values were monitored for stability using visual inspection. After stability of titrated delays was observed, acute determination began.
Drug Procedure

Nicotine ([-]-Nicotine Hydrogen Tartrate Salt; Sigma, St. Louis, MO) dissolved in a potassium phosphate buffered saline solution (KPO₄), which served as both vehicle and buffer against nicotine’s acidity with a 0.1 molar potassium phosphate buffer prepped with K+phos and NaCl to obtain a pH of 7.4. Dose-concentrations were based on previous research involving nicotine administration to pigeons (e.g., Chadman & Woods, 2004). On drug administration sessions, doses were administered by an intra-muscular injection in the pigeon’s breast. During chronic administration the site of successive injections was alternated between the two sides of the breast.

Acute Administration

Pigeons were administered several doses of nicotine acutely in the manner described in Experiment 1, that is, prior to a session once every 4 days. The doses were vehicle (potassium phosphate [KPO₄] 0.0), 1.3, 1.0, 0.3, 0.1 mg/kg nicotine (from base), examined in that order. Two acute determinations (5-dose series) were administered to insure a reliable and stable dose response function. Unlike the DMTS analyses in Experiment 1, however, it was not accuracy that was assessed, but daily mean titrated delays. Therefore, daily mean titrated delays under drug were compared with titrated delay values of preceding (control) sessions.

Chronic Administration

After a stable dose-response function was derived, a dose for chronic administration was chosen. The chronic dose was that which had the greatest change (increase or decrease) relative to control without eliminating responding. The chronic dose was administered for 30 consecutive sessions.
During-Chronic Assessment

Following 30 sessions of chronic administration, a re-determination of the dose-response function was conducted similar to the acute determination described above. That is, a determination was conducted with a different dose administered once every 4 days with the chronic dose continuing to be delivered on intervening sessions. The dose-response function was compared to that obtained prior to chronic administration, and again the vehicle was tested. This assessment identified any potential effects the chronic regimen had on the subject’s dose-response function (e.g., tolerance).

Post-Chronic Assessment

After completing the dose-response function during chronic exposure, subjects were exposed to a withdrawal phase in which chronic nicotine administration ceased and was replaced with daily injection of the vehicle (KPO₄) alone. Following 30 sessions of daily KPO₄, a final dose-response function was conducted in the manner described above to determine if changes in effects of nicotine had persisted.

Results and Discussion

Initial Training and Acquisition Training

Because the development of position or stimulus biases is often observed during initial MTS training and was also observed in Experiment 1, we exposed all subjects Experiment 2 to the trials-repeat correction procedure for 30 sessions directly after key-peck training. Figure 3-2 presents acquisition data under the correction procedure. Specifically, the total number of trials to complete the session is plotted as a function of successive sessions; the solid horizontal reference line represents the number of trials to complete the session with zero errors. All 5 subjects engaged in the simultaneous MTS task without biases and with few errors within approximately 10 sessions or less under the correction procedure (see Kangas & Branch, 2008,
for more details). Because all subjects were performing with consistently high accuracies after 30 sessions of exposure, all subjects were placed on the zero-delay MTS procedure on session 31. All subjects performed under these conditions with high accuracy and after 10 sessions were exposed to the TDMTS task.

**Titrating Delay Matching-to-Sample Training**

Figure 3-3 presents data from the TDMTS baseline conditions. Data points represent session-wide mean titrated delays and error bars indicate the full session range. For all 5 subjects, low values were observed during the 15 session exposures under the FR 1, 2, and 4 conditions with increased delay values under the FR 8 sample response requirement for 3 or 5 subjects. The terminal sample response requirement for all 5 subjects was FR 16 because as Figure 3-3 shows, during the first 15 sessions under FR 16 as well as the last 15 sessions before the acute administration phase, all subjects maintained high titrated delay values with ranges consistently above zero, imperative in order to see any possible drug effects in either direction. In addition, these results systematically replicated the results of our previous research (Kangas, 2005) described above.

**Acute Administration**

Figure 3-4 shows the relationship between session-wide mean titrated delays as a function of dose of nicotine. As Figure 3-4 shows, the mean titrated delay values across all subjects were not significantly different from vehicle suggesting that performance under all doses of nicotine was not affected in any significant fashion. If nicotine administration was related to improved remembering, mean titrated delay values under the effective doses would have been higher than those of vehicle. If there was any effect it was observed during the large dose (1.3 mg/kg nicotine) with diminished accuracy (see data of Subjects 682 and 848).
These acute data on TDMTS performance alone might, like accuracy data under the DMTS procedure in Experiment 1, suggest that the choice of dose of nicotine was not active in these subjects; however, the TDMTS procedure like DMTS revealed a significant and very reliable dose-related effect on the latency to complete the first trial, also observed under the larger doses of nicotine (i.e., 1.3, 1.0, and 0.3 mg/kg for all subjects). Figure 3-5 shows trial latency data from the session on which each subject received its first dose of nicotine (1.3 mg/kg [closed diamonds]) and the previous session (open squares). Like Figure 2-4, Figure 3-5 indicates there is a very long latency to complete the first trial – again sometimes as long as 30-40 min, and all other trial latencies well within control values. Also similar to Experiment 1, although these data are only from the first dose of nicotine administered, the first-trial pattern is highly representative of all high doses in all 5 subjects and the pattern did not change in any significant way across acute exposure.

The filled circles in Figure 3-6 show the dose-response relationship of latency on the first trial as a function of dose. A curvilinear dose-related increase was observed in all 5 subjects. The error bars represent the range across the two acute determinations demonstrating replication of this effect across determinations. A count of the two determinations (descending 5 dose series) across the 5 subjects revealed 14 instances in which the first determination yielded a higher latency value and 11 instances in which the second determination yielded a higher latency value providing, as in Experiment 1, evidence that there were no systematic changes across the two acute determinations. Like Figure 2-5, these data suggest that there was indeed a systematic drug effect, although not on our chief dependent measure of interest (i.e., titrated delay).

**Chronic Administration**

Because, similar to Experiment 1, there was no significant change in remembering during the acute nicotine administration, the dose chosen for chronic administration was that that had
the largest effect on latency to complete the first trial, viz. 1.0 mg/kg for all subjects. As accuracies were largely unchanged during acute administration, they were also similar to pre-chronic values during the 30 days of chronic administration. The latency to complete the first trial however, changed in a systematic fashion. Figure 3-7 shows the latency to complete the first trial across the 30 consecutive sessions of chronic administration. Although the magnitude of the effect varied across subject, all latencies decreased over the chronic administration condition. That is, 4 of the 5 subjects approached near control levels of latencies, and all 5 subjects displayed tolerance to the effects of 1.0 mg/kg nicotine on this measure of performance.

**During-Chronic Assessment**

To capture more fully the extent of tolerance to the latency effects of nicotine, the open circles in Figure 3-6 show the during-chronic dose-response relationship of latency to complete the first trial as a function of dose. The rightward shift of the dose-response function indicates tolerance.

Figure 3-8 presents the dose-response relation of mean titrated delays as a function of dose of nicotine. As with the acute determinations, no significant or reliable departures from control or vehicle levels were observed. In addition, the control range did not change in any appreciable way between acute and chronic phases providing evidence of nicotine’s null effect on accuracy during chronic exposure.

**Post-Chronic Assessment**

To determine the permanency of the tolerance observed during chronic administration on the latencies to complete the first trial, following the during-chronic determinations each subject was exposed to 30 consecutive sessions of daily administration of the KPO₄ vehicle (i.e., withdrawal phase). The open squares in Figure 3-6 show the post-chronic dose-response relationship of latency to complete the first trial as a function of dose. As the figure shows, for
most doses in all subjects, the function for post-chronic determination is between those from the acute and during-chronic determinations, suggesting a partial loss of tolerance following the 30+ sessions of withdrawal.

The Limits of Memory

Because as in Experiment 1, the effect of nicotine on remembering was essentially null, again, enhancement may have been impossible under these conditions due to the subject’s inability to respond better under their parameters of the procedure. Perhaps, for example, use of FR 16 as the sample-key response requirement may have led to maximal remembering under control conditions. To test this hypothesis under the TDMTS procedure, we attempted to engender higher accuracies by again manipulating a nonpharmacological variable – the response requirement on the sample. The role of the sample response requirement on titrated delay has been previously studied (Kangas, 2005) and has been shown to increase mean titrated delay values, although the specific value we chose for the subjects in the present study had not been examined. After completion of the post-chronic determination we stopped daily injections of KPO4 and conducted a 15-session baseline. On Session 16 we increased the response requirement on the sample from 16 to 24. The FR 24 condition was only 15 sessions instead of 30 sessions in Experiment 1. Figure 3-9 presents daily session-wide mean titrated delay values with the error bars indicating range. All 5 subjects began to exhibit consistently higher titrated delay values within 7 sessions under the higher response requirement. These results both replicate the findings of Kangas (2005) and provide evidence that these subjects could indeed maintain higher titrated delay values.
Figure 3.1. Titrating delay matching-to-sample procedural flowchart.
Figure 3-2. Number of total trials per session under the correction procedure. Solid horizontal line indicates number of correct trials required to complete a session. Y-axis is logged to normalize proportional change across subjects.
Figure 3-3. Mean titrated delays during baseline conditions of increasing FR sample response requirements. Error bars indicate range.
Figure 3-4. Acute determinations of titrated delays as a function of dose of nicotine. Error bars indicate range.
Figure 3-5. Latencies to complete each trial during the session on which the first dose of nicotine (1.0 mg/kg) was administered (closed diamonds) and the session before (open diamonds). Ordinate is logged to normalize proportional change across subjects and to accommodate the long latency in the first session under drug.
Figure 3-6. Relationships between dose of nicotine and latency to complete the first trial of the session during the acute (filled circles), chronic (open circles), and post chronic (open squares) conditions. Error bars indicate range.
Figure 3-7. Latency to complete the first trial during each of the 30 sessions of chronic exposure.
Figure 3-8. Chronic determinations of titrated delays as a function of dose of nicotine. Error bars indicate range.
Figure 3-9. Mean titrated delay under 15 sessions with a sample response requirement of FR16 followed by 15 sessions under FR24. Error bars indicate range.
CHAPTER 4
GENERAL DISCUSSION

The delayed matching-to-sample (DMTS) procedure has proven to be a useful tool for the study of short-term remembering in both human and nonhuman animals (For a panel discussion on equating behavior under the DMTS procedure with short-term remembering, see Paule, et al., 1998). The titrated delay matching-to-sample (TDMTS) procedure, albeit less employed, is also useful for similar ends, and has the benefits of avoiding problems of ceiling effects in accuracy by holding accuracy constant, and avoids the effects of arbitrary programming of delay values by allowing the subject to control delay values. In addition, as pointed out elsewhere (Cumming & Berryman, 1965), the TDMTS procedure is especially useful in the study of pharmacological effects on short-term remembering.

Nicotine’s enhancement of remembering with nonhuman primates has been illustrated and replicated in the experiments using DMTS by Buccafusco et al. described in Chapter 1. These studies along with other neurological assessments have provided an empirical foundation for others to explore nicotinic treatments in hopes that they may have similar enhancing effects on remembering in a clinical population, including those suspected to have Alzheimer’s disease (e.g., Jones, Sahakian, Levy, & Warburton, 1992; Knott, Engeland, Mohr, Mahoney, & Ilivisky, 2000; Newhouse, 1986; White & Levin, 1999). The present experiments, however, failed to add evidence to support these claims. That is, neither performance under the DMTS or TDMTS preparation was enhanced following the administration of nicotine. These null effects were reliable for all subjects in both experiments.

There remains a possibility that our preparation was inadequate to elucidate these enhancement effects. The reason may be related to choice of subject. That is, although the pigeon is a very common experimental subject in both the DMTS and TDMTS preparation, the
radial arm maze findings promulgated by Levin and colleagues were with rats, and the DMTS findings by Buccafusco and colleagues were with nonhuman primates. There have been, however, recent and significant changes in the way which some view the homology between mammalian and avian brains. Specifically the avian cerebellum has a large pallium territory that appears to perform similar functions to that of the mammalian cortex, supporting similar advanced cognitive abilities avian species have displayed including categorization, symbolic behavior, transitive logic, tool use, spatial, episodic, and other types of memory (see Reiner et al., 2004; Jarvis et al., 2005; for discussion). In addition, this possible shortcoming may also be tempered by the fact that, although operating primarily on different systems, cocaine’s effects on forgetting functions in the pigeon (e.g., Branch & Dearing, 1982) and monkey (e.g., Baron & Wenger, 2001) as well as d-amphetamine’s effects on forgetting functions in the pigeon (Spetch & Treit, 1984) and monkey (e.g., Baron & Wenger, 2001) are very similar in shape and dose-related effect. Indeed, there are no known significant qualitative differences in effects of operant conditioning procedures, or of drug effects on behavior under such procedures, between pigeons, rats, or monkeys.

Another possible variable responsible for the failure to replicate Buccafusco and colleagues memory enhancing effect with nicotine is the limited robustness of the effect. That is, although a 5-10% increase in accuracy as a function of nicotine administration might be a very important finding, it has been realized only after selectively choosing specific accuracy changes in particular retention intervals under particular dose concentrations. If the reader is as unconvinced as the authors are of the absence of a dose-related effect in Figure 2-3, see Table 4-1. Table 4-1 shows the accuracy values of each of the 6 subjects in Experiment 1 for the 8 s and 16 s retention intervals (long delays) under each of the doses of nicotine as well as control (i.e.,
mean accuracies during intervening sessions of the acute determinations). The shaded cells highlight instances in which accuracy of a long retention interval under a dose of nicotine is higher than that of control. As the table shows, all subjects had sessions in which nicotine administration was associated with higher accuracies under long retention intervals relative to control, and all 6 subjects had numerous instances. This is almost assuredly the case because of the inherent variability in DMTS performance demonstrated in these subjects during baseline (see Figure 2-2). Moreover, because in all DMTS experiments each session is limited in the number of trials a given retention interval is programmed, getting one additional trial correct or incorrect can have a relatively large impact on overall accuracy. For example, in Experiment 1, there were 60 trials of 5 retention intervals (0, 2, 4, 8, and 16 s) evenly dispersed exactly 12 times. When examining the accuracy under only one of the retention intervals, getting one more trial type correct relative to control will increase accuracy by approximately 8%. The protocol employed by Buccafusco et al. typically involves 108 trials per session and 6 delays per session (i.e., 18 occurrences of each retention interval trial type). Therefore, and interestingly, an additional trial correct relative to control would increase accuracy by approximately 5%. If the measurement and reporting of these percent changes were constant as the variability is, these percent changes would probably cancel each other out; however, when the researcher selectively decides which retention intervals are “long” and which doses are “moderate” and only pools together those accuracy effects a robust finding may appear to emerge simply as a result of the analysis tactics.

We may carry this idea one step further and use the percent changes in accuracy highlighted in Table 4-1 and present them in a fashion very similar to the way Buccafusco et al. present their data. Specifically, we first arranged our accuracy results into three groups of
functionally similar retention intervals – Retention Interval 1 included those that led to performances yielding 95-100% correct, Retention Interval 2 included delay intervals that yielded 80-85% correct, and Retention Interval 3 included those that resulted in 65-75% correct. These values in the percent-correct spectrum are exactly those assessed in all of the Buccafusco studies discussed above. Then a dose was chosen for each subject that led to the greatest increase, relative to control, on accuracy. Control values were derived as mean accuracy levels during the sessions in between acute nicotine-administration sessions, again exactly as Buccafusco calculated. Figure 4-1 shows percent change from control under each of the three retention interval groups as defined above during the acute determinations of a single dose (the same dose within-subject but different between-subject). As the figure indicates, a positive percent increase from control is found during the functionally defined Retention Interval 3 for 5 of 6 subjects. Again, the analysis techniques used to derive Figure 4-1 are identical to those used by Buccafusco et al. in all ways with the minor exception that Buccafusco and colleagues report conducting 3-5 acute determinations whereas we only conducted two. Despite the seemingly null effect suggested in Figure 2-3, by functionally defining retention interval categories, choosing the nicotine dose that had the largest effect on Retention Interval 3 (which is usually the longer delay values [but excludes those at which the subject is performing at chance]) we can show a modest enhancement of accuracy on Retention Interval 3 during moderate nicotine dose administration (i.e., 0.1 and 0.3 mg/kg nicotine). In addition, our percent change from control data (i.e., Figure 4-1) looks very similar in magnitude to results presented by Buccafusco et al (e.g., Elrod, Buccafusco, & Jackson, 1988).

In an attempt to see if we could present the findings suggested in Figure 4-1 within the framework and format of Figure 2-3 we derived a dose-response function for each subject but
limited the data to values to those of Retention Interval 3. The circled data points indicate the nicotine dose used to comprise Figure 4-1. As Figure 4-2 shows, accuracy values under delay values defined as Retention Interval 3 (i.e., values slightly above chance during control sessions) are elevated during sessions in which moderate doses of nicotine were administered, however, it is limited to a very selective number of doses and retention intervals and moreover isn’t an effect in every subject (Figure 4-2 mirrors Figure 4-1 well in that Subject 268 and 939 show no effect or very little dose-related effect, respectively.

So the question remains – does nicotine enhance remembering? Our results suggest that the answer may be yes if we limit ourselves to a very circumscribed dimension of the DMTS task – accuracy values during retention intervals that are slightly above chance. In addition, to answer yes we must be satisfied with very modest increases – approximately 5-10% which usually only represents one additional trial correct in an albeit functionally-defined yet small category of DMTS trial types. Is this, however, what most people mean when they think memory-enhancement? If these effects prove reliable, it means that nicotine isn’t related to remembering a stimulus longer (Experiment 2 showed no evidence of that), but remembering a certain subset of a stimulus class with slightly higher accuracy, but only when the baseline conditions permitted remembering at fairly abysmal, but better than chance, levels.

To conclude, no single experiment, or series of experiments for that matter, can conclusively prove or falsify a purported effect. It is only successful replication that can add confidence to the reliability and validity of an effect. Memory deficits that develop in the aging organism are a very real and significant problem and experimental research towards attenuating those dementias will always remain a laudable activity. These results suggest some evidence supporting the notion that nicotine can have effects that include memory enhancement but also
serve to highlight the extremely circumscribed nature and modesty of the effect – an effect that to us still remains dubious. In addition to calling into question the extent of the validity of these purported enhancements, we hope the present experiments will remind the reader of other non-pharmacological tactics that have been shown to enhance remembering in a much more robust and non-selective fashion; for example, extended response requirements on the sample stimulus as well as extended exposure to the task – two methods that have been shown here and elsewhere to have impact on all trial types and tested species.
Table 4-1. Accuracy of each retention interval under doses during the two acute determinations. Shaded cells indicate instances in which the accuracy for that trial type was higher than that of vehicle.

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Figure 4-1. Percent change from baseline control levels during each of three functionally defined retention intervals under a dose that was related to the highest percent increase during Retention Interval 3. The horizontal reference line indicates no change from baseline control.
Figure 4-2. Dose response functions under Retention Interval 3. Data points are the mean of the two acute determinations and the error bars represent range. The circled data point indicates data that comprised Figure 4-1.
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Brian D. Kangas decided to major in psychology for the most mundane reasons that aren’t worth mentioning here. Thankfully, he stumbled into some good direction early on and completed his Bachelor of Arts in psychology in 2003 at Southern Illinois University under the direction of Dr. Eric Jacobs studying issues related to human operant choice and decision-making. He completed his Master of Science in behavior analysis in 2005 at the University of North Texas under the direction of Dr. Manish Vaidya while focusing on issues related to complex conditional discriminations and game theory. A human behavioral pharmacology internship at the University of Chicago under the direction of Dr. Diana Walker made for close study with three Florida Gator doctorates so his next step was obvious. He completed his Ph.D. in 2009 under the mentoring of Dr. Marc Branch focusing on issues of drug/behavior interactions and the extent to which the study of pharmacological agents can inform issues related to basic behavioral processes.