SCOPOLAMINE EFFECTS UNDER A TITRATING-DELAYED-NONMATCHING-TO-POSITION PROCEDURE

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In a study of working memory, the performance of rats under titrating-delayed-nonmatching-to-position (TDNMTP) procedures was examined. Overall accuracy and the number of trials completed were inversely related to titration value, whereas the highest delay attained was directly related to titration value. When given intraperitoneally, scopolamine significantly reduced the highest delay attained without significantly affecting other performance measures for most administrations. Subcutaneous administrations significantly reduced all performance measures, suggesting that, as in most prior studies, the drug's disruptive effects were not limited to memory processes. These findings demonstrate the feasibility of using TDNMTP procedures with rats, but further research is necessary to determine if such procedures are especially valuable in studying working memory and drug effects thereon.

Discrete-trial delayed-matching-to-sample (DMTS) procedures and variants thereof have been used for decades to assess working memory in human beings and other animals (Paule et al., 1998; Pontecorvo and Clissold, 1992). A prototypical DMTS procedure involves presenting one of a number of sample stimuli (e.g., inserting the right or left lever in a rat chamber), requiring a response to that stimulus (e.g., a press of the lever, leading to its withdrawal), presenting two or more comparison stimuli (e.g., the two levers) immediately or after a delay, and rewarding (reinforcing) a response to the stimulus that matches the sample stimulus (e.g. delivering food after a press of the lever that was initially inserted as the sample). Many elaborations of the basic DMTS arrangement are possible. For example, a delayed-nonmatching-to-position (DNMTP) procedure is frequently used to study working memory, as well as the effects of drugs and other perturbations thereon, in rats. In DNMTP as usually arranged, lever location defines the sample stimulus and a response to the lever location not presented as the sample is designated as correct and is reinforced.

DNMTP has several advantages for the study of working memory in rats (Paule et al., 1998). In brief, it has face validity, it is readily automated, and it engenders relatively consistent performance that can be repeated within and across subjects. If an independent variable produces effects on

multiple dependent variables (e.g., accuracy during trials with different delays, number of trials completed), the procedure offers the advantage of potentially separating effects of independent variables on memory from general behavioral and motivational impairment. Finally, and importantly, published studies have demonstrated the value of DNMT for studying the effects of drugs and other variables (Paule et al., 1998).

Despite its strengths, several disadvantages of DNMT have been noted (Chudasama and Muir, 1997; Han, Pierre-Louis, Scheff, and Robinson, 2000; Nordholm, Moore, and Wenger, 1995). DNMT has ceiling effects due to high accuracy typically obtained at short delays. When delays are short, accuracy typically approaches 95%–100%; this phenomenon leaves little room for statistically significant improvement. Moreover, performance decrements are hard to detect at long delays, where baseline performance approaches chance and variability characteristically is large. In addition, response biases may develop in subjects at longer delays, especially in the presence of certain drugs, which artificially affect performance accuracy (Nordholm et al., 1995). Employing a nonmatching procedure can reduce such biases (Pache, Sewell, and Spencer, 1999). Also, behavioral effects typically interpreted as “memory impairment” may be due to disruption of behavioral strategies developed to “bridge” the delay interval (Chudasama and Muir, 1997; Paule et al., 1998). Finally, due to the binary nature of trials, Winters and Dunnett (2004) have argued that an arcsine transformation of overall percentage of correct responses is necessary for analysis-of-variance testing. This transformation, which typically reduces the power of analysis-of-variance tests (Han et al., 2000), appears to be rare in published studies.

DMTS procedures, including DNMT, typically utilize a set of fixed delay values from which a single value is selected pseudo-randomly for each trial (e.g., Winters and Dunnett, 2004). An alternative procedure is to arrange a performance-dependent delay value that is adjusted according to the accuracy of prior responses. For example, Han et al. (2000) arranged delays of 1 s or 11 s for rats exposed to DNMT. Within sessions, the delay on a given trial was 1 s if the running proportion of correct responses for the 1- and 11-s delays combined was 0.75 or lower. The delay was 11 s if this value was higher than 0.75.

The primary measure of working memory was the proportion of trials at the longer delay. Scopolamine, MK-801 (Dizocilpine), and WIN 55, 212-2 produced dose-dependent reductions in this measure. MK-801 is purported to disrupt working memory by acting as an NMDA receptor agonist, disrupting induction of long-term potentiation in the dentate gyrus and CA1 (carbonic anhydrase I) regions of the hippocampus, but it also produces behavioral deficits not classified as “memory impairment” (Mackes & Willner, 2006). WIN 55, 212-2 disrupts long-term potentiation in the hippocampus more specifically, by activating CB1 (cannabinoid) receptors (Mereu et al. 2003). Scopolamine and MK-801 reduced accuracy during trials with 1- and 11-s delays and did not reduce the number of trials completed during each session. WIN 55, 212, in contrast, decreased accuracy only when the delay was 11 s and reduced the total number of trials completed. Han et al. interpreted these data to indicate that the disruptions produced by scopolamine and MK-801, which were not delay-dependent, were not limited to working memory processes. This interpretation is based on the assumption that short delays (e.g., 1 s) do not involve remembering (Robinson, Scheff, Pierre-Louis, & Han, 2000).

Be that as it may, other studies involving DMTS procedures without titration have also suggested that scopolamine produces delay-independent effects and does not selectively impair working memory (Dunnett, 1985; Robinson and Mao, 1997; Tan, Kirk, Abraham, & McNaughton, 1989).

The adjusting delay procedure appears to have some advantages over nonadjusting procedures for studying drug effects (Han et al., 2000; Robinson et al., 2000). Another variation of DMTS, in which delay length is titrated continuously as a function of performance on prior trials, appears to do likewise. In general, under a titrating DMTS (TDMTS) procedure, a defined series of consecutive correct responses (e.g., three) causes the delay to be incremented by a set value (e.g., 1 s, with the value set at 0 s at the beginning of each session), whereas a defined series of incorrect responses (e.g., three) causes the delay to be decreased, usually by the same value (e.g., 1 s). Meaningful data that may be collected include number of trials completed, overall accuracy (i.e., percentage of correct responses), and the longest delay reached in each session. If in indexing working memory, attention is focused on the highest delay value reached during each session, instead of percentage of correct responses overall or as a function of delay length, this procedure avoids the floor effect referred to previously. TDMTS also produces higher-percentage accuracies at relatively long delays than do DMTS procedures (Poling, Temple, and Foster, 1996), avoiding the aforementioned ceiling effect, and results in more graded dose-response curves than nontitrating procedures (Paule et al., 1998).

TDMTS procedures have been used in published studies to evaluate drug effects in humans, pigeons, and squirrel monkeys (e.g., Dayer, Baron, Light, & Wenger, 2000; Nordholm et al., 1995; Wenger and Wright, 1990; Wysocki, Fuqua, Davis, & Breuning, 1981), but despite their potential advantages for studying working memory, the number of studies involving them is small. Given that rats are common subjects in psychopharmacological research and several studies reporting drug effects in rats exposed to DMTS procedures have appeared (e.g., Dunnett, 1985; Dunnett et al., 1999; Kirkby et al., 1995; Leanza et al., 1996; Robinson and Mao, 1997; Tan et al., 1989), it is noteworthy that there appears to be no published study in which drug effects were evaluated in rats exposed to a TDMTS procedure. The purpose of the present study was to examine the performance of rats exposed to three variations of a TDNMTP procedure, with the variations defined in terms of the length of the titration interval (step size), and to examine the effects of scopolamine under each variant. Prior studies have reported the effects of scopolamine, an anticholinergic drug known to produce working-memory deficits in rats performing under nonadjusting procedures (Dunnett, 1985; Robinson and Mao, 1997; Tan et al., 1989) and under the adjusting procedure described previously (Han et al., 2000), and it was of interest to compare the results of those studies with those of the present investigation.

Method

Subjects

Seven male Sprague-Dawley rats (Charles River, Portage, MI), each with previous lever pressing histories and ages of approximately 400 days at the

beginning of the study, served as subjects. Three of the rats had previously been exposed to acute doses of gamma-hydroxybutyrate (GHB) and its precursors. The rats were housed individually in plastic cages (24 cm long $\times$ 31.5 cm wide $\times$ 21 cm high) in a colony room maintained on a 12-hr light-dark schedule and kept at a relatively constant temperature (20–22°C). They were maintained at 80% of their free-feeding weights ($M = 407$ g) by means of daily feedings immediately after sessions concluded. Free access to water was provided in the home cages. Our Institutional Animal Care and Use Committee approved the study, which was conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* promulgated by the National Research Council (1996).

Apparatus

Experimental sessions were conducted in four operant conditioning chambers, each 28 cm long $\times$ 21 cm wide $\times$ 21 cm high (Med Associates, St. Albans, VT). Each chamber contained two retractable response levers 7 cm above the floor on the right and left sides of the front response panel. Complete lever retraction required approximately 700 ms from initiation, as did complete lever insertion. A stimulus light was above each lever, and a food cup, into which 45 mg of saccharine-sweetened food pellets (F0021, Bio-Serv, Frenchtown, NJ) could be delivered, was in the center of the panel. A photoelectric sensor recorded head entries into the food cup. An overhead 28-V chamber light provided ambient illumination throughout each experimental session. Each chamber was housed in a sound-and light-attenuating shell to which masking noise and ventilation were supplied. MED-PC software and instrumentation (Med Associates), operating on an IBM-compatible personal computer, were used to arrange experimental events and to record data.

Behavioral Procedure

During a previous experiment, the subjects were trained to respond on both levers. Training for the current study began with a DNMTTP procedure in which, after a 5-s intertrial interval, a randomly selected (left or right) sample lever was inserted into the chamber. The sample lever was retracted when pressed, and both levers were inserted after a 0.5-s delay. A response on the lever opposite the sample lever lit both stimulus lights and retracted both levers. A food pellet was delivered immediately after the levers were retracted. An incorrect response extinguished the chamber light and retracted the levers. After 5 s, the chamber light was lit and a new trial began. Training sessions were conducted until all subjects responded at 95% overall accuracy for three consecutive sessions (10 sessions, with 100–105 trials per session).

Thereafter, TDNMTTP training began, with procedures similar to those described by Poling et al. (1996). In brief, after the presentation of the sample lever, the first recorded head entry into the food cup after a delay initiated the insertion of the choice levers. This head entry requirement was imposed to limit behavioral positioning strategies and is usually somewhat effective in doing so (see Chudasama and Muir [1997] for an analysis). Correct and incorrect responses and the consequences thereof were identical to initial training. The delay value for each session began at 0 s. After three consecutive correct responses the delay was increased by a specified amount. It was

further increased by each correct response until an incorrect response was made. After an incorrect response, the delay did not increase again until three consecutive correct responses were made. If three consecutive incorrect responses occurred, the delay value decreased (and continued to decrease with each incorrect response) until a correct response was made or the delay value reached 0 s. Four different titration values were examined (0.2, 0.5, 1.0, and 2.0 s). Sessions, which lasted 1600 s, were conducted 6 days a week at about the same time each day.

Sessions were conducted at each of three titration intervals (0.2, 0.5, and 1.0 s) in ascending order until the highest delay attained during each session for individual rats showed no visually apparent trend over three consecutive sessions (10, 5, and 10 sessions, respectively). After performance was stable at the 1.0-s titration, pharmacological testing began.

Pharmacological Procedure

Scopolamine hydrochloride (Sigma, St Louis) was dissolved in sterile 0.9% saline solution prepared at an injection volume of 1 ml/kg; injection of the solution was either intraperitoneal (IP) or subcutaneous (SC) and occurred 20 min prior to behavioral testing. After responding was stable, injections were administered according to a BCDBC design, where B represents baseline sessions (no injection), C represents vehicle control sessions, and D represents drug sessions. Each dose was administered once. IP doses of 0.125 and 0.25 mg/kg were examined first for the 1.0-s titration in ascending order. These doses were then examined for the 2.0, 0.2, and 0.5 s titration intervals, respectively. An IP dose of 0.06 mg/kg was then examined at each titration value in ascending order. Finally, an SC dose of 0.06 mg/kg was examined at each titration value in ascending order. Three sessions of visually stable performance were required before pharmacological testing, when titration value changed. This requirement was three sessions in all cases. Each rat received 16 administrations of scopolamine; therefore, it is possible that tolerance developed over the course of the study. This possibility appears unlikely, in view of the relatively low doses given and the separation of administrations in time. Studies that demonstrate tolerance to the behavioral effects of scopolamine have used substantially higher doses administered daily for at least 2 weeks (e.g., Ortega-Alvaro, Gilbert-Rahola, and Micó, 2006).

Data Analysis

Each session, the number of trials completed, the overall accuracy (percentage of total responses that were correct), and the highest delay attained were recorded. These measures were analyzed via factorial analysis of variance followed, if significant effects were revealed, by Tukey's Honestly Significant Difference tests, which compared control means and means at each drug dose. Results were considered as significant if p was less than .05.

Results

Figure 1 depicts mean baseline and vehicle-control values ($\pm$ standard error of measurement) for overall accuracy, the highest delay attained at each titration value, and the number of trials completed. Baseline data rep-

resent sessions immediately prior to vehicle-control sessions; no injections were given during the former sessions. Visual inspection of Figure 1 suggests that performance did not differ during baseline and vehicle-control sessions. Moreover, overall accuracy was inversely related to titration value, the highest delay attained was directly related to titration value, and the number of trials completed was inversely related to titration value. Statistical analysis confirmed that in the absence of drug, baseline and vehicle-control means did not differ significantly with respect to overall accuracy, highest delay value reached, or trials completed per session, two-factor general linear model: dose $\times$ titration value; $F(1,160) = 0.23, 0.00, 0.00$, respectively, $p > .05$ for each;

lower than 1 F -values are due to nearly identical group means. Therefore, only vehicle-control data were considered during analysis of the effects of scopolamine dose and titration value. During vehicle-control sessions, the latter variable significantly affected mean overall accuracy, mean highest delay attained, and mean trials completed per session, $F(3, 80) = 97.89, 73.04$, and 38.20 , respectively, $p < .001$ for each.

Figure 2 shows for each titration value the effects of scopolamine on mean values for overall accuracy, the highest delay attained, and the number of trials completed, respectively. In these figures, data for sessions in which the indicated dose of scopolamine was administered are presented as a percentage of the values obtained during vehicle-control sessions (see Figure 1). Visual inspection indicates that scopolamine produced generally dose-dependent decreases in overall accuracy, with the largest decrease associated with 0.06 SC (see Figure 2). Statistical analyses confirmed that there was a significant effect of scopolamine dose, $F(4, 176) = 31.16$, $p < .001$, and titration value, $F(3, 176) = 14.94$, $p < .001$, on overall accuracy, and that the interaction between these factors was not significant, $F(12, 176) = 1.21$, $p = .283$. For the 0.2-, 0.5-, and 1.0-s titration values, overall accuracy was significantly below the vehicle-control level only at the 0.06 mg/kg SC dose of scopolamine. For the 2.0-s titration value, overall accuracy was significantly below the vehicle-

Figure 1. Mean ($\pm$ standard error of mean) overall accuracy (percentage of correct responses), number of trials completed, and highest delay reached by 7 rats during baseline (no injection) and vehicle-control sessions. Rats were exposed to four variations of a TDNMT procedure, with the variations defined in terms of the length of the titration interval, which is indicated.

control level at the 0.06 SC and 0.125 IP doses. It is important to note that statistical findings for drug administration were heavily influenced by the .06 mg/kg SC dose.

Visual inspection of Figure 2 suggests that scopolamine produced generally dose-dependent decreases in the highest delay attained. Regardless of titration value, the degree of impairment in this measure was larger than the impairment in overall accuracy. The effects of dose and titration value on the highest delay reached were statistically significant, $F(4, 176) = 45.74, p < .001$ and $F(3, 176) = 54.66, p < .001$, respectively, as was the interaction between these variables, $F(12, 176) = 2.01, p < .05$. At all titration values, all doses of scopolamine significantly reduced the highest delay attained relative to the vehicle-control mean, with one exception, 2.0-s titration, 0.06 mg/kg IP.

Scopolamine generally did not affect the number of trials completed (see Figure 2). However, statistical analysis confirmed that there was a significant effect of scopolamine dose, $F(4, 175) = 3.44, p = .01$, and titration value, $F(3, 175) = 19.89, p < .001$, on this measure, and that the interaction between the two factors was not significant, $F(12, 175) = .99, p = .463$. In only one case, 0.2 s, 0.06 mg/kg SC, was the total number of trials completed in the presence of drug significantly different from the number completed in vehicle-control sessions. It should be noted that statistical differences by dose, as in the percentage correct measure, were primarily due to the 0.06 mg/kg SC administration.

Figure 2. Effects of scopolamine on overall accuracy, trials completed, and highest delay reached. Data during sessions in which the indicated dose of drug was given are expressed as a percentage of vehicle control (see Figure 1). All doses were given intraperitoneally (IP) except for the indicated .06 mg/kg subcutaneous (SC) dose. Asterisks indicate doses where the drug mean differed significantly ($p < .05$) from the vehicle-control mean.

To quantify how titration value influenced the effects of scopolamine, effect sizes for the primary dependent variable, the highest delay attained, were calculated in the manner described by Cohen (1988). They are shown in Figure 3. In general, effect sizes for all scopolamine doses were inversely related to titration value. This difference is due primarily to the increase in variance associated with an increase in titration value.

Figure 3. Effect sizes (Cohen's *D*) for the highest delay reached, by dose of scopolamine. Data are presented separately for each of the four titration values. IP = intraperitoneal; SC = subcutaneous.

Route of administration (IP versus SC) was examined with a single low dose (0.06 mg/kg). Route of administration strongly influenced the effects of scopolamine, in that the 0.06 mg/kg SC dose not only produced larger effects than the same dose delivered IP; it also produced larger effects than the 0.125 and 0.25 IP doses (see Figure 3).

Discussion

Variations of the TDMTS procedure have been used successfully in previous published studies with pigeons, monkeys, chickens, and humans (e.g., Dayer et al., 2000; Nordholm et al., 1995; Poling et al., 1996; Wenger and Wright 1990; Wysocki et al., 1981) but not, to our knowledge, with rats. The data reported here indicate that one variation of the procedure, TDNMTP, quickly engendered consistent and repeatable performance in rats regardless of whether the titration interval was 0.2, 0.5, 1.0, or 2.0 s. Moreover, scopolamine significantly affected overall accuracy, the highest delay attained, and the number of trials completed at each titration value, although the latter two effects were evident only with the 0.06 mg/kg SC dose. As in prior studies performed with other procedures (e.g., Kirby et al., 1995), in the present study SC administration of scopolamine produced greater disruption than IP administration of the same dose. It is notable that statistically significant reductions in highest delay achieved were found at the 0.06 mg/kg IP administration, which did not disrupt accuracy in prior

studies performed with other variations of DMTS (Han et al. 2000; Chudasama & Muir, 1997; Kirkby et al. 1995). Therefore, it appears that TDNMTP may be especially sensitive to drug effects.

Han et al. (2000) previously examined the effects of scopolamine (as well as other drugs) under an adjusting delay procedure, which was programmed to hold overall accuracy at approximately 75% and to arrange 1- and 11-s delays, depending on accuracy on prior trials. At higher doses, scopolamine hydrobromide (0.06–0.5 mg/kg IP) reduced the proportion of trials at the longer delay, as well as the percentage of correct responses at both delay intervals. This finding suggests that scopolamine did not specifically disrupt working memory, which is consistent with the findings of a number of other investigations (Dunnett, 1985; Robinson and Mao, 1997; Tan et al., 1989). Our findings, too, suggest that the effects of scopolamine may not be confined to working memory, in that the most effective dose (0.06 mg/kg SC) disrupted all measures of performance, not just the highest delay attained.

Interestingly, Stanhope, McLenachan, and Dourish (1995) found that low doses of scopolamine (0.05 and 0.1 mg/kg IP) produced delay-dependent reductions in the accuracy of rats performing a DMTS task without reducing the number of trials completed. This finding is suggestive of selective impairment of working memory. It is noteworthy that Stanhope et al. used a matching-to-sample arrangement, whereas a nonmatching-to-sample procedure was used by Han et al. (2000) and in the present study. This difference may have accounted for the difference in findings (Han et al.). Pache et al. (1999) directly compared DNMTS and DMTP. Their results suggested that performance during DNMTS relies more on mediating behavioral strategies than does performance under DMTP, and that drug effects on such mediating strategies may lead to greater disruptions of accuracy under DNMTS. If this is the case, DNMTS should be more sensitive to general behavioral impairments produced by drugs, regardless of their effects on working memory.

As we have discussed elsewhere (Poling and Byrne, 2000), cognitive processes such as “working memory” and related behavioral processes such as “stimulus control” can be studied through the use of a variety of animal models, which do not necessarily yield comparable results with respect to the effects of drugs or other variables. Even if the same general procedure is used, the manner in which training and testing are arranged and the techniques used to quantify performance may powerfully affect results. For example, we have demonstrated that using food versus water as reinforcers did not significantly affect the development of a vehicle-GHB discrimination in rats but did significantly affect the results of subsequent generalization tests (Baker, Pynnonen, & Poling, 2004). As another example, we have also shown that whether 50% or 100% keylight-food pairings produced “stronger” autoshaped responding in pigeons depended on whether response rate or choice was used to index performance (Picker and Poling, 1982).

There are no best or standard ways to conduct drug discrimination studies, to study autoshaping, or to arrange other test procedures, including TDMTS and its variations. One purpose of the present study was to examine whether an aspect of the TDNMTP procedure, the titration interval, influenced control performance or drug effects. It clearly affected vehicle-control performance, in that overall accuracy and the number of trials completed were inversely related to titration value, whereas the highest delay attained was directly related to titration value. An interesting question that we did not examine is

whether similar results would have occurred if sessions were longer. In the interest of evaluating a procedure useful in examining the effects of short-acting drugs, we arranged relatively brief (1600 s) daily sessions. If our rats were perfectly accurate and responded as soon as the sample and comparison stimuli were presented, the maximal delays they could have attained were 25.7, 40, 55.5, and 78.5 s at the 0.2-, 0.5-, 1.0-, and 2.0-s delays, respectively. Actual mean attained delays during vehicle-control sessions were 11.55, 18.85, 27.11, and 32.75 s at these respective delays. That is, they were 45%, 47%, 49%, and 42% of theoretical maximums. Therefore, at all titration values there was room for substantial increases or decreases when the drug was administered (i.e., the floor and ceiling effects characteristic of performance with short and long fixed delays were not apparent). In general, dose-response curves for the maximum delay attained were similar across titration values, but the highest delay attained at a given dose was substantially higher with the 2-s titration value than with lower values. Because variability was greater, however, effect sizes were lower at the longest titration value. This outcome suggests that the 2-s titration value was inferior to the others under the conditions of the present study.

Drug effects not specific to memory, such as changes in motivation, sensory acuity, and motor activity, can easily confound results on working memory tests. Pure demonstrations of drug-induced deficits in working memory involve tests that have face validity, in that they involve responses under the control of stimuli recently, but not currently, present. They also demonstrate that drug-induced performance decrements are specific to those conditions (i.e., they do not occur under otherwise comparable conditions where responding is controlled by currently present or long-absent stimuli) or, alternatively, that these decrements cannot be attributed to nonspecific drug effects.

The highest delay attained under TDMTS procedures and variants thereof has face validity as a measure of working memory but has rarely been used in this capacity (for exceptions see Dayer et al., 2000; Nordholm et al., 1995; Wenger & Wright, 1990; Wysocki et al., 1981). Because, as discussed previously, the maximum delay that can be attained each session is directly related to the number of trials completed, a potential problem with this measure as an index of drug effects is that drug-induced decreases in the number of trials completed may reduce the maximal delay attained relative to control levels regardless of whether memory processes are directly affected. There are two general strategies for dealing with the problem. One is to end sessions only after a fixed number of trials is completed, which raises the issue of drug effects dissipating before the session ends.

The other strategy is to compare performance during drug sessions with vehicle-control performance for the number of trials completed during the drug session in question. That is, if 20 trials were completed at dose x of Drug y , the highest delay attained at that dose would be compared to the highest delay attained during the first 20 trials of vehicle-control sessions. Picker and Poling (1984) previously used such a strategy to tease apart the effects of several anticonvulsant drugs on the number of trials completed and the accuracy (learning) of pigeons exposed to a repeated acquisitions procedure. This strategy has not, however, been used previously to analyze TDMTS data.

Figure 4 shows how this approach could be used to analyze the effects of the 0.06 mg/kg SC dose of scopolamine in the present study. In this figure, the highest delay attained during drug sessions is compared with the highest delay

attained during the immediately prior vehicle-control sessions, with values for the latter sessions based on the number of trials completed in the former sessions. If, for instance, a particular rat completed 47 trials when it received the 0.06 mg/kg SC dose and the titration value was 0.2 s, the highest delay attained for that animal during the relevant vehicle-control session is the value arranged at the completion of the 47th trial, even if additional trials were completed.

Figure 4. Mean values for the highest delay reached when the 0.06 mg/kg subcutaneous (SC) dose of scopolamine was administered and in the immediately prior vehicle-control sessions when the same number of trials are considered. Data are presented for each of the four titration values.

Figure 4 shows that even when the number of trials completed was equivalent during drug and vehicle-control sessions, the highest delay attained was substantially higher in vehicle-control sessions than in sessions preceded by 0.06 mg/kg SC injections of scopolamine. This finding indicates that the effects of scopolamine on this measure of performance were not an artifact due to slower responding and a resultant decrease in the number of trials completed.

Rats used in this study had prior lever-press training, which undoubtedly affected the speed with which their performance initially stabilized under the TDMTS procedure and may have affected their terminal accuracy. Also, short (5-s) intertrial intervals were utilized in the present study, which might have resulted in substantial proactive interference within sessions and thereby reduced accuracy (see White, Parkinson, Brown, and Wixted, 2004). Future research that examines performance of experimentally naïve rats tested with a range of intervals appears to be warranted.

On the whole, the present findings demonstrate the feasibility of using TDNMTP to study drug effects in rats. They do not, however, demonstrate that the procedure is especially valuable for that purpose or as a general assay of working memory. Given, however, that despite their obvious limitations nonadjusting DMTS procedures and their variants have proven valuable (Paule et al., 1998; Pontecorvo & Clissold, 1992), further studies of titrating and adjusting procedures (e.g., Han et al. 2000) appear to be worthwhile.

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