

C O N F I D E N T I A L

**Delayed Matching-To-Sample Test in
Macaques**

DATE

This study was conducted under the terms of a Materials Transfer and Services Agreement between NeuroDetective International and Company dated.

1. Executive Summary

Purpose:	This study evaluated COMPOUND X for its ability to improve performance in a memory task in aged rhesus monkeys.
Design:	Six aged rhesus monkeys (21 yrs. +), previously trained to criterion in the Delayed Match to Sample (DMTS) test (see below), received a 2-dose regimen of COMPOUND X , and were tested on DMTS beginning 60 minutes following each dosing. The doses tested were 3.0 mg/kg (Dose 1) and 30 mg/kg (Dose 2). A soft ball-shaped cocoa mix was used as a vehicle (p.o. dosing) and was also tested separately.
Results:	<ul style="list-style-type: none">• COMPOUND X tended to increase accuracy of DMTS task performance, particularly during 0-Delay trials. This improved accuracy tended to be more consistent across animals treated with 30 mg/kg COMPOUND X , compared to the 3 mg/kg dose, although the magnitude of the drug effect was not different at the two doses. The tendency toward increased accuracy following drug treatment did not achieve statistical significance.• The tendency toward increased accuracy with compound X carried over to at least one day following drug administration.• The fact that task accuracy followed the same pattern with both doses of COMPOUND X (increased accuracy during days of drug administration, followed by sustained increases in accuracy on the first washout day, followed by return to baseline levels) indicates that a pharmacological response to the drug did occur, despite the absence of overall statistical significance.• COMPOUND X had no effect on task latencies, suggesting no effect on psychomotor speed.

2. Methods

2.1 Subjects

Six rhesus monkeys of mixed gender were well trained (>100 individual sessions) in the delayed matching-to-sample task (DMTS). The animals were maintained on tap water (unlimited) and standard laboratory monkey chow (Harlan Teklad Laboratory 20% monkey diet, Madison, WI) supplemented with fruits and vegetables. The animals were maintained on a feeding schedule such that approximately 15% of their normal daily food intake was derived from 300 mg reinforcement food pellets (commercial composition of standard monkey chow and banana flakes, Noyes Precision food pellets, P.J. Noyes Co., Lancaster, NH) obtained during experimental sessions. The remainder was made available following each test session. Each animal had participated in one or more pharmacological studies of drugs that were considered to have fully reversible actions. At least a 4 week washout period preceded the initiation of this study. The monkeys were maintained on a 12 hr light-dark cycle and were tested each weekday between 0900 and 1400 hrs. Room temperature and humidity was maintained at $72\pm 1^{\circ}\text{C}$ and $52\pm 2\%$, respectively. Subject demographics are presented in Table 1.

2.2 Drug administration

The solid compound COMPOUND X was stored in a tightly stoppered polypropylene vial in a desiccator cabinet at room temperature. No other precautions were used for compound storage. On each day of the experiment, appropriate amounts of drug were weighed to the nearest 0.1 mg and placed in a cocoa mixture for oral administration. Previous experience has shown that a cocoa mixture helps to disguise the potential taste and texture of test compound, and the animals readily consume the dose. To prepare the cocoa mixture: approximately 4 g baking cocoa (commercial supermarket brand) is combined with 9 g of confectioners powdered sugar. Water is added to consistency (1-1.5 ml). Test compound is added directly to the mixture which is formed into a ball (soft, non-sticky consistency) of about 2 cm in diameter. All animals readily consumed the cocoa mix, with or without added drug.

In each instance drug or vehicle-only administration occurred 1 hr prior to DMTS testing, according to the following schedule:

	Mon	Tues	Wed	Thur	Fri	Sat	Sun
Week 1	Vehicle	Dr1Ds1	Dr1Ds1	Dr1Ds1	Dr1Ds1	DMTS	DMTS
Week 2	DMTS	Dr1Ds2	Dr1Ds2	Dr1Ds2	Dr1Ds2	DMTS	DMTS
Week 3	DMTS	Vehicle	DMTS	DMTS	DMTS		

Dr1= COMPOUND X
Ds1=3 mg/kg
Ds2=30 mg/kg
Vehicle=cocoa mixture, no drug

2.3. Procedures

Animals were tested simultaneously in their home cages using a computer-automated training and testing system which measures and categorizes the percent correct responses at each time delay, and the latency of response at each step of each matching problem. The computer and operator were isolated from the subjects.

Daily sessions consisted of 96 trials conducted in front of the display panel in the home cage. A trial began by illumination of a sample key with one of three colored discs. Monkeys were trained to depress the illuminated sample key to initiate a trial. This action also terminated the illumination of the sample key during a computer-specified delay interval. Following the delay interval, the two choice keys, but not the sample key, were illuminated. One of the two choice keys is presented in the same color as the sample key while the other (incorrect) choice key is presented in one of the two remaining colors. If the monkey correctly matched (i.e., pressed the choice key whose color matched that of the stimulus key), that response was rewarded by delivery of a 300 mg banana-flavored food pellet. The inter-trial interval was always 5 sec.

Several testing precautions were incorporated into the presentation of the matching problem. First, the various combinations of stimulus color (red, green, yellow) were arranged so that each of the three colors appeared an equal number of times as a sample; each color appeared an equal number of times on the two choice keys; and each color appeared an equal number of times in combination with each other color. Likewise, when two colors (e.g., green/yellow) appeared in combination, each color was counterbalanced between left and right in a non-predictable pattern. Thus, correct responses were arranged so that simplistic strategies such as position preference, left/right alternation, or even double left/right alternation resulted in performance at precisely the chance (50%) level. Finally, all stimulus counterbalancing procedures were matched to length of delay. Monkeys exhibit individual capabilities to maintain matching performance following various delay (retention) intervals, and the longest delay chosen for a particular monkey is that which consistently allows correct matching at just above chance levels (approximately 60% correct). In general, the length of delay interval was adjusted until three levels of performance difficulty were found: 1) the least difficult zero delay (mean = 85-100% correct); 2) a short delay interval (means ranging from 75-84% correct); 3) a medium delay interval (means ranging from 65-74% correct) and 4) a long delay interval representing each animal's limit in terms of DMTS performance (55-64% correct). "Zero"-delay is included as a control to monitor for changes in reference memory and/or other potential non-mnemonic changes in task performance. Values obtained for each difficulty level were averaged and recorded as the mean percent correct for the respective interval. Baseline data were obtained following the administration of vehicle.

2.4 Statistics

The following parameters were recorded for all trials during all test sessions (96-trials per session): percent correct on trials with zero, short, medium, and long delay intervals, plus latencies. Data for percent correct were subdivided according to delay interval for each 24-trial delay component of the session. Four task latencies (collapsed across delays) for trials associated with correct and incorrect choices were recorded: sample latencies (time interval between presentation of the sample stimulus and the subject pressing the sample key) and choice latencies (time interval between presentation of the choice stimuli and the subject pressing a choice key). All statistical analyses were performed on raw data (% trials correct or median latencies in sec). Data were analyzed by use of a multi-factorial analysis of variance (ANOVA) with repeated measures (SAS, JMP statistical software package). The effects of drug, dose, delay interval, time of testing (i.e., the time elapsed between drug administration and DMTS testing), and all crosswise interactions were assessed. An orthogonal multi-comparison test was used to compare individual means. For each table (below) error values denoted by \pm indicates the standard error of the mean. Differences between means from experimental groups were considered significant at the $P < 0.05$ level (2-sided test).

3. Results

Performance efficiencies exhibited by the subjects who were tested 1 hr after administration of vehicle are presented in Table 2. Increasing duration of delay (retention) interval was associated with the expected decrement in performance efficiency. COMPOUND X was administered according to a two-dose (3 mg/kg and 30 mg/kg) sub-acute dosing schedule (see Table, above). When subjects were tested 1 hr after drug administration (Figure 1), no statistically significant effect of Experimental day alone ($F_{15,322}=1.14$, $P=0.32$), or as an interaction with delay interval ($F_{47,322}=1.20$, $P=0.19$) was obtained. However examination of the data (Fig. 1) suggests the possibility of a drug effect across the three treatment days and at least one day following treatment for each dose. This was more evident after examination of “average” (average of all completed trials) levels of task accuracy (upper most panel in Fig. 1). Further examination of Figure 1 indicates that the most consistent improvement in accuracy during drug sessions after drug administration occurred during trials associated with Zero delay intervals.

Because of the trends in the data that were visually (if not statistically) apparent, a separate analysis was performed as follows: statistical comparisons were made between “control” (combined vehicle and no-drug sessions) and (1) the 3 mg/kg COMPOUND X sessions collapsed over treatment days plus the first day after drug treatment (i.e., Days 1, 7, 8, 14-17 vs. Days 2-6); and (2) the 30 mg/kg COMPOUND X sessions collapsed over treatment days plus the first day after drug treatment (i.e., Days 1, 7, 8, 14-17 vs. Days 9-13). Using this approach, there was a nearly significant effect of drug administration in the

overall analysis, $F_{2,356}=2.47$, $P=0.086$. Post hoc tests indicated that virtually all of the drug effect was associated with the 30 mg/kg dose ($t=2.21$, $P=0.028$) rather than with the 3 mg/kg dose ($t=0.75$, $P=0.45$).

In addition to the effect of the test drug on aspects of working memory associated with the DMTS task, two task latencies are determined. Sample latencies (time interval between presentation of the sample stimulus and the subject pressing the sample key) and choice latencies (time interval between presentation of the choice stimuli and the subject pressing a choice key) were obtained for trials associated with correct and incorrect choices. These values are presented in Table 3. Comparisons were made between combined vehicle and no-drug sessions vs. the 3 mg/kg COMPOUND X sessions combined with one day after drug treatment (i.e., Days 1, 7, 8, 14-17 vs. Days 2-6) and between combined vehicle and no-drug sessions vs. the 30 mg/kg COMPOUND X sessions combined with one day after drug treatment (i.e., Days 1, 7, 8, 14-17 vs. Days 9-13). There were no statistically significant differences between vehicle/no-drug and DVD 742 treatment/1-day post treatment sessions ($F_{2,356}=1.20$, $P=0.301$).

4.0 Summary and Conclusions

1. COMPOUND X tended to increase DMTS task accuracy, particularly during trials associated with Zero delay intervals. The drug effect appeared to be dose-dependent, with the 30 mg/kg dose producing a more consistent, if not greater, response than the 3 mg/kg dose.
2. The effect of COMPOUND X on task accuracy appeared to carry over to at least one day following drug administration. The fact that task accuracy followed a pattern of increase, sustained response over the first washout day, then return to baseline levels, over the course of administration of both doses, indicates that a pharmacological response to COMPOUND X did occur despite the difficulty in finding a statistically significant effect of drug treatment.
3. COMPOUND X was without effect on psychomotor speed as the drug produced no effect on either of the task latencies.
4. No untoward effect of COMPOUND X was noted in any subject.

The results of this study are consistent with a moderate effect of sub-acute administration of COMPOUND X on working memory. The predominant effect of the drug evidenced during Zero delay intervals may indicate an effect on attentional components of memory. Since most of the potential cognition enhancing agents tested in this laboratory have evoked highly individualized responses in aged subjects, a more realistic test of the full efficacy of COMPOUND X awaits the use of additional doses for the determination of a Best Dose effect, where that “best dose” may be different for different animals.