Pharmacology Biochemistry & Behavior, Vol. 39, pp. 79–84. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Clonidine Enhances Delayed Matching-To-Sample Performance by Young and Aged Monkeys

WILLIAM J. JACKSON*[1] AND JERRY J. BUCCAFUSCO†‡

*Departments of *Physiology & Endocrinology, †Pharmacology & Toxicology, and ‡Psychiatry*
Medical College of Georgia
and Veterans Administration Medical Center, Augusta, GA 30912

Received 22 October 1990

JACKSON, W. J. AND J. J. BUCCAFUSCO. *Clonidine enhances delayed matching-to-sample performance by young and aged monkeys*. PHARMACOL BIOCHEM BEHAV **39**(1) 79–84, 1991.—Clonidine, an alpha-2 noradrenergic agonist, has been shown to alter cognitive performance in humans and animals. Included among the evidence are studies which differ in their conclusions regarding the question of whether clonidine administration improves delayed response (DR) performance by nonhuman primates. The present results indicated that clonidine administration to both young and aged monkeys results in a modest performance improvement as measured by one of the commonly employed versions of DR performance—delayed matching-to-sample (DMTS). The clonidine-induced enhancement of DMTS had a duration of at least 24 h in both age groups.

Aging Behavior Catecholamines Clonidine Delayed matching-to-sample Delayed response
Memory Monkey Nonhuman primate Noradrenergic drugs

NORADRENERGIC mechanisms constitute a portion of the neural substrate of learning and memory. Brain lesions or agents, which result in decreased cortical levels of norepinephrine, are associated with diminished capability to learn and remember (2,21). Ultimately, noradrenergic agonists may prove useful in the development of therapeutic agents for improving human memory, and pathology in the noradrenergic systems is associated with at least two categories of memory disorder—Alzheimer's disease and Korsakoff's syndrome (14, 15, 22).

Likewise, the memory deficits associated with these forms of human pathology can be detected by classic forms of delayed response (DR) performance, which are commonly used to test the mental status of nonhuman primates. Memory-impaired humans show significant performance deficits when tested by DR (11, 20, 24), and the fact that these tasks are sensitive to the impairments associated with human memory loss, supports the validity of using nonhuman primates performing DR tasks as models for the development of treatments designed to improve human memory.

The effects of noradrenergic agents upon DR performance by nonhuman primates have been studied. One series of experiments (3,4) reported that the alpha-2 noradrenergic agonist clonidine improved DR performance by aged monkeys. This enhancement was linked to putative binding at alpha-2 receptor sites within the prefrontal cortex, in the region of the principal sulcus. However, a subsequent paper (8) reported that similar dose levels of clonidine disrupted, rather than enhanced, DR performance. It is not clear why the findings of the two studies varied so dramatically; however, it was speculated (8) that the cognitive enhancement associated with clonidine administration might be specific to the testing environment or certain test procedures. The initial studies (3,4) had presented three-dimensional stimuli through the use of a Wisconsin General Test Apparatus (WGTA), while the subsequent study (8) had presented two-dimensional, spatially linked stimuli with an automated apparatus (6). The manually operated WGTA, relative to the typical automated test environment, involves greater opportunity for distraction to the test animal, especially through the rats (19).

In the present studies, a wide dose range of clonidine was administered, first to a group of young adult monkeys, and later to two monkeys of very advanced age. The test procedure was an automated version of the delayed matching-to-sample task (DMTS), which incorporates features of both the WGTA and the typical automated DR environment.

METHOD

A total of eleven monkeys, nine young monkeys and two aged monkeys, participated in the study. The young adult monkeys (>10 years) were of two species (four feral-reared *Macaca*

[1]Requests for reprints should be addressed to Dr. W. J. Jackson, Department of Physiology & Endocrinology, Medical College of Georgia, Augusta, GA 30912-3000.

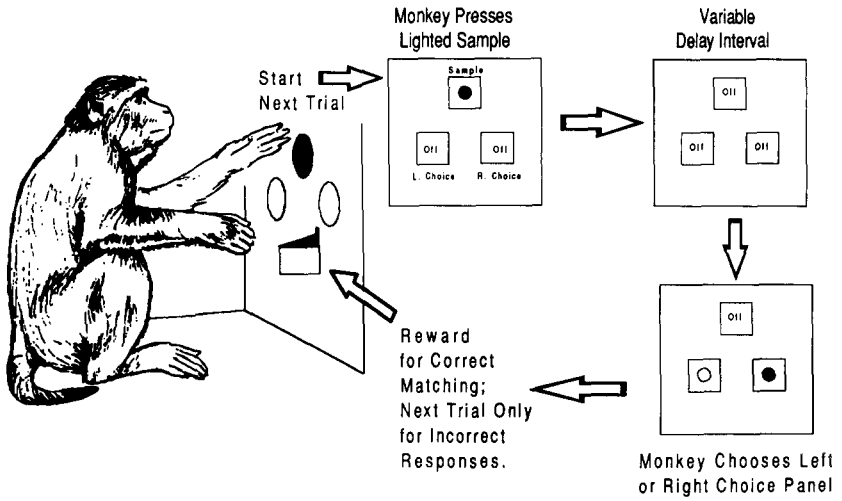

FIG. 1. The above figure illustrates a monkey in the DMTS test environment and the various steps which comprised an individual trial. To begin a trial, the upper sample panel was illuminated by one of three different hues. The sample panel remained illuminated until the monkey depressed it. That response darkened the sample panel, and all panels remained darkened during the delay interval. Following the delay interval, the two bottom choice panels, but not the upper sample panel, were then illuminated by two hues. One of the choice panels was illuminated by the same (matching) hue which had previously illuminated the sample, while the other choice panel was illuminated by one of the other (nonmatching) hues. Matching choice responses were rewarded. Monkeys were tested in their home cage and there were 96 trials per session.

fascicularis of Philippine origin, and five *Macaca iris*, reared in the colony of the University of Washington Regional Primate Center). The two aged *Macaca mulatta* were feral-reared males. Both aged monkeys had a history of nearly 30 years participation in a breeding program at the University of Pittsburgh and their estimated age at the time of testing was 35–36 years. Three additional aged monkeys were scheduled for this project, but two died before training was completed and the remaining aged animal has so far been unable to meet the cognitive requirements of the DMTS tests. Because of the difficulty in obtaining replacement aged monkeys and the long training period required to prepare for this study, it was decided to limit the aged population to the two available animals. All eleven monkeys were behaviorally naive prior to DMTS training. Initial DMTS training, which covered a period of several months for the young monkeys, and over a year for the aged monkeys, was conducted until the monkeys demonstrated criterion levels of matching performance. After initial training, but before the present study, the animals had participated in a test series in which the effects of nicotine upon DMTS were measured (12). Water was supplied on an ad lib basis, but the monkeys obtained approximately 15% of their food through performance of the DMTS tasks. The remainder of the food ration was provided following the test session. The animals were housed in rooms which sheltered as many as six monkeys, but the cages were arranged so that the animals could not observe one another.

For DMTS sessions the test panel was attached to the home cage, and while testing was in progress, the room lights were dimmed to a low level. Three animals were tested simultaneously using a computer-automated training and test system, which not only presented the DMTS task, but also measured and categorized a number of behavioral correlates for each DMTS trial. A schematic diagram of the DMTS behavioral paradigm is shown in Fig. 1; with additional details available in earlier papers (9,12). The apparatus and paradigm were designed to facilitate data analysis in a number of behavioral categories. Included in the list of behavioral categories were the percent correct performance at each of four delay intervals, the latency of response at each step of individual trials (both for correct and incorrect trials), the percent correct for left and right choice position, and the percent correct responses at each delay interval for the various combinations of stimulus colors. Software-controlled calculations of the daily session averages for these dependent variables were provided and stored in a data file, which was subsequently imported into an electronic spreadsheet. Stimuli were 1 in. diameter colored disks (red, yellow, and blue) presented on the screens of in-line-digital-display units. Each daily session consisted of 96 trials. A trial began by illuminating the sample key with one of the colored stimuli. The sample remained illuminated until the animal responded to the sample key. The response extinguished the sample light. Following a preprogrammed delay interval, during which no panel lights were illuminated, two choice lights located below the sample key were then illuminated. One of the choice stimuli always matched the previously presented sample light, while the nonmatching choice was one of the other two colors. The choice stimuli, but not the sample stimulus, remained illuminated until the animal depressed one of the choice panels. Responses to the choice panel illuminated by the colormatching the color of the previously presented sample panel were rewarded by a 300 mg banana-flavored pellet. Nonmatching choices were neither rewarded nor punished, but were simply followed by the next trial. A noncorrective procedure was used throughout the study; therefore, the next trial involved a different stimulus configuration. There were four possible delay intervals between a monkey's response to the sample stimulus and the presentation of the two choice stimuli: zero delay and three longer delay intervals, which hereafter will be referred to as short, medium, and long delays. Zero delay was not instantaneous, but involved darkening the sample stimulus (following a sample response),

and then a fraction of s later illuminating the choice stimuli. Each stimulus color configuration occurred in conjunction with each delay interval an equal number of times. The animals were trained until performance for "zero" delay trials averaged approximately 95% correct. Short, medium and long delays were progressively lengthened until correct-matching approximated the following performance levels: Short delay (70–80% correct); Medium delay (60–70% correct); and Long delay (50–60% correct). Because of intrasubject differences in skill-level, this procedure resulted in different lengths of delay intervals for each animal. However, the animals were thus equated for range of correct performance following short, medium and long delays. The range of delays for the younger monkeys ranged from as little as 0–20 s, to a maximum length of 140 s. For aged monkeys, these performance criteria were achieved within the 0–10 s range.

The order of the sample hues was carefully counterbalanced so that each hue and all combinations of hue occurred in conjunction with each delay and choice position an equal number of times. Reward patterns and delay intervals also followed counterbalanced sequences (9) to insure exactly 50% correct (chance) reward ratios for nonmatching strategies, including position habits, double or single alternation, and picture memory.

Clonidine was administered IM in the gastrocnemius muscle within a volume of 0.3 ml saline. After clonidine or vehicle injection, test panels were attached to the front of the animal's home cage, and the session began 10 min following the injection. Each monkey received a clonidine dose series including 0.5, 1.0, 5.0, 10.0, 20.0, 30.0 or 50.0 μg/kg, unless the dose appeared to elicit such undesirable side effects as excessively blue skin-coloring, sedative effects, or lack of interest in performing the DMTS task. A minimum drug "wash-out" period of two days was maintained between clonidine injections. For statistical analysis, each animal was used as its own control. The performance values obtained during sessions preceded by clonidine administration were compared by one-way ANOVA to the values obtained from the same animal during the previous days testing (saline injections) and to the following session 24 h later. Individual analysis was performed for each delay length. Scheffe tests were used to compare individual means, when the ANOVA indicated a minimum 95% level of confidence.

RESULTS

Young Monkeys

All young monkeys exhibited modest enhancement of DMTS following at least one of the lower doses of clonidine; however, the window of enhancement was narrow, since most individuals showed improved matching following only one of the doses. For five animals, the best dose was 0.5 μg/kg, for two animals the best dose was 1.0 μg/kg, and for the remaining two animals, the best dose was 10.0 μg/kg. Clonidine doses greater than 10 μg/kg either totally stalled the DMTS session or reduced the percentage of correct DMTS performance. During the session conducted 10 min following best dose clonidine administration, correct matching for the overall session (mean of all 96 trials) improved an average 3.5 percentage points (from 73.4 to 76.8%). During the session conducted 24 h after the clonidine injection, improved performance (+3.0 percentage points/session) remained evident, but performance had returned to baseline values by the session conducted 48 h after injection (Fig. 2). These percentage point increases correspond to an overall enhancement of 4.8 and 4.0%/session respectively.

Further analysis revealed that the performance improvement

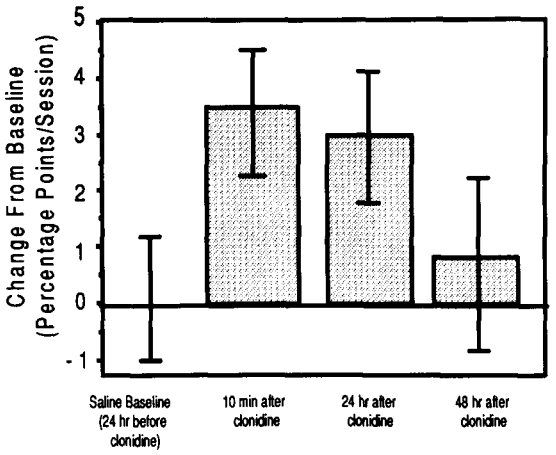

FIG. 2. Clonidine-mediated enhancement lasts for a period of at least 24 h, but less than 48 h. Data points reflect the overall means of all 96 trials/session, regardless of delay interval or stimulus color configuration. Data points also represent the best dose replicated three times for nine young adult monkeys. Error bars represent the s.e.m.

associated with the best dose of clonidine occurred in conjunction with trials involving the medium to long delay intervals. ANOVA revealed significant differences, especially for the long delay interval, $F(2,71)=4.26$, $p<0.017$. DMTS performance improved an average 6.5 percentage points above the saline control sessions (from 67.9% to 74.5%) for the sessions which began 10 min following the best dose of clonidine, and performance remained at a mean 6.8 percentage points/session above baseline during those sessions which began 24 h following the best dose of clonidine. These values represent an improvement of 9.5 and 10.0 percent improvement respectively. Performance associated with trials involving zero delay intervals was consistently impaired by clonidine at all dose levels. Figure 3 shows the effects of the best dose of clonidine upon performance associated with trials involving each of the delay intervals.

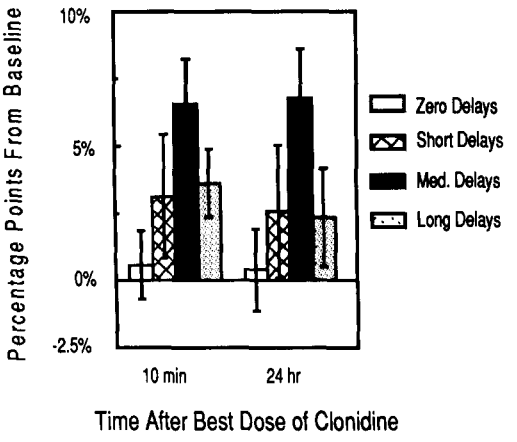

FIG. 3. Clonidine administration increased correct matching following all delay intervals; however, the only statistically significant differences were attributable to improvement during the 24 trials/session which involved the medium length delay intervals. Data points also represent means of the best dose replicated three times for nine young adult monkeys. Error bars represent the s.e.m.

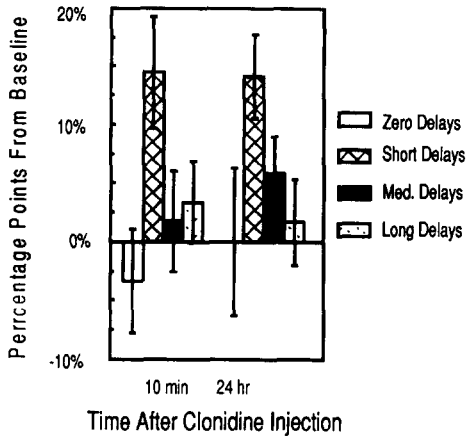

FIG. 4. Clonidine administration increased the overall average of correct matching by aged monkeys; however, because of the small test population, there were no statistically differences attributable to any individual delay interval. The only statistically significant index for discriminating between baseline sessions and clonidine sessions was the overall session average. Enhanced matching performance remained evident 24 h following clonidine administration. Data points represent means of all 96 trials/session following saline or clonidine administration replicated three times for both aged monkeys. Error bars represent the s.e.m.

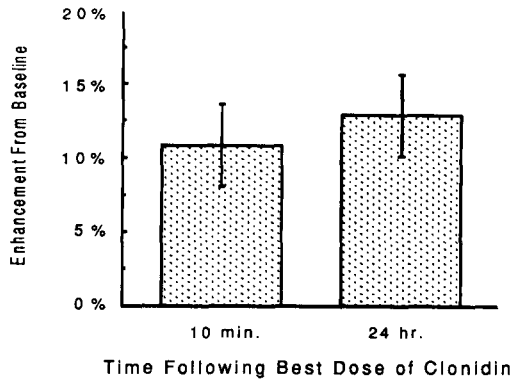

FIG. 5. Much of the improvement in DMTS performance manifested by the aged monkeys was due to better performance on that choice position which had been assoicated with the lowest percentage of matching during baseline saline sessions. Data points represent means of the best dose replicated three times for both aged monkeys. The data points show only those 48 trials/session associated with the nonpreferred choice position. Error bars represent the s.e.m.

Latency of response was not significantly altered following clonidine administration at doses which enhanced matching performance. Latencies were analyzed for time of response to both sample and choice, for trials which were matched correctly, and also for trials which were not associated with correct matching; however, these analyses did not reveal significant differences attributable to clonidine.

Aged Monkeys

DMTS performance of both aged monkeys was improved by low doses of clonidine. The best dose for one aged monkey was 0.5 μg/kg and 1.0 μg/kg for the other. Despite large increases associated with the short delays on the day of clonidine administration, the small degrees of freedom prevented these values from being statistically significant (Fig. 4). The only statistically significant measure of enhancement following clonidine administration was for mean overall performance considering all 96 trials/session, regardless of delay interval, $F(2,12)=4.3$, $p<0.039$. DMTS enhancement remained evident during the sessions conducted 24 h following injection. As with younger monkeys, analysis of the various latency categories failed to uncover significant differences attributable to clonidine.

Both aged monkeys were prone to form nonmatching strategies such as position preference and preferences regarding stimulus color combinations. Since for each session, exactly one-half of the correct matches were on the left and one-half on the right, it was possible to compare the behavior of the monkeys in regard to position preferences during saline and subsequent clonidine sessions. An analysis of the effects of clonidine upon position preference revealed that some of the improved performance of the aged monkeys following clonidine administration was due to an 11 percentage point/session increase (10 min following clonidine) and a 13 percentage point/session increase (24 h following clonidine) over baseline saline values regarding percent correct matching on the nonpreferred choice side; i.e., the side which was associated with the lowest level of performance following saline administration (Fig. 5). As with other indices of clonidine-mediated improvement, this statistically significant 11 percentage point/session improvement of nonpreferred spatial performance remained for at least 24 h following clonidine administration, $F(2,12)=4.51$, $p<0.034$. These figures represent a 17.8% increase for the session 10 min following clonidine and a 21.2% increase for the session 24 h following clonidine administration.

Both aged monkeys appeared to be more sensitive to the sedative effects of clonidine than younger monkeys; for example, neither aged monkey was able to complete a full 96 trials session following a clonidine dose greater than 10.0 μg/kg. Following larger doses, the aged monkeys appeared "drowsy."

DISCUSSION

It is remarkable that clonidine administration had similar effects upon monkeys of varying subspecies, age, and gender. Since the previous studies of clonidine upon DR had been conducted upon aged monkeys, these findings present new information regarding the enhancement in young adult monkeys of DR performance by clonidine. Although the different species composition of the young vs. the aged groups inhibits our statement regarding the interaction of clonidine with age group, the robust improvement of the aged group following clonidine administration is noteworthy.

In regard to the question of whether clonidine enhances DR performance of aged monkeys, our findings confirm those studies that clonidine administration did improve DMTS performance; however, the most effective doses were lower than those previously reported (3,4). However, the somewhat slower reaction times, and general sedative effects associated with higher doses of clonidine are compatible with the study (8) which found clonidine to disrupt DMTS performance. It remains a possibility that differences in test environment interacting with various dose-levels of clonidine accounts for the varying results of previous studies. By means of comparison, the study which had found clonidine to be disruptive to DR (8) had employed an automated spatially oriented DR task, while the initial studies, which had shown enhancement following clonidine (3,4), had used a manually operated WGTA to present DR problems. The WGTA involves more human contact, and increased numbers of potentially distracting visual and auditory stimuli. The raising and lowering of the opaque screen is a particularly prominent part of WGTA-mediated DR testing. Along that line, it has

been speculated that clonidine-mediated enhancement of DR performance could be mediated through the abundant cluster of alpha-2 adrenergic receptors located in the region of the principal sulcus of the prefrontal cortex (3,4). It is well-established that lesions of the principal sulcal region diminish the ability of monkeys to perform a variety of behavioral tasks within the DR category, and it was demonstrated many years ago that sedative drugs (e.g., phenobarbital) improve DR performance by monkeys with prefrontal lesions (18). Such sedative-mediated enhancement is thought to result from attenuation of the characteristic hyperreactivity, which follows lesions of this brain region; thus monkeys with prefrontal lesions perform DR more efficiently when slightly sedated or when placed in distraction-free test environments. Part of the variance among previous reports of the effects of clonidine upon DR is possibly a result of different levels of potentially distracting stimuli. The sedative effects of clonidine may improve DR performance to a greater extent in the presence of distracting stimuli. The "distraction-quotient" of the present test environment was probably intermediate to the previously reported studies, and may explain the somewhat intermediate results. Our automated procedure reduced direct human contact before and during testing, did not involve such distractions as the elevation of opaque screens, and, therefore, reduced extraneous stimulation compared to the typical WGTA environment. However, our testing did occur within a room housing other animals, and thus allowed a potential for more uncontrolled extraneous stimulation than the study (8) that had found clonidine to be disruptive of DR performance. Contrary to this hypothesis regarding possible interaction between DMTS performance and the sedative effects of clonidine is the finding (5) that the alpha-2 agonist guanfacine improved memory in aged monkeys independent of sedative or hypotensive side effects. Based on this and other findings, it was hypothesized that the memory-enhancing effects of alpha-2 agonists may be due to actions at a different receptor subtype than the sedative effects of these compounds. Regardless of the mechanism, this work supports the growing body of literature that has found alpha-2 adrenergic stimulation to result in improved delayed response performance.

In addition, these results provide information about the longevity of clonidine-mediated enhancement. Neither previous study mentioned enhancement beyond the day of injection, although in this study, a consistent feature of the data was the continued strength of the enhancement for a period somewhere between 24 and 48 h. Similar long-lasting effects have been observed following guanfacine administration (6). It is unlikely that this effect is due to rebound or withdrawal effects. Rebound or withdrawal effects to clonidine are difficult to produce in experimental animals because of the short half-life of the drug. Usually very high doses are required and/or continuous administration. In the present study, one dose of clonidine was administered every 3 days; therefore, the dosage range was too low and the frequency of administration too low to expect significant rebound effects. Furthermore, at the end of the protocol and after the highest doses were administered, no untoward signs or symptoms of rebound were noted in the animals. Although the biological basis of this extended enhancement remains unclear, it apparently is not unique to noradrenergic stimulation, since similar observations have been reported following nicotine administration (13). Apparently the behavioral enhancement far outlasts the duration of measurable quantities of these compounds in the body.

ACKNOWLEDGEMENTS

The authors thank Ms. Dianne Cox for expert technical assistance. This work was supported by funds from the Callaway Foundation, the regents of the University System of Georgia, and Veterans Administration Medical Center.

REFERENCES

1. Adolfsson, R.; Gottfries, C. G.; Roos, B. E.; Winblad, B. Changes in brain catecholamines in patients with dementia of Alzheimer type. Br. J. Psychiatry 135:216–223; 1979.
2. Anzelark, G.; Crow, T.; Greenberg, T. Impaired learning and decreased cortical norepinephrine after bilateral locus coeruleus lesions. Science 181:682–684; 1973.
3. Arnsten, A. F. T.; Goldman-Rakic, P. S. Alpha-2-adrenergic mechanisms in prefrontal cortex associated with cognitive decline in aged nonhuman primates. Science 230:1273–1276; 1985.
4. Arnsten, A. F. T.; Goldman-Rakic, P. S. Noradrenergic mechanisms in age-related cognitive decline. Neural Transm. (Suppl.) 24:317–324; 1987.
5. Arnsten, A. F. T.; Cai, J. X.; Goldman-Rakic, P. S. The alpha-2 adrenergic agonist guanfacine improves memory in aged monkeys without sedative or hypotensive side effects: Evidence for alpha-2 receptor subtypes. J. Neurosci. 8:4287–4298; 1988.
6. Bartus, R. T.; Johnson, H. R. Short-term memory in the rhesus monkey: disruption from the anticholinergic scopolamine. Pharmacol. Biochem. Behav. 5:539; 1976.
7. Bondareff, W.; Mountjoy, C. Q.; Roth, M. Loss of neurons of origin of the adrenergic projection to cerebral cortex (nucleus locus ceruleus) in senile dementia. Neurology 32:164–168; 1982.
8. Davis, R. E.; Callahan, M. J.; Downs, D. A. Clonidine disrupts aged-monkey delayed response performance. Drug Dev. Res. 12:279–286; 1988.
9. Elrod, K.; Buccafusco, J. J.; Jackson, W. J. Nicotine enhances delayed matching-to-sample performance by primates. Life Sci. 43:277–287; 1988.
10. Gellerman, L. W. Chance orders of alternating stimuli in visual discrimination experiments. J. Genet. Psychol. 42:207–208; 1933.
11. Irle, E.; Kessler, J.; Markowitsch, H. J. Primate learning tasks reveal strong impairments in patients with presenile or senile dementia of the Alzheimer type. Brain Cogn. 6:429; 1987.
12. Jackson, W. J.; Pegram, G. V. Acquisition, transfer and retention of matching by rhesus monkeys. Psychol. Rep. 27:839; 1970.
13. Jackson, W. J.; Elrod, K.; Buccafusco, J. J. Delayed matching-to-sample in monkeys as a model for learning and memory deficits: Role of brain nicotinic receptors. In: Meyer, E. M.; Simpkins, J. W.; Yamamoto, J., eds. Advances in behavioral biology, vol. 36. Novel approaches to the treatment of Alzheimer's disease. New York: Plenum Press; 1989:39–52.
14. McEntee, W. J.; Mair, R. G. Memory enhancement in Korsakoff's psychosis by clonidine: Further evidence for a noradrenergic deficit. Ann. Neurol. 7:466–470; 1980.
15. McEntee, W. J.; Mair, R. G. Memory impairment in Korsakoff's psychosis: A correlation with brain noradrenergic activity. Science 202:905–907; 1978.
16. Mohr, E.; Schlegel, J.; Fabbrini, G.; Williams, J.; Mouradian, M. M.; Ulrike, M. M.; Claus, J. J.; Fedio, P.; Chase, T. N. Clonidine treatment of Alzheimer's disease. Arch. Neurol. 46:376–378; 1989.
17. Perry, E. K.; Tomlinson, B. E.; Blessed, G.; Perry, R. H.; Cross, A. J.; Crow, T. J. Neuropathological and biochemical observations on the noradrenergic system in Alzheimer's disease. J. Neurol. Sci. 51:279–287; 1981.
18. Pribram, K. H. Some physical and pharmacological factors affecting delayed response performance of baboons following frontal lobotomy. J. Neurophysiol. 13:373–382; 1950.
19. Pribram, K. H.; Gardner, W. W.; Pressman, G. L.; Bagshaw, M. An automated discrimination apparatus for discrete trial analysis (DADTA). Psychol. Rep. 11:247; 1962.
20. Rice, D. C. Primate research: Relevance to human learning and development. Dev. Pharmacol. Ther. 10:314; 1987.
21. Roberts, R. B.; Flexner, J. B.; Flexner, L. B. Some evidence for the involvement of adrenergic sites in the memory trace. Proc. Natl.

Acad. Sci. 66:310–313; 1970.
22. Rossor, M.; Iversen, L. L. Non-cholinergic neurotransmitter abnormalities in Alzheimer's disease. Br. Med. Bull. 42:70–74; 1986.
23. Sawaguchi, T.; Matsumura, M.; Kubota, K. Catecholaminergic effects on neuronal activity related to a delayed response task in monkey prefrontal cortex. J. Neurophysiol. 63:1385–1400; 1990.
24. Squire, L. R.; Zola-Morgan, S.; Chen, S. K. Human amnesia and animal models of amnesia: Performance of amnesic patients on tests designed for the monkey. Behav. Neurosci. 102:210; 1988.