BOOK CHAPTER

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CHOICE REACTION TIME IN AGED RHESUS MONKEYS AS A MODEL OF AD

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INTRODUCTION

Alzheimer's disease (AD) has been a recognized clinical entity for 80 years, yet has still not been reproduced in the laboratory. The only factor clearly associated with the general incidence of this disease is increasing age (Horner, 1987; Shoenberg et al., 1987). (Note: Down's syndrome, history of head trauma and autosomal dominant inheritance apply clearly only to small portions of the population). Therefore, an important line for developing a model of AD is the investigation of aged animals.

Several animal models show neuropathological changes similar to those of AD, but none are identical (Wisniewski et al., 1970). However, the aged monkey is susceptible to the development of senile plaques (Wisniewski et al., 1973) and these plaques show a distribution similar to that of the aged human (Struble et al., 1985). The senile plaques seen in aged animals of many higher mammalian species are immunologically similar to those of aged humans and Alzheimer patients (Selkoe et al., 1987). The consistent pathological distinction is that the neurofibrillary tangles, which are characteristic of AD and are commonly seen in elderly humans (Ulrich, 1985), are not seen in the monkey; understanding this distinction may be a key to elucidating the etiology of AD.

While the aged monkey does not have the complete picture of Alzheimer neuropathology, this model also shows memory deficits which are comparable to impairments seen in aging humans and AD patients (Bartus et al., 1978; Bartus and Dean, 1987; Davis et al., 1982; Presty et al., 1987). Short-term memory in the young adult monkey has been further shown to be modified by cholinergic (Bartus, 1979) and anticholinergic manipulations (Bartus and Johnson, 1976; Penatar and McDonough, 1983; Pontecorvo and Evans, 1985; Levin and Bowman, 1986) in a manner similar to that seen in humans (Aigner and Mishkin, 1986). However, short-term memory in monkeys shows only a small impairment after damage to the basal forebrain cholinergic system (Aigner et al., 1987).

Underlying the memory deficit of AD, there is likely to be a basic deficit of neuroplasticity (Ashford and Jarvik, 1985). This link suggests that all information processing functions which access memory will be impaired by AD, while those processes associated strictly with perception will be preserved. In support of this concept, choice reaction time is slowed by AD while simple reaction time is not (Pirozzolo et al., 1981; 1985). Therefore,

these two types of information processing pathways should be differentially affected in an Alzheimer model. We chose to study the relation between simple and choice reaction time in the aged monkey model, adding the challenge of cholinergic and anticholinergic agents to more extensively test for a similarity to the Alzheimer syndrome (Bartus et al., 1982).

Cholinergic neurons function in several different brain systems including the nucleus basalis of Meynert and the basal ganglia. Those neurons in the nucleus basalis of Meynert are highly affected in AD (Whitehouse et al., 1982) though acetylcholine neurons in other areas, such as the basal ganglia, are also affected (Rossor et al., 1982). The existence of neurons using the same neurotransmitter but anatomically localized in vastly different situations has made it difficult to explore the pharmacologic properties of cholinergic neurons. Yet, the possible role of cholinergic drugs is of importance in the treatment of AD (Ashford et al., 1981; Brinkman and Gershon, 1983; Jorm, 1986), and the effect of antimuscarinic agents on memory has been advocated as a model of AD (Drachman, 1977).

METHODS

Subjects

This study used three male rhesus monkeys, Macaca mulatta, ages over 22 years. The animals, retired breeders, were experimentally naive prior to behavioral training. Animals were maintained on a diet of biscuits (Purina monkey chow) and fresh fruit daily. To enhance performance motivation, fluid was restricted for up to 23 hours prior to testing. This schedule of fluid deprivation has been used with adequate motivational effects and without harmful health effects. Fluid intake was closely monitored daily. The animals were housed individually in a room with automatically controlled lighting (light:dark, 12:12).

Behavioral paradigm

The paradigm assesses the speed of simple and complex (choice) information processing and the accuracy of binary memory during task performance in the context of a simultaneous simple and choice reaction time task.

The first step in training the monkeys was to adapt them to the restraining chair and testing booth. As the animals learned to sit quietly, facing the video screen in the testing booth, they were introduced to the reward delivery system. The monkeys quickly learned to manipulate the joystick lever to obtain a squirt of sweetened grape juice for reward. The next step was to pair the correct motor response (a lever press) to its corresponding screen color. A red screen required a downward deflection, while green demanded an upward deflection. The task was entirely computer automated (Tandy Radio Shack Color Computer). Once all three monkeys had mastered the choice reaction time task, they were shown a blue screen as a cue stimulus. Correct response to the blue stimulus was a downward deflection, a simple reaction. Once the second task was well learned, the two tasks were combined. Criterion for training completion was performance for 5 consecutive days above 90%

correct. (Training these animals took about 8 months.)

In the combination task used for drug testing, the monkey was first presented the blue video screen. Correct response was required for the trial to continue. After a 2-3 second delay, the red or green screen was presented. Correct response was then rewarded (1 cc of juice). The response to each stimulus was recorded by the computer (correctness and reaction time).

Anticholinergic agent study

Each monkey was entered into a counterbalanced order of drug (scopolamine hydrobromide, .4 mg/cc, Elkins-Sinn, Cherry Hill, NJ), active placebo (glycopyrrolate, .2 mg/cc, Elkins-Sinn, Cherry Hill, NJ), and inactive placebo (saline). The drug and the placebo sessions were separated by sessions in which no agent was administered. Drug doses were determined in an earlier dose-finding phase. The maximum tolerable dose (dose 5) was used as a high dose in the experiment. In these monkeys, the maximum tolerable dose at which some performance occurred was approximately 50 micrograms per 2/3 root of body weight in kilograms (surface area). In the experiment, 1/5, 2/5, 3/5, 4/5, and 5/5 of the maximum dose was given. Each alternating week for 9 weeks, monkeys received the drug and complementary placebos in a specific order, unique to that monkey. The doses were also given in an order unique to each monkey. The dose of scopolamine and glycopyrrolate was the same for a given monkey in a given week. For drug/placebo sessions, each monkey received an intramuscular injection of either scopolamine, glycopyrrolate, or saline while in his home cage. All injections were brought to a constant volume of 3 cc with sterile saline. Two hours following the injection, the monkey was placed in the testing booth and performance tested for 1 hour (160 trials). Therefore, the measured reaction times occurred 2-3 hours following drug administration, giving the scopolamine sufficient time to develop its behavioral effects (Safer and Allen, 1971).

Performance of the animals during scopolamine administration was analyzed and compared to both control conditions (glycopyrrolate and saline) for responsiveness (was the animal working), accuracy (generally the performance should exceed 90% correct for each aspect of the task) and reaction time: simple and choice. These aspects of behavioral performance test for effects of general motor function and allow for different aspects of behavior to be distinguished.

Cholinergic Agent Study

During the weeks between scopolamine, glycopyrrolate and saline testing, the animals were studied following administration of a cholinergic drug (physostigmine salicylate, 1 mg/cc, Forest Pharmaceutical, St. Louis, MO), active placebo (pyridostigmine bromide, 12 mg/cc, Roche Laboratories, Nutley, NJ) and inactive placebo (saline). The cholinergic drug testing was carried out in the same manner as the anticholinergic study. In the dose-finding phase, the maximum tolerable dose of physostigmine was determined to be 160 micrograms per 2/3 root of body weight in kilograms. The maximum dose of pyridostigmine was determined to be 5 milligrams per 2/3 root of body weight in kilograms. As in the

anticholinergic study, doses 1-5 and placebo agents were given in a specific, unique order to each monkey. The cholinergic agents and saline were administered orally one hour prior to testing. Each dose was brought to a constant volume of 5 cc with sweetened grape juice. The animals were tested for 1 hour, 160 trials. Performance for each monkey during cholinergic trials was analyzed in the same manner as the anticholinergic data.

RESULTS

Glycopyrrolate, a polar drug, which is not supposed to enter the brain, may have had some effect on the animals' performance, but this was not consistent. One strictly peripheral effect would be a dryness of the mouth, which should increase the animals' thirst and enhance performance. Other peripheral side-effects could distract the animals (e.g.: dry eyes, blurred vision). Glycopyrrolate is thought to have twice the peripheral potency of scopolamine, but some of the compound may enter the brain as does methscopolamine (Bohdanecky et al., 1967).

Scopolamine showed obvious effects, even at the lower doses. The 2/5 dose of scopolamine caused a substantial decrease in responsiveness. This effect clearly contrasted with the inactive and active placebo data. In fact, across the three elderly monkeys, the major effect of scopolamine was to reduce responsiveness. Little fluctuation of responsiveness was noted for the placebo conditions, levels varying for two animals between 85-95%, and for the third, 65-75 %. The two more responsive animals were more sensitive to low doses of scopolamine, dropping below 20% responsiveness at the 3/5 dose. The responsiveness problem was mainly manifest as a failure to respond to the blue stimulus. If the animal made that initiating step, even in the presence of higher doses of scopolamine, responses to the blue stimulus may actually have been more rapid than usual. When the animal made that initial response, the response correctness and the choice response latency were minimally affected. Because of the decrease of responsiveness, the effect of scopolamine on reaction time relative to the placebos could not be assessed.

Pyridostigmine, the active control, had no consistent effect on either simple or choice reaction time. Similarly, in all but the highest dose (dose 5), no consistent effects were seen on correctness or responsiveness relative to the saline control. At the highest dose, pyridostigmine had an adverse effect on the performance of two of the monkeys, perhaps due to cholinergic side-effects.

Physostigmine generally had no consistent effect on either reaction time measure compared to saline and pyridostigmine controls. No major effects of the drug were detected in responsiveness or correctness at the doses administered. Following the initial study, a higher dose of physostigmine (192 ug/2/3 root body weight) was administered to each monkey in two separate weeks. Again, no effect on behavior or reaction time was observed.

CONCLUSIONS

Scopolamine toxicity has been advocated as a model for

Alzheimer's disease because of its severe effect on memory function in normal humans. Further, scopolamine has been shown to slow choice reaction in normal adults (Caine et al., 1981; Nissen et al., 1987). Choice reaction time has been shown to be an index of the severity of Alzheimer's disease. However, in this experiment, scopolamine made its major impact on responsiveness, masking an effect on either simple or choice reaction time (in some cases a more rapid reaction time was noted). Failure to respond might be due to effects on any of the numerous central cholinergic pathways, and might best be related to the delirium which occurs with toxic anticholinergic serum levels in humans (Miller et al., 1988). The monkeys showed erratic behavior, more aggressiveness and less coordination at the higher doses. Though failure to recall the rules of the task and psychosis may be compared to deficits seen in severe Alzheimer's patients, we were not able to document a scopolamine-induced prolongation of choice reaction time in aged monkeys as is seen in mild AD.

There has been considerable difficulty in the literature with documenting a beneficial effect from physostigmine (Ashford and Jarvik, 1981; Jorm, 1986). However, some primate studies have shown positive effects in spatial attentive memory (Bartus and Johnson, 1976), in the delayed match-to-sample task (Penatar and McDonough, 1983) and in a short-term memory task (Aigner and Mishkin, 1986; Aigner et al., 1987). Moreover, bethanechol can improve reaction time in AD patients (Davous and Lamour, 1985). The findings in these positive studies have generally been small and have represented extremely diligent work. AD affects many brain systems other than the cholinergic system, and some of the behavioral deficits of AD may be related more to those other deficits than the cholinergic deficits.

The possibility that refinements of cholinergic enhancement may lead to more substantial benefits for AD patients has served as an impetus to a host of scientific studies. To more clearly understand the positive effect of cholinergic agents, more simple models must be designed to demonstrate the value of these drugs. In the course of these studies, a better animal model for AD is likely to emerge. The approach of analyzing information processing utilizes easily measured biological phenomena related to AD (Ashford and Fuster, 1985) and is one approach which warrants further study.

SUMMARY

Choice reactions are slowed relative to simple reactions in Alzheimer's disease (AD) patients (Pirozzolo et al., 1981; 1985). To further elucidate this behavioral component of information processing as a model of AD, we administered anticholinergic and cholinergic agents to three elderly monkeys (>22 years) performing a concurrent simple and choice reaction time task. Scopolamine was administered (doses of 10, 20, 30, 40, 50 ug/2/3 root of body weight in kg) once a week. Inactive placebo (saline) and active placebo (glycopyrrolate) were given on the other days (drug administered 2 hours before testing). At low doses, scopolamine slightly impaired responsiveness but not correctness, while reaction time was slightly improved. However, at higher doses, scopolamine caused a major decrease of animal responsiveness and correctness. Only at the highest dose was reaction speed slowed

below the saline mean. Low doses of scopolamine may improve motor function by affecting the basal ganglia as such drugs benefit Parkinsonian patients. However, the behavioral disruption at the higher doses is similar to that seen with AD patients performing related tasks. Glycopyrrolate showed no effect on performance, though delayed effects were suspected. Oral physostigmine (5 graded doses up to a maximum of 160 ug/2/3 root of body weight) and an active control (pyridostigmine) were tested using the same paradigm in another study using the same elderly animals. No significant effects of either drug were observed in the monkeys at the doses administered. Two additional trials with a 20% higher dose still showed no effects on behavior. Thus, we were unable to demonstrate selective effects of cholinergic drugs on choice response latencies, even though this measure is impaired in AD.

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