The effect of physostigmine on spontaneous alternation in infant rabbits*

RONALD H. BAISDEN, ROBERT L. ISAACSON,
MICHAEL L. WOODRUFF, and CAROL VAN HARTESVELDT
Departments of Psychology and Neuroscience
University of Florida, Gainesville, Fla. 32601

Beginning about 10 days after birth, infant rabbits were tested for spontaneous alternation while under single dosages of physostigmine and dl-amphetamine, as well as after saline injections or without any injection. Only under physostigmine did the animals alternate at levels which were above chance and which approximated levels found in normal adult animals of other species. This indicates that the capacity for spontaneous alternation exists in the infant rabbit, even though it is normally not expressed in behavior.

Spontaneous alternation refers to the tendency of animals to alternate responses to spatial locations of the goalboxes on consecutive trials in a two-choice maze. Apparently this is a rather ubiquitous aspect of mammalian life, but it has been most extensively studied in the rat (see Douglas, 1966). Under various experimental conditions, the results of the utilization of this tendency as a behavioral task have provided insights into some of the neural mechanisms underlying this response; for example, it is eliminated by bilateral destruction of the hippocampus (Douglas & Isaacson, 1964). After such a lesion, responses to the two goalbox locations drop to chance level. The same effect can be produced by the administration of anticholinergic drugs (Douglas & Isaacson, 1966).

The tendency of normal adult animals to exhibit spontaneous alternation is not found in very young animals, at least in certain species. For example, Douglas¹ has found that the tendency to spontaneously alternate develops rather abruptly in rats at about 28 days of age. Douglas¹ has found that rats which do not alternate at the usual rate (of about 80%) early in life can be improved to this level by administering .01 mg/kg of physostigmine. He has also shown that physostigmine can improve the alternation rates of DBA mice which, as a strain, show low rates of alternation. These studies, along with the earlier report of disruption of spontaneous alternation by an anticholinergic drug, implicate cholinergic mechanisms in the mediation of the neural mechanisms underlying this behavior.

In the present study, we wanted to determine whether or not

physostigmine could "release" spontaneous alternation behavior in rabbits, a species in which spontaneous alternation has not been found. If it could, we felt that this would indicate that the neural mechanisms underlying spontaneous alternation behavior were present in the nervous system of these animals, even though they are not expressed. This behavior could be elicited by a change in the balance of the neurochemical systems underlying it. In addition, we felt it important to determine the effects of an adrenergic stimulant, dl-amphetamine, on spontaneous alternation in the same animals.

METHOD

Fifteen New Zealand albino and six Dutch Belt rabbits (*Oryctolagus cuniculus*) bred in the laboratory were used for this study. Female rabbits were exposed to the male, and both were placed in a single cage overnight. Females were removed from the males on the following morning and returned to their individual cages for the duration of pregnancy. At the proper gestational time (total of 30 days), straw-filled nests were placed in the females' cages. Approximately 10 days postnatally, when the Ss had opened their eyes and could locomote freely, the infant rabbits were numbered and testing was begun.

Ss were run in a T-maze of the following dimensions: 6.5 in. high, 4.5 in. wide, and corridor length of 18 in. Animals were placed at the base leg of the T behind a sliding door. When the door was raised, the rabbit was allowed to travel along the remaining length of the corridor and into either the left or right alley. Five trials per S were run each day, with an intertrial interval of 10-30 sec. In returning the animal to the starting position, care was taken not to rotate the S more than necessary. Throughout the experiment, none of the Ss received reinforcement for any response.

The rabbit is a difficult animal to study in mazes since its tendency to go down the enclosed arms of a maze seems related to the size of the alley relative to its own body size. Rabbits grow, of course, at a very rapid rate early in life, and their willingness to enter arms of the maze may vary from one day to the next. In some instances, the animal refused to enter either alley. In these cases, the response recorded was determined on the basis of the direction in which the S turned. On those trials in which the animals refused to make even this response, the S was returned to its cage and a "no response" was recorded.

On the first day of testing, the animals were numbered and then placed in the maze. After 4 days of testing, the animals were divided into three groups for the purposes of drug treatment. One group was injected IP with a solution containing 0.06 mg/kg body weight of the anticholinesterase physostigmine. A second group was injected with 0.5 mg/kg body weight of dl-amphetamine. Both of these drugs were given dissolved in a 0.9% saline solution. Fifteen minutes after the injections, the animals were tested in the T-maze. After 2 consecutive days of testing after drug administration, the physostigmine and dl-amphetamine groups were both transferred to saline injections before their daily testing. These animals then received saline injections and testing for 2 days. The animals were then tested for 2 more days under the other drug. Thus, an animal first given physostigmine for 2 days would receive saline injections over 2 days and the dl-amphetamine for 2 days. The animals initially receiving dl-amphetamine for 2 days would have a 2-day period of testing under saline and then a final 2 days' testing under physostigmine. The Ss given saline injections initially were given either dl-amphetamine or physostigmine after the first 2 days. Then they were given 2 days more of testing under saline before the change to the other drug for 2 days of testing. After all animals had been subjected to all drug and saline treatments, they were run for 4 more days, and their rate of alternation was determined again.

RESULTS

The percentage of times that each rabbit alternated goalboxes was calculated by dividing the observed number of alternations by the total number of opportunities to alternate and multiplying the result by 100. This was done for the number of alternations observed between the first two trials of each testing session and among all of the trials given in each session. The mean percent alternations

*This research was supported in part by National Science Foundation Grant NSF GB 17345 and by National Institute of Mental Health Grant MH 10320-07.

Table 1
Mean Percent Alternation Following Drug and Control Treatments

Administration	Percent Alternation First Two Trials	Percent Alternation Total Trials
Predrug	44.5	52.0
Postdrug	54.4	48.0
First Amphetamine	55.5	50.0
Combined Amphetamine	48.0	50.0
First Physostigmine	80.0*	61.3
Combined Physostigmine	76.5*	57.3
First Saline	47.6	48.5
Combined Saline	60.0	58.5

*Indicates statistically significant difference

under each condition are shown in Table 1. From this table it can be seen that physostigmine substantially increased the rate of alternation between the first two trials, both for the first day of drug administration and for the 2 days of drug administration; a Friedman two-way analysis of variance by ranks (Siegel, 1956) demonstrated a statistically significant increase in alternation rate ($p < .01$) for these two measures. However, no significant change was found in the percentage of alternations made among all five trials on each day after physostigmine injection. Saline and amphetamine injections had no significant effect on any of the measures used in this study.

DISCUSSION

The results indicate that young rabbits can exhibit spontaneous alternation behavior at a level approximate to that found in rats (note: the rabbit is a rodent) when under the influence of the anticholinesterase physostigmine. While our results do not describe

parametric effects of physostigmine on spontaneous alternation behavior, they do show that there is at least one level of physostigmine that will produce spontaneous alternation and there is at least one level of amphetamine that does not. We do not know if there is a level of amphetamine that would enhance spontaneous alternation behavior. Whether there is or not, the physostigmine results do indicate a behavioral, and presumably, neural capability not normally demonstrated by the young rabbit.

It has been postulated that spontaneous alternation behavior results from the habituation of the initial preferred stimuli associated with one arm or goalbox area. This habituation may be dependent upon cholinergic systems (Carlton, 1969). This would suggest a reason for the effectiveness of physostigmine, which enhances the action of acetylcholine by inhibiting the enzyme which destroys it.

Douglas & Peterson (1969) have suggested that the development of

spontaneous alternation is correlated with the development of specific neural systems. There does seem to be a temporal relationship between the postnatal development of microneurons in the hippocampus (Altman, 1970) and the time at which spontaneous alternation develops. Whether or not there is a causal relationship between the development of hippocampal neurons and whether such neurons participate in the cholinergic systems which seem to be important for spontaneous alternation behavior remains to be decided by future laboratory investigations.

REFERENCES

- ALTMAN, J. Postnatal neurogenesis and the problem of neural plasticity. In W. A. Himwich (Ed.), *Developmental neurology*. Springfield, Ill: Charles C Thomas, 1970. Pp. 197-240.
- CARLTON, D. L. Brain acetylcholine and inhibition. In J. T. Tapp (Ed.), *Reinforcement and behavior*. New York: Academic Press, 1969. Pp. 288-328.
- DOUGLAS, R. J. Cues for spontaneous alternation. *Journal of Comparative & Physiological Psychology*, 1966, 62, 171-183.
- DOUGLAS, R. J., & ISAACSON, R. L. Hippocampal lesions and activity. *Psychonomic Science*, 1964, 1, 187-188.
- DOUGLAS, R. J., & ISAACSON, R. L. Spontaneous alternation and scopolamine. *Psychonomic Science*, 1966, 4, 238-284.
- DOUGLAS, R. J., & PETERSON, J. J. The sudden development of spontaneous alternation in baby rats. Paper presented at Western Psychological Association, 1969.
- SIEGEL, S. *Nonparametric statistics for the behavioral sciences*. New York: McGraw-Hill, 1956.

NOTE

1. Douglas, R. J. Personal communication, 1971.