Pharmacology Biochemistry & Behavior, Vol. 16, pp. 805–809, 1982. Printed in the U.S.A.

Detection of Cholinergic Mediation of Behavior in 7-, 9-, and 12-Day Old Rat Pups

GREGORY J. SMITH,[1] LINDA PATIA SPEAR AND NORMAN E. SPEAR

State University of New York at Binghamton, Binghamton, NY 13901

Received 5 June 1981

SMITH, G. J., L. P. SPEAR AND N. E. SPEAR. *Detection of cholinergic mediation of behavior in 7-, 9-, and 12-day old rat pups.* PHARMAC. BIOCHEM. BEHAV. **16**(5) 805–809, 1982.—A training procedure that permitted infant rats (7, 9, and 12 days old) to acquire a T maze discrimination to escape footshock (Experiment 1) was used to study the effects of the cholinergic antagonist, scopolamine hydrobromide (0, 0.2 mg/kg and 0.8 mg/kg) (Experiment 2). These results indicated that (1) Scopolamine had no effect on T maze choice behavior, unlike previous results for 15- and 23-day old rats, but (2) scopolamine did increase latency to choice in the discrimination task at all ages, an effect previously seen in 15-day old animals, but opposite that seen after scopolamine administration to 23-day old animals or adults. This "paradoxical" effect of scopolamine on response latency is present approximately two weeks prior to earlier estimates of the time course of maturation of the cholinergic system.

Developmental psychopharmacology Scopolamine Acetylcholine Rat pups T-Maze discrimination Latency to choice

IT has been suggested that the cholinergic neurotransmitter system may attain functional maturity in rats and mice during the third week of life, around 15–20 days postnatally [3]. It is not until that age that animals respond in the typical adult fashion to anticholinergic drugs such as scopolamine with an increase in locomotor activity [3], and to cholinergic drugs with catalepsy [1]. Yet, an earlier functional maturation of the cholinergic system has been suggested by studies that have assessed behaviors other than those typically seen in adult animals upon modification of their cholinergic systems. For example, we [13] observed that while 15- and 23-day old animals, like adult animals, responded to scopolamine with a decreased probability of a correct choice in a T maze escape task, scopolamine-treated 15-day old rats exhibited increased latency to choice while 23- day old rats exhibited the adult-typical response of a decreased latency to choice. These latency results supported the observation made by others that, ontogenetically, the earliest locomotor response to scopolamine is a paradoxical decrease in activity rather than the increase in activity seen later [2, 5, 10, 12].

In spite of these interesting "paradoxical" responses to anticholinergic agents at the beginning of the rat's third postnatal week, there have been few reports of the effects of drugs affecting the cholinergic system in younger pups. Such work is critical to discern the ontogeny of cholinergic function in mediating infant behaviors. Hölmgren and Urbá-Hölmgren [7] have reported that cholinergic agonists induce yawning and head-shaking that is most evident during the first and second weeks of life, respectively. Electrophysiologically, Le Blanc and Bland [9] have reported that an anticholinergic drug inhibited theta rhythms in rat pups at the beginning of the second postnatal week. Our intention was to examine possible cholinergic effects on instrumental learning during this period. In our previous work examining scopolamine responsiveness in 15- and 23-day old animals, we did not test younger animals on the same T maze discrimination task due to their typical failure to show significant instrumental learning within a reasonable period [13]. Subsequent work in our laboratory, however, indicated that with modification, this task potentially could provide an effective testing paradigm for rat pups during the second week of life [12]. In Experiment 1, the effectiveness of this task for training animals of these ages was further refined and verified. Then, this task was applied in Experiment 2 to assess the influence of scopolamine on choice behavior and latency to choice in 7-, 9-, and 12-day old rat pups.

EXPERIMENT 1

This experiment was designed to verify the reliability of the spatial discrimination procedure for use with 7-, 9-, and 12-day old rats tested in the presence or absence of litter shavings.

METHOD

Subjects and Experimental Design

The subjects in this experiment were 60 Sprague-Dawley

[1]Present address: Department of Psychology, Matherm Memorial Building, Case Western Reserve University, Cleveland, OH 44106.
[2]Submit reprint requests to N. E. Spear, Department of Psychology, State University of New York at Binghamton, Binghamton, NY 13901.

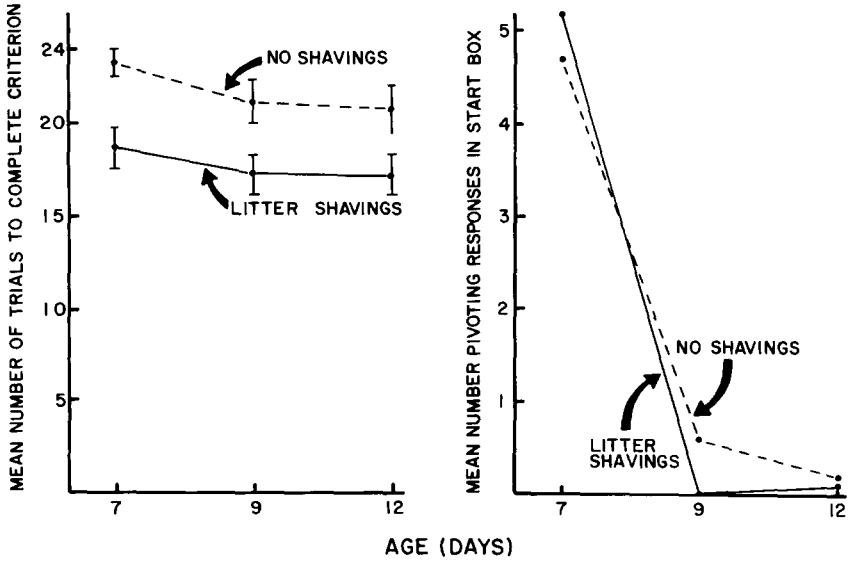

FIG. 1. Acquisition of a spatial discrimination to escape footshock (left panel) and the incidence of 180° pivoting responses (right panel) as a function of age (7 days vs 9 days vs 12 days) and training environment (home nest odors present or absent).

rats born and raised in our colony and maintained under a 0700 (lights on)–2300 (lights off) cycle. In a 3×2 factorial fashion, 3 ages (postnatal days 7, 9, and 12) × 2 training conditions (presence or absence of litter shavings), rats were assigned in a quasi-random split-litter design to one of six orthogonal treatment groups (10 rats per group). The assignment of rats was restricted in that an equal number of littermates was represented in each treatment group. Throughout the experiment rats were housed with their parents and littermates except for expressed training periods. All training occurred between 0800 and 1200 hours.

Apparatus and Procedures

The training apparatus was made from opaque Plexiglas (startbox: 8 cm long × 3 cm wide × 3 cm high; each arm: 24 cm long × 3 cm wide × 3 cm high) and mounted on a grid floor that could be electrified (0.2 mA) by a Grason-Stadler shock generator and scrambler (Model No. E 1065 GS).

The procedures have been described in detail elsewhere [12]. Briefly, rats at each age were placed in the startbox facing the choice point. After 5 seconds, the guillotine door separating the startbox from the choice point was raised simultaneously with initiation of the footshock and a latency timer. Once the rat left the startbox, the door was lowered behind it to prevent retracing. The first trial was considered the preference trial. On all remaining trials, rats were trained to escape the footshock by running to that arm of the maze not visited on the preference trial. Training occurred in 3 blocks, 8 trials per block, with approximately 15 minutes separating each block. After each block of trials the rat was returned to its home nest cage to await the next block of trials. Training continued for a maximum of 24 trials or until the rat made 7 correct choices in any 8 consecutive training trials beginning after trial 9. If a rat failed to acquire the discrimination in 24 trials, a maximum score of 24 was assigned to this animal. A 24-trial limit was chosen as the upper limit for training since escape performance sharply declines with additional trials. Twenty pups were tested at each age with half of the animals in each age group trained in the presence of home litter shavings and the remainder with no shavings beneath the grid floor of the training apparatus.

RESULTS AND DISCUSSION

Figure 1 presents the results of Experiment 1. The left panel of this figure indicates the mean number of trials to complete criterion training and the right panel shows the mean number of 180° pivoting responses in the startbox during the final eight training trials for each animal. A 3 (ages) × 2 (shavings) analysis of variance was conducted for both the trials to criterion and pivoting responses. Within each age group, rats trained with home litter shavings beneath the apparatus acquired the spatial discrimination faster than littermates trained in the absence of shavings (a main effect of shavings: $F(1,54)=17.95$, $p<0.001$). There was no main effect of age on the number of training trials required to attain the present criterion, which is in agreement with previous results from our laboratory [12]. Analysis of the pivoting responses indicated only a main effect of age, $F(2,54)=15.32$, $p<0.001$, reflecting the tendency for the youngest age group to pivot more in the apparatus than the older pups. Others have also reported that the incidence of pivoting responses decreases after 7 days of age [6]. The presence or absence of home shavings apparently had no effect on pivoting behaviors in the maze.

These results confirm the reliability of this training procedure for use with rats as young as 7 days postpartum. Note, however, that learning was substantially poorer in the absence of home nest odors. Considering all ages tested, 33% of the rats trained in the absence of shavings, compared to

77% of those trained in the presence of home nest odors, achieved the training criterion. At these ages, learning of this kind apparently is facilitated when it occurs in the context of the home nest environment (e.g., [10,11]). Another important point in these results is that there was in fact no discernible difference in the rate of acquisition of the response across the three ages when measured in terms of trials to criterion. A similar conclusion was also reached when examining the proportion of rats attaining the training criterion, particularly when examining the performance of rat pups trained in the presence of home nest odors. For rats trained in the absence of shavings, 10% of the 7-day olds, 50% of the 9-day olds, and 40% of the 12-day olds achieved the training criterion. For rats trained in the presence of home nest shavings, 70% of the 7-day olds, 80% of the 9-day olds, and 80% of the 12-day olds reached criterion. Thus, although rats trained in the presence of nest odors were equivalent in their performance across the ages examined, rats trained in the absence of shavings showed some tendency toward a positive relationship between performance and age.

In the present study, the presence of litter shavings enhanced the performance of 7-, 9-, and 12-day old rat pups in this discrimination learning task. Moreover, in the presence of litter shavings there were no age differences in the rate of acquisition of the response. Consequently, use of litter shavings in this test would be particularly useful for the assessment of age-related effects of scopolamine because a particularly unequivocal examination of drug-related effects on performance in an instrumental-learning task can be accomplished if learning is equated across the ages in question. In Experiment 2, therefore, this procedure was used to assess the effects of scopolamine on choice behavior and latency to choice.

EXPERIMENT 2

Experiment 2 used the procedure developed in Experiment 1 where rats were trained in the presence of home environmental stimuli, to assess the behavioral responsiveness of 7-, 9-, and 12-day old rats to the cholinergic blocking agent, scopolamine hydrobromide.

METHOD

Subjects and Experimental Design

The subjects were 72 Sprague-Dawley rats born and raised in our colony. Housing and maintenance were identical to that in Experiment 1. Rats were assigned in factorial fashion to one of nine orthogonal treatment conditions represented by the 3 × 3 design: 3 ages (7-, 9-, and 12-days old at training) × 3 drug treatments (0.2 mg/kg, 0.8 mg/kg scopolamine hydrobromide (Sigma Chemical Co.), or an equal volume of 0.9% saline).

Apparatus and Procedures

The apparatus described in Experiment 1 was used in the present experiment. Ten minutes prior to training each rat was injected subcutaneously with the appropriate drug. As in Experiment 1, training occurred in 3 blocks of 8 trials per block or until the rat reached the training criterion of 7 correct in 8 consecutive trials beginning with trial 9. All rats were trained with shavings from their home nest beneath the entire training apparatus.

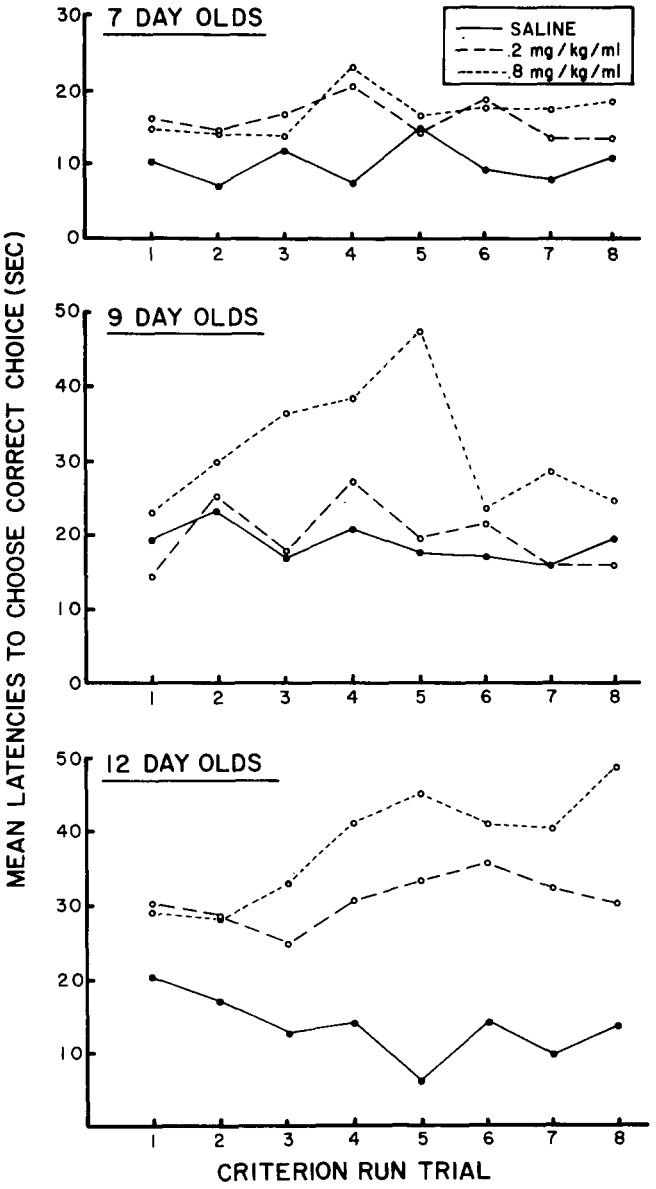

FIG. 2. Mean latencies to choose the correct arm of the maze during the last 8 training trials. Solid lines represent the saline injected control treatment; dashed lines represent groups injected with either 0.2 mg/kg or 0.8 mg/kg of scopolamine hydrobromide.

RESULTS

Analysis of the number of trials required to complete the training criterion indicated that all groups performed comparably. Seven-day olds acquired the discrimination in an average of 21.9 trials (saline: 20.8 trials; 0.2 mg/kg scopolamine: 23.0 trials; 0.8 mg/kg scopolamine: 22.0 trials); 9-day olds acquired the response in an average of 22.1 trials (saline: 21.3 trials; 0.2 mg/kg scopolamine: 22.8 trials; 0.8 mg/kg scopolamine: 22.3 trials); and 12-day olds required an average of 21.9 trials (saline: 22.8 trials; 0.2 mg/kg scopolamine: 19.9 trials; 0.8 mg/kg scopolamine: 23.1 trials). Administration of scopolamine did not significantly influence

the rate at which rats of these ages acquired the spatial discrimination ($F < 1$). Thus, although at 15 and 23 days of age scopolamine decreased the rate of acquisition of a spatial discrimination task [13], scopolamine did not affect probability of a correct choice among rats 7, 9, and 12 days of age.

Figure 2 reports the mean latency to reach the goal box during the criterion run (the last 8 training trials, 7 of which were correct choices). Analysis of these data indicated the existence of a reliable main effect of drug, $F(2,63)=7.47$, $p<0.001$, which did not interact with age. Planned comparisons indicated that the main effect of drug was largely due to animals given 0.8 mg/kg scopolamine exhibiting reliably slower running speeds across test trials than saline animals (7-day olds: $F(1,63)=9.24$, $p<0.01$; 9-day olds: $F(1,63)=26.08$, $p<0.001$; 12-day olds: $F(1,63)=113.06$, $p<0.001$). Generally speaking, animals given 0.2 mg/kg scopolamine performed intermediate to saline and 0.8 mg/kg scopolamine groups.

GENERAL DISCUSSION

In the present study, a simple spatial, discriminated-escape task was used to determine the effects of scopolamine in 7-, 9-, and 12-day old animals. At all ages, scopolamine increased latency to escape from footshock in the discrimination task, without affecting probability of a correct choice. However, since our analysis in Experiment 2 failed to measure pivoting responses, we cannot unequivocally assert that scopolamine did not affect latencies by increasing pivoting behaviors. We feel confident that pivoting was not a major contributor to the increases in escape latencies since a similar scopolamine-induced increase in escape latencies has been observed in 15-day olds in our laboratory [13], an age well beyond that when pivoting disappears from the rat's repertoire [6]. Furthermore, it is unlikely that this increased latency to escape after scopolamine is due to an alteration in footshock sensitivity thresholds. Scopolamine did not alter shock sensitivity thresholds in 15-day old rats who exhibit a similar increased escape latency [13].

Scopolamine is hydrolyzed in the body by esterases. While there has been little investigation of the ontogeny of these esterase enzymes, there is evidence that oxidative, reductive and conjugative enzymes used in drug metabolism develop only slowly during postnatal life (e.g., [8]). Thus, if hydrolysis enzymes, like other major types of drug metabolizing enzymes, develop only slowly during postnatal life, then metabolism of scopolamine may occur more slowly in the younger animals. This might result in higher brain levels of scopolamine in younger animals. Yet, in our investigations, younger animals generally appear to be less sensitive to the effects of scopolamine. For example, in the present study, 7-, 9-, and 12-day old animals did not respond to scopolamine with an alteration in the probability of a correct choice, whereas it has been previously reported that older preweanling and weanling (15- and 23-day old) animals do exhibit an adult-typical scopolamine-induced disruption of choice performance [12]. Thus, it is unlikely that these age-related differences in the effects of scopolamine are related to ontogenetic alterations in drug metabolism.

It is interesting to compare the responses to scopolamine in the spatial discrimination task at the different ages. While an increase in latency to correct choice after scopolamine was seen in the present study by postnatal Day 7, a scopolamine-induced disruption in the rate of acquiring the spatial discrimination is not seen until around 15 days of age, and the typical adult response of a decreased latency to choice in this task was seen in 23- but not in 15-day old animals [13]. There are several possible interpretations of these results. For example, perhaps scopolamine induces a biphasic effect on arousal with a depressant effect seen at higher brain levels and an excitatory effect evident at lower central levels of scopolamine. Given the potential immaturity of scopolamine hydrolysis systems in young animals, brain levels of scopolamine might be greater at the time of test than those of older animals. While this interpretation would explain the scopolamine-induced behavioral depression seen in pups 15 days of age and younger as compared with the scopolamine-induced hyperactivity seen at older ages, there are problems with this interpretation. First, there is no evidence that scopolamine induces such a biphasic effect in developing or adult animals. In addition, the lack of influence of scopolamine on acquiring the spatial discrimination seen in infant (7-, 9-, and 12-day old) rat pups is also difficult to reconcile with this interpretation.

An alternative explanation of these results is that the maturation of the cholinergic system may not be an "all-or-none" phenomenon. The scopolamine-induced increase in choice latency seen in the present experiment appears several weeks prior to the typical adult response of a decreased latency to choice in this task. Such results suggest the hypothesis that the cholinergic neurotransmitter system may be comprised of several distinct subsystems, each of which matures at its own characteristic rate during ontogeny. Behaviorally, with the differential ontogeny of these subsystems, animals may exhibit various age-specific responses to drugs affecting the cholinergic system prior to the development of adult-typical behavioral drug responses.

ACKNOWLEDGEMENTS

Preparation of this article was supported by Grant BNS 78-02360 from the National Science Foundation and Grant MH 35219-01 from the National Institute of Mental Health to Norman E. Spear. Thanks to Norman Richter, Robin Weiss, and Teri Tanenhaus.

REFERENCES

1. Baez, L. A., N. K. Eskridge and R. Schein. Postnatal development of dopaminergic and cholinergic catalepsy in the rat. *Eur. J. Pharmac.* **36:** 155–162, 1976.
2. Blozovski, D. and M. Blozovski. Effects de l'atropine sur l'exploration, l'apprentissage et l'activite electrocorticale chex le rat au cours du dévelopment. *Psychopharmacologia* **33:** 39–52, 1973.
3. Campbell, B. A., L. D. Lytle and H. C. Fibiger. Ontogeny of adrenergic arousal and cholinergic inhibitory mechanisms in the rat. *Science* **166:** 635–636, 1969.
4. Concannon, J. T., G. J. Smith, N. E. Spear and S. R. Scobie. Drug cues, drug states, and infantile amnesia. In: *First International Symposium on Drugs as Discriminative Stimuli*, edited by F. C. Colapert and J. A. Rosencranz. Amsterdam: Elsevier, 1978.
5. Fibiger, H. D., L. D. Lytle and B. A. Campbell. Cholinergic modulation of adrenergic arousal in the developing rat. *J. comp. physiol. Psychol.* **72:** 384–389, 1970.

6. Gard, C., E. Hard, K. Larsson and V. Petersson. The relationship between sensory stimulation and gross motor behavior during the postnatal development in the rat. *Anim. Behav.* **15:** 563–567, 1967.
7. Hölmgren, B. and R. Urbá-Hölmgren. Cholinergic mechanisms involved in headshaking of infant rats. *Pharmac. Biochem. Behav.* **15:** 563–567, 1977.
8. Jondorf, W. R., R. P. Maickel and B. B. Brodie. Inability of newborn mice and guinea pigs to metabolize drugs. *Biochem. Pharmac.* **1:** 352–354, 1959.
9. LeBlanc, M. D. and B. H. Bland. Developmental aspects of hippocampal electrical activity and motor behavior in the rat. *Expl Neurol.* **66:** 220–227, 1979.
10. Murphy, J. M. and Z. M. Nagy. Development of cholinergic inhibitory capacities in the hyperthyroid mouse. *Pharmac. Biochem. Behav.* **5:** 449–456, 1976.
11. Smith, G. J. and N. E. Spear. Home environmental effects on withholding behaviors and conditioning in infant and neonatal rats. *Science* **202:** 327–329, 1978.
12. Smith, G. J. and N. E. Spear. Home environmental stimuli facilitate learning of shock escape discrimination in rats 7–11 days of age. *Behav. Neural Biol.* **31:** 360–365, 1981.
13. Smith, G. J., L. P. Spear and N. E. Spear. Ontogeny of cholinergic mediation of behaviors in the rat. *J. comp. physiol. Psychol.* **93:** 636–647, 1979.