Pharmacology Biochemistry & Behavior, Vol. 7, pp. 567–570. Copyright © 1977 by ANKHO International Inc.
All rights of reproduction in any form reserved. Printed in the U.S.A.

BRIEF COMMUNICATION

Technique for Assessing Visual Discrimination Learning in Mice[1]

TINA E. LEVINE,[2] ROBERT L. BORNSCHEIN AND I. ARTHUR MICHAELSON

Department of Environmental Health, University of Cincinnati, Cincinnati, OH 42567

(Received 18 April 1977)

LEVINE, T. E., R. L. BORNSCHEIN AND I. A. MICHAELSON. *Technique for assessing visual discrimination learning in mice.* PHARMAC. BIOCHEM. BEHAV. 7(6) 567–570, 1977. – An automated technique for the study of visual discrimination learning in mice has been developed. The technique utilizes a nose-poke as the operant response. The nose-poke response requires no shaping, has a relatively high operant level and can be used to measure preacquisition exploratory behavior. CD–1 mice acquired a simultaneous brightness discrimination readily but a successive brightness discrimination proved more difficult. A 20 sec intertrial interval was optimal for acquisition of the simultaneous discrimination. Reversal learning was slow. This procedure should prove useful in the study of the effects of pharmacologic and toxic agents on learning and performance in both weanlings and adult mice.

Visual discrimination Operant behavior Nose-poke

MICE have long been a favorite species for toxicological investigations. The reasons include cost, ease of housing and care, short life span, and a large body of available data pertaining to biochemistry, physiology, immunology, and genetics. Unfortunately, for those interested in behavioral indices of toxicity, there are only a limited number of methodologies available for assessing behavior of mice. Methodologies currently available include reflex testing, water or shock escape/avoidance learning and general measures of locomotor activity. For numerous methodological reasons, automated appetitive operant conditioning procedures such as schedules of reinforcement and discrete trial procedures, have rarely been applied to the behavioral assessment of mice. (For an extensive bibliography of behavioral studies in mice, see [5]). Those investigators who have attempted appetitive operant studies in mice report varying degrees of difficulty with the force requirement of conventional operant levers and with the maintenance of adequate motivation [2,4].

Recently several investigators have used a head movement, rather than a lever press, as the required response in operant studies involving mice [1,6]. We have successfully used a similar response, the nose-poke, to study acquisition and reversal of visual discrimination problems in mice. The nose-poke is monitored by a photocell assembly mounted behind two holes in the front panel of the operant chamber. The response is a very natural one for the mouse and has been used previously to measure exploratory activity [3]. It requires no shaping and can easily be brought under the control of the reinforcement contingencies. The 0.01 cc milk reward used in our studies is highly palatable and animals maintain a high rate of performance at 90–95% free-feeding weight (or after 24 hr deprivation), thus avoiding motivational problems.

Because of the paucity of methodological data pertaining to automated conditioning techniques in mice, we have found it necessary to direct our attention to the development of an adequate understanding of control variables. We report here the results of our initial investigation of the effect of varying the intertrial interval on acquisition and reversal of both a simultaneous and a successive discrete-trial brightness discrimination in mice.

METHOD

Animals

Sixty mice of the CD–1 strain served as experimental animals. They were obtained from the supplier (Charles River, Wilmington, MA 01887) at 35 days of age and started in the experiment at 44 days of age. They were maintained on a water deprivation schedule throughout the

---

[1] This work was supported in part by National Institute of Health Postdoctoral Fellowships ES–02364 (T.E.L.) and ES–02614 (R.L.B.), in part by USPHS Grant ES–NE–01077 (I.A.M.), and in part by a grant from the International Lead and Zinc Research Organization LH–245. The authors wish to acknowledge the technical assistance of Ms. Martha McPherson and Mr. Denny Webb.

[2] Present address: Department of Pharmacological and Physiological Sciences, The University of Chicago, 947 East 58th Street, Chicago, IL 60637.

study with only 30 min access to water each day, two hours after completing the daily session.

Apparatus

The experimental chamber (Fig. 1) consisted of a Plexiglas frame. The front wall of the chamber was painted flat black to prevent reflected light from triggering the photocells. A houselight and two photocell and stimulus assemblies were mounted on the front wall. A dipper hole was located on the floor of the chamber. The dipper was located to the right with the trough extending under the front of the chamber.

The photocell and stimulus assembly is also diagrammed in Fig. 1. A 3/8 in. Plexiglas washer, painted flat black, was mounted behind each hole in the front wall of the chamber. Two holes were drilled in the washer along the vertical axis. A Clairex photoresistor (CL 903L) was mounted in the top hole and a sub-mini lamp (T 1–3/4) was mounted in the bottom hole. The photoresistor circuit was amplified by a Coulbourn solid state switch input. In the simultaneous discrimination task, the stimulus lamp was mounted behind the washer housed in a solid Plexiglas cylinder. The front of the solid cylinder was sanded to diffuse the light from the stimulus lamp. A Dialco incandescent lamp cartridge and holder (No. 507–3912; 28 V, 40 ma) served as the stimulus lamp. The bright stimulus was generated by passing 28 V through the lamp; the dim stimulus was generated by passing 28 V through a 430 Ω resistance in series with the lamp. This procedure generates a bright stimulus of 40–62 ft L and a dim stimulus of 1–5 ft L as measured by a Spectra brightness Spotmeter No. SB 1–1/2 (Photo Research Corporation, Hollywood, CA). In the successive discrimination task, the houselight served as the stimulus. The houselight was a 28 V, 40 ma T 3–1/4 miniature lamp with white translucent cap. The houselight emitted 190–260 ft L. Again, the bright stimulus was generated by passing 28 volts through the lamp; the dim stimulus was generated by passing 28 V through a 430 Ω resistance in series with the lamp.

Each experimental chamber was housed in an 80 quart Coleman camping cooler equipped with a ventilating fan. The fan provided masking noise for the chamber. Coulbourn solid state equipment controlled events in the chamber and recorded data.

Procedure

Animals were randomly assigned to the simultaneous or successive task and to one of three intertrial intervals (ITIs) within that task (10 mice/group). The three ITIs were 10, 20 and 40 sec. Prior to the start of training, the animals were placed in the appropriate chamber and nose-pokes were monitored for 15 min. The lighting conditions in the chamber were the same as those in the ITI of the task – houselight on in the simultaneous box, houselight off in the successive box. There were no stimulus lights or reinforcements during this time. The animals were then deprived of water for 24 hr before initiation of training. For the first day of training, all animals were run with a

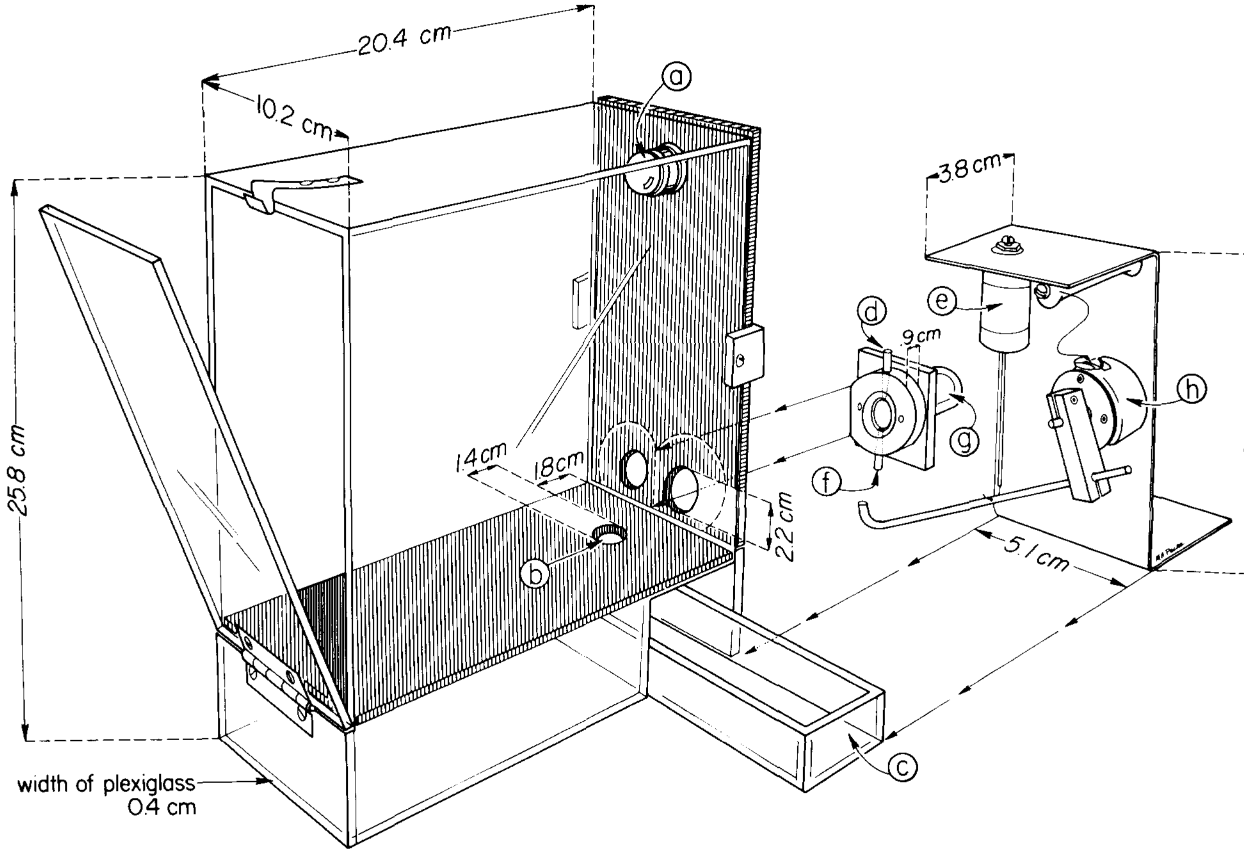

FIG. 1. Mouse Operant Chamber: (a) house light, (b) dipper access hole, (c) dipper trough, (d) photocell, (e) airpot, (f) light sources, (g) stimulus light, (h) rotary solenoid. The left stimulus assembly is omitted for clarity.

20 sec ITI and a 15 sec feeder cycle. On the second day of training, the feeder cycle was shortened to 5 sec and the ITI was adjusted to the value assigned to each animal.

The simultaneous discrimination began with the presentation of the bright and dim stimuli to the animal. If the animal poked its nose into the brighter hole, the stimuli went off, the houselight came on, and the dipper was activated. If the animal poked the dim hole, the stimuli went off, the houselight came on, and there was no reinforcement. During the ITI, responses had no programmed consequences but were, nonetheless, counted. After the ITI, two stimuli were again presented. The position of the positive stimulus varied randomly after correct responses. To prevent the formation of position habits, the position of the positive stimulus remained the same if the animal made an error until a trial ended with a correct response. In the successive task, the animal was required to poke its nose into the left hole when the houselight was bright and into the right hole when the houselight was dim. All other conditions for the two tasks were equated. Preliminary experiments indicated that these two problems were easier to acquire than the converse problems (dim light positive for the simultaneous discrimination; bright-right, dim-left for the successive discrimination). The animals were run for 25 trials per day, six days per week. They were reversed to the more difficult converse problems after attaining a criterion of 92% accuracy (23 of 25 trials correct) on 3 of 4 consecutive sessions. This comparatively strict criterion was chosen to assure a consistent high level of performance in all animals before reversal training.

RESULTS

The level of nose-poking prior to training is shown in Fig. 2. The overall rate of nose-poking is quite high even with no programmed consequences for the response. The animals made about 3–6 responses per minute. The animals in the dark chamber emitted significantly more nose-pokes than those in the illuminated chamber ($t = 3.65$, $df = 58$; $p<0.005$). There were no preferences for left or right response holes in the dark chamber ($t_d = 0.09$, $df = 29$; $p>0.450$). However, there was a highly significant preference for the right hole in the light-on condition ($t_d = 3.70$, $df = 29$; $p<0.005$). Subsequent studies have revealed no position preferences in any of the operant chambers in the light-on condition.

At the termination of the study on Day 47, all but four animals had reached criterion on the simultaneous problem. Figure 3 shows the cumulative number of animals in each ITI group reaching criterion over the course of the study. The 20 sec ITI was optimal for the acquisition of the simultaneous problem: all 10 animals reached criterion. In the 40 sec group, 9 of the 10 animals reached criterion by Day 47 and in the 10 sec group, only 7 of the 10 animals reached criterion by the termination of the experiment. Arbitrarily, a value of 50 days was used as the learning score for those animals that did not reach criterion. Analysis of variance showed that the ITI effect was not significant ($F(2,27) = 2.74$; $0.05<p<0.10$).

The successive discrimination was a much more difficult problem. None of the 30 animals had reached criterion by Day 47. The 40 sec group had the highest median accuracy at the termination of the experiment (10 sec – 56%, 20 sec – 52%, 40 sec – 68%).

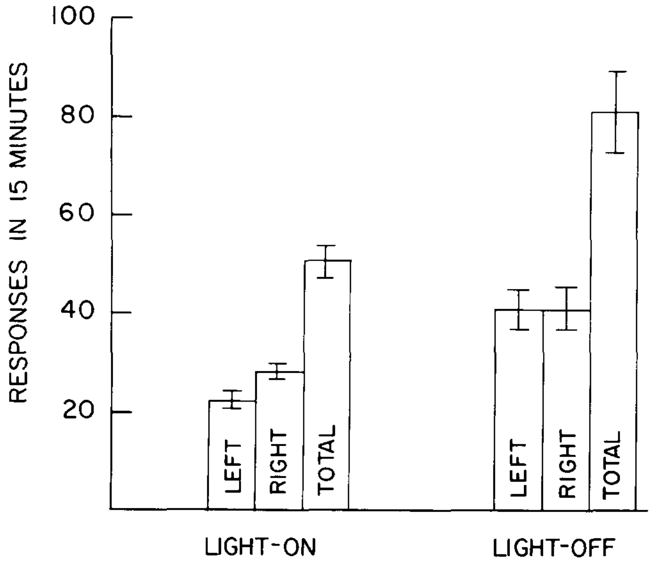

FIG. 2. Preacquisition level of nose-pokes in light-on and light-off conditions. Mean of 30 animals ± standard error of the mean is shown for each condition.

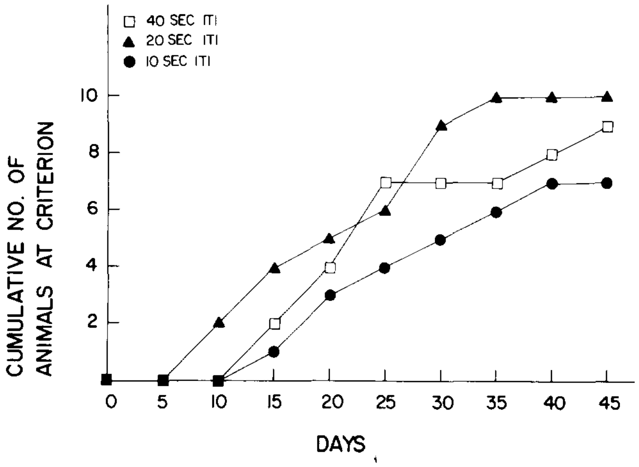

FIG. 3. Cumulative number of animals reaching criterion (23 of 25 trials correct on 3 of 4 consecutive sessions) on the simultaneous discrimination with three values of intertrial interval. There were 10 animals in each ITI group.

Mean latency of response decreased rapidly in both conditions. Figure 4 shows the mean latencies and standard deviations for both the simultaneous and the successive task at selected times during the experiment. The animals' latencies decreased markedly within a day and were close to stability by Day 6. There was little change after Day 9 in the simultaneous task and Day 18 in the successive task. For the simultaneous task, efficiency of responding, computed as number of correct responses/total number of responses (trial + ITI responses) was correlated with accuracy, computed as number of correct responses/number of trials (25) [10 sec, $r = .87$; 20 sec, $r = .90$; 40 sec, $r = .90$]; that is, as the animals acquired the task, they learned to discriminate both the positive stimulus and the trial condition; therefore making fewer intertrial responses.

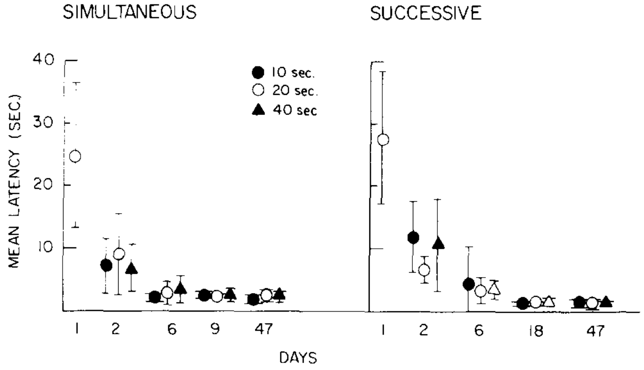

FIG. 4. Mean latency to respond (± SD) on the simultaneous (left) and successive (right) task at selected times during the experiment. There were 10 animals in each ITI group. Day 1 data are based on 30 animals.

Reversal learning was slow. Only one of the animals had reached 90% accuracy on the reversal problem by the termination of the experiment, and this animal took 24 days to reach 90% accuracy for the first time. On initial acquisition, some animals reached 90% accuracy by the fifth day of training. Because of the differences in the rate of initial acquisition, the number of animals performing on the reversal problem and the number of days of reversal training varied considerably among groups. The median accuracy for reversal following 2 weeks of training was as follows: 10 sec – 32% (n = 6); 20 sec – 38% (n = 10); 40 sec – 38% (n = 6). During initial acquisition, the median accuracy following two weeks of training was as follows: 10 sec – 78%; 20 sec – 90%; 40 sec – 88%.

DISCUSSION

The results of our initial investigation of automated brightness discrimination in the mouse indicated that the acquisition of a simple simultaneous brightness discrimination problem was sufficiently slow that a learning deficit due to exposure to a toxic agent would be easily detected. The successive discrimination task appears to be extremely difficult for the mouse under the conditions studied.

We have been interested in developing behavioral indices of toxicity in mice for many of the reasons mentioned above. In particular, mice offer economy in collection of the correlative chemical data essential in any toxicological investigation. They are also more active than rats and have been trained as early as 21 days of age. The technique described above offers several advantages over previous methodologies. First of all, it is not necessary to subject the animals to the stress of a high level of deprivation in order to maintain adequate motivation (18 to 24 hr of water deprivation is sufficient). Such stress might interact with the direct effects of the agent in question. Secondly, acquisition of the operant is rapid and requires no shaping, thereby decreasing the experimenter-subject interaction which could bias the results of behavioral studies. Thirdly, the nose-poke response can be studied in very young animals allowing the assessment of developmental variables. Fourthly, the level of nose-poke in the animals before training can be used to assess baseline exploratory activity. Finally, the task difficulty may be manipulated readily by selecting: (1) the number of trials per day, (2) the ITI, (3) the stimulus intensity or type of pattern, (4) the problem type (visual discrimination vs. position habit; simultaneous vs. successive discrimination; acquisition vs. reversal).

REFERENCES

1. Carlson, N. R., F. W. El-Wakil, L. J. Standish and D. L. Ormond. DRL performance extinction and secondary reinforcement: Effects of appetitive value of food in mice with septal lesions. *J. comp. physiol. Psychol.* **90**: 780–789, 1976.
2. Freund, G. and D. W. Walker. Operant conditioning in mice. *Life Sci.* **11**: 905–914, 1972.
3. Shillito, E. E. A method for investigating the effects of drugs on the exploratory behavior of mice. *Br. J. Pharmac.* **40**: 113–123, 1970.
4. Sprott, R. L. and J. P. Symons. Operant performance in inbred mice. *Bull Psychon. Soc.* **4**: 46–48, 1974.
5. Sprott, R. L. and J. Staats. Behavioral studies using genetically defined mice – a bibliography. *Behav. Genet.* **5**: 27–81, 1975.
6. Wenger, G. R. and P. B. Dews. The effects of phencyclidine, ketamine, d-amphetamine and pentobarbital on schedule-controlled behavior in the mouse. *J. Pharmac. exp. Ther.* **196**: 616–624, 1976.