Instrumental escape learning in neonatal C3H mice

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Five groups of C3H mice, 3-11 days of age, were given 25 trials in a straight-alley shock-escape task. Improved performance was indicated by a decrease in the number of competing responses made over training trials by all age groups and by an increase in the number of trials on which no competing responses occurred for groups 5-11 days of age. Running speeds appeared to reflect differences between age groups in the development of motor abilities rather than in learning capacities. These results support previous research indicating successful escape learning in neonatal mice and rats 5 days of age and extend this general finding to C3H mice as young as 3 days of age.

Evidence for instrumental learning of an escape response has recently been reported for neonatal Wistar rats (Misanin, Nagy, & Weiss, 1970) and Swiss-Webster mice (Nagy, Misanin, & Newman, 1970) as young as 5 days of age. In these studies, running speed, the measure typically used to demonstrate escape learning in adult animals, did not reflect an improvement in performance over trial blocks for most of the ages tested. Instead, these studies used the number of 180-deg turns made away from the direction of the goal as the main indicator of learning. Previous studies have demonstrated that such turning or pivoting occurs in young rats (Bolles & Woods, 1964) and mice (Fox, 1965) when they are removed from the nest and increases in frequency during aversive stimulation (Gard, Hård, Larsson, & Petersson, 1967). In the Misanin et al (1970) and Nagy et al (1970) studies, the number of turns made by the neonates decreased over the shock-escape training session, suggesting that neonates can learn to inhibit this turning response. Improved performance over the session was also made evident by an increase in the number of trials on which these turns failed to occur (Nagy et al, 1970).

The purpose of the present study was to examine escape behavior in another strain of mouse, C3H, and at a younger age, 3 days, to test further the generality of these findings.

SUBJECTS

The Ss were 100 C3H mice, born and reared in 30.4 x 18 x 12.8 cm polyethylene cages with wire-grid tops and wood-chip shavings on the floor. Separate groups of 10 males and 10 females were tested at 3, 5, 7, 9, or 11 days of age. A modified split-litter design was used in which each litter was represented by each sex in two or more age groups. The mothers were on

ad lib food and water and remained with the mice at all times until testing.

APPARATUS

The apparatus consisted of a straight alley, 22.8 x 3.2 x 6.4 cm, constructed of clear Plexiglas. One-millimeter-diameter stainless steel grids spaced 3 mm apart, center to center, ran parallel with the length of the alley and formed the floor. A removable Plexiglas door was placed in the alley 5 cm from one end, forming the startbox. The alley was marked into three 7.6-cm sections (start, center, and goal). A scrambled ac shock source (Harvard Instrument, Model H.I. 3121) provided .1-mA 60-Hz ac current to the grid floor.

PROCEDURE

At one of the selected ages, a mouse was removed from the home cage and placed in the startbox for 5 sec. The trial began with the simultaneous removal of the door and the onset of shock. When the mouse had reached within approximately 6 mm of the goal end, shock was turned off and the mouse was removed and held in the E's hand for a 45-sec intertrial interval. Following this interval, the mouse was returned to the startbox for the next trial. This procedure was repeated for 25 trials. The time required for the mouse to reach the goal end of the alley was recorded to the nearest .1 sec and was considered the response latency. A maximum latency of 300 sec was allowed on each trial. Mice not reaching the goal end within that time were gently forced to the goal and assigned a 300-sec response latency score for that trial. Shock-offset also occurred as they reached the goal end in the forced trials.

The number of 180-deg turns away from the goal end and the section of the alley in which these turns occurred were also recorded. Such turns were considered to be competing responses.

Fig. 1. Mean reciprocal latencies (running speeds) over blocks of five trials as a function of age.

RESULTS

Response latencies were converted to speed scores by computing the reciprocal of the latency on every trial. The mean reciprocal latency for each block of five trials was then determined for individual mice, and a 5 by 2 by 5 analysis of variance with one repeated factor was performed on these data. The factors were age, sex, and trial block. The analysis showed age ($F = 3.98$; $df = 4,90$; $p < .01$), trial block ($F = 5.01$; $df = 4,360$; $p < .0005$), and the Age by Trial Block interaction ($F = 2.18$; $df = 16,360$; $p < .01$) to be significant. Sex had no significant effect on running speed and did not interact with any of the factors. Figure 1 presents the mean running speeds for the five age groups over trial blocks. With the exception of the 5-day-old group, overall running speed generally increased with age. Over trial blocks, the 3-day-old group showed a slight decrease in running speed, while the 5- and 7-day-old groups showed reliable decreases ($F_s = 3.10$ and 2.10 ; $df = 16,360$; $p < .01$, respectively). Although running speed on the first and last trial block did not differ for both the 9- and 11-day-old groups, both groups showed a reliable decrease in speed from the first to the second trial block and a reliable increase from the second to the last block (all $ps < .01$).

Figure 2 presents the mean number of competing responses made by each age group in each of the three sections of the alley over trial blocks. These data were analyzed by a 5 by 2 by 3 by 5 analysis of variance, with the last two factors repeated measures. The factors were age, sex, place of turn, and trial block, respectively. Sex was not a significant factor ($F = 3.34$; $df = 1,90$) and did not interact with any other factor. As is apparent from the figure, total turns

Fig. 2. Mean number of competing responses over blocks of five trials and place of turn in the alley as a function of age.

decreased with an increase in age ($F = 20.04$; $df = 4,90$; $p < .0005$) and over trial blocks ($F = 60.98$; $df = 4,360$; $p < .0005$). There were also differences in the place in the alley where the turns occurred ($F = 449.34$; $df = 2,180$; $p < .0005$), with all age groups making the greatest number of turns in the start section of the alley, the second greatest number of turns in the center section, and the least number of turns in the goal section. The Age by Place of Turn ($F = 21.93$; $df = 8,180$; $p < .0005$) and Place of Turn by Trial Block ($F = 29.75$; $df = 8,720$; $p < .0005$) interactions were also highly reliable. Individual comparisons within age groups between the first and last block

of trials indicated that the 3-day-old group decreased competing responses only in the start section ($F = 44.84$; $df = 32,720$; $p < .0005$), while all other age groups showed decreases in both the start and center sections (all $F_s \geq 7.55$; $df = 32,720$; all $ps < .0005$). There were no reliable changes in the number of competing responses made in the goal section at any age (all $F_s < 0.60$; $df = 32,720$). The figure inset depicts the Age by Place of Turn interaction so that turns per section may be easily compared across age. At every age, more competing responses were made in the start section than in the center and more in the center than in the goal section (all $F_s \geq 2.60$; $df = 8,180$; all $ps < .05$). Across ages, the 3-day-old group made more competing responses in the start section than all other groups (all $F_s \geq 63.13$; $df = 8,180$; all $ps < .0005$), and each successive age group made fewer start turns than all other younger groups (all $F_s \geq 2.68$; $df = 8,180$; all $ps < .05$). The 3-day-old group made more center turns than all other age groups (all $F_s \geq 4.47$; $df = 8,180$; all $ps < .0005$), none of which differed among themselves (all $F_s < 1.00$; $df = 8,180$). There was no difference among age groups in the total number of competing responses made in the goal section.

The number of trials on which the mice made no turns (errorless trials) was analyzed by a 5 by 2 by 5 analysis of variance with one repeated measure. The analysis showed age ($F = 24.81$; $df = 4,90$; $p < .0005$) and trial blocks ($F = 24.40$; $df = 4,360$; $p < .0005$) to be the only significant factors. Figure 3 presents the mean number of errorless trials made by each age group

Fig. 3. Mean number of errorless trials over blocks of five trials as a function of age.

over blocks of five trials. Although the Age by Trial Block interaction was not statistically reliable ($F = 1.39$; $df = 16,360$), the figure indicates that the 3-day-old group did not increase the number of errorless trials made over trial blocks, whereas all other age groups did. This was confirmed by individual comparisons between the first and last trial blocks for each age. The 3-day-old group did not differ in the number of errorless trials ($F = 1.03$, $df = 16,360$), whereas all other groups showed reliable increases (all $F_s \geq 16.51$; $df = 16,360$; all $ps < .0005$).

DISCUSSION

The results of the present study confirm previous work with Wistar rats and Swiss-Webster mice and indicate that the finding of an intrasession improvement in the shock-escape performance of neonatal animals is neither species- nor strain-specific. All of the C3H groups between 3 and 11 days of age showed reliable decreases in the total number of competing responses made during the training session, suggesting their ability to inhibit the turning response. This ability appears to increase with age, as indicated by the rates of decreases in the number of turns within the training session. Older animals show a greater rate of decrease earlier in the session than do younger animals. The increase in the number of trials without turns in Swiss-Webster mice as young as 5 days of age (Nagy et al, 1970) was also replicated with C3H mice in the present study, where all age groups, with the exception of the 3-day-old group, reliably increased the number of errorless trials over trials. Finally, running speeds did not prove to be a useful measure of learning with the C3H strain, as it did not with either neonatal rats (Misanin et al, 1970) or Swiss-Webster mice (Nagy et al, 1970). On the escape task, running speeds appear to reflect differences in maturation levels rather than differences in the learning capacities of neonatal mice and rats.

In a previous report with Swiss-Webster mice (Nagy et al, 1970), it was found that the total number of competing responses made as a function of age formed an inverted U-shaped function, with 9-day-old mice making the greatest number of turns and both younger and older mice making fewer turns. In the present study, however, the 3-day-old mice made the greatest number of turns and each older group made successively fewer turns. This strain difference, in the function relating competing responses to age, may reflect a differential rate of development of locomotor ability and coordination or,

possibly, a difference in sensitivity levels between strains.

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Chlordiazepoxide and aversive conditioning: Effects of acquisition and performance of the conditioned nictitating membrane response in the rabbit*

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A 12-mg/kg dosage of chlordiazepoxide was shown to disrupt acquisition and postasymptotic performance of the classically conditioned nictitating membrane response of rabbits induced by pairing a tone with eyeshock. This result makes more plausible the hypothesis that chlordiazepoxide interferes with avoidance learning in rabbits by suppressing the conditioned fear component of avoidance learning.

Although chlordiazepoxide (CDP) reduces shuttle-avoidance performance in the rabbit (Chisholm & Moore, 1970a, b), it is not apparent how the drug affects avoidance behavior. Rescorla & Solomon (1967) have analyzed avoidance by studying the effects of classically conditioned cues associated with shock upon the instrumental avoidance behavior. Their two-process learning theory emphasizes that the classically conditioned cues play a major role in controlling the instrumental avoidance responses. Chisholm & Moore (1970) showed that CDP does not interfere with formation of a conditioned association between a tone and inescapable footshock. In that study, rabbits learned a shuttle-avoidance response to a light signal. Some rabbits then experienced tone-shock pairings under a 12-mg/kg dosage of CDP. Saline-injected control animals

received the same treatment. Rabbits, which experienced the tone-shock pairings in the drugged state, showed transfer of this fear conditioning when subsequently tested in the nondrugged state for avoidance to the tone. Transfer in this case did not differ from that demonstrated by the control animals. A differential conditioning procedure precluded the possibility that nonassociative factors (e.g., sensitization) were responsible for this transfer. Thus, there were actually two tones of equal intensity in the fear conditioning phase of the experiment, but only one was systematically paired with shock. In the transfer phase, the tone paired with shock elevated the speed of avoidance over that to the original avoidance cue (the light), whereas the other nonpaired tone systematically increased response latency in a similar, but opposite, fashion.

Since CDP in the dosage employed in the previous experiments does not seem to impair learning, it would seem most likely that the drug affects the

avoidance response by removing the motivational substrate for the response. This could come about either by blocking the expression of fear (without fear there might be no "reason" for the animal to shuttle on cue) or by reducing whatever reward value a successful avoidance might have. It is our hypothesis that CDP suppresses the expression of conditioned fear: that it is the "performance" of the classically conditioned component of the avoidance task, not the actual learning, which is affected by the drug. As a more direct test of whether or not CDP does suppress aversive conditioning in rabbits, we thought it appropriate to investigate another independent classical conditioning situation. Accordingly, the present experiment was designed to investigate the effect of 12 mg/kg of CDP on the conditioned nictitating membrane response (NMR). This is a widely used aversive conditioning preparation adapted for rabbits by Gormezano and his associates (Gormezano, 1966) and is known to be suitable for pharmacological investigation (e.g., Elliott & Schneiderman, 1968).

SUBJECTS

The Ss were 20 New Zealand albino rabbits, approximately 110 days old.

APPARATUS

The conditioning apparatus and the recording equipment have been described elsewhere (O'Malley, Hupka, & Moore, 1969). Essentially, four rabbits were run simultaneously in a four-drawer fireproof filing cabinet. Each drawer was illuminated by two 4-V dc lights located behind translucent white plastic. Independent ventilation for each S was provided by centrifugal blowers. Continuous white noise of 70 dB SPL and the tones were presented via speakers located directly in front of each animal. Clear Plexiglas boxes, similar to those developed by Gormezano (1966), were used to restrain the rabbits. The movement of the nictitating membrane was recorded by having one end of a thread hooked to a nylon loop sutured in the membrane and the other end attached to a Giannini minitorque potentiometer. Movements of the right nictitating membrane were amplified and recorded on a Grass 5D oscillograph. A CR was defined as a 1-mm deflection of the recording pen within the interstimulus interval.

PROCEDURE

The rabbits were assigned randomly to one of two groups. Each group received intraperitoneal injections of CDP (12 mg/kg) or saline (1 cc/kg) 30 min before the training session and were run using an E-blind procedure. On the days before the first conditioning session, the nictitating

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