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Hypothermia-produced retrograde amnesia in young and adult rats

CHARLES F. HINDERLITER and DAVID C. RICCIO
Kent State University, Kent, Ohio 44242

Although the phenomenon of retrograde amnesia has received extensive investigation in adult animals, relatively little attention has been given to the effects of amnestic agents on retention performance of young animals. In the present experiment, 50-66- and 25-31-day-old rats were given one-trial passive avoidance training followed immediately by hypothermia treatment. Adult and young rats cooled to a temperature at or below 21° C within 8-9 min showed little evidence of retention 24 h later. In contrast, 25-31-day-old rats cooled in half the time to the same temperature showed evidence of good retention. The implications of these findings for further studies examining the relationship between age and short-term changes in memory traces were considered.

Numerous studies have shown that long-term retention is better in adult than in immature rats (see Campbell & Spear, 1972, for review). Less attention has been given to the question of whether these age-related changes in retention are paralleled by differences in vulnerability of newly acquired memories to disruption by amnestic treatment. While there is lively debate over the extent to which memory loss after these various treatments represents impairment of storage or retrieval processes (cf. Gold & King, 1974; Miller, & Springer, 1973), the empirical phenomenon of retrograde amnesia (RA) is well established.

Earlier research by Thompson and his colleagues (Thompson, 1957; Thompson, Haravey, Pennington, Smith, Ganon, & Stockwell, 1958) suggested that ECS impaired retention of a brightness discrimination in both young and adult rats but that the younger animals (30-40 days old) were more severely affected. It is not clear, however, whether this outcome reflected ontogenetic differences in memory sensitivity (e.g., rate of "consolidation") or simply a greater magnitude of CNS disruption produced by ECS in immature animals. More recently, memory disruption for a shock escape task in 9-day-old rats has been demonstrated using hypothermia as the amnestic agent, but comparison with adults was not included (Misanin, Nagy, Keiser, & Bowen, 1971).

We report here a preliminary study utilizing a retrograde amnesia paradigm to assess the immediate postacquisition stability of memory in young and adult animals. Single-trial passive avoidance training was employed, as this procedure permits relatively accurate specification of the interval between acquisition and amnestic treatment as well as control over possible punishing effects of the amnestic agent. While acquisition of the passive avoidance task is reported to vary with age (Riccio, Rohrbaugh, & Hodges, 1968), these differences appear minimal beyond 25 days of age. Hypothermia was chosen as the amnestic agent, partly because of its demonstrated effectiveness in producing memory loss (e.g., Beitel & Porter, 1968; Misanin et al., 1971; Riccio, Hodges, & Randall, 1968) and partly because the severity of treatment could be readily indexed in terms of colonic temperature change. It was hoped that the functional intensity of the amnestic agent could be roughly matched for both age groups by equating the extent of body temperature loss (cf. Campbell & Riccio, 1966).

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METHOD

Subjects

Subjects were albino rats purchased from the Holtzman Co., Madison, Wisconsin. A total of 12 females and 135 males were quasirandomly assigned to treatment groups. All rats were weaned at 22-26 days of age and were kept in colony cages of 6-8 animals throughout the experiment. Subjects were maintained on ad-lib food and water schedule. Four of the 50-66-day-old rats died following hypothermia treatment and were replaced.

Apparatus

The passive avoidance apparatus was a 14 x 15.5 x 35 cm wooden box divided by a partition with an 8 x 7.5 cm door into one chamber with white walls and a clear Plexiglas lid and another chamber with walls and lid painted black. The entire apparatus was placed on a grid of 1-mm stainless steel, spaced 2 mm apart. Suspended 25 cm above the white chamber was a 25-W light bulb. A Foringer Model SC-901 scrambler and a matched-impedance ac shock source (Campbell & Teghtsoonian, 1958) were used to deliver a 150-V shock trhough the grid floor. A Model 2095 Forma Temp. Jr. bath and circulator was used to produce hypothermia. Rectal temperatures were taken with a Shick X-ray Company, Ellab Instruments thermometer. Probes were inserted 1.5 and 2.5 cm into the anus of the 25-31- and 50-66-day-olds, respectively.

Procedure

The general plan of this experiment was to give young and adult rats a single passive avoidance trial, followed immediately

by cold-water (3-4°C) immersion and to test for retention 24 h later. Preliminary work indicated that immersion durations of 4-5 and 8-9 min for the young and adult groups, respectively, were necessary to produce temperatures at or below 21°C. In previous research with older adult rats, Riccio, Hodges, and Randall (1968) have shown that a drop in body temperature to 21°C produces extensive disruption of retention performance. When it became apparent after several replications that little, if any, RA was produced in the immature rats under these conditions, we added a young group matched with adults in terms of exposure duration. In order to produce the necessary rate of drop in body temperature over an 8-9-min period, the water temperature was raised to 13-14°C for the second group of younger animals.

More specifically, 2 h prior to experimental treatments, each rat was handled 2-3 min, each was ear punched for the purpose of identification, and rectal temperatures were recorded. Rats which received training (FS) were placed in the white chamber facing away from the door. Ten seconds later, the door separating the two chambers was opened. When the rat placed all four feet in the black chamber, a 1-sec inescapable shock was presented. Step-through latencies were recorded to the nearest .1 sec.

Three FS-HYPO groups received hypothermia immediately after training. The 50-66-day-old subjects (n = 16) were placed in wire cloth or plastic cylinders and immersed to the ears in 3-4°C water for approximately 8-9 min. Subjects in one group of 25-31-day-olds (n = 22) were immersed in 3-4°C water for a period of 4-5 min. A second group of 25-31-day-olds (n = 22) was immersed in 13-14°C water approximately 8-9 min. For all groups, if rectal temperatures were above 21°C when initally checked, rats were reimmersed until temperatures were at or below 21°C.

Two additional groups of 25-31-day-old rats (n = 22 for each group) and one group of 50-66-day-olds (n = 16) also received training (FS-NoHYPO). Immediately after training, these subjects were restrained in cylinders and suspended in air for times matched to subjects in each of the respective FS-HYPO groups.

Two groups of 25-31-day-old rats were treated identically as the FS-HYPO groups except that no footshock was presented when rats crossed into the black chamber. One of these NoFS-HYPO groups (n = 12) was immersed in 3-4°C water approximately 4-5 min, the other group (n = 15) into 13-14°C water approximately 8-9 min.

After restraint or hypothermia, rectal temperatures were recorded for all animals 0, 2.5, and 5.0 min later. Rats were then placed in individual cages located in a room separate from the colony. Temperatures were again recorded 50-60 min and 170-175 min later. Rats were then returned to their group cage located in the colony.

Twenty-four hours after training, the time to enter the black chamber was recorded during testing. If a rat did not cross within 5 min, it was assigned a test latency of 300 sec.

RESULTS

No significant differences were found between the groups' training latencies as indicated by the results of a Kruskal-Wallis one-way analysis of variance ($H = 6.28$, $p > .3$, $df = 5$). Training latencies were subtracted from test latencies for each animal and are presented as difference scores in Figure 1. Because of the large number of ceiling scores obtained during testing, nonparametric two-tailed comparisons were made.

As indicated in Figure 1, both age groups retained the passive avoidance response equally well. No significant differences were found between any of the

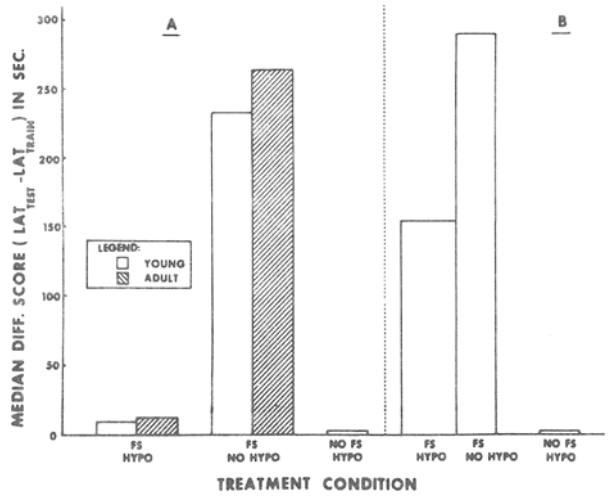

Figure 1. Median difference scores for all groups. In Panel A, the HYPO groups were cooled to a mean colonic temperature of 20.5°C in 8-9 min. In Panel B, scores are from young rats also cooled to 20.5°C but at a faster rate of temperature loss.

three FS-NoHYPO groups ($H=2.88$, $p > .3$, $df=2$). Thus, 24-h retention, as measured in this experiment, appeared to be equal in both age groups.

Rats in both age groups showed significant memory impairment when duration of hypothermia treatment was 8-9 min. Both FS-HYPO groups which were immersed 8-9 min had significantly lower latencies than their respective FS-NoHYPO control groups ($Z = 2.65$, $U = 80$, $ps < .02$, $n_1/n_2 = 22/22$ or $16/16$). Alternatively, the FS-HYPO group which was immersed 4-5 min was not significantly different from the FS-NoHYPO group which was restrained for 4-5 min ($Z = 1.75$, $p > .08$, $n_1/n_2 = 22$), indicating that little amnesia was produced by hypothermia in this group of 25-31-day-olds.

Although the two 25-31-day-old FS-HYPO groups did not differ significantly ($Z=1.58$, $p > .1$, $n_1/n_2=22$), subjects in the 50-66-day-old FS-HYPO group had significantly lower latencies than the younger animals which were immersed 4-5 min ($Z = 2.22$, $p < .03$, $n_1/n_2=22/16$). In addition, no significant difference was found between the two different age groups when immersed for 8-9 min ($Z=.89$, $p > .1$, $n_1/n_2=22/16$). These results appear to indicate that hypothermia was equally effective for both the 25-31- and 50-66-day-old rats when the total duration of the hypothermia treatment was equal.

That hypothermia did not completely disrupt 24-h retention was indicated by the fact that both the FS-HYPO groups which were immersed 8-9 min had significantly longer latencies than the NoFS-HYPO group, also immersed 8-9 min ($Zs \geq 2.63$, $ps < .02$, $n_1/n_2 = 22/15$). Significant differences were also found between the 25-31-day-old FS-HYPO and NoFS-HYPO groups immersed 4-5 min ($Z = 3.19$, $p < .002$, $n_1/n_2 = 22/12$).

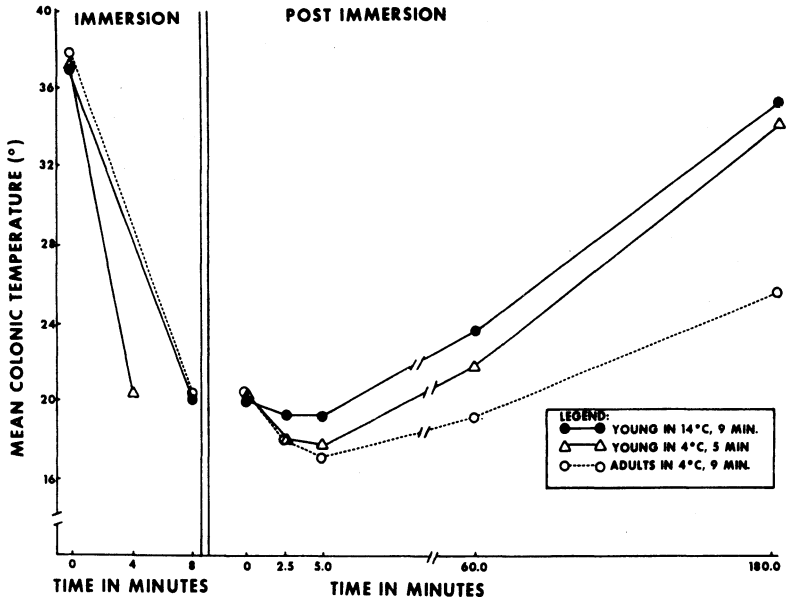

Figure 2. Mean colonic temperature (°C) for rats which received training-hypothermia treatment.

Figure 2 depicts the time course of temperature changes for the FS-HYPO subjects. As is evident from the figure, matching the duration of exposure across ages (with water temperature varied) failed to produce an equivalent colonic temperature drop. Immature rats remained substantially warmer than adults. In contrast, when young rats were exposed to the colder water for 4-5 min, their body temperatures upon removal, and 5 min later, closely approximated that of adults, although subsequent warming rate was slightly faster in the immature group.

DISCUSSION

These data are in agreement with the findings of Misanin et al. (1971) in demonstrating that hypothermic treatment shortly after acquisition can produce RA in young as well as adult rats. But whether there are age-related differences in vulnerability of newly acquired memories remains unresolved. When immersion times were matched for the two age groups, the amount of amnesia produced was comparable and nearly complete for both groups. (It is possible, of course, that floor effects could have obscured any age differences in RA.) When young rats were exposed to the same water temperature as adults (but of necessity for a briefer duration), they showed relatively little, if any, retention loss. Under this condition, then, it would appear that memory was more resistant to disruption in immature than in adult rats.

These behavioral outcomes, considered in conjunction with the body temperature data, pose an interesting but perplexing finding. In young animals, rapid induction of hypothermia appears less effective in producing RA than more prolonged exposure to less severe conditions. While it might have been desirable to attempt to include a more rapidly cooled adult group, the mature rats cooled to 20.5°C in 8-9 min did, in fact, show profound RA. In any event, it is difficult to incorporate the anomalous data of the young animals into the various models of RA. If storage processes are being disrupted, then one would expect faster cooling to be a more effective amnestic treatment. Retrieval-oriented models might account for the outcome by suggesting that the longer exposure period with the milder immersion temperature provides more opportunity for extraneous stimuli to scramble "tagging" processes (Misanin & Hoover, 1971) or for memory to be encoded in terms of contextual cues of the treatment (Hinderliter, Webster, & Riccio, 1975). But the finding that the total duration of the hypothermic state (onset of treatment until return to normothermic level) in the young group showing little memory loss was intermediate between the other two groups which showed evidence of RA poses difficulty for these latter explanations.

On a more positive note, these findings should prove methodologically useful in further developmental studies of time-dependent changes in the memory trace. For example, the importance of equating the degree of original learning is well recognized in research on retention (Underwood, 1964), and the problem of achieving this goal has received careful attention in a number of developmental studies of animal memory (cf. Campbell & Spear, 1972). Similarly, we suggest that to compare RA gradients in different age groups, it is important to utilize conditions which produce equal "learning loss" at the shortest training-treatment interval. Using the minimal training-treatment interval feasible, we have obtained a set of treatment conditions which appear to produce comparable RA in young and adult rats. These conditions should then provide an appropriate basis for examining the amount of RA as function of the delay between training and treatment in different age groups.

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