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'Return to Home Cage' as a Reward for Maze Learning in Young and Old Genetically Heterogeneous Mice

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Recent studies have shown that 'return to home cage' can serve as a reward for maze learning in adult male mice. The present study examined whether the same reward is an effective motivator of learning in young and old mice and included females in the study design. We tested 25- and 65-d-old HS mice and 85- and 800-d-old B6D2F$_2$ mice in a Lashley III maze. Return to home cage motivated maze acquisition in all groups. Compared with 65-d-old HS mice, 25-d-olds acquired the maze more slowly, took longer to achieve the test criterion, and showed increased latency to reach the goal box. There was no difference between 85- and 800-d-old B6D2F$_2$ mice in rate of acquisition. This reward procedure may reduce the potentially confounding effects of deprivation or aversive stimuli on maze performance and may be suitable as a motivational procedure for a wide range of subject groups.

Abbreviations: D, defecation; GBL, latency to reach goal box; LI, learning index; SL, latency to start; T, trial; TTC, number of trials to reach test criterion; U, urination

We previously demonstrated that 'return to home cage' (or, more accurately, return to a familiar cage) is an effective motivator of learning of a Lashley III maze.[3] Adult male mice derived from 2 genetically heterogeneous stocks (HS and Swiss–Webster) acquired the maze in approximately the same number of trials as did mice from those genetic groups deprived of food and rewarded with food pellets.

We suggested that the procedure represented an alternative to traditional motivational procedures (for example, food or water deprivation, electric shock), any of which might interact with the independent variable of interest (for example, age, strain, sex) and confound interpretation of group differences in performance. In particular, comparisons of learning behavior of inbred strains of mice whose performance is motivated by food deprivation could be based on differential effects of food restriction.[8,13]

However, it is still unclear whether 'return to home cage' is a suitable procedure for a broader range of subject groups. In the present experiment we set out to assess the utility of the procedure for young and old mice and tested both males and females of 2 genetically different heterogeneous populations, HS mice[11] and F_2 animals derived from a cross of C57BL/6J and DBA/2J strains (B6D2F$_2$). Demonstrating the broad applicability of the procedure is an important 1st step toward establishing the general value of the protocol for studies of learning and memory.

Materials and Methods

Maintenance conditions. All animals were maintained on a reversed 12:12-h light:dark cycle (lights off, 0600; lights on, 1800) for at least 2 wk prior to use in this experiment. This arrangement permitted the test of cognition to be conducted in the dark phase of the circadian cycle when mice are more active and responsive to external stimuli. Fresh food (LabDiet 5010 Autoclavable Rodent Diet, which is nutritionally equivalent to LabDiet 5001 after autoclaving; PMI Nutrition International, Brentwood, MO) and water were available ad libitum. To facilitate incorporation of the home cage into the Lashley III maze-learning protocol,[9] mice were adapted to modified home cages for 2 wk before testing. An aperture (6 × 6 cm) was cut in the long side of the home cage (dimensions, 18 × 28 × 13 cm) under the food hopper. When the cage was in the colony room, the aperture was closed with a clear acrylic insert that was fitted between the food hopper and the side of the cage, to prevent the resident from escaping. When used in conjunction with the maze, the insert was raised and the aperture juxtaposed to the end of the goal box. When animals entered their home cage after traversing the maze, the insert was lowered to prevent re-entry into the goal box. Animal care and experimental procedures were in accordance with guidelines approved by the Pennsylvania State University Animal Care and Use Committee and the National Research Council.[12]

Experimental groups. Two genetically heterogeneous mouse stocks were tested. Animals of the HS strain, which originally was developed at the Institute for Behavioral Genetics at the University of Colorado by McClearn and colleagues[11] from a systematic intercross of 8 inbred strains (more than 60 generations of structured outbreeding), comprised the 25- and 65-d-old groups (10 male and 10 female mice in each age group). The other stock was derived from a cross of C57BL/6J (B6) and DBA/2J (D2) to produce the F_2 generation (B6D2F$_2$) and consisted of an 800-d-old group (9 male and 7 female mice) and an 85-d-old group (10 male and 10 female mice). The 800-d-old mice were raised in a barrier facility in the same building as the colony rooms previously described. Before testing, old mice were adapted to the new colony

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Table 1. Correlation coefficients of learning index (LI) between successive trials (LI-1, LI-2, and so forth) calculated using the combined data set

	LI-2	LI-3	LI-4	LI-5	LI-6	LI-7
LI-1	–0.074	0.014	0.216	0.151	0.120	–0.168
LI-2		0.217	0.066	0.134	0.007	0.070
LI-3			0.384[b]	0.353[a]	0.413[c]	0.257
LI-4				0.572[c]	0.559[c]	0.238
LI-5					0.607[c]	0.434[b]
LI-6						0.532[c]

For early trials, n = 76. For later trials, as animals reached the acquisition criterion, n = ~50.
[a]$P < 0.002$.
[b]$P < 0.001$.
[c]$P < 0.0001$.

Table 2. Correlation coefficients of start latencies (SL) between successive trials (SL-1, SL-2, and so forth) as calculated using the combined data set

	SL-2	SL-3	SL-4	SL-5	SL-6	SL-7
SL-1	0.38[a]	0.13	0.17	0	–0.03	–0.02
SL-2		0.91[b]	0.89[b]	0	0.05	0.05
SL-3			0.87[b]	0.12	0.07	0.13
SL-4				0.14	0.12	0.04
SL-5					0.12	0.09
SL-6						0.07

For early trials, n = 75. For later trials, as animals reached the acquisition criterion, n = ~50.
[a]$P < 0.001$.
[b]$P < 0.0001$.

environment for at least 2 wk. All animals were housed individually in the modified home cages described earlier.

Maze protocol. The maze protocol has been described in detail elsewhere.[3] Mice were tested once per day starting 1 h after the start of the dark phase under dim illumination supplemented by infrared light to facilitate behavioral observation. Immediately before testing, food was removed from the cage in the colony room and the water bottle inverted to prevent access to water. Test batches of 4 to 6 cages were brought from the colony room on a laboratory cart with an opaque plastic bag covering the cages during the 4-m journey, to prevent exposure to bright illumination. In the test room, the plastic bag was removed, and the cages remained on the cart until testing was completed. During acquisition, mice were given 1 trial per day until they performed the maze with 0 or 1 error per trial for 2 successive days, a more lenient criterion than that previously used (0 or 1 error per trial for 3 successive trials[3]).

To initiate testing, each animal was picked up by the tail, allowed to cling to the front of the experimenter's laboratory clothing, and then placed in the start box of the maze—a procedure that lasted approximately 10 s. Each animal's home cage then was placed at the end of the goal box, with the acrylic door raised so that the animal could enter it after traversing the maze. The trial was initiated by raising the start box door when an animal approached it. A stopwatch was used to record start latency (SL) and time to reach the goal box (GBL). An error was scored when an animal made a four-paw entry into a blind alley or retraced to alleys previously traversed. The frequencies of defecation (D) and urination (U) were recorded. After the animal entered its home cage, the door was lowered, and the home cage was returned to the cart. After each trial, the maze was cleaned with a dilute bleach solution, rinsed with water, and wiped dry in preparation for the next animal. Approximately 30 min after return to the animal room, food and water were returned to the home cages of all mice; the total time that animals were deprived of food and water was usually less than 1 h. The Lashley III maze dimensions have previously been described.[3] Retention was tested in $B6D2F_2$ mice 5 wk after completion of the acquisition criterion; each animal underwent a single trial for retention testing.

Data analysis. ***Maze scoring.*** Scoring was conducted using logic functions in an Excel (Microsoft, Redmond, WA) spreadsheet according to the conventions of Denenberg and others.[5] Correct entries, forward errors (entries into blind alleys towards the goal box), and backward errors (entries into alleys in the direction of the start box) were accumulated for each daily trial. Learning index (LI) was defined as the number of correct entries divided by the total number of entries. The number of trials mice needed to reach the criterion (TTC) was recorded.

Statistical analysis. The data were analyzed by analysis of variance after partitioning the variance of between- and within-group factors as appropriate by using SPSS 11.0 (SPSS, Chicago, IL). All tests were 2-tailed, and the threshold for statistical significance was set at $P = 0.05$.

Results

As detailed in the following paragraphs, all groups (25- and 65-d-old HS mice, 85- and 800-d-old $B6D2F_2$ mice, and both sexes of animals) acquired the Lashley III maze. Therefore, home cage reward appears to be an effective motivator for a range of subject groups. First, we show the results of correlation analyses, which estimate the stability of individual differences of learning indices by using the combined data set. Each index of learning (LI, SL, GBL) was correlated across trials (T1 to T2, T2 to T3, and so forth) to estimate the trial-to-trial stability of each measure. The lack of significant correlation between scores for trial 1 (LI-1) and later trials shows that there was no relationship between LI in the maze on trial 1 and LI after animals had found a path to the home-cage (Table 1). As trials progressed, a positive relationship emerged (note correlation between LI values for trials 3 and 4) and was maintained between scores on adjacent trials thereafter.

The pattern of intertrial correlation in SL was somewhat different. There was significant positive correlation ($r = 0.38$, $P < 0.001$) between SL-1 and SL-2, indicating that individual differences in this index influenced behavior on trials before and after experience with the maze (Table 2). There was also a strong positive relationship between SLs for trials 2 through 4 ($r > 0.80$ for all trials). However, as SL decreased on later trials, the relationship between SL for adjacent trials became nonsignificant, presumably because of the truncation of range in this index (after trial 4, mean SL was less than 3 s).

The pattern of intertrial correlation for GBL (Table 3) was similar to that described for LI. GBL-1 did not correlate with GBL-2 or GBL on subsequent trials, but from trial 2 onward, GBLs for consecutive trials were significantly correlated ($r = 0.28$ to 0.80). As demonstrated by these findings, individual differences in learning indices in the Lashley III maze were characterized by intermediate levels of trial-to-trial stability, which provides encouragement that the test protocol represents an orderly procedure for studying phenotypic variations in learning and memory.

Table 3. Correlation coefficients of goal box latency (GBL) between successive trials (GBL-1, GBL-2, and so forth) calculated using the combined data set

	GBL-2	GBL-3	GBL-4	GBL-5	GBL-6	GBL-7
GBL-1	0.08	0.04	–0.02	0.29	0.13	0.03
GBL-2		0.28[a]	0.05	0.03	0.01	–0.04
GBL-3			0.84[c]	0.47[c]	0.22	0.39[b]
GBL-4				0.41[b]	0.27[a]	0.45[c]
GBL-5					0.56[c]	0.52[c]
GBL-6						0.65[c]

For early trials, n = 76. For later trials, as animals reached the acquisition criterion, n = ~50.
[a]$P < 0.05$.
[b]$P < 0.001$.
[c]$P < 0.0001$.

Correlations among selected indices of maze behavior. Defecation and urination frequently are used to measure fear in rats and mice.[2,7] Therefore, it was of interest to calculate the relationship between these indices and maze-learning (the level of fear evoked by the maze might predict the degree to which escape to home cage was rewarding). Correlations between time to reach the goal box during the first 5 trials and defecation and urination frequency for those same trials were low and did not reach statistical significance. For this reason, raw D and U scores (unadjusted for time in the maze) were used for subsequent analyses. D scores were positively and significantly correlated across all of the first 5 trials: of the 10 *r* values, all but 1 were statistically significant, ranging from 0.37 to 0.76 (the correlation between D scores on trials 1 and 5 was the exception). A similar positive relationship was found among U scores for trials 1 through 5. However, there was generally a low and statistically insignificant correlation between D and U scores on individual trials, supporting the idea that although individual differences in these scores across maze-learning trials have some stability, they reflect different behavioral and physiologic processes. Finally, D and U scores for trials 1 through 5 were not significantly correlated with LI for those same trials or with TTC, providing little evidence of a relationship between levels of fear (as estimated by these indices) and ability to acquire the maze. The only evidence of a relationship between D and U scores and maze behavior was that for trials 3 through 5, for which D and U scores were negatively correlated (*r* = approximately –0.3) with SL.

Maze acquisition in adult versus aged B6D2F$_2$ mice. Analysis of LI was restricted to the first 4 d of training in order to include all animals (after that trial, some animals would be excluded from the analysis because they had reached criterion). LI progressed from approximately 0.40 (40% correct choices) on day 1 to nearly 0.70 on day 4 (combining data across age and sex). The change across days was highly significant ($F_{3,96} = 31.04$, $P < 0.001$), and the trend across days was compatible with a linear trend ($F_{1,32} = 69.71$, $P < 0.001$). There were no statistically significant differences between groups (age and sex), and no interaction among age, sex, and days (Figure 1 C). The 800-d-old B6D2F$_2$ mice reached criterion in 10.06 ± 1.71 trials compared with 11.0 ± 1.52 d for the 85-d-old group; analysis of variance of TTC revealed no age or sex differences and no interaction between them. Although the absolute range of TTC scores was higher for the 800-d-old mice, this increase was primarily due to the extreme score of a single male that required 34 trials to achieve criterion. With this animal excluded, TTC variability of old animals was lower than that of the 85-d-old group (standard deviations were 1/3 to 1/2 of those of same-sex 85-d-old mice).

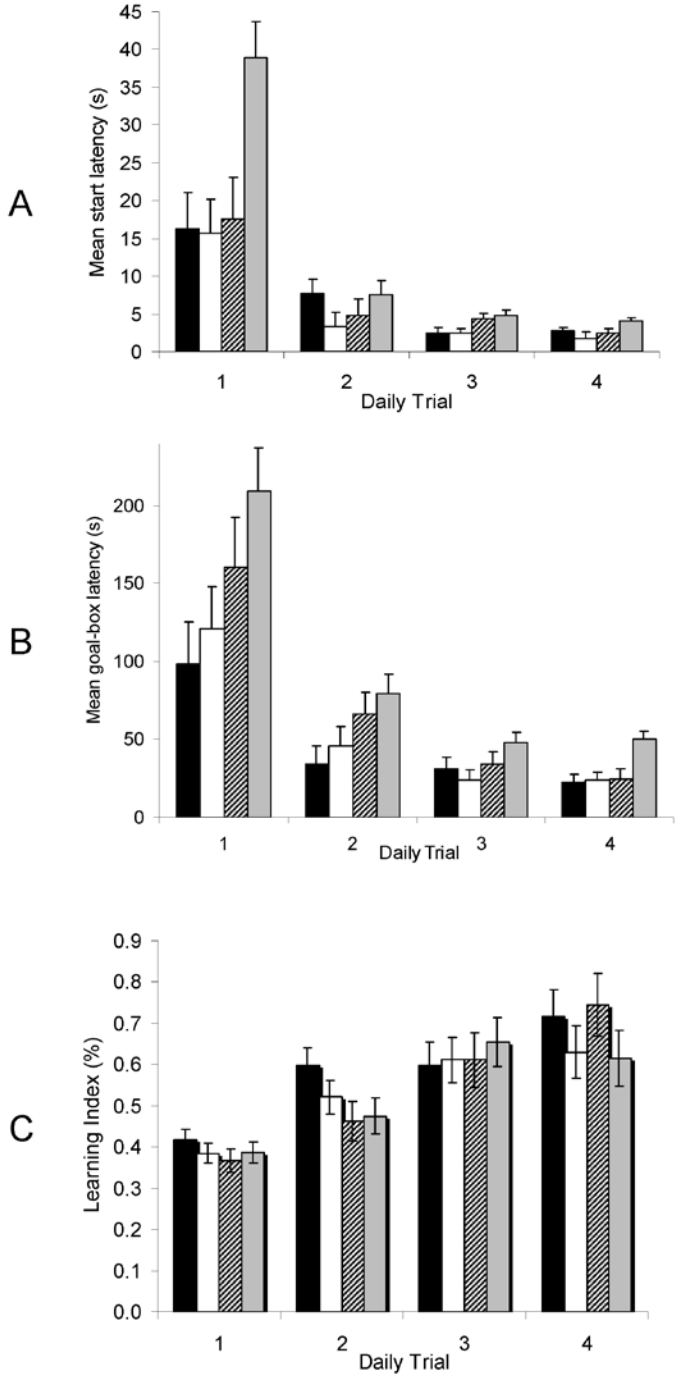

Figure 1. Home-cage reward in 85-d-old (female, ■; male, □) and 800-d-old (female, ▨; male, ▩) B6D2F2 mice trained on a Lashley III maze. (A) Mean start latency. (B) Mean goal box latency. (C) Mean maze acquisition. Bar, standard error.

Retention. Performance on the single retention trial at 5 wk was excellent. Approximately 50% of the animals performed the maze with 2 errors or fewer. There were no age or sex differences and no age × sex interaction.

Maze acquisition in adult versus young HS mice. Combining data across age and sex, LI in the HS mice progressed from 0.42 to

0.67 between days 1 and 4, closely resembling the rate of acquisition of the B6D2F$_2$ mice. The change across days was highly significant ($F_{3,105} = 24.9$, $P < 0.001$), with the change being accounted for by a significant linear trend ($F_{1,35} = 77.1$, $P < 0.001$). In addition, 25-d-old animals had significantly lower LI values across the 4 d than did 65-d-old mice ($F_{1,35} = 4.4$, $P < 0.05$). There was no sex difference and no interaction between age and sex or between days and these variables (Figure 2 C). Paralleling the group differences in LI, 25-d-old HS animals achieved TTC in 10.35 ± 0.72 trials, performing significantly worse ($F_{1,36} = 13.5$, $P < 0.001$) than did the 65-d-old animals, which required only 6.60 ± 0.72 d.

Additional indices of maze performance. Although all groups successfully acquired the Lashley III maze, behavioral indices such as SL, GBL, and D did show significant differences between the groups.

Adult versus aged B6D2F$_2$ mice. SL decreased across the first 4 daily trials ($F_{3,93} = 45.7$, $P < 0.001$; Figure 1 A), with the major decrement being seen from day 1 (day 1, mean of all groups = 22.2 ± 2.5 s) to subsequent days (day 2, 5.9 ± 1.0 s; day 3, 3.5 ± 0.4 s; day 4, 2.8 ± 0.2 s). Old mice had greater SL values than adults (85-d-old mice, 6.6 ± 0.9 s, 800-d-old, 10.5 ± 1.0 s, $F_{1,31} = 9.5$, $P < 0.004$); but the finding was related to a higher-order interaction (days × age × sex; $F_{1,31} = 4.93$, $P < 0.03$), which reflected the finding that old male mice had significantly increased SL on day 1 than did old female animals (800-d-old males on day 1, 38.9 ± 4.8 s; female, 17.6 ± 5.4 s), with no sex difference for adult mice on day 1 (males, 15.7 ± 4.6 s; females, 16.2 ± 4.8 s). On days 2 through 4, when SL values were much lower, sex differences at both ages were small or absent.

GBL for the first 4 trials was analyzed (Figure 1 B). Like SL, GBL decreased across trials ($F_{3,96} = 44.2$, $P < 0.001$), with the major change being seen from day 1 (mean of all groups, 147.3 ± 14.1 s) to subsequent days (day 2, 56.6 ± 6.2 s; day 3, 34.6 ± 3.5 s; day 4, 30.3 ± 2.8 s). Old mice had longer GBL than young adults (85-d-old mice, 50.3 ± 5.0 s, 800-d-old, 84.1 ± 5.6, $F_{1,32} = 20.2$, $P < 0.001$), and males (75.3 ± 5.1 s) had longer GBL than females (59.1 ± 5.5 s; $F_{1,32} = 4.6$, $P < 0.05$). There was no interaction between age and sex.

Analysis of D scores was restricted to days 1 to 3 because scores were usually 0 after that point in training. Because time in the maze changed dramatically as training proceeded, we do not report changes in D or U scores across trials. Old mice defecated significantly less than adults (85-d-old mice, 1.8 ± 0.3; 800-d-old, 0.2 ± 0.3; $F_{1,32} = 11.6$, $P < 0.01$). There was no sex-associated difference or sex × age interaction. Old mice urinated less frequently than adults (800-d-old, 0.1 ± 0.2; 85 d-old, 1.1 ± 0.2; $F_{1,32} = 14.1$, $P < 0.001$), and there was an age × sex interaction ($P < 0.01$) reflecting the fact that 85-d-old males (1.7 ± 0.2) urinated more than females (0.4 ± 0.2), with an absence of a sex difference in old mice (U close to 0 in both males and females).

Adult versus weanling HS mice. SL decreased across the first 4 daily trials ($F_{3,102} = 4.9$, $P < 0.003$), with the major change being seen from day 1 (mean of all groups, 23.8 ± 8.8 s) to subsequent days (day 2, 7.3 ± 1.4 s; day 3, 4.2 ± 0.5 s; day 4, 2.7 ± 0.2 s). As shown in Figure 2 A, young mice had longer SL than adults (25-d-old mice, 14.2 ± 3.4 s; 65-d-old, 4.8 ± 3.2), but the difference just failed to reach statistical significance ($P < 0.052$). There were also no statistically significant sex differences. Figure 2 A appears to provide evidence of an age × sex × day interaction with regard to SL (see mean differences between relevant groups on day 1). However, due to extreme variation within subgroups, there were no statistically significant interactions between age and sex or between those 2 variables and day of testing.

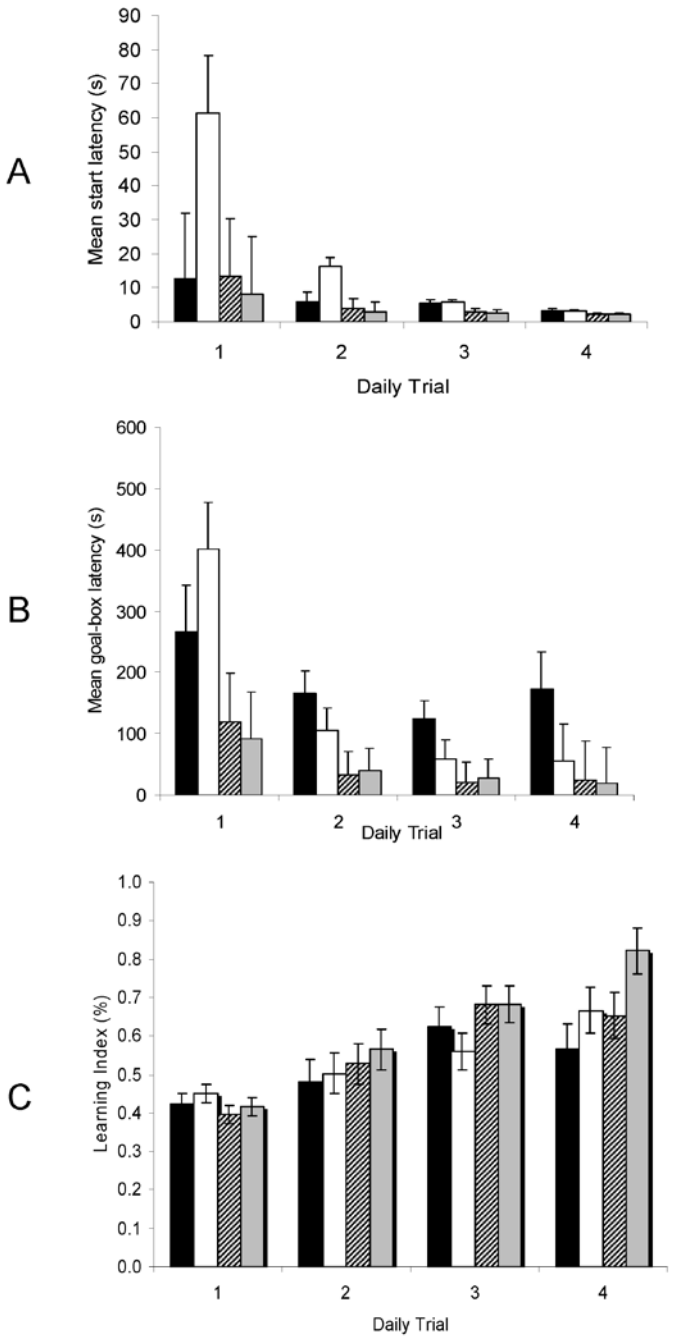

Figure 2. Home-cage reward in 25-d-old (female, ■; male, □) and 65-d-old (female, ▨; male, ▩) HS mice trained on a Lashley III maze. (A) Mean start latency. (B) Mean goal box latency. (C) Mean maze acquisition. Bar, standard error.

Like SL, GBL decreased across the first 4 daily trials ($F_{3,105} = 7.5$, $P < 0.001$; Figure 2 B), with the major change being seen from day 1 (mean of all groups, 219.2 ± 38.7 s) to subsequent days (day 2, 86.5 ± 18.2 s; day 3, 58.2 ± 15.2 s; day 4, 67.9 ± 30.5 s). In addition, 25-d-old mice had longer GBL than adults (25-d-old mice, 168.8 ± 19.1 s; 65-d-old, 47.0 ± 19.6; $F_{1,35} = 19.8$, $P < 0.001$). There were no sex-associated differences or interaction of age × sex.

Discussion

Our previous experiment[3] demonstrated that 'return to home (or familiar) cage' was an effective method of motivating Lashley III maze acquisition by adult male mice of HS and Swiss-Webster (Tac:SW) derivations. In the present experiment, we expanded the range of mice assessed by this procedure to include a greater age range (25 to 800 d), both sexes, and an F_2 population derived from C57BL/6J and DBA/2J (2 of the most commonly used inbred strains). As evidenced by the systematic change in learning index across the first 4 daily trials (Figures 1 and 2, Panel C) and the attainment of the acquisition criterion by all groups of animals, it is clear that the home-cage reward procedure is an effective tool for a broad range of subject groups. The lack of difference in maze acquisition between 65- and 800-d-old $B6D2F_2$ mice should not be taken as the final word on the potential of the home cage reward procedure to explore age differences in cognition. Only a single iteration of the protocol was presented to young and old mice. Many different training and retention protocols can be presented using the same reward paradigm. Differences in cognition between adult and old animals may well be subtle and require careful exploration to tease out the nature of the differences. The present study with genetically heterogeneous animals should be supplemented with studies with inbred strains, which may provide a sensitive test of age-related cognitive changes

The changes in SL and GBL across trials were also of interest. In particular, the large decrease in SL and GBL between trials 1 and 2 (Figures 1 and 2, panels A and B) illustrates the striking influence of a single exposure to the maze and a change from exploratory behavior in a novel environment to goal-directed behavior after animals have learned the association between the novel environment and 'return to home-cage.' Change in latency scores between trials 1 and 2 may represent a useful index of the effects of the 1st day's exposure, in addition to the more traditional indices discussed above. Our correlational analyses of the relationship between LI, SL, and GBL scores for consecutive trials also provide valuable information regarding the intertrial changes mentioned previously. Supporting the idea that behavior during trial 1 reflects a different motivation than does behavior during subsequent trials, LI and GBL scores for trial 1 did not correlate positively with the same scores for trial 2 (Tables 1 and 3). However, positive correlations between consecutive trials (for LI from trial 3 and for GBL from trial 2) illustrate substantial trial-to-trial stability of goal-directed behavior in the maze (Tables 1 and 3). This finding is noteworthy because intertrial correlation of behavioral scores by heterogeneous mouse stocks in an extensively used test, the Morris water maze, have often been found to be low,[6] necessitating the conduct of an extensive series of trials to obtain a reliable index. In our study, there *was* a positive correlation between SL scores for trials 1 and 2 (Table 2), supporting the idea that there is some motivational commonality underlying this behavior during the first 2 trials.

Information on latencies will also be useful to plan efficient scheduling of the procedure. Whereas 3 min were required to complete a trial on day 1 with the $B6D2F_2$ mice (SL + GBL), on day 4 an average trial lasted only 30 s. HS took longer to complete the maze, averaging 4 min on day 1 (SL + GBL), with the corresponding time on day 4 somewhat longer than 1 min; the rapid adjustment of mice to the test situation is shown by the fact that more than 90% of all mice had SLs of 5 s or less on trial 4. There was also variation in different subgroups within HS and $B6D2F_2$ mice: 800-d-old $B6D2F_2$ and 25-d-old HS mice had longer GBLs than their young adult counterparts. Using a food deprivation protocol, Matzel and others[10] systematically adapted animals to the apparatus before conducting Lashley III acquisition trials, perhaps to reduce the impact of emotional factors on maze acquisition. Introduction of such a procedure into the home-cage reward protocol would not save the overall time required to carry out the test; in any case it would be important to establish that such an adaptation procedure did not interfere with the efficacy of the reward system. More generally, careful recording of SL, GBL, and latency to enter the home cage (once an animal has entered the goal box) could shed light on the processes that are involved in home cage reward by providing information on key transitions in motivational dynamics during maze acquisition.

We found that 25-d-old animals acquired the maze more slowly than did 65-d-old HS mice (Figure 2 C) and required more trials to achieve criterion. This difference in performance was correlated with the time it took to traverse the maze (GBL), which was significantly longer in 25-d-old mice. The group difference in SL between 25- and 65-d-old mice just failed to reach statistical significance but it was in the same direction as the difference in GBL (longer latency in 25-d-old mice). This finding is consistent with the possibility that the differences in LI and TTC between 25- and 65-d-old HS mice are related to emotional reactivity. The longer GBLs were associated with periods of immobility in individual weanling mice both in the start box and within the maze. The role of emotional factors in maze acquisition by young and old mice deserves further attention.

In general, indices such as SL and GBL behaved in a systematic manner: both decreased across the first 4 d of testing, particularly from day 1 to subsequent days in both $B6D2F_2$ and HS groups (Figures 1 and 2, panels A and B). It should, of course, be recognized that, on day 1, mice have not had the opportunity to learn that they can exit from the maze, whereas on subsequent trials, they have had that experience. However, some of the measures gave evidence of differences in the manner in which animals of the various groups behaved in the maze. In the $B6D2F_2$ groups, old males had significantly longer SL on day 1 than did old females (males and females in the young adult age group did not differ in this regard) but did not differ on days 2 to 4. In addition, old $B6D2F_2$ animals, regardless of sex, had longer GBL than adult $B6D2F_2$ mice. Males also had longer GBL than did females. Defecation and urination frequency also showed differences between the groups: old animals defecated and urinated less frequently than adults, and adult males urinated more frequently than adult females, with no sex-associated difference in old mice.

Whereas use of home cage reward was successful in motivating maze acquisition in all groups, group differences in SL, GBL, D, and U support the idea that exposure to the maze-testing protocol was perceived differently by some groups. As noted, individual differences in D and U scores from trial to trial were relatively stable but, although it seems reasonable to hypothesize that levels of fear in the maze influence the ability of return to home cage to be rewarding, neither D nor U as putative indices of fear in mice[2] were significantly associated with LI or TTC. Likewise, there was no clear association among group differences in maze acquisition and the other behavioral indices in this group of mice: age and sex did not influence maze acquisition in $B6D2F_2$ mice, whereas SL, GBL, D and U did exhibit differences between these groups, a distinction between learning and performance that mirrors one previously made by Crady and Quinton.[4] In the HS groups, the slower rate of maze acquisition by 25-d-old adult

mice and its possible relationship to the longer GBL of 25-d-old mice was discussed earlier. It will be interesting in future studies to see whether the same relationship between latency measures and maze acquisition is confirmed in comparisons of young and adult mice.

The present study amply attests to the efficacy of 'return to home cage' in motivating acquisition of a Lashley III maze by genetically heterogeneous mice. The procedure works well in both male and female mice and is an effective instrument for study of maze learning in animals ranging from 25 to 800 d of age. Concerns have frequently been expressed about the use of deprivation procedures with subject populations at each extreme of the age continuum; 'return to home cage' may prove to be a useful alternative for use with such groups. The efficacy of the protocol now needs to be tested in subject groups that are also known to be unsuited to commonly used test or motivational procedures. For example, Wolfer and others[14] found that the performance of inbred mouse strains in the Morris water maze was so poor that it was impossible to detect the effects of a deleterious genetic mutation (transferred to the inbred strain) on learning, whereas the effect of the same mutation was detectable on a heterogeneous genetic background. Warren[13] also showed that the use of food deprivation with certain inbred strains resulted in substantial animal attrition when applied to old mice. 'Return to home cage' may represent a superior protocol for the study of learning in diverse apparatuses as well as for a variety of groups that respond poorly to traditional motivators: aged animals, inbred strains, genetically manipulated mice, as well as other at-risk groups already identified. As noted, the influence of age should not be assumed to parallel the findings of the present study (lack of effect on LI) until a variety of protocols have been explored using home cage reward. In addition, the potential existence of sex differences should also be carefully examined using a variety of protocols and within diverse subject groups (see previous paragraph).

Why 'return to home cage' is reinforcing is not known. The Barnes maze protocol[1] has some similarities to the present procedure. However, that procedure deliberately attempts to increase the aversiveness of the test situation (high illumination, loud noise, and so forth) to motivate escape. Our protocol attempts to minimize stress by conducting tests in dim illumination. It will be interesting to discover whether 'return to home cage' can be integrated with other maze protocols and to explore other aspects of the procedure to examine its flexibility as a general tool for the study of learning. In this regard, it is especially important to discover whether the procedure will work if a massed rather than distributed trial procedure is adopted. If so, this attribute would confer additional flexibility on what appears to be a very promising experimental protocol.

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