

## Learning Performance Varies with Brain Weight in Heterogeneous Mouse Lines

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Three lines of unselected heterogeneous stocks of mice were tested for learning and activity in active avoidance acquisition and extinction, water-maze discrimination learning and reversal learning, operant discrimination, and passive avoidance acquisition tasks. Ambulation in the open field was also measured. Small to moderate correlations (absolute values of .17 to .42) between brain weight and learning measures were obtained for all tasks except passive avoidance. A moderate correlation between brain weight and activity was found only in the open field ( $r = .39$ ). Partialing out differences in operant level and body weight had little effect on the magnitude of the correlations between brain weight and learning performance. When ambulation in the open field was partialled out, however, all correlations between brain weight and learning performance decreased. Previous research has suggested a positive relation between brain weight and learning scores across mammalian orders and species. The results reported here extend this relation to within-species variation in brain size. The results also emphasize the limitations of estimating genetic associations between brain and behavior from comparisons between small numbers of inbred strains or selected lines.

In his recent summary of vertebrate brain evolution, Jerison (1973) concluded that selection pressure has existed for bigger brains. Henderson's (1970) report that brain size in mice shows heterotic inheritance is consistent with this conclusion since heterotic inheritance presumably occurs with those traits that have provided some selective advantage to the species (Bruell, 1967). That increased brain size may be

associated with increased learning performance across species is supported by reports from Rensch (1956) and Riddell, Corl, and Gravetter (1976). Rensch compared the performance of closely related species with different brain sizes on the acquisition and retention of learned tasks and also compared the complexity of their instincts. Although task difficulty and body size were not controlled, Rensch concluded that there is a positive across-species relation between brain size and learning ability. More recently, Riddell et al. have supported Rensch's conclusion both within the order Primates and across orders. With task difficulty controlled statistically for a variety of species, performance on an extradimensional shift problem improved with increased "extra" cortical neurons as calculated from the data of von Bonin (1937) and Crile and Quiring (1940).

Attempts to find a positive relation between brain size and learning performance within a single species have typically not been successful. Positive evidence for such a relation comes from studies of the effects of environmental enrichment upon learning performance. For 22 experiments involving

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This research was supported in part by Grant BMS 73-01499 from the National Science Foundation to John L. Fuller and represents a new analysis of a portion of a dissertation submitted by the first author to the State University of New York at Binghamton in partial fulfillment of the requirements for the PhD degree. The assistance of committee members C. James Scheirer and Stanley R. Scobie is gratefully acknowledged. The authors also wish to thank David A. Blizard and Richard G. Burrig for comments on an earlier version of the manuscript and Thomas H. Roderick for supplying mice from the SEL 19 B and SEL 16 C lines. The support of the Pace University Committee on Scholarly Research is also gratefully acknowledged. A previous report of this research was presented at the 1977 meeting of the Behavior Genetics Association in Louisville, Kentucky.

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10 rat strains raised under enriched conditions, Bennett and Rosenzweig (1968) reported a correlation of  $-0.53$  ( $p < .02$ ) between the 22 means for cortical/subcortical ratio of brain weight and mean errors per reversal in a Krech Hypothesis Apparatus. In a recent summary of the effects of environmental enrichment on learning performance, Davenport (1976) concluded that enrichment, which increases brain weight (Diamond, 1976), is usually associated with improved learning performance.

Evidence that at first appears to contradict a positive within-species relation between brain size and learning performance comes from studies using mice selected for brain weight. Two independent selections for brain size in mice have produced separate high and low (Roderick, Wimer, & Wimer, 1976) and high, medium, and low (Fuller & Herman, 1972) brain-weight lines. Although the lines differ on learning performance, no consistent relation between brain size and learning performance has been found for either selection (Collins, 1970; Jensen, 1977; Roderick et al., 1976). These negative results, however, are probably irrelevant to the question of whether learning ability increases with brain size since random within-line fixation of alleles unrelated to brain weight could produce between-line differences on a variety of performance variables affecting the learning scores.

Theoretically, one method of controlling potentially confounded performance variables in behavior-genetic investigations is to measure them independently and then equate subjects on them prior to assessment of learning performance (cf. Satinder, 1976; Wahlsten, 1972). Unfortunately, it is impossible to know all the variables that could confound the results, and equating on more than a few is prohibited by practical considerations.

A second control for confounded performance variables is to assess the correlation between the variables of interest in heterogeneous, unselected stocks of mice (cf. Wimer, Wimer, & Roderick, 1971). In such populations, truly independent variables are expected to be randomly distributed across subjects; associations that persist are deemed to be functional. This strategy was

used here in assessing relations between brain weight and learning performance in active avoidance, water-maze discrimination, operant discrimination, and passive avoidance tasks by three unselected, heterogeneous stocks from the Fuller and Roderick brain-weight selections. To determine whether brain weight might be related to activity level, we also obtained correlations between brain weight and activity measures from the four learning tasks and the open field.

## Method

General summaries of the subjects and procedures used for the various tasks are given below. For the learning tasks, further details concerning the experimental procedures can be found in Jensen (1977). Further details regarding the open-field testing are also available (Jensen, 1974). The five tasks were given to the mice in the following order: open field, active avoidance, water maze followed by operant discrimination for half of the mice and vice versa for the other half, and, finally, passive avoidance.

## Subjects

Six males and six females from each of the three unselected lines of the Fuller and Roderick selections served as subjects. Mice from the Fuller HET stock and the Roderick SEL 19 B stock came from nine litters each, and mice from the Roderick SEL 16 C stock came from six litters. All mice were raised from weaning in individual enriched environments. Behavioral testing began at approximately 7 wk of age. After active avoidance testing, the "toys" in the enriched cages were no longer changed daily but remained in the cages until the end of behavioral testing. One female from the HET stock was added to replace an animal that died during active avoidance training.

## Apparatus and Procedure

*Open field.* The open field was a circular Lehigh Valley Electronics Model 1497 Q activity cage (52.5 cm high  $\times$  70.0 cm in diameter). The field was divided by six photobeams, with three of them intersecting at right angles with the other three. Two fluorescent tubes were located 30.0 cm above the floor on the lid of the chamber. Together, the 32- and 22-W tubes provided 235-ftc. (2,530-lx) illumination to the floor of the chamber. In four consecutive daily sessions, each mouse was lowered into the center of the field, the lid was put in place, and the number of photocell crossings in a 1-min period was recorded.

*Active avoidance.* During the first four daily sessions (Days 1-4), each mouse was placed in a shuttle chamber for 20 min, and the number of grid crossings in the absence of any conditional stimulus (CS) presentations

was recorded. Following Day 5 when crossings in the presence of 40 CS-only presentations (a white cue light) were assessed, training commenced by presenting 40 CS-shock trials (CS-shock interval = 3 sec). All CS presentations were made according to a 31-sec variable interval (VI) tape. During the 12 training sessions (Days 6–17), a response during the CS prevented shock from occurring. If no shuttle response was made during the initial 3 sec of the CS, a .75-mA electric shock came on, and both the CS and shock remained on until a shuttle response was made or 3 sec without a response had elapsed. The use of a 3-sec CS was based on Royce's (1966) report that this is the "optimal" CS-shock interval to use in finding differences in avoidance acquisition among inbred strains of mice. The number of daily avoidance responses (Days 6–16) was expressed in units relative to both initial operant level of avoidance responding (number of "avoidance" responses on Day 5) and terminal acquisition performance (number of avoidance responses on Day 17) by the use of Anderson's (1963) shape function. The mean of the 11 shape-function values was used as the measure of active avoidance learning performance. Lower values reflect superior performance.

After training, resistance to extinction of the avoidance response was assessed. During the first extinction session (Day 18), each mouse was given 10 avoidance training trials before the shock source was disconnected for the remaining 30 trials of the session. Extinction was continued during four subsequent daily sessions (Days 19–22) of 40 trials each. As during training, the CS automatically terminated after 6 sec if a shuttle response had not been made. The number of avoidance responses for extinction days was expressed in units relative to terminal acquisition (Day 17) and terminal extinction (Day 22) levels by the shape function, and the mean of the four shape-function values was used as the measure of extinction of active avoidance responding. Higher values of the shape function reflect increased resistance to extinction.

**Water maze.** The water maze was made of sheet metal and consisted of two arms that curved back toward the start alley to prevent the escape ladder in the correct arm from being seen from the choice point (Waller, Waller, & Brewster, 1960). One arm was painted black and the other was painted white. Water temperature in the maze was maintained at  $20 \pm 1^\circ\text{C}$ .

On Day 1, 10 escape trials were given. For the next 29 daily sessions of 10 trials each, each mouse was trained to swim to an arm of a given color. After the mice met the learning criterion of 9 correct trials in a block of 10, the problem was reversed on the following day. Swimming time on each Day 1 escape trial, trials to criterion on the original problem, and number of times meeting criterion were recorded.

**Operant discrimination.** The active avoidance chamber was converted to an operant chamber by adding a lever and a fountain for dispensing .025 ml of tap water. Following water deprivation to 80% of ad lib body weight, the mice were trained to respond according to a VI 1-min schedule of reinforcement for 10 daily sessions followed by 30 daily 40-min sessions of discrimination training in which a light-on/light-off condition was changed every 1, 2, or 3 min in random order.

Reinforcement during light-on was programmed according to a VI 1-min schedule, and the light-off condition was correlated with extinction. Operant level of activity was assessed in three pretraining sessions, and discrimination performance was measured by relative response rate during S– (response rate during S–/nominal S– response rate during prediscrimination training). Relative S– response rate was used as a learning measure because it has a higher loading on a factor-analytically determined learning factor than does a discrimination ratio (Jensen, Schmitt, Scheirer, & Cochrane, 1978).

**Passive avoidance.** Passive avoidance training was given according to the procedure described in Jensen (1977). In each daily session, time to step through to the dark side of the passive avoidance apparatus was recorded. Learning performance was measured by trials to meet the learning criterion of remaining on the lighted side of the apparatus for 5 min.

**Determination of brain weights.** After behavioral testing, the wet brain weight of each mouse was determined according to the procedure described by Wimer, Roderick, and Wimer (1969). Dura mater, flocculonodular lobes, and hypophysis were removed. The optic nerve was cut at the chiasma, the trigeminal nerve was cut at the surface of the brain, and the spinal cord was cut at the base of the medulla approximately 5 mm caudal to the cerebellum.

## Results

### *Brain Weight and Learning Correlations*

Correlations between brain weight and learning performance for the six measures from the four learning tasks are presented in Table 1, along with the proportion of variation in learning scores that may be attributed to variation in brain size (coefficient of determination). Small to moderate correlations were found for all measures except passive avoidance acquisition. In acquisition, the signs of the correlations for the shape function from active avoidance training and trials to first criterion and total criteria in the water maze are all in a direction that indicates superior performance with increased brain weight.

Brain weight was positively correlated with resistance to extinction in both the active avoidance and operant discrimination tasks. These positive correlations are interesting because in the water maze the sign of trials to criterion on the first reversal problem was negative ( $r = -.23$ ,  $r^2 = .05$ ,  $ns$ ). Since performance on the reversal problem probably reflects to some degree extinction of responding to previously positive stimuli,

Table 1  
*Correlations Between Brain Weight and Learning Performance*

Partialed variable	Active avoidance				Water maze				Operant discrimination		Passive avoidance	
	Training (shape function)		Extinction (shape function)		Trials to first criterion		Total criteria		Relative S- response rate		Trials to criterion	
	<i>r</i>	<i>r</i> <sup>2</sup>	<i>r</i>	<i>r</i> <sup>2</sup>	<i>r</i>	<i>r</i> <sup>2</sup>	<i>r</i>	<i>r</i> <sup>2</sup>	<i>r</i>	<i>r</i> <sup>2</sup>	<i>r</i>	<i>r</i> <sup>2</sup>
None	-.17	.03	.37*	.14	-.24	.06	.40**	.16	.42**	.18	.07	.00
Operant level	-.17	.03	.38*	.14	-.22	.05	.38*	.14	.48***	.23	.11	.01
Body weight	-.21	.04	.39*	.15	-.29	.08	.44***	.19	.39*	.15	.06	.00
Open-field activity	-.13	.02	.26	.07	-.20	.04	.33	.11	.32	.10	.02	.00

\*  $p < .05$ .

\*\*  $p < .02$ .

\*\*\*  $p < .01$ .

it is necessary to account for the differences in the signs of the correlations found in the active avoidance and operant discrimination tasks versus the water maze. These differences may be due to differences in the consequences of responding. An incorrect response in the water maze leads to a delay in escape from water. A response during active avoidance extinction or during S- has no direct punishment. Therefore, the correlation between brain size and extinction performance may be negative when punishment follows a response and positive in the absence of punishment.

#### *Brain Weight and Activity Correlations*

Brain weight and activity correlations for the open field and the four learning tasks are presented in Table 2. Only the open-field correlation is statistically significant. The other measure typically obtained in the open field (defecation) was unrelated to brain

weight ( $r = -.05$ ,  $r^2 = .00$ , *ns*). Since mice with bigger brains would also tend to have bigger bodies, body weight was partialled out of the correlations between brain weight and activity. The effect of partialing out body weight was to increase the magnitude of all the correlations between brain weight and activity, but significance levels were unaffected.

#### *Partial Brain Weight and Learning Correlations*

The correlations between brain weight and learning performance in Table 1 are potentially confounded with other variables that may be correlated with brain weight. Two of these potentially confounding variables are operant level of responding and body weight. To eliminate the contribution of these variables to the correlations between brain weight and learning performance, we used partial correlations to hold these vari-

Table 2  
*Correlations Between Brain Weight and Activity*

Partialed variable	Open field		Active avoidance		Water maze		Operant discrimination		Passive avoidance	
	Photocell crossings		Grid crossings (Days 1-4)		Swin speed (Day 1)		Grid crossings (operant level)		Step-through latency (Day 1)	
	<i>r</i>	<i>r</i> <sup>2</sup>	<i>r</i>	<i>r</i> <sup>2</sup>	<i>r</i>	<i>r</i> <sup>2</sup>	<i>r</i>	<i>r</i> <sup>2</sup>	<i>r</i>	<i>r</i> <sup>2</sup>
None	.39*	.15	-.02	.00	.14	.02	-.11	.01	.11	.01
Body weight	.41**	.17	-.15	.02	.22	.05	-.30	.09	.16	.02

\*  $p < .05$ .

\*\*  $p < .02$ .

ables constant. Reading across Table 1, the operant level variables partialled out were number of crossings during the 4 days prior to avoidance training, number of avoidances on the final day of training, median speed of swimming during the 10 escape trials on Day 1 (used for both water-maze learning measures), mean response rate during nominal S— over the final 5 days of prediscrimination training, and step-through latency on Trial 1, respectively. In all six cases, the partial correlations were very similar to, or identical with, the original ones.

Partialing out body weight from the correlations between brain weight and learning performance did not produce a consistent increase or decrease in the magnitude of the correlations coefficients. Therefore, the correlations between brain weight and learning performance are not seriously confounded with body weight.

The highest of the five correlations between brain weight and activity in Table 2 is for photocell crossings in the open field. Walsh and Cummins (1976) have reviewed evidence indicating that ambulation scores in an open field are not synonymous with activity and that such scores partially reflect learning and habituation. The correlation of brain weight with ambulation in the open field and not with other scores is consistent with both conclusions. Moreover, when ambulation in the open field was partialled out of the correlations between brain weight and learning performance, the magnitude of the original correlations decreased in all cases, as expected. Because the temporal course of learning and habituation in an open field is poorly understood (Walsh & Cummins, 1976), this precludes a more precise analysis and interpretation of the relation between ambulation in an open field and learning on other tasks.

#### *Brain Weight Correlations in Males and Females*

To evaluate possible sex differences in the correlations between brain weight and learning performance, we recalculated the correlations of Table 1 separately for males and females. There were no consistent sex differences in the signs or magnitudes of the

correlations. The mean absolute values of the six correlations between brain weight and learning performance (Fisher's  $r$  to  $z$  transformation) is .28 for males and .32 for females. Similarly, there were no consistent sex differences in the sign or magnitude of the correlations between brain weight and activity (mean Fisher's  $r$  to  $z$  transformation is .30 for males and .18 for females).

## Discussion

### *Relations to Previous Correlational Studies*

The results provide support for the existence of a positive relation between brain weight and learning performance in *Mus musculus*. Previous reports of correlations between brain weight and learning performance in unselected stocks of mice are summarized in Roderick et al. (1976). Although statistically nonsignificant, the signs of three of their four correlations between brain weight and performance on original learning in a water maze with spatial or brightness cues indicate superior performance with increased brain weight.

In addition to confirming the previously found correlations between brain weight and learning in a water maze, the present results extend this relation to other tasks. The signs of the correlations between brain weight and learning performance for both the shape function during active avoidance training and total criteria in the water maze are in a direction indicating superior performance with increased brain weight. The generality of the correlations is not an artifact of task similarity. With a factor analysis of the four learning tasks for 93 mice of the original investigation (Jensen, 1974), Jensen et al. (1978) found low communality of the active avoidance and operant discrimination tasks with either the water-maze or passive avoidance task. It is not surprising that the passive avoidance results show essentially no relation with brain weight. The passive avoidance results reflect the smallest sample of behavior of the six measures of learning performance, and performance on a passive avoidance task has

not reflected neural state in other investigations (cf. Davenport, 1976).

At present, the positive correlations between brain weight and resistance to extinction in the active avoidance and operant discrimination tasks simply indicate a consistent relation between brain weight and extinction performance. Whether high response levels during extinction of unpunished responding and low response levels during extinction of punished responding reflect "superior" performance requires further investigation.

### *Implications for Selection and Strain-Difference Studies*

In an attempt to relate brain weight to learning performance, Jensen (1977) tested mice from five lines selected for brain weight (Fuller & Herman, 1972; Roderick et al., 1976) and the three unselected heterogeneous stocks of the present report on the same tasks used in the present experiment. No relation between brain weight and learning performance was found either within selections and across tasks or within any one task and across selections. The discrepancy between the relations between brain weight and learning performance for the heterogeneous stocks and for the lines selected for brain weight is likely due primarily to random fixation of alleles within the various selected lines. Under laboratory conditions selection usually involves small populations and consequent inbreeding. Thus, the selected lines probably differed on traits other than brain size that were important determinants of task performance.

The correlations reported here provide supporting evidence for a positive within-species relation between brain size and learning performance. Thus, this study joins other investigations as diverse as those represented by Henderson (1970) and Riddell et al. (1976) in being consistent with Jerison's (1973) theory of the evolution of brain size and intelligence. Negative results from selection and small-scale strain-difference studies are at least potentially confounded with unassessed differences in performance variables and should not be used to support the argument that brain size

and learning ability are not positively related. A similar statement regarding negative evidence can be made for most behavioral genetic studies. Correlations between the variables of interest should be obtained for heterogeneous stocks or across several unrelated inbred strains before concluding that a relation does or does not exist. Obtaining such correlations is a very efficient method of controlling for unequal distribution of alleles responsible for factors other than the one of primary interest that affect the behavior under investigation.

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Received August 19, 1977 ■