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Brain Weights Correlate with Behavioral Parameters in Individual Inbred Mice Housed in a Common and Enriched Environment

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Fifty 8-week-old Balb/c mice were individually identified and housed together in a large and enriched environment for 5 months. Maze and open field exploration, response to an aversive noise, swimming, and induced grooming tests were applied to each mouse in an initial search for possible relationships between brain morphology and spontaneous behavior in isogenic individuals living in a complex social and physical environment. The tasks generated 39 quantitative behavioral indices which include locomotion, rearing, still, and grooming bout frequencies, latencies, total, and mean bout durations. At the end of the tests, the 7-month-old mice were sacrificed and the fresh weights of their whole brain, cerebellum, brain stem, diencephalon, telencephalon, and prosencephalon were rapidly obtained. Behavioral data have wide variations and do not adjust to normal population distributions. Means of the same parameter differ between tests. A Spearman correlation matrix of all data yielded many significant correlations between indices of the same task which can be interpreted in terms of time budget and sequence probability. Significant correlations between indices of different tests suggest diverse emotionalities, exploratory strategies, and motor skills. The correlations between body and brain weights and among separate brain regions were not significant. There were several low but significant correlations between brain weights and behavioral indices. Such correlations, the resulting factors, and significant behavioral differences between mice with large and small brains suggest that mice displaying low motor activity in novel environments have larger brains and forebrain/hindbrain ratios than mice with high activity, and that animals with high scores of some specific behaviors have larger brain areas physiologically related to such behaviors. Since mice from a pure inbred strain were used and the same enriched housing condition was maintained during

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the last 5 months of their lives, intra-individual variability in brain weights and behavior may be attributed to differential experience due to social factors.

The functional hierarchy of some brain regions in the expression of specific behaviors has been widely confirmed since the early neuropathological findings and more recent neurophysiological experiments involving lesions and electrical stimulation of discrete brain sites. Lately it has been possible to image brain regional activity in correlation with specific sensorial and motor tasks (Lassen, Ingvar, & Skinoj, 1978; Sokoloff, 1979). There is also evidence for considerable brain plasticity in the rehabilitation of lost functions (Lenneberg, 1967) and the acquisition of new ones (Merzenich, Kaas, Wall, Sur, Nelson, & Fellmand, 1983). The functional plasticity of the brain is manifested by asymptomatic subjects with hydrocephalic volumes which occupy 95% of their cranial capacity (Lewin, 1981). Moreover, rat brain is responsive to environmental complexity in terms of enzymatic activity, dendrite sprouting, synaptogenesis, cortex/subcortex weight ratio, occipital cortex thickness, and brain weight (Greenough, Hwang, & Gorman, 1985; Rosenzweig, Bennett, & Diamond, 1972). All this evidence suggests that individual brain size and form are reciprocally related to behavioral characteristics. Brain morphology and function as well as motor expression of spontaneous behavior are regulated by genetic, developmental, and environmental influences linked in a single epigenetic process (Wilson, 1978).

Some anatomical brain features have been shown to have consequences in behavior. Wimer, Wimer, and Roderick (1971) found behavioral differences related to natural variations of hippocampal volume in random-bred mice. Hahn, Haber, and Fuller (1973) showed differential agonistic behavior in mice selected for brain weight. Macrocephalic mice develop earlier and more abundant behaviors (Henderson, 1979; Mikulik & Nash, 1979; Wahlstein, 1975) and solve T-mazes faster than controls (Herman & Nagy, 1977). About half of the patients diagnosed as schizophrenic (Johnstone, Crow, Frith, Husband, & Kreel, 1976) and some of their healthy relatives (DeLisi, Goldin, Mamovit, Maxwell, Kurtz, Nuremberg, & Gershon, 1985) have larger cerebral ventricles. There are differences in the size of corpora callosa in both mice and humans according to hand preference (Ward & Collins, 1985; Witelson, 1985). It has been shown that the seasonal expression of birdsong is correlated with the gross volume and neuronal endowment of the nucleus hyperstriatum ventrale (Nottebohm, Kelley, & Patton, 1982). These evidences follow the prevailing notion that behavioral variability is due to differences in the structure and function of the central nervous system which, in turn, result from underlying genetic variation. Except for the experiments of Rosenzweig

et al. (1972), the converse possibility that behavioral experience may affect brain features has been less explored.

The studies of brain–behavior relationships designed to separate genetic and environmental influences usually compare several inbred strains or, less frequently, intra-individual differences in random-bred mice. In both cases the intention is to evaluate the genetic contribution taking advantage of the genetic uniformity within a strain or the genetic variability in random-bred individuals. Differences between individuals belonging to the same inbred strain are presumably nongenetic in origin (Wimer, Wimer, & Roderick, 1969) and, therefore, provide a potential model to analyze environmental influences on brain and behavior. Accordingly, both brain size and morphometric features show significantly less environmental variation in hybrid compared to inbred mice (Leamy, 1985).

In the present paper 39 behavioral indices and 6 brain weights were obtained and correlated in 50 individually identified mice belonging to the Balb/c inbred strain. Therefore, any intra-individual differences can be attributed to environmental influences. Since the enriched physical environment and food sources were the same through the mice's lives and the sole uncontrolled variable was the social system, it can be suggested that brain–behavior correlations may be a consequence of social influences.

METHODS

Animals

Fifty 8-old-week male Balb c/AnN mice were used. At the beginning of the experiment their weight was 25 g on the average. At the time of sacrifice they were 32 weeks old and weighed 32 g. The 50 mice were marked for individual recognition and housed in a single glass cage (100 × 30 × 25 cm) provided with two running wheels, balancing beams, plus vertical and horizontal wire structures. Water and food pellets were available ad libitum. The woodshaving bed was changed and the animals were weighed and re-marked twice a week. The cage was located near a south-facing window and therefore submitted to a natural light–dark cycle (12 ± 1 h at 19° latitude) and a temperature ranging from 18 to 22°C.

The enriched cage soon became a complex mouse-living unit with specific places for feeding, sleeping, grooming, and urinating. Two obvious socially dominant mice dwelled in different corners. Thus, the environment allowed for individual and social differences to develop and consolidate.

Apparatus

A maze, an open field, a sound-proof chamber, and a swimming tank were used. The maze was a black-painted wood cage (60 × 60 × 10 cm) with attached exit and entry corner chambers. The inner arrangement provided for a single solution path 100 cm long and four dead ends. The

open field consisted of a 40 × 40 × 40 cm black wood cage with a glass front and an opposite mirror to allow for direct observation. The floor was divided in nine squares 13 × 13 cm each. The open field was located inside the sound-proof chamber (100 × 100 × 70 cm) with lateral and superior windows. In its ceiling this cage contains two circular 35-W fluorescent lights and a buzzer that delivers a 100-dB sound. The swimming tank was made of glass, was 40 × 20 × 25 cm, and was half-filled with cold tap water.

Procedure

All mice were recorded in the same sequence of behavioral tasks. Maze exploration was carried out on 3 consecutive days between 1000 and 1200 h when the mice were 10 weeks old, and consisted of locating each mouse at the entrance chamber for 60 s. The departure gate was then opened and the chronometer was started. The test ended when the mouse entered at the arrival cage chamber. In order to avoid odor cues, the maze was thorougly washed after each test.

The second phase consisted of a battery of tests lasting for 60 min, and was performed on one mouse per day between 1700 and 1800 h. The mouse was introduced into the same corner of the open field in a small wooden cage where it remained for 60 s. The cage was removed and the chronometer was started. The exploratory behavior of the novel environment was recorded in videotape for 10 min. At this time a noise (2 s, 100 dB) was delivered by the buzzer and the behavior after this aversive sound was recorded for a period of 5 min. At the end of this phase, the mouse was removed from the open field and placed in the swimming tank for 60 s. Swimming behavior was recorded at three frames per second on videotape. After this test the animal was returned to the open field and the grooming behavior induced to dry the fur was recorded on videotape for 30 min. After each observation the open field cage and swimming tank were thorougly cleaned.

Behavioral Events and Indirect Behavioral Indices

Previous mouse ethograms (Mackintosh, Chance, & Silverman, 1977) modified in our laboratory (Diaz and Santis, 1983) were employed to define the behaviors recorded. Several indirect indices (Eysenck & Broadhurst, 1964) were also used. The following indices were recorded either by direct observation or through videotape analysis.

Capture order. Ordinal sequence (1–50) of capture and marking of individual mice by a single person from the common transport cage.

Maze departure latency. Time in seconds elapsed from exit gate opening to full entrance into maze and gate closing.

Maze arrival latency. Time in seconds elapsed from entrance into maze to entrance into arrival cage.

Maze grooming bouts. Frequency of grooming episodes during maze exploration. Grooming periods comprise several self-cleaning actions such as washing, licking, scratching, nibbling, and shaking of body segments in a variety of postures described under *Induced grooming bouts.*

Open field departure latency. Time in seconds elapsed from removal of the introduction cage to departure from the initial placement square.

Open field traveled distance. Number of floor lines crossed in 10 min.

Open field grooming. Frequency, total duration (s), mean bout duration (average seconds of grooming episode), and preferred site (duration in maximal site/total duration; Santis and Diaz, 1983) of grooming activity.

Open field rearing bouts. Frequency of vertical standings on rear legs, including vertical wall-leaning acts.

Open field still behavior. Frequency (number of episodes), total duration (s), and mean duration (s) at least 5-s periods of awake immobility.

Some of these indices were also recorded after the aversive noise was delivered.

Swimming traveled distance. Displacement (cm) during 60 s in the tank.

Swimming wall-jumping attempts. Frequency of episodes of vertical impulse action at walls and corners.

Induced grooming bouts. Grooming behavior after swimming was dissected into nine specific action/postures according to the body segments that established contact: hands–face, hands–ears, mouth–hands, mouth–flanks, mouth–abdomen, mouth–genitals, mouth–tail, mouth–feet, and feet–head. Lateral actions were recorded separately. *Brief* and *long pauses* were also recorded when grooming was interrupted for less or more than 5 s, respectively. A long pause includes other actions besides grooming and separated two grooming episodes.

Brain Dissection and Weights

The sacrifice and brain dissection of the 44 mice that completed all the tasks was performed in 1 week. Each mouse was killed in a chloroform-saturated chamber and its brain was rapidly removed after a sagital section of the skull and separation of the bones. The brain was detached from the spinal cord by sectioning at the level of the pyramids and the olfactory bulbs were discarded. Surface blood was removed and the brain was weighed. All brain weights were measured to the nearest milligram with an accuracy of ± 0.5 mg in a mechanical August Sauter scale. The brain was then rapidly dissected. The cerebellum was separated from the pons by cutting the brachia. The brain stem was obtained by a coronal cut at the rostral border of the anterior corpora cuadrigemina. The basal surface of the anterior remanent, comprising the cerebral hemispheres or "prosencephalon," was exposed and the hypothalamic area was dissected in a cone-shaped piece taking as basal limits the medial borders

of the hippocampi, the optic chíasma, and the cerebral peduncules. This section was called "diencephalon" and the rest of the hemispheres "telencephalon."

The dissection of a single animal was performed on a filter paper soaked with saline solution and took less than 4 min, a time short enough to minimize tissue weight loss through evaporation. Such fresh brain weights, commonly used in neurochemistry, are devoid of the problems involved in techniques of perfusion and fixation.

Data Analyses

At the end of the experiment all mice had 39 quantitative indices of behavior and 6 brain weights. From the many body weights available, those from the battery test day and maximal weight were also included in the analysis. Descriptive statistics (mean, standard deviation, standard error, and coefficient of variation) were initially undertaken in order to assess the performance of the population, the variance of the data, and to infer some of the physiological implications of the figures. Since not all indices had normal distributions, a Spearman correlation matrix was used to provide information about the relationship between each pair used. A factor analysis (SPSS; Kim, 1975) was performed on the Spearman correlation matrix in order to generate hypotheses for further testing (Pimentel & Frey, 1978). An initial factor analysis was performed on all the variables and a restricted analysis was later done on eight sets of 14 variables each, since this is the reliable proportion for this sample (Short & Horn, 1984). The final factors were selected for eigenvalues superior to 1 and the inclusion of r coefficients $>.25$ in at least one behavioral and one brain variable. Student t-tests were applied to compare the behavioral performance of the 10 mice with the heaviest brains to the 10 with the lightest brains.

RESULTS

The descriptive statistics of the numerical indices obtained in the 44 mouse sample is shown in Table 1. The capture order is the only cardinal index used and, therefore, the mean does not have any physiological significance. The departure latency in the maze was 12 s and 6 s in the open field. The arrival latency in the maze was near 250 s and the frequency of grooming bouts was very scarce (1 on average) and highly variable. In the open field the frequency of grooming bouts was four times higher (six episodes in 10 min), and the animals crossed 116 floor lines indicating an active exploratory locomotion of about 6 m per animal. Grooming behavior was preferentially performed in a particular place for each mouse (96% of the grooming time). The exploratory significance of grooming and the selection of a preferred grooming site have been described previously by this laboratory (Santis & Diaz, 1983). Vertical

TABLE 1
Descriptive Statistics of Behavioral and Cerebral Parameters

Behavioral and cerebral parameters	Mean	SD	SE	CV
Capture order (ordinal index 1–50)	26.2	14.1	2.1	.54
Maze departure latency (s)	12.3	7.8	1.1	.64
Arrival latency (s)	249	172	27	.69
Grooming bouts	1.00	1.36	.2	1.36
Open field departure latency (s)	5.9	13.2	2.0	2.22
Traveled distance (squares)	116	39	6.0	.34
Grooming bouts	5.8	1.8	0.3	.30
Total duration (s)	238	88	14	.37
Mean bout duration (s)	42.9	16.0	2.5	.37
In preferred site	.96	.84	.13	.88
Rearing bouts	51.3	21.9	3.4	.43
Still bouts	1.95	2.53	.39	1.30
Total duration (s)	39.2	81.1	12.5	2.07
Mean bout duration (s)	7.69	13.1	2.0	1.70
Aversive noise still total duration (s)	128	112	17	.87
Grooming bouts	1.86	1.66	.26	.89
Total duration (s)	117	114	18	.97
Mean bout duration (s)	38.7	43.7	6.7	1.13
Rearing bouts	11.2	12.2	1.9	1.09
Swimming traveled distance (m)	13.4	1.40	0.2	.10
Wall jumping attempt bouts	21.9	10.4	1.6	.48
Induced grooming bout number	2.67	1.10	.17	.41
Total duration (s)	997	476	74	.48
Mean bout duration (s)	295	176	27	.60
Action bout number	148	121	19	.81
Hands–face bouts	8.64	12.4	1.9	1.44
Hands–ears bouts	12.4	7.7	1.2	.62
Mouth–hands	28.2	11.0	1.7	.39
Mouth-right flank bouts	20.1	10.9	1.7	.54
Mouth–left flank bouts	19.4	10.1	1.6	.52
Mouth–abdomen bouts	6.38	11.0	1.7	1.71
Mouth–genitals bouts	.88	1.48	.2	1.68
Mouth–tail bouts	.40	1.23	.2	1.68
Mouth–right foot bouts	3.52	2.51	.4	.72
Mouth–left foot bouts	2.26	2.23	.3	.73
Right foot–head bouts	1.60	1.71	.3	1.07
Left foot–head bouts	2.26	2.23	.3	.99
Brief pauses (number)	28.8	11.9	1.9	.41
Long pauses (number)	1.76	1.12	.2	.64
Weight, body test day (g)	29.8	2.34	.4	.08
Maximal (g)	31.8	1.74	.3	.05
Whole brain (mg)	435	14.6	2.3	.03
Cerebellum (mg)	55.7	3.05	.5	.05
Brain stem (mg)	87.0	6.03	.9	.07
Diencephalon (mg)	35.6	4.53	.7	.13
Telencephalon (mg)	257	12.7	2.0	.05
Prosencephalon (mg)	293	12.7	2.0	.04

Note. Data for 44 mice. SD, standard deviation; SE, standard error: CV, coefficient of variation (SD/mean).

postures were also abundant in the open field test (five per min), whereas still behavior was scarce and highly variable (two episodes, 40 s total duration on the average). The mean duration of still bouts was 7.7 s and it does not correspond to the product of dividing the total duration/frequency since only the episodes that were observed from beginning to end were considered for computation.

The same behavioral parameters yielded different data after the aversive noise was delivered. Still behavior increased to 130 s in 5 min and probably expresses a "freezing" after the unexpected sound. As previously described, after this noise freezing occurred predominantly at the preferred grooming site (Santis & Diaz, 1983). Vertical postures decreased to about half those of the initial exploratory period. These changes may also be a normal temporal tendency in an open field of these characteristics. In contrast, grooming parameters did not differ in the two periods.

The swimming test yielded data with very low variability. The distance traveled in 1 min was more than 13 m per mouse and there was an average of 22 attempts to jump off the walls.

Grooming activity induced by being wet after swimming was dissected in 17 behavioral parameters. The number of episodes was low (2.7), but their duration was very long (1000 s; 60% of the analysis time). This activity comprises 12 different postural actions (considering the lateral actions separately) and amount to 150 per mouse. These results indicate that grooming activity was strongly induced by being wet and suggest that it is a very appropriate test to study this behavior. The most frequent actions were mouth–hands and brief pauses with 28 bouts each. Mouth–flanks occurred 20 times for each side, and it would have been the most frequent action if considered as a single one. In decreasing order of frequency there were the actions of hands–ears (12 bouts), hands–face (9), mouth–feet (7), mouth–abdomen (6), foot–head scratchings (4), and, finally, mouth–genitals and mouth–tail with less than one episode each. These data express the fact that induced grooming is an organized activity of different actions. The sequence matrix of the recorded actions has been presented elsewhere (Diaz and de la Vega, 1985), and showed a rostrocaudal progression pattern usually beginning with mouth–hands, continuing with cleaning of a single side, and interrupted with brief pauses. In contrast to behavioral indices, body and brain weights had low coefficients of variation (0.3–.13) and normal distributions, a usual pattern for biological parameters. Body weight was 30 g and whole brain weight was 435 mg. Thus, brain weight represents 1.45% of body weight (cerebral index), a well-known high proportion in the phylogenetic scale (Jerison, 1973).

The Spearman correlation matrix of all numercial indices showed that significant correlations ($r > .259$, $p < .05$) were most frequently found among parameters of a single behavioral task. Such correlations can

usually be interpreted in terms of time budget or sequence probability. For example, there is an expected negative correlation between traveled distance and still behaviors in the open field (−.50), and strongly positive correlations between induced grooming actions (.7–.9).

Very low correlations were found between body weight and whole brain (.06) or regional weights. This is not surprising since heavy and lean individuals should not differ in brain weights. Moreover, there were no significant correlations among the weights of brain stem, cerebellum, diencephalon, and telencephalon (−.15 to .15) indicating a relative independence in the development of the main brain areas. The correlations obtained between whole brain weight and regional weights were in direct proportion to the weight of the region (with telencephalon .82, cerebellum .40, brain stem .33, and diencephalon .19).

The correlation matrix between brain weights and behavioral parameters is shown in Table 2. Whole brain weight is correlated mainly with behavioral indices of the open field test. There are negative correlations with movement actions (distance traveled −.33 and rearing bouts −.26) and positive ones with still indices (.27–.40). Accordingly, the total number of induced grooming actions has a negative correlation with whole brain weight (−.26).

The weight of the cerebellum shows only a few correlations with a tendency to reach significance ($.1 < p > .05$). The highest and most interesting is with capture order (.25), an index of evasivennes. The brain stem is correlated with some induced grooming indices, especially with mean bout duration (.38). Since the telencephalon comprises most of the prosencephalon, and both amount to more than half the whole brain weight, their correlations with behavior show the same tendency and order as those of the whole brain.

A factor analysis was performed on sets of 14 parameters of the Spearman correlation matrix and significant factors are shown in Table 3. Only the factors with eigenvalues above 1 and that include both brain weights and behavioral indices with coefficients above 25% are considered of interest. Whole brain and telecephalon weights are negatively clustered with traveled distance and rearing bouts in the open field (factors 1, 12) and positively with still duration and grooming bouts in the same test (factors 4, 8, 12). The weight of the brain stem is positively associated with traveled distance and rearing bouts in the open field (factor 2) and, especially, with spontaneous and induced grooming indices (factors 3, 5, 8, 11, 20). The cerebellum is clustered with capture order (factors 5, 14), swimming traveled distance (factor 6), and, in contrast to the brain stem, negatively with induced grooming parameters (factors 10, 14). The diencephalon is positively associated with still durations in the open field and after the aversive noise (factors 7, 15, 17, 18), with grooming parameters after the aversive noise (factor 16), and negatively with induced grooming

TABLE 2
Correlation Matrix among Brain Weights and Behavioral Parameters in Balb/c AnN Mice

Behavioral test and parameter	Whole brain	Cerebellum	Brain stem	Diencaphalon	Telencephalon	Prosencephalon
Capture order	−.10	.25†	−.11	.04	−.17	−.15
Maze exploration						
Departure latency	.13	−.05	−.05	−.02	.15	.10
Arrival latency	.09	.24†	.05	.00	.01	.05
Grooming bouts	.13	.16	−.14	−.11	.16	.17
Open field exploration						
Departure latency	.06	−.01	.08	−.09	−.04	.00
Traveled distance	−.33**	−.03	.07	−.23†	−.25†	−.35**
Grooming bouts	.24†	.22†	−.10	−.21	.34**	.26*
Total duration	.17	−.02	.07	−.14	.20†	.14
Mean bout duration	−.03	−.23†	.13	.01	−.04	−.06
In preferred site	.24†	.03	.10	.03	.22†	.24†
Rearing bouts	−.26*	.09	.12	−.02	−.27*	−.32**
Still bouts	.27*	.24†	−.17	.35**	.15	.28*
Total duration	.32**	.24†	−.14	.34**	.19	.31*
Mean bout duration	.40***	.21†	−.06	.31*	.26*	.39***
Aversive noise						
Still total duration	−.04	−.14	.01	.19	−.14	−.08
Grooming bouts	−.09	−.01	.10	−.10	−.11	−.09
Total duration	−.10	−.04	.03	.16	−.17	−.10
Mean bout duration	−.07	−.13	.07	.14	−.11	−.06
Rearing bouts	−.09	.11	.13	−.07	−.17	−.13
Swimming						
Traveled distance	.01	.18	.14	.06	−.12	−.14
Wall-jumping attempts	.12	−.02	.05	−.02	.14	.11
Induced grooming						
Bout number	−.26*	−.13	.03	−.44***	−.09	−.24*
Total duration	.18	.09	.28*	−.01	.06	.05
Action bout number	.17	.09	.38***	.10	−.07	.02
Hand–face bouts	.08	−.17	.07	.19	.01	.09
Hand–ears bouts	.13	.03	.27*	−.10	.07	.06
Mouth–hands bouts	.21†	.09	.15	.11	.08	.13
Mouth–right flank	−.02	.13	.21*	−.13	−.02	−.10
Mouth–left flank	.07	.01	.04	−.25†	.09	.07
Mouth–abdomen	.15	−.21	−.05	.04	.23†	.20
Mouth–genitals	−.11	.16	−.03	−.23†	−.04	−.12
Mouth–tail	.00	.21†	.03	.02	−.03	.00
Mouth–right foot	.06	.10	.11	−.36***	.13	.00
Mouth–left foot	.12	−.03	.04	−.19	.25†	.16
Right foot–head	−.14	−.22†	.40***	.01	−.23	−.23
Left foot–head	−.15	−.16	.14	−.17	−.17	−.20
Brief pauses	.07	.23†	.08	−.16	.08	.03
Long pauses	.25†	−.14	.06	−.38***	−.11	−.25*

Note. Data represent Spearman's r correlation coefficients among values.
$^{+}p = .1$–$.05$; $^{*}p < .05$; $^{**}p < .02$; $^{***}p < .01$.

TABLE 3
Factor Analyses for Behavioral and Cerebral Variables

Behavioral and cerebral variables	I		II					III		
	1	2	3	4	5	6	7	8	9	10
Capture order	—	—	27*	−19	77*	07	18	—	—	—
Maze departure latency	10	14	—	—	—	—	—	—	—	—
Arrival latency	—	—	—	—	—	—	—	—	—	—
Grooming bouts	—	—	—	—	—	—	—	11	03	60*
Open field departure latency	−04	12	—	—	—	—	—	—	—	—
Traveled distance	−28*	34	—	—	—	—	—	—	—	—
Grooming bouts	—	—	—	—	—	—	—	52*	29*	22
Total duration	—	—	39*	−08	10	−61*	24	—	—	—
Mean bout duration	—	—	—	—	—	—	—	—	—	—
In preferred site	—	—	—	—	—	—	—	—	—	—
Rearing bouts	−26*	39*	—	—	—	—	—	—	—	—
Still bouts	—	—	—	—	—	—	—	—	—	—
Total duration	—	—	−21	40*	47*	08	49*	—	—	—
Mean bout duration	—	—	—	—	—	—	—	—	—	—
Aversive noise still duration	−17	10	—	—	—	—	—	—	—	—
Grooming bouts	—	—	—	—	—	—	—	−08	−15	−10
Total duration	—	—	65*	−30*	07	−09	21	—	—	—
Mean bout duration	—	—	—	—	—	—	—	—	—	—
Rearing bouts	−13	24	—	—	—	—	—	—	—	—
Swimming traveled distance	—	—	09	−18	−11	71*	14	—	—	—
Wall juming attempts bouts	—	—	—	—	—	—	—	—	—	—

Induced grooming bout number	—	—	—	—	—	—	—	−13	80*	−05
Total duration	—	—	85*	06	−23	10	−08	—	—	—
Mean bout duration	—	—	—	—	—	—	—	—	—	—
Action bout number	—	—	—	—	—	—	—	—	—	—
Hands–face bouts	−06	06	—	—	—	—	—	—	—	—
Hands–ears bouts	—	—	80*	16	−04	−15	−08	—	—	—
Mouth–hands bouts	11	20	—	—	—	—	—	—	—	—
Mouth–right flank bouts	—	—	—	—	—	—	—	80	28*	−01
Mouth–left flank bouts	—	—	—	—	—	—	—	—	—	—
Mouth–abdomen bouts	—	—	—	—	—	—	—	—	—	—
Mouth–genitals bouts	—	—	—	—	—	—	—	—	—	—
Mouth–tail bouts	—	—	—	—	—	—	—	—	—	—
Mouth–right foot bouts	—	—	—	—	—	—	—	23	48*	19
Mouth–left foot bouts	—	—	—	—	—	—	—	—	—	—
Right foot–head bouts	—	—	—	—	—	—	—	−27*	−01	−26*
Left foot–head bouts	—	—	—	—	—	—	—	—	—	—
Brief pauses (number)	—	—	76*	10	37*	17	−22	—	—	—
Long pauses (number)	—	—	—	—	—	—	—	—	—	—
Weight, body test day	—	—	—	—	—	—	—	—	—	—
Maximal	—	—	—	—	—	—	—	18	−17	−72*
Whole brain	91*	19	13	90*	−13	14	26*	88*	−26*	07
Cerebellum	46*	12	12	35*	33*	65*	10	35*	−25*	62*
Brain stem	07	88*	37*	03	−58*	18	18	04	03	−07
Diencephalon	−07	−08	−05	00	01	04	88*	−04	−79*	−08
Telencephalon	92*	−16	−01	91*	−03	−11	−16	90*	08	−04
Prosencephalon	—	—	—	—	—	—	—	—	—	—

TABLE 3—*Continued*

Behavioral and cerebral variables	IV		V	VI				VII	VIII
	11	12	13	14	15	16	17	18	19	20
Capture order	—	18	62*	38*	—	—	—	—	—	—
Maze departure latency	—	—	—	—	−01	−18	81*	−20	—	—
Arrival latency	—	00	−57*	—	—	—	—	—	—	—
Grooming bouts	—	14	—	—	—	—	—	—	—	—
Open field departure latency	—	—	—	—	00	19	09	−83*	−06	—
Traveled distance	—	−81*	03	—	−81*	−13	−06	25*	−23	—
Grooming bouts	−36*	—	—	—	—	—	—	—	—	—
Total duration	—	—	—	—	—	—	—	—	—	—
Mean bout duration	—	—	—	—	—	—	—	—	−03	13
In preferred site	—	—	—	—	—	—	—	—	—	—
Rearing bouts	—	—	—	05	−73*	−05	−15	37*	01	—
Still bouts	—	83*	35*	—	90*	−12	02	26*	—	—
Total duration	—	85*	31*	—	91*	−19	08	26*	27*	—
Mean bout duration	—	—	—	—	91*	−11	02	23	26*	—
Aversive noise still duration	—	—	—	—	12	−26*	66*	46*	—	—
Grooming bouts	−18	−21	07	—	−20	73*	−43*	−09	04	—
Total duration	—	00	13	—	−01	95*	−08	−12	—	—
Mean bout duration	—	—	—	—	07	91*	−05	−01	34*	—
Rearing bouts	—	—	—	03	−31*	59*	−43	−07	01	—
Swimming traveled distance	—	—	—	13	—	—	—	—	—	—
Wall jumping attempt bouts	—	−08	−58*	−11	—	—	—	—	—	—

Induced grooming bout number	08	−54*	20	—	—	—	—	—	−82*	—
Total duration	—	—	—	—	—	—	—	—	—	—
Mean bout duration	—	—	—	—	—	—	—	—	01	73*
Action bout number	—	—	—	—	—	—	—	—	—	—
Hands–face bouts	—	38*	10	—	—	—	—	—	—	06
Hands–ears bouts	—	04	−16	—	—	—	—	—	—	52*
Mouth–hands bouts	—	—	—	—	—	—	—	—	—	16
Mouth–right flank bouts	29*	—	—	06	—	—	—	—	—	13
Mouth–left flank bouts	—	—	—	−11	—	—	—	—	—	—
Mouth–abdomen bouts	—	—	—	−51*	—	—	—	—	—	—
Mouth–genitals bouts	—	—	—	24	—	—	—	—	—	—
Mouth–tail bouts	—	—	—	—	—	—	—	—	—	—
Mouth–right foot bouts	13	—	—	—	—	—	—	—	—	08
Mouth–left foot bouts	—	—	—	—	—	—	—	—	—	—
Right foot–head bouts	36*	—	—	−44*	—	—	—	—	−15	69*
Left foot–head bouts	—	—	—	−31*	—	—	—	—	—	44*
Brief pauses (number)	—	−06	23	—	—	—	—	—	—	08
Long pauses (number)	—	—	—	—	—	—	—	—	—	—
Weight, body test day	—	—	—	02	—	—	—	—	—	−03
Maximal	09	—	—	—	—	—	—	—	—	—
Whole brain	28*	56*	−46*	—	—	—	—	—	—	15
Cerebellum	18	—	—	86*	—	—	—	—	—	—
Brain stem	87*	—	—	—	—	—	—	—	—	75*
Diencephalon	10	—	—	—	31*	32*	30*	48*	81*	—
Telencephalon	−11	—	—	—	—	—	—	—	—	—
Procencephalon	—	—	—	—	—	—	—	—	—	—

parameters (factors 9, 19), with traveled distance, rearing bouts, and departure latency in the maze (factors 15, 17, 18).

In order to test whether mice with large and small brains in this sample differed in behavioral performance, Student's t tests were applied to compare the behavioral means of the 10 highest and 10 lowest brain weights (Table 4). The animals with the largest brains had significantly less ambulation and rearing in the open field and spent more time immobile and grooming in the same place. There was a trend to reverse such relationship after the aversive noise was delivered. In general they had higher values of induced grooming activity, but only mouth–abdomen bouts reached significance.

DISCUSSION

Direct and indirect indices of spontaneous and induced behavior show great variability between individuals and log-normal Poisson distributions (Fagen, 1978). This was the case for many of the behavioral parameters obtained in the present work as evidenced by the coefficient of variation, and the differences between medians and means. These characteristics are usually a problem in experimental designs but they are advantageous for our objectives, since they allow for individual differences to be expressed and clusters of parameters to be established. Individual differences in all the tests probably indicate peculiar traits such as stress, fear, physical strength, and motor coordination.

During exploration of a novel environment mice divide their time between gathering sensory information (exploratory behaviors: locomotion, rearing) and selecting places for fixed behaviors (grooming in a preferred site, still behavior). It seems that in this sample there are two types of mice differing in behavioral performance and brain weights. The correlations and significant factors establish that "fast" or "active" mice with high indices of locomotion, rearing, and spontaneous grooming activity, and low indices of still behaviors and induced grooming actions show small brains and a smaller forebrain/hindbrain ratios than "slow" mice with the opposite profile. It would be of interest to select and breed populations of mice differing in exploratory activity to test whether they would also differ in brain weight. The hypothesis could also be tested in mice selected for 30 generations for high and low open field activity by DeFries, Cook Gervais, and Thomas (1978) and in the Fuller Brain Weight selection lines which, after 12 generations, the high and low lines differed by over 120 mg brain weight (Fuller, 1979). Wimer, Wimer, and Roderick (1971) reported that the ratio of hippocampal volume to total forebrain volume was negatively related to open field activity in random-bred mice with naturally varying hippocampal size. Thus, mice with larger hippocampi tended to be less active and a significant relation was found between

TABLE 4
Behavioral Comparison between Mice with Large and Small Brains

Behavioral variables	Brain weights 450 (1.4)	427 (0.7)	***
Capture order	27.4 (4.0)	26.5 (3.9)	
Maze departure latency	14.6 (2.5)	10.3 (2.2)	
Arrival latency	247 (55)	208 (28)	
Grooming bouts	1.3 (.5)	0.5 (.3)	
Open field departure latency	4.1 (.8)	3.0 (.5)	
Traveled distance	94 (6.3)	126 (13)	*
Grooming bouts	6.1 (.4)	5.4 (.5)	
Total duration	296 (25)	229 (25)	†
Mean bout duration	48.7 (2.9)	45.9 (6.9)	
In preferred site	.87 (.05)	.73 (.05)	†
Rearing bouts	38 (4.6)	64 (8.3)	**
Still bouts	2.7 (.7)	1.2 (.7)	
Total duration	66.3 (27.0)	11.4 (7.9)	†
Mean bout duration	23.5 (6.4)	7.9 (1.4)	†
Aversive noise still duration	137 (30)	95 (25)	
Grooming bouts	1.3 (.4)	2.3 (.4)	
Total duration	74 (26)	127 (24)	
Mean bout duration	64 (19)	67 (13)	
Rearing bouts	9.2 (5.2)	14.3 (3.9)	
Swimming traveled distance	13.4 (.4)	13.4 (.5)	
Wall jumping attempt bouts	21.3 (1.1)	19.7 (1.2)	
Induced grooming bout number	2.7 (.3)	3.3 (.2)	†
Total duration	1076 (105)	975 (46)	
Mean bout duration	458 (131)	311 (31)	
Action bout number	153 (15)	142 (10)	
Hands–ears bouts	16.2 (2.8)	15 (3.4)	
Mouth–hands bouts	32.9 (4.0)	27.9 (3.2)	
Mouth–right flank bouts	20.4 (2.5)	22.9 (1.7)	
Mouth–left flank bouts	21.6 (2.2)	18.9 (1.3)	
Mouth–abdomen bouts	6.6 (1.3)	3.9 (.4)	*
Mouth–genitals bouts	.8 (.4)	.9 (.3)	
Mouth–tail bouts	.3 (.2)	.6 (.4)	
Mouth–right foot bouts	4.6 (.9)	4.3 (.3)	
Mouth–left foot bouts	4.4 (.8)	4.4 (.8)	
Right foot–head bouts	1.4 (.5)	2.4 (.6)	
Left foot–head bouts	2.1 (.8)	2.0 (.5)	
Brief pauses (number)	31.9 (4.0)	31.2 (2.2)	
Long pauses (number)	1.7 (.3)	2.4 (.3)	†
Body weight, test day	30.4 (.8)	30.0 (.8)	
Maximal	32.4 (.7)	32.0 (.6)	

Note. Numbers represent means and standard errors in parentheses ($n = 10$).
† $p = .05–.1$; *$p < .05$; **$p < .025$; ***$p < .01$ (Student's $t \pm$ test).

squares entered in a 5-min open field trial and the relative volume of the neocortex. Since the relative volume index is inversely related to total volume, their results agree with the present data.

High indices of locomotion and rearing activity in novel, open field situations are usually related to exploration and low emotionality while the performance of emotional rodents is slow with long periods of still and freezing behaviors (Eysenck & Broadhurst, 1964; Archer, 1973). The specific meaning of these terms in the context of spontaneous behaviors in laboratory settings is still unclear and the emotionality interpretation is debatable. Several emotional traits, such as assertion and care, may be manifested by these behavioral indices instead of fear or stress. For example, it has been reported that dominant mice produce multiple urinary marking patterns in novel environments in contrast to single large ones for subordinates (DesJardins, Maruniak, & Bronson, 1973). Accordingly, in this laboratory it has been found that dominance rank strongly affects maze exploration; the dominant mice being much slower during the initial contacts with the apparatus (Lopez-Lujan, Mondragon, Mayagoitia, & Diaz, 1982). Moreover, mice with different social ranks differ in their use of their living environment and in novel surroundings. For example, it was reported that social rank imposes long-lasting differential sets of behavior upon individual mice including a higher wheel-running activity for dominant mice (Mondragon, Mayagoitia, Lopez-Lujan, & Diaz, 1987). Therefore, some emotional traits related to social hierarchy could modify the weight of the brain through a differential use of the environment. The experiments of Rosenzweig et al. (1972) compared groups of rats exposed to enriched and unfurnished environments but never included intra-individual comparisons within a group. Their report that isolated rats in enriched environments did not show brain changes supports the hypothesis that social factors may indirectly influence brain features.

The possibility that two types of individual from the same species differ in brain biology is also suggested by experiments with humans. Using the Standarized Personality Inventory, Mathew, Weinman, and Barr (1984) found significant inverse correlations between the extraversion/introversion score and brain blood flow in 51 women, and Robinson (1986) found differences in thalamo-cortical arousability. In general, the hypothesis of a relationship between somatic types and behavioral profiles is central in constitutional psychology (Sheldon and Stevens, 1944), but it has been difficult to prove in humans due to the unreliability of the measurements (Pivinicki, 1964). This type of hypothesis can be more adequately tested in animals since both spontaneous behavior and brain parameters can be measured with relative precision.

The present results also suggest that individuals displaying high incidences of certain behaviors that can be an expression of the activity of discrete brain sites would have differences in the size of such sites. It is interesting

to recall that the correlations among the weights of the four dissected brain areas (brain stem, cerebellum, diencephalon, and telecephalon) were lower than .15 and all negative in the case of the brain stem. Wimer et al. (1969) reported a correlation of .43 between hippocampal volume and total forebrain volume in mice and suggested a genetically independent regulation system for specific brain structure size in addition to a system controlling the general size and structure in the entire cerebrum. The present results emphasize the importance of an independent regulation system and, furthermore, suggest a nongenetic component in the development of specific brain structures. Thus, the correlations and clusters between specific behavioral parameters and regional brain weights suggests a dynamic relation between the size of discrete brain sites and the expression of particular behaviors.

High values for freezing, still behaviors, and departure latencies—which are seemingly more related to emotionality in terms of stress and fear than locomotion—correlate with the weight of the diencephalon. The weight of the diencephalon is more specifically related to poststartle grooming behaviors (Factor 16) and departure latencies (Factor 18) than the weight of the telencephalon. In general, the cerebellum follows the correlation patterns of the whole brain, except for specific clusters with distance traveled swimming and capture order, two behaviors that may indicate motor coordination. The weight of the brain stem is specifically related to induced grooming durations and some posterior induced grooming actions. The functional role of hypothalamic and diencephalic structures in emotional behavior, of the cerebellum in motor coordination, and the brain stem in basic and reflex activities have been widely documented, but these results would suggest a morphological relationship. Such a relationship was one of the four main claims in Gall's Phrenology (Gall, 1825) which was initially strengthened with qualitative descriptions of cerebral cortex shape and size in necropsy brains of unusual individuals made by pioneer neuroanatomists (Nieto & Nieto, 1978) but later rejected because it conflicted with a long prevailing notion of an anatomically static brain. At present this hypothesis is also feasible since the weight of some discrete brain sites undergoes gross changes in relation to the occurrence of certain behaviors (Nottebohm et al., 1982).

Since a single strain of mice was used, care was taken to submit them to the same environment throughout their lives, and a long time elapsed between obtaining behavioral indices and necropsy; individual differences in behavioral profiles and brain weights may be attributed to social variables. Thus, the relations among (a) exploratory behavior, (b) whole and regional brain weights, and (c) social dominance rank in isogenic individuals seem worthy of further analysis.

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