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Multivariate Analysis of Temporal Descriptions of Open-field Behavior in Wild-derived Mouse Strains

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The open-field test is a commonly used apparatus in many behavioral studies. However, in most studies, temporal changes of details of behavior have been ignored. We thus examined open-field behavior as measured by both conventional indices and 12 ethograms supported by detailed temporal observation. To obtain a broader understanding, we used genetically diverse mouse strains: 10 wild-derived mouse strains (PGN2, BFM/2, HMI, CAST/Ei, NJL, BLG2, CHD, SWN, KJR, MSM), one strain derived from the so-called fancy mouse (JF1), and one standard laboratory strain, C57BL/6. Conventional measurements revealed a variety of relationships: some strains did not show the hypothesized association between high ambulation, longer stay in the central area, and low defecation. Our ethological approach revealed that some behaviors, such as freezing and jumping, were not observed in C57BL/6 but were seen in some wild-derived strains. Principal component analysis which included temporal information indicated that these strains had varied temporal patterns of habituation to novelty.

KEY WORDS: Open-field behavior; wild mice; ethological; temporal analysis; principal component analysis; strain differences.

INTRODUCTION

The open-field test is widely used apparatus in psychology, pharmacology, and genetics. In this test, an animal is simply placed into a novel, brightly lit arena from which escape is barred: the ambulation and the number of defecation in the field are often measured (Hall, 1934). The open-field test has been mainly validated in the rat, and it has been expected that the ambulation and the defecation have negative correlation (Broadhurst, 1957; Hall, 1934, 1936). Nevertheless, the test has also been widely used in mouse studies. In both cases, either via selection experiments (Broadhurst, 1960; Defries and Hegman, 1970; Hall, 1951; Makino, 1983; Ramos *et al.*, 2003) or QTL analyses (Fernandez-Teruel *et al.*, 2002; Flint *et al.*, 1995; Gershenfeld *et al.*, 1997; Gershenfeld and Paul, 1997; Talbot *et al.*, 1999; Turri *et al.*, 2001a, 2001b; Ramos *et al.*, 1999), genetic factors have been shown to make a large contribution to phenotypic variation in various measures of open-field behavior. Recently, the reverse genetic approach, such as use of transgenic or knock out/in mice, has shown that modification of specific genes affects this behavior. In spite of its popularity, however, interpretation of open-field indices is not fully validated in species other than

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rat, such as chicken (Gallup *et al*., 1976), guinea pig (Suarez and Gallup, 1982), and mouse (Blizard, 1971; Bruell, 1969; Collins, 1966). For example, it has been suggested that the defecation response, which has been shown to decrease over trials in the rat (Hall, 1934), changes in the opposite direction in specific mouse strains (Collins, 1966). Also, the relationships among open-field indices vary according to strain (Thompson, 1953). Thus, the interpretation of the results of this test in mice requires careful consideration.

When the animals are placed into the open-field, they show a series of behaviors such as sniffing, rearing, running and grooming. An animal's behavior is complex, consisting of many particular patterns of bodily movement, and these patterns of behavior change with time. However, these behaviors are ignored in many tests. Some researchers have stressed the importance of describing the animals' behavior in detail (Bindra, 1961; Gray, 1965; Streng, 1971; van Abeelen, 1963). They demonstrated that the frequency and pattern of the temporal changes of each behavior (sniffing, rearing, grooming, freezing, etc.) were strain-dependent (Crusio *et al*., 1989; Makino *et al*., 1991; Oortmerssen, 1971; Streng, 1971; Vadasz *et al*., 1992), and differed by sex (Gray, 1965) or context (Bindra and Spinner, 1958, Oortmerssen, 1971) in both the rat and the mouse.

To deal with this complexity, an ethological approach has been adopted and applied to elucidate drug effects (Antoniou *et al*., 1998; Blanchard *et al*., 1993; Choleris *et al*., 2001). This is thought to be a more sensitive way of detecting differences in pharmacological effects, since drugs that have a similar effect on overall levels of ambulation showed different effects on discrete behaviors (Antoniou and Kafetzopoulos, 1991). The manner in which behavior changes over time also needs to be considered. Animals change their behavior as they become more familiar with the situation; these intra-session changes are believed to reflect psychological changes in the animal, just as has been previously argued with regard to inter-session changes (Vadasz *et al*., 1992, Walsh and Cummins, 1976). Makino and his colleagues performed a principal component analysis of ethological measurements of open-field behavior using four mouse strains, C57BL/6, DBA/2, C3H, and BALB/c, and demonstrated that the temporal changes in those component scores clearly differed in those strains (Makino *et al*., 1991). Nevertheless, most studies tend to use the summation of the frequency of each behavior in the test period as the primary index for this test.

We have been continuing work on the Mishima battery of wild mouse strains. These mice were established as inbred strains derived from wild mice. These inbred strains are known to have a wide genetic diversity by examining the variation of microsatellite markers (Koide *et al*., 1998, 2000) or triplet repeats (Ogasawara *et al*., 2005), since they were captured from many countries and belong to several different subspecies (Bonhomme and Guenet, 1996; Moriwaki, 1994). Wild-derived strains have been shown to exhibit enormous behavioral differences from standard laboratory inbred strains and also within wild-derived strains (Fernandes *et al*., 2004; Furuse *et al*., 2002a, 2002b, 2003; Holmes *et al*., 2000; Koide *et al*., 2000), and are expected to be a major resource for the genetic study of behavior. In the present study, we profiled their open-field behavior using detailed temporally based observations and performed principal component analyses to identify the relationship between ethological measurements and conventional variables, and revealed the temporal patterns of their open-field behavior.

METHODS

Animals

Twelve inbred strains were used in this study. Ten inbred mouse strains, PGN2, BFM/2, HMI, NJL, BLG2, CHD, SWN, KJR, MSM and JF1, were established as inbred strains after 20 generations of brother–sister mating at the National Institute of Genetics (NIG, Mishima, Japan). The place of collection and establishment of these strains has been reported previously (Furuse, 2002a; Koide *et al*., 1998, 2000). Because the coat color, *s*, gene which JF1 possesses is known to relate to auditory disability, we used a spontaneous revertant at this allele with a black coat color, JF1-s^+, in this study. C57BL/6J and CAST/Ei were secured from The Jackson Laboratory (Bar Harbor, ME, USA). All strains are maintained at NIG under a 12-h light/dark cycle (light from 8:00 to 20:00) in a temperature-controlled room (23 ± 2°C). The mice were housed in same sex groups (2–5 per cages) in standard sized plastic cages on wood shavings, and one week before the test, they were housed individually. Food and water were available *ad libitum*. Ten males and 10 females from each strain were used in this test at the age of 10 weeks.

Apparatus

The open-field consisted of a square arena (60×60 cm) made of white polyvinylchloride plastic board with walls 40 cm high. The arena was lit by incandescent lighting placed 90 cm above the arena. The light level was 365 lux at the center of the arena. The open-field was surrounded by a black curtain except for one side from where the experimenter directly observed the animal's behavior. For analyzing ambulation, the arena was continuously recorded by a video camera placed over its center and relayed to a video tracking system (Image OF, O'hara & Co. Ltd., Tokyo, Japan).

Procedure

At least 1 h before the beginning of the test, animals were brought into the test room to minimize the effect of transfer. Each mouse was gently picked up by its tail with tweezers and placed in the same corner of the open-field. During the 10-min trial, their behavior was observed directly and recorded using the multi-dimensional sampling method (Makino *et al.*, 1991). The behaviors collected included the following 12 behavioral items. The presence or absence of each behavior was recorded as 1/0 in each 5-s period.

Sniffing: sniffing the arena and air, identified by characteristic movements of nose and whiskers.
Locomotion: walking and running around the arena.
Stretched attend posture (stretching): stretching its whole body forward while keeping the hindlimbs in place.
Leaning-against-wall (leaning): standing on the hindlimbs with the forelimbs against the wall.
Rearing: standing on the hindlimbs without touching the wall.
Grooming: licking and/or scratching its fur, licking its genitalia and tail.
Face-washing: scrubbing its face with the forelimbs, not followed by grooming.
Digging: trying to dig a hole in the arena or the corner of the wall.
Gnawing: gnawing mainly on the corner of the wall.
Jumping: jumping vertically.
Pausing: a brief moment of inactivity.
Freezing: stationary state lasting more than 3 s regardless of posture.

At the end of the sessions, the number of defecations and presence of urination were recorded, after which the arena was cleaned with a damp cloth. All tests were carried out during the light period (13:00–17:00).

Statistical Analysis of Behavioral Data

The arena was divided into 16 squares (4×4), and the number of squares transited was counted as ambulation by the computer software (Image OF). Ambulation in the central area has also been used, since rodents naturally avoid staying in open spaces. This is known as thigmotaxis (Treit and Fundytus, 1989). We also calculated the percentage of central ambulation (the number of transitions in the central 4 blocks/total 16 blocks $\times$ 100). The three measures —ambulation, percentage of central ambulation, and the number of fecal pellets—and the frequencies of behavioral items were subjected to one-way ANOVA. Most variables did not show any sex differences, thus, we decided not to deal with the effect of sex in the analysis, and combined data across males and females. More detailed data including sex differences are available on web site (http://www.nig.ac.jp/labs/MGRL/eMGRL_behavior_OF.html). Because individual differences with respect to urination were very large, statistical analysis was not performed on this index. *Post hoc* comparisons were carried out using the HSD test ($p < 0.05$).

The frequencies of behavioral items for 10 min were also subjected to one-way ANOVA. In some behavioral items, locomotion, stretching, leaning, and rearing, a minute-by-minute frequency was calculated to analyze the temporal factor, and 1-way ANOVA for repeated measures of continuous variable (time) was also performed on their frequency.

Principal Component Analysis

A principal component analysis (PCA) was performed using SYSTAT version 9 (SPSS) software packages. Means were calculated for each minute in each variable, and those minute-by-minute scores were subjected to the PCA. That is, 10 (min) $\times$ 11 (behavioral items) matrix was made in each animal. By using two main components extracted from the PCA, open-field behaviors in each 12 mouse strains were evaluated. The multiple-dimensional representation of PCA score has been performed often in morphological study (e.g. Pan and Oxnard, 2004),

and this is a useful way to compare the characteristics of each strain. The average of the one-minute component score of each sex in a strain was calculated and plotted in two-dimensional coordinate space made by two main components.

RESULTS

Conventional Variables

Data in three conventional variables (open-field ambulation, percentage of central ambulation, and the number of defecations over 10 min) are summarized in Table I. ANOVA revealed significant main effects of strain on all three measures [all $F(11,228) \geq 12.982$, $p < 0.0001$]. In the ambulation, two wild strains, KJR and SWN, and a laboratory strain C57BL/6 were characterized as high activity strains, whereas the wild strain MSM and the fancy strain JF1 were low activity strains. Central ambulation was higher for the wild strains BLG2 and BFM/2, and the lowest in JF1. The wild KJR also showed relatively low central ambulation. The wild KJR and the fancy JF1 showed a high frequency of defecation, while it was low in the wild BFM/2 and the laboratory C57BL/6. The correlations between each conventional variable were calculated, and they were just modestly correlated. (Concerning phenotypic correlation: $r = -0.22$ for ambulation and defecation, $r = 0.43$ for ambulation and % central ambulation, and $r = -0.27$ for % central ambulation and defecation, $p < 0.001$ for all correlations). MSM, BFM/2 and BLG2 showed urination in many animals, while C57BL/6 and PGN2 rarely urinated.

Behavioral Measures

Total Frequency of Each Behavioral Measure

ANOVA indicated a significant effect of strain on the all 12 behavioral items [$F(11,228) \geq 6.766$, $p < 0.0001$]. Some of those results were summarized in Table I (for locomotion, stretching, leaning, and rearing, see Fig. 1). Sniffing was the most frequently observed behavior. Especially C57BL/6 showed this behavior very often, while CHD exhibited it less. C57BL/6 showed higher locomotion, while JF1 and MSM were lower. This was closely paralleled the results of total ambulation. CAST/Ei, NJL and JF1 showed higher stretching, while PGN2, BLG2, SWN, and KJR seldom showed this behavior. KJR exhibited the highest frequency of leaning. On the other hand, CHD and JF1 showed significantly lower leaning than other strains. Rearing was higher for PGN2, while JF1 seldom showed rearing. MSM showed the highest frequency of grooming, while six strains—C57BL/6, PGN2, BFM/2, HMI, CAST/Ei, and BLG2—showed this behavior infrequently. Face-washing was higher for BLG2, while it was low in the NJL, KJR, MSM, and JF1. Digging and gnawing appeared less frequently than other behavioral items. BFM/2 and BLG2 showed the highest frequency of digging and gnawing, respectively. On the other hand, HMI, CHD, and C57BL/6 seldom showed digging, and JF1 rarely exhibited either digging or gnawing. PGN2 showed the highest frequency of jumping, while C57BL/6, MSM, and JF1 seldom showed this behavior. Pausing was higher for MSM, JF1, and CHD, while it was low in the C57BL/6. In the freezing, JF1 showed the highest frequency of freezing, while five strains—CAST/Ei, NJL, BLG2, KJR, and C57BL/6—seldom or never showed freezing.

Temporal Changes of the Behavioral Measurements

The temporal changes in some behavioral items, locomotion, stretching, leaning, and rearing, are summarized in Figure 1. Repeated-measure ANOVA indicated significant strain $\times$ time interactions on those four measurements [$F(99,2052) \geq 2.126$, $p < 0.0001$].

Locomotion: Ten strains, except for the C57BL/6 and CAST/Ei, showed significant changes across time (for all except NJL, $p < 0.01$; NJL, $p < 0.05$). Most exhibited an initial increase in frequency, which then stabilized or in some showed a gradual decrease.

Stretching: Ten strains, except for KJR and PGN2, exhibited a significant effect of time (for all except BLG2, $p < 0.01$; BLG2, $p < 0.05$). Most showed the highest frequency during the first minute, which did not recur, but some strains, such as CAST/Ei, CHD and JF1, exhibited stretching later in the session.

Leaning: Eleven strains, except for CAST/Ei, showed significant changes across time ($p < 0.01$). The temporal changes were similar to those for locomotion, with most strains showing an initial increase, which then stabilized or gradually decreased.

Rearing: Ten strains, with the exception of CHD and JF1, showed a gradually increasing frequency (for all except CAST/Ei, $p < 0.01$; CAST/Ei, $p < 0.05$).

We had also analyzed the temporal changes in the conventional variables (ambulation and

Table I. Open-field Measures of a Total of 10 min

Strain	Total crossing	Central section crossing (%)	Defecation	Urination
C57BL/6	378.3 ± 8.8	16.6 ± 0.7	0.5 ± 0.3	2
PGN2	228.4 ± 6.3	13.7 ± 1.0	1.1 ± 0.4	1
BFM/2	324.5 ± 9.8	19.1 ± 1.4	0.9 ± 0.5	7
HMI	310.6 ± 11.3	12.0 ± 1.0	1.4 ± 0.4	4
CAST/Ei	328.6 ± 10.9	12.1 ± 1.6	3.2 ± 0.7	5
NJL	328.8 ± 12.2	12.3 ± 1.3	5.7 ± 0.8	7
BLG2	314.8 ± 8.3	19.9 ± 0.9	4.9 ± 0.6	13
CHD	247.4 ± 11.3	16.0 ± 2.1	2.2 ± 0.6	8
SWN	360.9 ± 12.6	14.9 ± 1. 6	2.5 ± 0.6	10
KJR	378.8 ± 12.0	9.4 ± 1.7	9.3 ± 1.0	8
MSM	200.2 ± 8.7	10.5 ± 1.0	3.9 ± 0.6	15
JF1	93.3 ± 9.1	2.1 ± 0.9	7.1 ± 0.6	9
Strain	Sniffing	Grooming	Face-wash	Digging
C57BL/6	118.4 ± 0.3	1.7 ± 0.5	5.3 ± 0.8	0.2 ± 0.1
PGN2	107.9 ± 2.1	1.3 ± 0.4	6.9 ± 0.9	1.7 ± 0.4
BFM/2	114.0 ± 1.8	1.4 ± 0.7	8.1 ± 1.0	7.2 ± 1 .7
HMI	109.0 ± 1.9	0.2 ± 0.1	9.6 ± 0.8	0.1 ± 0.1
CAST/Ei	117.6 ± 0.4	2.4 ± 0.7	4.7 ± 0.5	1.7 ± 0.7
NJL	103.6 ± 2.2	13.9 ± 2.7	3.9 ± 0.6	2.5 ± 0.8
BLG2	116.2 ± 0.8	2.0 ± 0.6	13.5 ± 1.4	2.7 ± 0.7
CHD	96.7 ± 3.7	12.5 ± 2.5	4.5 ± 0.8	0.3 ± 0.2
SWN	110.9 ± 2.0	3.0 ± 0.8	7.1 ± 0.9	4.8 ± 1.4
KJR	108.5 ± 1.5	4.7 ± 1.0	3.7 ± 0.6	4.3 ± 0.9
MSM	100.8 ± 2.8	18.8 ± 2.2	3.7 ± 0.5	1.6 ± 0.6
JF1	105.3 ± 2.2	9.2 ± 1.7	4.3 ± 0.6	0.1 ± 0.1
Strain	Gnawing	Jumping	Pausing	Freezing
C57BL/6	0.8 ± 0.2	0.1 ± 0.1	3.9 ± 0.8	0.0 ± 0.0
PGIM2	3.7 ± 0.8	46.3 ± 2.8	21.6 ± 1.4	10.9 ± 2.3
BFM/2	4.4 ± 0.8	3.7 ± 1.0	7.4 ± 1.1	4.2 ± 1.8
HMI	1.2 ± 0.3	19.4 ± 3.6	15.4 ± 1.9	8.2 ± 2.3
CAST/Ei	4.5 ± 0.6	2.1 ± 0.7	12.9 ± 1.7	0.2 ± 0.1
NJL	4.9 ± 0.9	11.1 ± 2.4	12.4 ± 1.3	1.4 ± 0.6
BLG2	6.3 ± 1.3	3.0 ± 0.8	12.3 ± 1.7	1.0 ± 0.3
CHD	2.5 ± 0.8	1.0 ± 0.5	26.1 ± 3.4	1 1.0 ± 2.7
SWN	2.4 ± 0.7	9.0 ± 1.5	14.5 ± 2.1	5.5 ± 1.5
KJR	3.0 ± 0.8	19.9 ± 2.9	12.2 ± 1.5	1.8 ± 0.5
MSM	1.8 ± 0.4	0.2 ± 0.1	28.6 ± 3.3	5.0 ± 1.5
JF1	0.1 ± 0.1	0.0 ± 0.0	28.2 ± 2.0	16.3 ± 3.7

All data, without urination, presented as mean values ± SEM. For urination, the digit shows the number of animals urinating. For calculating the frequency of the behavioral items, presence or absence of each behavior was recorded as 1/0 in each 5-s period. Number of animals = 10 male and 10 female in each columns.

percentage of central ambulation) and all other behavioral measurements, and found there were significant strain×time interactions for most of them. Temporal data of those behaviors are also available on the web site: http://www.nig.ac.jp/labs/MGRL/eMGRL_behavior_OF.html

Principal Component Analysis

To identify the relationship between these behavior items and conventional variables, and to extract some common components underlying the open-field behavior of all strains, we performed principal component analysis (PCA). To add a temporal dimension to each component, we included minute-by-minute scores for each variable. Some variables which showed a low frequency (digging, gnawing) and which had no temporal information (defecation) were excluded. Because ambulation (number of crossings) and locomotion (instantaneous assessment of ambulation) were closely correlated ($r = 0.84$), we chose only ambulation to represent

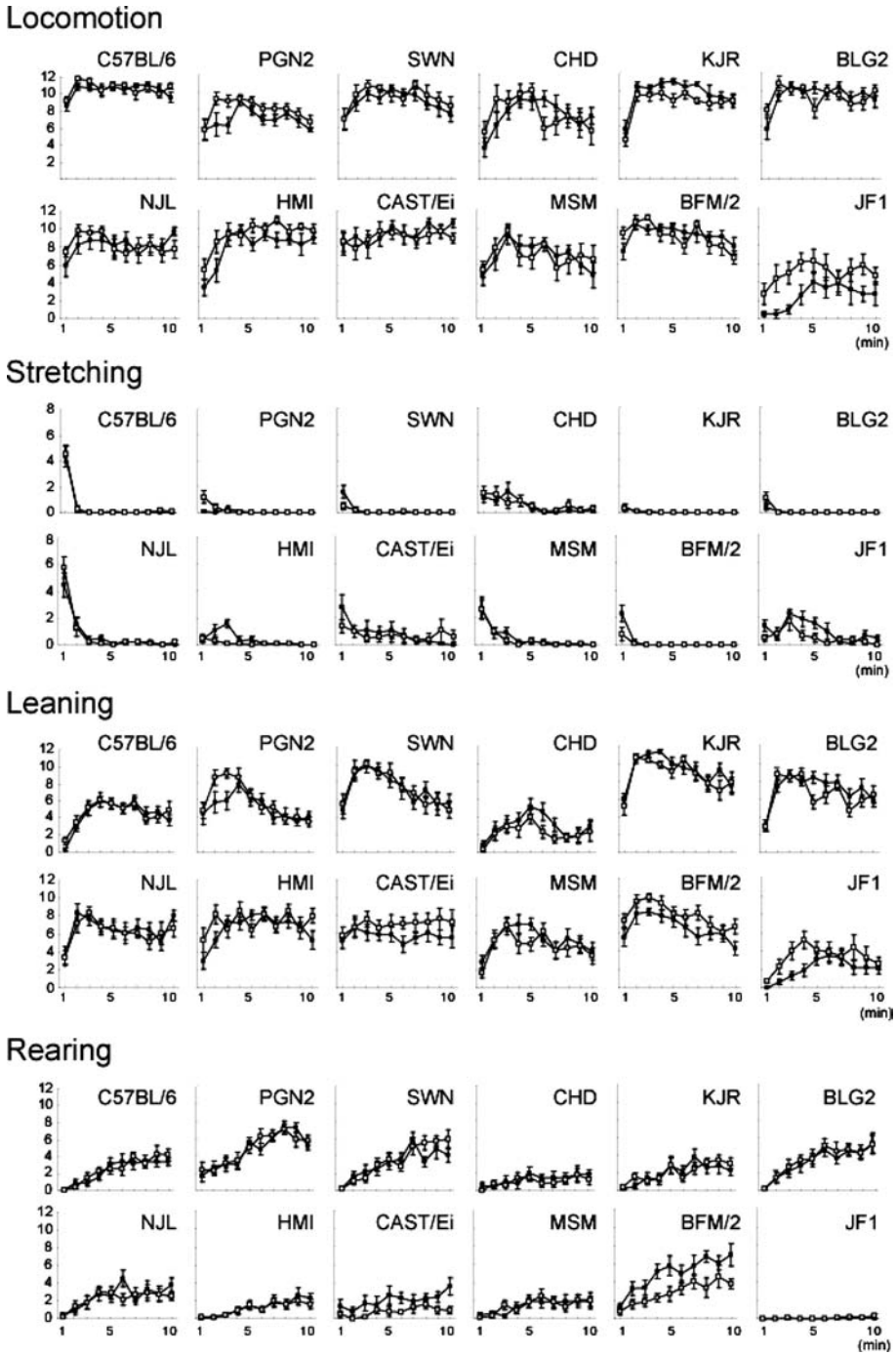

Fig. 1. The temporal changes of each behavioral component. For calculating the frequency, the presence or absence of each behavior was recorded as 1/0 in each 5-s period, and summed for each minute. Filled boxes and open boxes indicate the frequency of each one-minute period of behavior in males and females, respectively. Temporal patterns of other behavioral data are available on the web site: http://www.nig.ac.jp/labs/MGRL/eMGRL_behavior_OF.html.

locomotor activity. Therefore, a matrix containing 11 variables (in columns) and the frequency of each behavioral variable in each 1 min (that is, 10 rows for each animal) were subjected to analysis.

Five components (PC) were extracted with an eigenvalue higher than 1, which together accounted for 72.3% of the total variance (Table II). The significant variables that positively loaded highly on PC1 were freezing, whereas ambulation, sniffing and leaning loaded negatively. PC2 had positive loadings from sniffing and stretching, but negative loadings from rearing and grooming. PC3 had a positive loading from leaning, but negative loadings from rearing and percentage of central ambulation. PC4 had simple negative loadings from pausing and jumping. PC5 had positive loadings from grooming and face-washing, but a negative loading from freezing.

Some of these measurements indicated heteroscedasticity in a statistical analysis, since several behaviors were never observed in some strains or in certain periods. For example, stretching was not exhibited later in the session. However, for applying PCA, it is desirable that each behavioral data have homogeneity of variance. To deal with this controversial point, we decided to confirm the relation between behavioral items that were loaded by the same components, because PCA relies on the correlations between behavioral items. To test the relationships, Kendall's rank correlations were calculated on the mean frequency of each behavioral item in each strain for each one-minute period. This statistical analysis was performed on both sexes. It was revealed that ambulation and leaning were positively correlated (males: $\tau = 0.50$, $p < 0.01$; females: $\tau = 0.42$, $p < 0.01$) whereas ambulation and freezing exhibited negative correlation (males: $\tau = -0.51$, $p < 0.01$; females: $\tau = -0.41$, $p < 0.01$), as expected from the structure of PC1. Furthermore, stretching and rearing (males: $\tau = -0.57$, $p < 0.01$; females: $\tau = -0.57$, $p < 0.01$) or grooming in females ($\tau = -0.19$, $p < 0.01$) were negatively correlated, which also supports the relation in PC2. We therefore decided to use these components from the PCA for the following analysis.

To evaluate the character of the 12 mouse strains, we next represented their open-field behaviors using two main components, PC1 and PC2. The average of the one-minute component score of each sex in a strain was calculated and plotted in two-dimensional coordinate space made by PC1 and PC2 (Fig. 2). In this way, we were able to view the temporal structures as a pattern.

C57BL/6 first showed a high PC1 and PC2 score. Both substantially decreased from one through 2 min. Their behavior then stopped changing at a particular state, designated a "stabilized state", until the end of the session. That is, the open-field behavior of C57BL/6 consisted of a high stretching tendency at first, which quickly diminished, then continued to show locomotor activity such as ambulation and leaning. Many strains showed a similar temporal pattern, with some important alterations in the level of behavior at first, then holding a "stabilized state" for the remainder of the time. However, PGN2, SWN, KJR, and BLG2 seldom exhibited any initial decrease in PC2 score, but showed a very modest

Table II. Eigenvectors and Eigenvalues from Principal Component Analysis

	Component				
Items	1	2	3	4	5
Ambulation	**−0.45**	−0.01	0.20	0.20	−0.17
% of central ambulation	−0.28	−0.29	**−0.45**	0.27	−0.22
Sniffing	**−0.42**	**0.42**	−0.20	0.02	0.10
Stretching	0.18	**0.44**	−0.34	0.25	−0.11
Leaning	**−0.39**	0.14	**0.49**	−0.11	0.06
Rearing	−0.29	**−0.42**	**−0.35**	−0.13	−0.08
Grooming	0.29	**−0.40**	0.24	0.29	**0.39**
Face-washing	−0.09	−0.23	−0.32	−0.17	**0.53**
Jumping	−0.17	−0.17	0.11	**−0.65**	−0.13
Pausing	0.18	0.31	−0.26	**−0.46**	0.27
Freezing	**0.35**	−0.12	−0.04	−0.22	**−0.60**
Eigenvalue	2.95	1.59	1.28	1.10	1.02
Variance %	26.83	14.48	11.62	10.09	9.23

Factor loadings >0.35 are boldfaced.

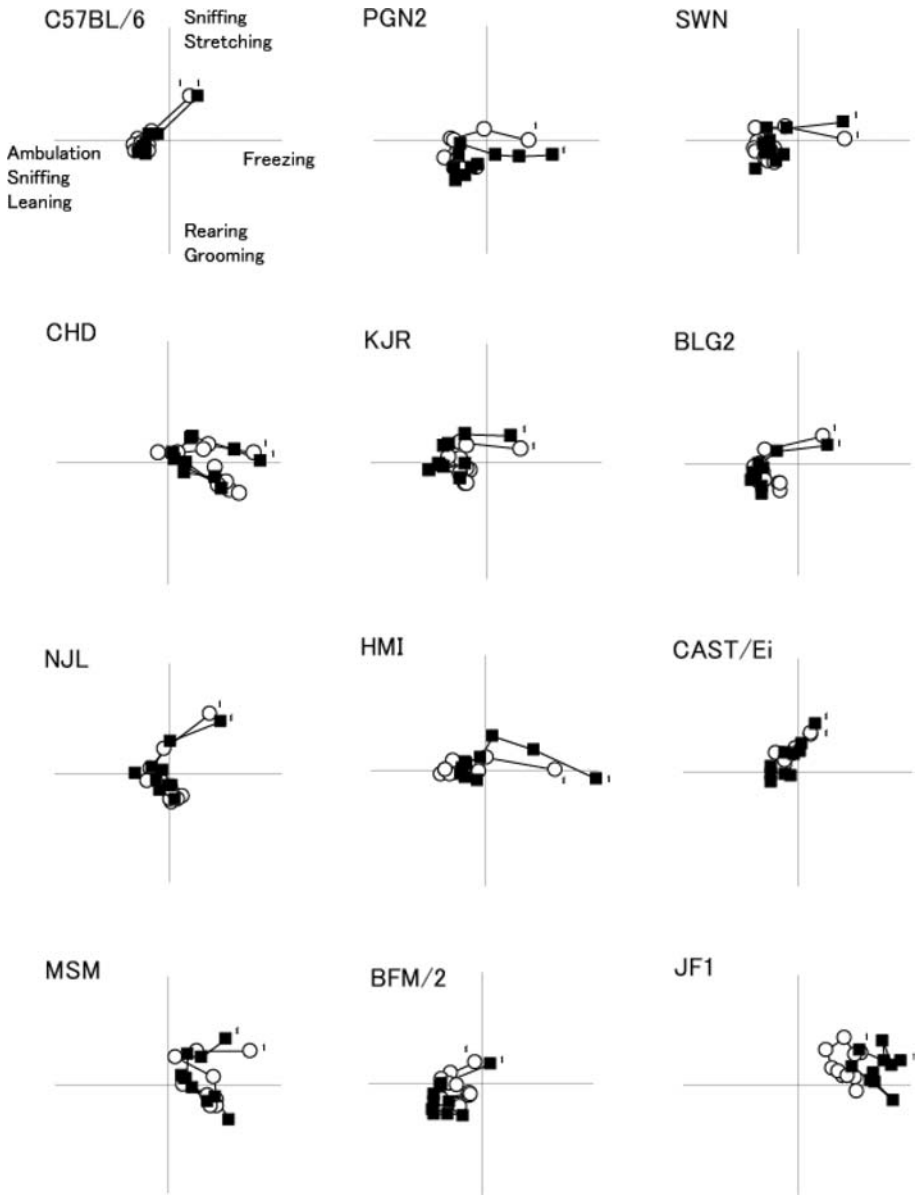

Fig. 2. Two-dimensional representation of open-field behavior. Each axis was derived by principal component analysis (*x*: PC1, *y*: PC2; see Table II). Each point represents mean of the component scores of each one-minute block. Initial one-minute blocks are indicated "1" by the side of the point.

decrease toward the end. There were also differences in the latency of achieving the stabilized state, PGN2, NJL and HMI took longer than C57BL/6. Moreover, CAST/Ei showed a gradual decrease throughout the session.

CHD exhibited an entirely different temporal structure. They showed a marked decrease in the first half of the session in PC1 score but not in PC2, just like PGN2. However, they did not subsequently reach a stabilized state, and instead continued to change their behavior in a different direction, showing increased PC1 scores and decreased PC2 scores. That is, the behavior of CHD was characterized by a major freezing tendency at first, but shifted toward locomotor activity through the middle of the session, and then began to show a grooming tendency with decreased locomotor activity. The same pattern was found in MSM and JF1. However, MSM had a higher PC2 score than CHD at the start of the session, and JF1 continued to maintain a higher PC1

score throughout the session. Sex differences were not prominent in most strains.

DISCUSSION

In open-field tests, it has been expected that animals that show relatively low total ambulation have low central ambulation and high defecation in the rat (Hall, 1934, 1936). Although this test has not been thoroughly validated in the mouse (Blizard, 1971; Collins, 1966), these indices are widely used in mouse studies as well. We first investigated the relationship of these traditional variables in the present battery of strains. The ambulation data indicated that KJR, SWN, and C57BL/6 were characterized as high activity strains, while MSM and JF1 were low activity strains. A number of studies have reported that C57BL/6 shows higher activity in the open-field when compared with other laboratory strains (Crawley *et al*., 1997; Logue *et al*., 1997; Marks *et al*., 1986; Streng, 1971; Thompson, 1953). The present study suggests that C57BL/6 showed high ambulation even compared to wild-derived mouse strains. This strain also showed higher frequency of central ambulation and lower defecation in the field. Meanwhile, JF1 showed the opposite pattern: low activity, low central ambulation, and high defecation. These results are consistent with the traditionally expected relationships between the open-field indices. However, there were many exceptions. Such as, wild-derived KJR mice, which were also high activity strain in the open-field, exhibited a lower level of central ambulation and the highest defecation of all strains. Some other strains also exhibited inter-relationships between those measurements that are inconsistent with Hall's original proposal (Table I). Because some strains showed the expected relationship between those measurements but some strains did not, there were only modest correlations between them. While the traditional association between key indices of open-field behavior has already been questioned (Archer, 1973), the present study provides ample evidence of a variable relationship between these measures within strains in the Mishima battery.

Detailed observations of open-field behavior revealed the temporal character of each behavioral component. In most strains, stretching was exhibited during the first few minutes but was seldom evident later in the session. This behavior was considered related to risk assessment and approach-avoid conflict (Blanchard *et al*., 1991; Carola *et al*., 2002; Rodgers and Johnson, 1995); therefore C57BL/6 and NJL, who were characterized by a high level of stretching early in the session, were thought to have a high risk-assessment tendency at the beginning of the session. By comparison, CAST/Ei and JF1 kept this behavior throughout the session, so these strains might need a longer time to reduce the strong tension that induced their risk assessment behavior. In other words, they took longer to get used to the situation. Leaning and rearing, both standing postures, had different temporal changes. The temporal pattern of leaning was fairly similar to the behavioral component of "locomotion". These two behaviors, leaning and locomotion, were frequently observed concurrently in a short segment of time (5 s) in the open-field (Makino *et al*., 1991). Thus, leaning is thought to have a close relationship to locomotor activity. In contrast, rearing showed a gradual increase throughout the session. A similar temporal pattern has been reported in previous studies, and rearing has been viewed as a behavior expressed when animals habituate to the environment (Vadasz *et al*., 1992). Most strains demonstrated an increased pattern of rearing over time, whereas JF1 never showed this behavior. This again suggests the difficulty of characterizing habituation in JF1. However, we need longer periods of observation before concluding this, as there is a possibility that the JF1 strain exhibits different behavioral patterns in the habituated situation.

Some behavioral components were characteristic of or specific to several strains. Grooming was characteristic in MSM, and they exhibited long bouts of grooming late in the session (data not shown). Meanwhile, jumping was especially pronounced in PGN2. This strain also exhibited a high frequency of rearing but not locomotion; therefore, PGN2 mice were very active only on the vertical axis. It was previously reported that some behaviors, freezing and jumping, were observed in many wild-derived strains but not in laboratory strains (Fernandes *et al*., 2004; Holmes *et al*., 2000). In the present findings, again, laboratory strain C57BL/6 seldom exhibited these behaviors. There may be some differences in the behavioral patterns exhibited in a novel situation between laboratory and wild-derived strains. Thus, it seems that the effect of domestication is associated with a change in their behavior, however, there was also large variety within the wild strains. Some sophisticated studies proposed that the behavioral differences between strains reflect the ecological context in which the strains evolved (Dudek *et al*., 1983; Oortmerssen, 1971). We may conclude therefore that the strain differences of the open-field

behavior reflects both the process of domestication as well as their natural ecological context. Taking these data together, it is assumed that each behavioral component reflects a particular psychological state (especially when considered within a temporal context) but the expression of particular behaviors must also be considered within their strain-specific context.

To find the fundamental structure that underlies the open-field behavior of various mouse strains, we conducted a principal component analysis (PCA) of ethological measurements. To include temporal information in the structure, we performed PCA of minute-by-minute data. PC1, the first component was described as "locomotor activity" because it correlated negatively with ambulation and leaning and positively with freezing. Some factor analyses of open-field behavior interpreted the factor describing these behaviors in terms of general motor activity (Carola *et al*., 2002; Pardon *et al*., 2000; Royce, 1977; Trullas and Skolnick, 1993). However, because of the direction of loading, a high PC1 score means low "locomotor activity". PC1 explains the greatest variance, and is thought to be the main component of open-field behavior in the present battery of strains. A similar factorial structure, though pausing is negatively loaded instead of freezing, was reported of using four laboratory inbred mouse strains (Makino *et al*., 1991). Sniffing and stretching loaded positively on PC2 and rearing and grooming negatively. The positively correlated stretching tended to occur during the first few minutes but was seldom seen later. On the other hand, both rearing and grooming rarely appeared at first but gradually increased toward the end of the session. It is therefore important to consider temporal information when interpreting PC2. An animal adjusts gradually to a novel environment over time. PC2 thus describes the behavioral pattern of "habituation". Also, sniffing is a basic behavior in response to novelty in rodents (Espejo, 1997), and, as previously mentioned, stretching represents risk assessment. Both are exploratory behaviors for gathering information on the environment. PC2 is also believed to be associated with the exploratory tendency. Because mice are heavily reliant upon olfaction, sniffing is a principal element of mouse behavior in novel situations (Espejo, 1997). Thus, this behavior was heavily loaded on both PC1 and PC2. Leaning (observed beside the wall) loaded positively on PC3 and the frequency of central ambulation and rearing (often seen in the central area of the field) negatively. This component was considered as simply reflecting the place an animal was in, so we describe it as "thigmotaxis". PC4 was hard to interpret and PC5 might simply reflect the behavior characteristics seen when animals stay in one place (grooming or freezing). In the present study, it was revealed that many psychological factors are included in the open-field behavior. This idea makes it possible to explain the previous non-existence of correlation among the conventional measurements.

The temporal pattern of each mouse strain was also examined by representing their open-field behavior in two-dimensional space, derived from principal component analysis. Some strains, such as C57BL/6, showed a substantial behavioral change during the first few minutes, which then stabilized. When an animal is placed in the open-field, it experiences strong stress as a result of being picked up by the tail and placed in a novel situation. Thus, they are in the most agitated state at the beginning of the session. It is hypothesized that the first behavioral change reflects the reduction of emotion following initial intensive exploration of the environment. Along with the exploration, they gather information about the environment which causes animals to habituate to it. The stabilized behavioral state after the initial reaction may therefore reflect the state of animals after habituation to the situation. C57BL/6 exhibited comparatively higher locomotor activity in this state, although it actually showed lower activity in its habitual environment (Koide *et al*., 2000). This is because even if an animal adjusts to the environment, the aversive property of the open-field, a brightly illuminated open space, remains constant (Dulawa *et al*., 1999). We assume that in each strain, the time taken to arrive at the stabilized state reflects the time required to attenuate strong emotions and habituate to the novelty. From this point of view, C57BL/6 was categorized as one of the fastest strains to habituate, whereas some strains (PGN2, NJL, and HMI) needed a longer time.

Some strains, such as CHD and MSM, did not enter a stabilized state but rather showed different temporal changes between the first half and last half. They first showed increased locomotor activity (PC1), but it decreased later in the session. They also exhibited a reduction of PC2 (i.e., increased grooming tendency) with the latter change, especially in MSM. The first behavioral change is thought to be due to the same psychological changes as exhibited by C57BL/6, i.e., a reduction of intense emotional response. However, they did not maintain that behavioral state throughout, so we hypothesize that they arrive at a stabilized state at a different point. In

a preliminary study, we used a longer period of examination (30 min) of open-field behavior, and revealed that MSM reached a stabilized state after around 10 min, while C57BL/6 displayed the same behavior during the extended session. Thus, CHD and MSM are strains characterized by freezing or grooming behavior in an aversive situation.

Finally, by describing open-field behavior in detail and examining temporal changes, we were able to identify the prominent behavioral features of each strain. The results of this study make it possible to assert that conventional simple measurements lose substantial information, such as temporal changes and the variety of behaviors that can be displayed, and the use of too few indices might easily lead to confusion in interpreting the genetic mechanisms underlying open-field behavior or "emotionality". In any case, inclusion of wild-derived strains in our behavioral battery has provided a broader understanding of the behavior of mice, recognition that many behavioral responses have been differentiated during domestication or adaptation to the ecological context, and the need to take such differences into account when considering the exploratory behavior/fear measured under standard test conditions.

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