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The third component, or intangible variation, is relatively consistent along time in mice of the C56Bl/6J strain

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C57Bl/6J mice displayed considerable inter-mice variation in some behaviors in the open-field and the elevated plus-maze (coefficients of variation from 30% to 200%). Those individual differences were not entirely random, because four successive measurements of each behavior were positively correlated. Application of the aggregation principle revealed that (i) in males and females, odd-even correlation coefficients for ambulation (in the open-field and in the plus-maze) were of the order of 0.60, (ii) the odd-even correlation coefficient for defecation in the open-field was 0.74 for males and 0.23 for females (not significant), (iii) the odd-even correlation coefficient for the antibody response (to immunization with aggregated bovine serum albumin) was 0.43 for males and 0.60 for females. In males, but not in females, these variables were intercorrelated: ambulation in the open-field, ambulation in the enclosed arm of the plus-maze, and defecation in the open-field; the antibody response was uncorrelated with behaviors.
Keywords: *Open-field, elevated plus-maze, intangible variation, C57Bl/6, ambulation, antibody response, defecation.*

El tercer componente, o variación intangible, es relativamente consistente a lo largo del tiempo en los ratones de la cepa C57Bl/6J

Los ratones de la cepa C57Bl/6J muestran considerable variación entre ellos en las conductas en el campo abierto y en el laberinto elevado en forma de + (coeficientes de variación de 30% a 200%). Esas diferencias no son aleatorias, ya que cuatro medidas de la misma conducta correlacionan positivamente

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entre sí. La aplicación del principio de agregación muestra que (i) en machos y en hembras, el coeficiente de correlación par-impar de la deambulación, en el campo abierto y en el laberinto elevado, es aproximadamente 0,60; (ii) el coeficiente de correlación par-impar para la defecación en el campo abierto es 0,74 en los machos y 0,23 para las hembras (no significativo), (iii) el coeficiente de correlación par-impar para la respuesta de anticuerpos (a la inmunización con seroalbúmina bovina) es 0,43 en los machos y 0,60 en las hembras. En los machos, pero no en las hembras, las variables siguientes están correlacionadas: deambulación en el campo abierto, deambulación en el brazo cerrado del laberinto elevado, y defecación en el campo abierto. La respuesta de anticuerpos no correlaciona con las conductas.

Palabras clave: *campo abierto, laberinto elevado, variación intangible, C57Bl/6, deambulación, respuesta de anticuerpos, defecación.*

Introduction

Rodents of an inbred strain display large variations in several characteristics: body weight (Dawson, 1970; Gärtner, 1990; Tamashiro et al., 2003), kidney weight (Gärtner, 1990), mandible shape (Festing, 1976), sucrose preference (Strekalova & Steinbusch, 2010), fear conditioning (Siegmund, Kaltwasser, Holsboer, Czisch, & Wotjak, 2009), decrease in social interaction after social defeat (Krishnan et al., 2007). This variability, which cannot be ascribed to genetics or environment, has been termed intangible variation, developmental noise (Falconer, 1989; Blewitt, Chong, & Whitelaw, 2004) or third component (Gärtner, 1990). In previous studies (Vidal, 2013, 2014), it was noticed that (i) mice of a given inbred strain (Balb/c or C57Bl/6) showed large differences in ambulation and defecation in the open-field, and (ii) those individual differences held along time. Besides, some variables measured in the open-field were not correlated with an immunological variable (antibody response; Vidal & Rama, 1994).

Animals may have personality (Gosling, 2001), and the expression of that personality could be a set of correlated behaviors (behavioral syndromes; Sih, Bell, & Johnson; 2004). Accordingly, it was expected that (i) ambulation of the mice in a relatively safe environment (near the wall of the open-field) correlated with ambulation in another safe environment (enclosed arms of the elevated plus-maze), and (ii) ambulation of the mice in an exposed environment (in the inner part of the open-field, away from the wall) correlated with ambulation in another exposed environment (open arms of the elevated plus-maze). In fact, thigmotaxis (i.e., the tendency of the mouse to stay close to the wall) is used to assess anxiety (Choleris, Thomas, Kavaliers, & Prato, 2001; Simon, Dupuis, & Costentin, 1994; Treit & Fundytus, 1989), and time spent by the animal in the open arms of the elevated plus-maze is used as a measure of anxiety (Hogg, 1996; Walf, & Frye, 2007).

The goals of the present experiment were: (i) to verify the occurrence of individual differences among the mice of the C57Bl/6J strain in some behaviors in the open-field (ambulation, defecation), or in the elevated plus-maze (ambulation in the open and enclosed arms), (ii) to find out if those individual differences held along the time (e.g., if the more active mice in the first trial were also the more active mice in successive trials), (iii) to find out if some behaviors were intercorrelated and correlated with the antibody response. The results reported here support the stability of the individual differences in all behaviors and the antibody response, but support the occurrence of a behavioral syndrome only in males.

Method

Subjects

Male and female mice of the C57Bl/6J strain were purchased from Harlan Iberica (Barcelona, Spain). Three C57Bl/6J females were mated with three C57Bl/6J males, and the offspring were the subjects of replication 1 below; five females were mated with five males, and the offspring were the subjects of replication 2 below. The males were removed from the females 1 week before parturition.

Adult mice of the same sex were housed 3-5 per cage, at 21±1 ºC, under a 12 h light-dark cycle (lights on at 8:00 hours). Food and water were available ad libitum. At the time of the first test, the mice were approximately 8 weeks old. The illumination on the floor of the mouse room was 220 lux approximately.

The experimental procedures were approved by the University of Barcelona Ethics Committee on Animal Experimentation.

Open field

The open field was a square enclosure made of gray plastic, 100.0 x 100.0 x 30.0 cm; the floor was divided by black lines in 400 squares (dimensions of each square: 5 x 5 cm). An inner and an outer zone were defined: the inner zone was a (90 x 90 cm) square situated at 5 cm from each wall, and the outer zone was the remaining of the open-field; Vidal, 2014). The apparatus was lit by a neon tube that yielded about 160 lux in the center of the field. The open-field test was performed in silence.

Elevated plus-maze

The apparatus, purchased from Ugo Basile (Varese, Italy), was made of metal, had two (36.5 x 5.0 cm) open arms, and two (35.0 x 5.0 cm) enclosed arms whose

walls were 15.5 cm high; the apparatus was elevated 53 cm above the ground. This plus-maze was modified thus: (i) a plastic ledge, 0.25 cm high, was placed around the open arms, and (ii) seven (5 x 5 cm) squares were drawn on the floor of each arm. The apparatus was lit by a neon tube that yielded about 260 lux on the open arms. The mice took the plus-maze test in silence.

Immunization and antibody measurement

Each mouse was injected intraperitoneally with 1 mg of aggregated bovine serum albumin in 0.10 ml of saline; the albumin was aggregated by heating at 67 ^{o}C for one hour (Passos, et al., 1977). Serum concentration of antibodies of the IgG class to bovine albumin was measured by diffusion-in-gel enzyme-linked immunosorbent assay (DIG-ELISA; Nilsson, Björck, & Ouchterlony, 1985; Vidal, 2002).

Procedure

Mice took the first behavioral test when they were about 8 weeks old. For each sex, the order of (open-field and plus-maze) tests was counterbalanced.

Each mouse was placed in a corner of the open-field and allowed to move freely for 5 minutes. These variables were recorded: ambulation (number of squares crossed) in the inner zone, ambulation (number of squares crossed) in the outer zone, and defecation (number of fecal boli) (Vidal, 2014). Each session, held between 15:00 and 19:00 hours was videotaped. The field was washed with disinfectant soap between two mouse sessions.

Each mouse was placed in one open arm of the elevated plus-maze, facing the center and close to it, and allowed to move freely for 5 minutes. These variables were recorded: ambulation (number of squares crossed) in the enclosed arms, and ambulation (number of squares crossed) in the open arms. Each session, held between 15:00 and 19:00 hours was videotaped. The plus-maze was washed with disinfectant soap between two mouse sessions.

Mice were immunized with bovine serum albumin two or three days after the last behavioral session, and bled 10 days after immunization.

The above sequence of tests was repeated when the mice were about 12, 17, and 22 weeks old.

Statistical analysis

The Spearman correlation coefficient was used to calculate the correlation between variables; this nonparametric coefficient was chosen because the goal was to find out if the mice that scored higher in the first measurement were also

the mice that scored higher in the successive measurements. Before computing the correlation coefficients, scatterplots of the appropriate variables were produced: the shapes of the plots were either amorphous or suggested a straight line. To compute the correlation coefficient between aggregates of the same variable, the raw scores of each of the four measurement of that variable were ranked: on the one hand, the ranks of measurements 1 and 3 were averaged, on the other hand, the ranks of measurements 2 and 4 were averaged, and the Pearson correlation coefficient between odd aggregates and even aggregates was computed.

Combination of a correlation coefficient in replication 1 with its homologous coefficient in replication 2 was performed via transformation of the correlation coefficients in *z* statistics (Shadish & Haddock, 2009).

The statistical package STATISTICA v12 (Tulsa, Oklahoma) was used to calculate the correlation coefficients and to produce the scatterplots.

Results

Variability of measurements

Table 1 shows the coefficient of variation of the variables recorded in the first trial: variability was considerable, with most coefficients of variation being larger than 30%, and some were larger than 100%.

TABLE 1. VARIABILITY OF THE VARIABLES IN THE FIRST TRIAL.

Variable	**Coefficient of variation (%)**			
	Replication 1		***Replication 2***	
	males	*females*	*males*	*females*
amb outer OF	31.8	16.9	28.8	26.3
amb inner OF	40.7	28.8	43.6	50.7
amb maze closed	14.8	50.2	39.1	38.4
amb maze open	118.6	100.0	110.3	135.3
def OF	167.3	244.9	263.9	204.9
abs	95.0	135.7	30.2	38.4

Note. Variables: *amb outer OF*: ambulation in the outer zone of the open-field; *amb inner OF*: ambulation in the inner zone of the open-field; *amb maze closed*: ambulation in the enclosed arms of the elevated plus-maze; *amb maze open*: ambulation in the open arms of the elevated plus-maze; *def OF*: defecation in the open-field; *abs*: concentration of antibodies to aggregated bovine serum albumin. Number of mice: in replication 1, 6 males and 6 females; in replication 2, 15 males and 13 females.

Consistency of measurements

Table 2 shows the correlation coefficients between the four measurements of the same variable (data are provided for replication 2 only given the scarcity of subjects in replication 1 [6 males and 6 females]): most correlation coefficients were positive although of varying magnitude.

TABLE 2. CORRELATIONS BETWEEN REPEATED MEASURES OF THE SAME VARIABLE IN THE OPEN-FIELD (REPLICATION 2).

C57Bl/6 Males

	V1a	*V2a*	*V3a*	*V4a*		*V1b*	*V2b*	*V3b*	*V4b*		*V1c*	*V2c*	*V3c*	*V4c*
V1a	1				*V1b*	1				*V1c*	1			
V2a	0.39	1			*V2b*	0.70*	1			*V2c*	0.37	1		
V3a	0.24	0.32	1		*V3b*	0.56*	0.76*	1		*V3c*	0.78*	0.55*	1	
V4a	0.39	0.20	0.47	1	*V4b*	0.20	0.56*	0.51	1	*V4c*	0.53*	0.39	0.68*	1

C57Bl/6 Females

	V1a	*V2a*	*V3a*	*V4a*		*V1b*	*V2b*	*V3b*	*V4b*		*V1c*	*V2c*	*V3c*	*V4c*
V1a	1				*V1b*	1				*V1c*	1			
V2a	0.37	1			*V2b*	0.63*	1			*V2c*	0.36	1		
V3a	0.37	0.56*	1		*V3b*	0.77*	0.45	1		*V3c*	-0.36	-0.41	1	
V4a	0.12	0.12	0.31	1	*V4b*	0.55*	0.18	0.54	1	*V4c*	0.66*	0.10	0.08	1

Note. Figures in the table are Spearman correlation coefficients. V1a-V4a: ambulation in the outer zone of the open-field, trials 1-4. V1b-V4b: ambulation in the inner zone of the open-field, trials 1-4. V1c-V4c: defecation in the open-field, trials 1-4. Males: N=15, females: N=13. *: p=<0.05.

Table 3 (see next page) shows the correlations between the four measurements of each variable in the plus-maze, as well as the correlations between the four measurements of the antibody concentration: the pattern was made up of positive correlation coefficients of varying magnitude.

This pattern of positive correlations suggested consistency of behavior (i.e., the mice that scored high in the first trial tended to score high in the other trials), and Kendall's coefficients of concordance bore out that supposition: coefficients of concordance ranged from 0.47 [$\chi^2(14)=16.60$, p=0.021, for antibody concentration in males] to 0.83 [($\chi^2(14)=46.40$, p=0.00002, for ambulation of males in the enclosed arms of the plus-maze], the only exception being the coefficient of concordance for defecation of females in the open-field (0.30) that did not reach statistical significance [$\chi^2(12)=14.53$, p=0.27].

TABLE 3. CORRELATIONS BETWEEN REPEATED MEASURES OF THE SAME VARIABLE IN THE PLUS-MAZE, AND BETWEEN REPEATED MEASUREMENTS OF THE ANTIBODY CONCENTRATION (REPLICATION 2).

Males

	V1a	V2a	V3a	V4a		V1b	V2b	V3b	V4b		Ab1	Ab2	Ab3	Ab4
V1a	1				V1b	1				Ab1	1			
V2a	0.71*	1			V2b	0.82*	1			Ab2	0.42	1		
V3a	0.35	0.15	1		V3b	0.79*	0.76*	1		Ab3	0.25	0.34	1	
V4a	0.15	0.22	0.56*	1	V4b	0.76*	0.80*	0.72*	1	Ab4	0.04	0.41	0.33	1

Females

	V1a	V2a	V3a	V4a		V1b	V2b	V3b	V4b	Ab1	Ab1	Ab2	Ab3	Ab4
V1a	1				V1b	1				Ab1	1			
V2a	0.52	1			V2b	0.47	1			Ab2	0.84*	1		
V3a	0.47	0.73*	1		V3b	0.56*	0.42	1		Ab3	0.01	0.06	1	
V4a	0.21	0.28	0.42	1	V4b	0.38	0.47	0.52	1	Ab4	0.58*	0.76*	0.10	1

Note. Figures in the table are Spearman correlation coefficients. V1a-V4a: ambulation in the enclosed arms of the maze. trials 1-4. V1b-V4b: ambulation in the open arms of the maze. trials 1-4. Ab1-Ab4: IgG antibodies to aggregated bovine serum albumin, measurements 1 to 4. Males: N=15, females: *N*=13.
*: p=<0.05.

One reason for the variability of the correlation coefficients in tables 2 and 3 could be the imprecision occurring when two single measurements of a given behavior are correlated (Epstein & O'Brien, 1985): averaging some of the measurements could cancel out the influence of random vagaries that affect the score (Epstein & O'Brien, 1985). Therefore, scores in trials 1 and 3 of the same variable were averaged (odd scores), scores in trials 2 and 4 were averaged (even scores), and odd and even scores were correlated. Table 4 (see next page) shows the odd-even correlation coefficients: in males, for ambulation (in the open-field and in the enclosed arms of the plus-maze) and defecation, the coefficients were comparable between replications, although they did not reach statistical significance in replication 1; in females, the same pattern seemed to emerge (except for defecation in the open-field), although the consistency was lower. For the antibody concentration, replications 1 and 2 showed comparable correlation coefficients, although only the coefficient corresponding to the second replication, in females, reached statistical significance.

TABLE 4. ODD VS. EVEN CORRELATION COEFFICIENTS.

	r	95% C.I.	*r*	95% C.I.	*r*	95% C.I.	*r*	95% C.I.
	Replication 1				***Replication 2***			
	Males		*Females*		*Males*		*Females*	
periph OF	0.68	-0.24; 0.94	0.70	-0.21; 0.94	0.64[b]	0.18; 0.86	0.55[a]	0.00; 0.83
center OF	0.72	-0.18; 0.95	0.74	-0.14; 0.95	0.69[b]	0.27; 0.88	0.75[b]	0.33; 0.91
+maze closed	0.67	-0.26; 0.94	0.37	-0.56; 0.87	0.61[a]	0.14: 0.84	0.68[b]	0.20; 0.88
+maze open					0.86[c]	0.61; 0.95	0.59[a]	0.06; 0.85
def OF	0.88[a]	0.23; 0.98	-0.25	-0.84; 0.63	0.69[b]	0.27; 0.88	0.36	-0.23; 0.74
abs	0.61	-0.34; 0.93	0.60	-0.35; 0.92	0.38	-0.16; 0.76	0.60[a]	0.07; 0.85

Note. Odd scores are obtained by averaging the ranks in trials 1 and 3; even scores are obtained by averaging the ranks in trials 2 and 4. Variables: *periph OF*: ambulation in the outer zone of the open-field: *center OF*: ambulation in the inner zone of the open-field; *+ maze closed*: ambulation in the enclosed arms of the open-field; *+ maze open*: ambulation in the open arms of the open-field, *def OF*: defecation in the open-field; *abs*: antibody concentration. Number of mice: in replication 1, 6 males and 6 females; in replication 2, 15 males and 13 females. *r*: Spearman correlation coefficient, C.I.: confidence interval. Mice did not enter the open arms of the plus-maze in trials 3 and 4 of replication 1.
a: $0.05>p>0.010$; b: $0.01>p>0.001$; c: $p<0.001$

The combined odd-even correlation coefficients of replications 1 and 2 are shown in table 5 (see next page): (i) in male mice, correlation for ambulation (in the open-field and in the enclosed arms of the plus-maze) was 0.65 approximately, correlation for defecation in the open-field was 0.70 approximately, and correlation for antibodies was of the order of 0.40 (this correlation showed a trend toward statistical significance, $p=0.08$),(ii) in female mice, correlation for ambulation, in the open-field and in the plus-maze, was of the order of 0.60, correlation for defecation was about 0.20, and did not reach statistical significance, and correlation for antibodies was about 0.60. Correlation coefficients for ambulation in the open arms of the plus-maze could not be combined because mice did not enter the open arms in trials 3 and 4 of the first replication.

Correlation between different variables

To find out whether the different variables were consistently correlated, the ranks of the four measurements of each variable were averaged, and the resulting averages were correlated by the Pearson correlation coefficient. Table 6 (see next page) shows the correlation matrix of combined averages (i.e., the correlation coefficients of replications 1 and 2 combined). Because no mice entered the open arms of the plus-maze in trials 3 and 4 of the first replication, table 6 does not show the correlation of ambulation in the open arms with the other variables.

TABLE 5. ODD VS. EVEN CORRELATION COEFFICIENTS (REPLICATIONS 1 AND 2 COMBINED).

	Males		***Females***	
	r	*CI*	*r*	*CI*
amb outer OF	0.65	0.26; 0.86	0.59	0.13; 0.84
amb inner OF	0.70	0.34; 0.88	0.75	0.40; 0.91
amb maze closed	0.62	0.22; 0.84	0.62	0.18; 0.85
def OF	0.74	0.42; 0.90	0.23	-0.30; 0.65
abs	0.43	-0.04; 0.75	0.60	0.15; 0.84

Note. Variables: *amb outer OF*: ambulation in the outer zone of the open-field; *amb inner OF*: ambulation in the inner zone of the open-field; *amb maze closed*: ambulation in the enclosed arms of the open-field; *amb maze open*: ambulation in the open arms of the open-field; *def OF*: defecation in the open-field; *abs*: antibody concentration. Number of mice: in replication 1, 6 males and 6 females; in replication 2, 15 males and 13 females. *r*: Spearman correlation coefficient; C.I.: 95% confidence interval of *r*.

TABLE 6. CORRELATIONS OF AGGREGATED VARIABLES (REPLICATIONS 1 AND 2 COMBINED).

			Males		
	periph	*centre*	*closed*	*def*	*abs*
periph	1				
centre	0.39 (-0.09; 0.72)	1			
closed	0.74 (0.42; 0.90)	0.48 (0.02; 0.78)	1		
def	-0.78 (-0.91; -0.49)	-0.51 (-0.79; -0.05)	-0.46 (-0.76; 0.01)	1	
abs	-0.19 (-0.60; 0.31)	-0.07 (-0.52; 0.41)	0.11 (-0.38; 0.55)	0.46 (-0.01; 0.76)	1
			Females		
	periph	*centre*	*closed*	*def*	*abs*
periph	1				
centre	0.31 (-0.21; 0.70)	1			
closed	0.05 (-0.45; 0.54)	0.37 (-0.15; 0.73)	1		
def	-0.50 (-0.80; -0.01)	-0.10 (-0.56; 0.42)	0.39 (-0.13; 0.74)	1	
abs	0.03 (-0.47; 0.52)	-0.10 (-0.57; 0.41)	-0.66 (-0.87; -0.25)	-0.35 (-0.72; 0.17)	1

Note. Aggregated variable: mean of the ranks of the four measurements of a given variable. Variables: *periph*: ambulation in the outer zone of the open-field; *centre*: ambulation in the inner zone of the open-field; *closed*: ambulation in the enclosed arms of plus-maze; *def*: defecation in the open-field; *abs*: antibody concentration. Figures in the table indicate mean correlation coefficient (95% confidence interval). Homogeneity statistic: periph vs. closed in females, Q(1)=4.04; p=0.04; for the other correlations, $p>0.10$.

Discussion

The coefficients of variation displayed in table 1 confirm previous findings; i.e., the occurrence of phenotypic variability in inbred strains of mice, even when the mice were raised in controlled conditions (Introduction). This finding gave rise to the concept of intangible variation, developmental noise, or third component. The large coefficients of variation shown in table 1 suggested the existence of individual differences within the mice of the C57Bl/6J strain.

The next question was whether those individual differences were occasional or whether they persisted along the life of the mice. The correlation matrices shown in tables 2 and 3 (which represent the correlations of four measurements of the same behavior taken along the life of the mice) display a pattern: most of the correlation coefficients are positive (with the exception of defecation in female mice, that included two negative coefficients out of six ones; table 2). This pattern of positive correlations suggested some consistency of behavior, e.g., the most active mice in the first trial were generally the most active mice in successive trials. This consistency of behavior was tested by computing Kendall's coefficient of concordance for the four trials of each variable (the trials being the "judges"): in all instances, except defecation of females in the open-field, the coefficient of concordance was higher than 0.45 and statistically significant (Results).

Nevertheless, consistence of behavior was expressed to various degrees; for instance, in males, the correlation coefficient for ambulation in the inner open-field was 0.70 between trials 1 and 2, and 0.20 between trials 1 and 4 (table 2). The question was: Why is it so? The explanation put forward here is that the tendency to behave in a given manner is modulated by different environmental factors. On the one hand, the predisposition to score high or low in a given behavior was inferred from the correlation matrices shown in tables 2 and 3, and from the magnitude of Kendall's coefficients of concordance (Results); on the other hand, several factors influence the score of a tests in a particular occasion: maternal effects, littermate effects, diet, handling, etc. (Henderson, 2005; Lewejohann, Zipser, & Sachser, 2011; Wahlsten, 2001); therefore, the combination of predisposition and environmental influences might account for the actual correlations (tables 2 and 3).

The next step was to estimate the magnitude of the predisposition. This was approached by supposing that some of the environmental factors that influenced the score in a particular test were random, and therefore, could be cancelled out by averaging the measurements of the same variable. This same approach was used to reveal the presence of a trait from measurements of several behaviors purporting to measure that trait (the principle of aggregation; Epstein, 1979; Epstein & O'Brien, 1985). Consequently, ranks in trials 1 and 3 were averaged on the one hand, and ranks in trials 2 and 4 were averaged on the other. Table 4 shows the correlation coefficients between averages in each replication, whereas

table 5 shows the correlations in both replications combined: the coefficients were of the order of 0.60 (and statistically significant) for most variables, except for defecation of females in the open-field, whose coefficient was 0.23, and antibody concentration in males, whose coefficient was 0.43 (table 5). Thus, the magnitude of the predisposition to behave in the open-field, or in the plus-maze, was of the order of 0.60. A note is in order: combination of correlation coefficients by the Schmidt and Hunter method (Hunter & Schmidt, 1990) yielded comparable coefficients, but (i) the confidence intervals were narrower, and thus the figures (mean and confidence interval) for the antibody concentration in males were 0.44 (0.30, 0.58), and (ii) most of the variability encompassed by the confidence intervals was sampling error.

The next question concerned the occurrence of behavioral syndromes. Sih et al. (2004) termed a set of correlated behaviors behavioral syndromes, and those correlated behaviors have been proposed as expression of animal personality (Gosling, 2001; Lewejohann et al., 2011). Correlation coefficients of aggregated variables are displayed in table 6, and interpretation of results has to be done for males and females separately: (i) in males, these variables were intercorrelated (or showed a trend toward significant correlation): ambulation in the periphery of the open-field, ambulation in the inner zone of the open-field, ambulation in the enclosed arms of the plus-maze, and defecation; therefore, the data support the occurrence of a behavioral syndrome for ambulation that includes defecation. Two comments are in order: (a) the negative correlation between ambulation and defecation in the open-field has been known for some time (Walsh & Cummins, 1976), and the correlations in table 6 confirm that correlation; and (b) the positive correlation between ambulation in the periphery of the open-field and ambulation in the inner part of the open-field supports the difficulty in dissociating activity from anxiety (Milner & Crabbe, 2008); the antibody concentration was uncorrelated with any ambulation (which agrees with previous results; Vidal & Rama, 1994); (ii) in females, the picture was different: ambulations in different apparatuses were not significantly correlated, and the only significant correlations were (a) between defecation and ambulation in the periphery of the open-field and (b) between antibodies and ambulation in the enclosed arms of the plus-maze: it is not clear that those correlated variables constitute a behavioral syndrome (because they are conceptually different).

There remains a question: if the individual differences reported here occurred in mice of an inbred strain raised in the same environment, how were those differences generated? The results reported in this article do not provide an answer to that question, although it has been proposed that those differences have an epigenetic origin (Blewitt et al., 2004; Wong, Gottesman, & Petronis, 2005).

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