Genes, Brain and Behavior (2005) **4:** 431–443

Techniques

Texture of locomotor path: a replicable characterization of a complex behavioral phenotype

N. Kafkafi* and G. I. Elmer

Maryland Psychiatric Research Center, University of Maryland School of Medicine, Department of Psychiatry, MD, USA
**Corresponding author: N. Kafkafi, Maryland Psychiatric Research Center, University of Maryland School of Medicine, Department of Psychiatry, Maple & Locust Sts., PO Box 21247, Baltimore, MD 21228, USA. E-mail: nkafkafi@mprc.umaryland.edu*

A database of mouse locomotor path in spatial tests can be used to search *in silico* for behavioral measures that better discriminate between genotypes and are more replicable across laboratories. In this study, software for the exploration of exploration (SEE) was used to search a large database for a novel behavioral measure that would characterize complex movement paths. The database included mouse open-field behavior assessed in 3 laboratories, 7 inbred strains, several pharmacological treatments and hundreds of animals. The new behavioral measure, 'path texture', was characterized using the local curvature of the path (the change of direction per unit distance, in degrees/cm) across several spatial scales, starting from scales smaller than the animal's body length and up to the scale of the arena size. Path texture analysis differs from fractal dimension analysis in that it does not assume self-similarity across scales. Path texture was found to discriminate inbred strains with relatively high broad-sense heritability (43%–71%) and high replicability across laboratories. Even genotypes that had similar path curvatures in some scales usually differed in other scales, and self-similarity across scales was not displayed by all genotypes. Amphetamine decreased the path curvature of C57BL/6 mice in small and medium scales, while having no effect on DBA/2J mice. Diazepam dose-dependently decreased the curvature of C57BL/6 mice across all scales, while 2 anxiogenic drugs, FG-7142 and pentylenetetrazole, increased it. Path texture thus has high potential for behavioral phenotyping and the study of drug effects in the mouse.

Keywords: Amphetamine, cocaine, curvature, diazepam, exploratory behavior, FG-7142, genotype × laboratory interaction, inbred strains, pentylenetetrazole, SEE

Received 5 November 2004, revised 18 January 2005, accepted for publication 24 January 2005

Many paradigms in behavioral neuroscience and behavioral genetics depend on the accuracy and consistency of behavioral measurements. The proper characterization of specific behaviors, sometime referred to as behavioral phenotyping, is important for eventually associating them with neurobiochemical events or particular gene loci. The need for behavioral phenotyping has resulted in the design of behavioral and physiological test batteries for mice (e.g. Crawley *et al*. 1997; Rogers *et al*. 1999). Considerable effort has been made to automate these tests in order to increase the throughput needed for testing large numbers of animals and to avoid the effect of subjective human judgement. The proper characterization, however, becomes more difficult when complex behavioral phenotypes are involved.

The open-field test is included in many test batteries and can be conducted efficiently using commercially available video tracking or photobeam systems. Strategy for the Exploration of Exploration (SEE, see Drai & Golani 2001) is a software-based strategy, embedded in the programming environment of Mathematica™ (Wolfram 1999), for the analysis of locomotor path measured automatically using any kind of tracking system. It is based on ethologically oriented studies in rats (Drai *et al*. 2000; Eilam & Golani 1989; Eilam *et al*. 1989; Golani *et al*. 1993; Kafkafi *et al*. 2001; Tchernichovski & Golani 1995; Tchernichovski *et al*. 1996; Tchernichovski *et al*. 1998) and was recently shown to be useful for the behavioral phenotyping of mice (Benjamini *et al*. 2001; Drai *et al*. 2001; Kafkafi *et al*. 2003a; Kafkafi *et al*. 2003b; Kafkafi *et al*. 2005; Lipkind *et al*. 2004). These studies found that, in contrast to a common view of open-field behavior as an essentially stochastic phenomenon, it is structured and consists of typical behavior patterns. These patterns have found utility in psychopharmacological and psychobiological studies (Cools *et al*. 1997; Gingras & Cools 1997; Szechtman *et al*. 1999; Wallace *et al*. 2002; Whishaw *et al*. 1994; Whishaw *et al*. 2001). The most basic of these patterns, which serve as the foundation from which more elaborate patterns are constructed, are progression segments and lingering episodes. Progression segments consist of locomotor movement, while lingering episodes (stops in their generalized sense) consist of arrests and local, non-locomotor movements such as scanning, rearing, stretch-attend, etc.

doi: 10.1111/j.1601-183X.2005.00126.x

An experienced observer can frequently identify the genotype in the path plots of progression segments from different inbred strains (Fig. 1). It is frequently difficult, however, to explicitly define the features that differentiate the genotypes, because they do not necessarily relate to simple properties of segments such as their number, their typical length or speed. Rather, it seems that each genotype has its own general 'style' or 'handwriting' of drawing the path. One optional way to quantify the general quality of a meandering path is to use Fractal Theory. The idea is to measure a certain variable in different scales. If the relation between the scale and the value of the variable measured in this scale is linear when plotted in a log–log scale, this implies similarity across scales, also called fractal structure (Mandelbrot 1982). That is, the path will look identical (at least with respect to this specific variable) in any scale it is observed. In such a case, the slope (termed the fractal dimension) of this linear relation can be used to characterize the scale-independent structure of a complex process using a single number.

Fractal dimension analysis was previously applied by Paulus and Geyer (1991; 1993a; 1993b) to characterize the locomotor path of rats and recently also of mice (Ralph *et al*. 2001; Ralph-Williams *et al*. 2003) with the spatial coefficient *d*. *d* is measured by taking path pieces of different lengths (i.e. scales) and measuring the Euclidian distance between the two ends of the piece. The relation between the log of the scale and the log of the distance is approximately linear and is characterized by its slope *d*.

The approach in the current study is similar but is embedded in the different framework of SEE: within progression segments alone, we consider the curvature of the path within a neighborhood of a given length (i.e. scale) around every data point (Fig. 2). The curvature is defined as the change in path direction that occurred within this neighborhood. The curvature as a function of the scale in which it is measured is then considered.

Apart from the slightly different definition of the measured variable, there are two important differences between our framework and the framework employed in Paulus and Geyer (1991). First, the analysis in our study is not performed over the whole path but only within progression segments, isolated according to the intrinsic categorization applied in SEE (Drai *et al*. 2000). This is important because the structure and dynamics of lingering is very different from that of progression, hence concentrating only on a component of behavior geared towards progression may highlight a dimension of behavior previously obscured in the combined analysis. Secondly, rather than considering only the slope in the log–log plot of curvature as a function of scale, we consider also the absolute values of curvature in each scale and deviations from linear dependence. Although we found that the dependence of log-curvature on the log-scale is roughly linear (Fig. 3), meaning that there is indeed certain similarity across scales in the path, there were also significant deviations from this linearity that may be informative. Rather than assuming a fractal structure, we therefore consider the whole profile of path curvature across a range of distance scales. This profile may be referred to as the 'texture' of the path.

A critical consideration in behavioral phenotyping is the ability to replicate results in additional laboratories. Crabbe *et al*. (1999) demonstrated that this constitutes a problem in many behavioral tests, even when using genetically identical animals and standardizing housing and testing conditions to a much higher degree than is currently practiced in the field. Kafkafi *et al*. (2005) have recently suggested using an approach based on the mixed ANOVA statistical model (e.g. McCulloch & Searle 2001) as opposed to the commonly used fixed ANOVA. In this approach, several phenotyping laboratories in a behavioral experiment, conducted with the usual level of standardization, are treated as a sample representing the population of many potential phenotyping laboratories. According to this approach, the replicability of behavioral measures should be estimated in such a multi-laboratory experiment using the standard deviations (SD) of the laboratory effect and especially the laboratory × genotype interaction, as computed in the mixed ANOVA model. These behavioral measures may then be used in any single-laboratory experiment as long as the previously measured SD of laboratory and interaction are taken into account.

Kafkafi *et al*. (2005) suggested that the advantages of the mixed ANOVA approach and an open-ended strategy such as SEE may be effectively combined by constructing databases of raw behavioral data, in our case path coordinates, gathered by many experimenters in many laboratories. These databases can then be systematically mined with the express purpose of designing and testing replicable and informative measures of behavior. The capacity of SEE to

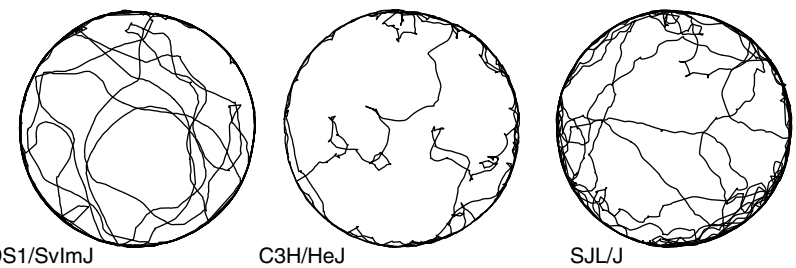


Figure 1: Path plots of progression segments in three mice of different inbred strains in a 2.50 m circular arena, demonstrating different styles of progression path.

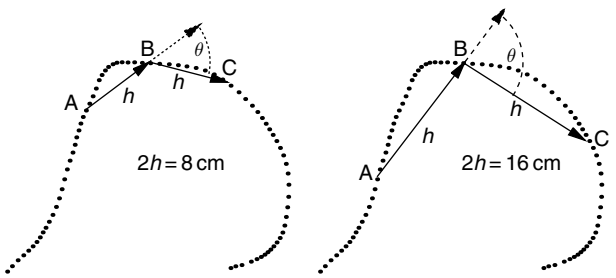


Figure 2: Computation of path curvature. Examples of computing the curvature of the path in one data point B of a progression segment in two scales: 8-cm scale (left) and 16-cm scale (right). The curvature in degree/cm is defined as $\theta/(2h)$. The process is repeated in all data points belonging to progression segments and in several scales. See text for more details.

use such a multi-laboratory database to construct more replicable measures has already born fruit in the complex analysis of phenotypes such as darting and thigmotaxis (Kafkafi 2003; Kafkafi *et al*. 2003b; Lipkind *et al*. 2004). In this study we use our database, which currently includes some additional genotypes and treatments, to test the utility and replicability of path texture.

Materials and methods

Data sets

In accord with our proposed approach, the path texture was explored in a large database of raw open-field data. Three data sets were used. The experiments used to obtain all the three data sets were conducted in accordance to each institute's animal care and use policies and the animals used in these studies were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

Data set 1

Included mice from seven common inbred strains: BALB/cByJ, C3H/HeJ, C57BL/6J, DBA/2J, FVB/NJ, SJL/J and 129S1/SvImJ, each tested across three laboratories: National Institute of Drug Abuse (NIDA) – IRP in Baltimore, Maryland Psychiatric Research Center (MPRC), University of Maryland and the Department of Zoology in Tel Aviv University. This experiment was used by Kafkafi *et al*. (2005) to evaluate the replicability of genotype differences with previously designed behavioral measures. The A/J inbred strain, which also took part in the original experiment, was discarded from the present study because most mice from this strain were very inactive and did not use the progression modes. Methods of housing and testing in this experiment are detailed in Golani *et al*. (2004). There were 11–12 animals in each group, for a total of 247 animals. Session time was 30 min.

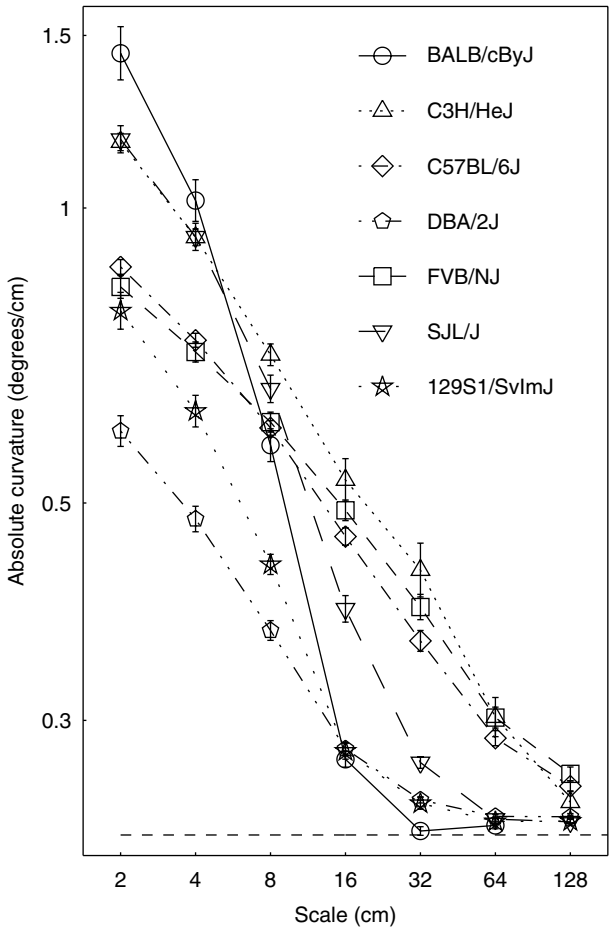


Figure 3: The path texture of seven inbred strains. A log–log graph of the median absolute curvature as a function of scale presents the texture profiles of seven inbred strains. Each symbol represents the strain mean pooled over three laboratories. Error bars represent strain standard errors. The horizontal dashed line at the bottom is the curvature of the arena wall. The curvature of the BALB/cByJ in the 128-cm scale is not shown, because only few animals of this strain had 128-cm length progression segments.

Data set 2

This included mice from the inbred strains C57BL/6J and DBA/2J, injected intraperitoneally (i.p.) with one of four doses (including vehicle) of amphetamine and cocaine. This experiment was performed at NIDA. Methods of housing and testing can be found in Kafkafi *et al*. (2003b), where this data set was used to evaluate the previously designed measure of darting. There were five to eight animals in each group, for a total of 92. Session time was 90 min.

Data set 3

This was a newly generated data set, obtained at MPRC. The subjects were C57BL/6J males 60–80 days old at the time of

the experiment. They were shipped from Jackson Laboratories and housed in the animal colony in MPRC for at least 2 weeks before they were tested. They were kept in standard conditions of 12 : 12 light cycle, 22 °C room temperature, water and food *ad libitum* and housed four per cage. Animals were injected with diazepam (1 or 2 mg/kg, i.p.), FG-7142 (10 mg/kg, i.p) or pentylenetetrazole (PTZ, 20 mg/kg, i.p.) and immediately placed in the 250-cm circular arena. Doses were assigned so that no two animals from the same cage received the same drug and dose. There were four to seven animals in each drug treatment group, for a total of 34. Animals were tested between 0900 and 1500 h, and session time was 60 min. Diazepam was dissolved in saline. FG-7142 (*b*-Carboline-3-carboxylic acid *N*-methylamide) was put in solution using 17.5 mM (2-Hydroxypropyl)-*b*-cyclodextrin (Sigma, St. Louis, MO) and 5% Tween. PTZ was dissolved in saline. The experimental protocols followed the 'Principles of Laboratory Animal Care' (NIH publication no. 86–23, 1996).

Path analysis

All path and curvature analysis was done with SEE as described in previous phenotyping studies (Kafkafi *et al*. 2003a; Lipkind *et al*. 2004). The first stage is smoothing the path using a specialized algorithm implemented in the standalone program SEE PATH SMOOTHER (Hen *et al*. 2004). The second stage is the segmentation of the path into progression segments and lingering episodes using the intrinsic properties of the data in each session, as implemented in the standalone program SEE PATH SEGMENTOR (the algorithm is detailed in Drai *et al*. 2000 and slightly updated in Kafkafi *et al*. 2001; 2003a). The statistical discrimination of the path into progression and lingering is a critical aspect of the investigation, because it confines the texture analysis to directly relevant components of behavior with distinctly different biological and behavioral underpinnings (Drai *et al*. 2000). The segmented path was then analyzed with the SEE package (Drai & Golani 2001) with the help of the specialized packages SEE EXPERIMENT EXPLORER and SEE ENDPOINT MANAGER (Kafkafi 2003). All these are open-source programs and are available from the authors.

Curvature computation

Curvature was computed for all data within progression segments only (i.e. specifically excluding lingering episodes). Curvature was measured in seven distance scales: $2h = 2$, 4, 8, 16, 32, 64 and 128 cm (h denotes 'half scale', for a reason that will be immediately clarified). For each scale $2h$, the algorithm for computing the curvature was as follow (Fig. 2): For each data point B, find the data points that occur immediately prior to and after this point (points A and C, respectively) that are at least h cm distant from B. The direction change θ between lines AB and BC is the direction change at point B. This direction change is thus defined as zero when A, B and C are on a straight line (i.e. the animal is moving straight forward). Positive direction changes imply curving to the left, while negative direction changes imply curving to the right. The curvature in degree/cm for data point B was defined as $\theta/(2h)$. Data points that are nearer than h cm to the beginning or end of a progression segment were not assigned a curvature value and are thus excluded from the analysis in the specific scale $2h$.

In a previous study (Kafkafi *et al*. 2003a), we used the measure of radius of turn during progression. This measure in fact estimated the curvature within a time window, 0.4 seconds from beginning to end, constructed around each data point, multiplied by $180/\pi$ in order to get the result in terms of the momentary radius of turn, and further standardized by dividing by the radius of the arena. We found, however, that computing the curvature using a time window has a disadvantage when the animal is moving slowly, because the distances AB and BC in Fig. 2 are likely to be too small for a meaningful estimation of the direction change, given that the resolution of the tracking system and the level of measurement noise are approximately 1 cm. In fact, measuring the curvature using a time window means that it is measured in different distance scales depending on the animal's momentary speed. While the radius of turn did reveal replicable differences between inbred strains, we show here that more information may be gleaned by explicitly measuring curvature in different scales of distance.

Lipkind *et al*. (2004) also measured the curvature using a distance window but divided the direction change by the cumulative distance actually traveled by the animal within the window (e.g. the distance along the path between A and C in Fig. 2). In the context of the present study, it was more proper to divide by the Euclidian distance $2h$, because the scale is used here as an independent variable that should be defined regardless of the actual path data. Furthermore, when measuring the curvature in one scale, if the path is more meandered in a smaller scale, this would increase the distance traveled. This means that, when dividing by the distance traveled along the path, measurement in the larger scale would not be independent of the measurement in the smaller scale.

The typical curvature of a particular progression segment, in each scale, was measured by the median of the absolute (i.e. no distinction between left and right turning) curvature in this scale, pooled over all points in this progression segment. Figure 4 shows four segments demonstrating different combinations of low and high median absolute curvature in different scales. The typical curvature of a particular animal, in each scale, was measured by the median absolute curvature in this scale, pooled over all progression segments in the session.

Statistical methods

All graphs in this study are presented using the curvature computed in seven different scales: 2, 4, 8, 16, 32, 64 and

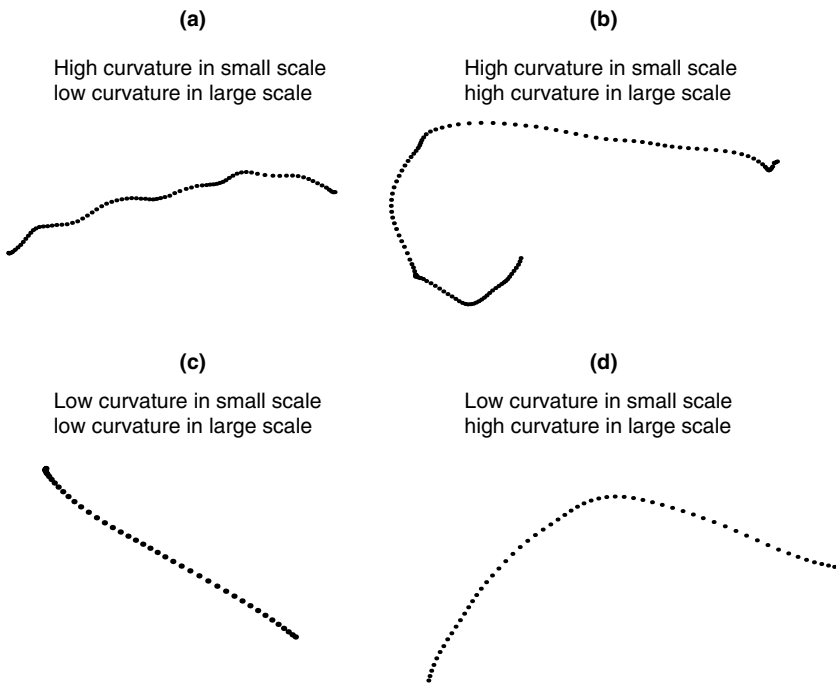


Figure 4: Examples of different path textures. Four progression segments of the path database demonstrate four different path textures as captured by the median absolute curvature in a smaller scale (4 cm) and a larger scale (32 cm).

128 cm. The 128-cm curvature is not shown for the BALB/ByJ strain in Fig. 3, because only 26 of 35 animals in this strain performed segments of 128-cm length. In the C3H/HeJ strain, 11 of 35 animals did not perform 128-cm length progression segments, and their 128-cm curvature (Fig. 3) was calculated from the remaining 24 animals. In all other strains and treatments the proportion of discarded animals was lower, usually 0 or close to it.

For all statistical analysis, however, we used only three scales: 4, 16 and 64 cm (see the results section for the rationale of this decision). For each animal, the median absolute curvature was pooled from data points belonging to progression segments over the whole session.

The results from data set 1 were analyzed in each scale separately, using the mixed model ANOVA of strain (fixed factor) × laboratory (random factor) in order to assess the significance of strain differences over the background of laboratory and interaction effects in addition to the within-group variability (Kafkafi *et al.* 2005). The effect size (i.e. the proportion of variance attributed to each factor of the total variance) of genotype and laboratory effects, as well as their interaction, can be estimated using the mixed ANOVA. As in Kafkafi *et al.* (2005), the genotypic effect size is used as an estimation of broad-sense heritability. Note that this is a conservative estimation, relative to estimations from single-lab experiments, because some of the interaction with the laboratory might also be genetic. The mixed model was fitted in the statistical software S-PLUS 2000 using the 'lme' function. The SD of the laboratory × strain interaction was calculated using the 'Restricted Maximum Likelihood' method (see McCulloch & Searle 2001), which is the more conservative method, and is reported here (Table 1). According to the mixed model approach, this SD should be considered in future experiments when measuring the curvature in yet additional laboratories, in order to ensure that the results of these experiments are also replicable across laboratories (Kafkafi *et al.* 2005). Note that the relevant statistic for comparison with the strain differences across the laboratories in the experiment (Fig. 5) is the standard error, which is even smaller than the SD; but unlike the SD, it was different for the pairwise comparison of each of two strains, because it involves *n*, which was not equal for all strains.

Table 1: Mixed ANOVA analysis of path curvature across laboratories

Scale (cm)	Genotype		Laboratory		Interaction		Within-group		*n*
	V (%)	*P* value	V (%)	SD	V (%)	SD	V (%)	SD	
4	65	<0.0001	2	0.0600	6	0.11	27	0.24	211
16	71	<0.0001	0	0.0009	2	0.11	28	0.44	211
64	43	<0.0001	0	0.0006	2	0.09	55	0.51	210

The percent of total variability (%V) attributed to genotype, laboratory, genotype × laboratory interaction and within-group, with the P value or the standard deviation (SD) as calculated using mixed ANOVA analysis in each of the scales 4, 16 and 64 cm. Standard deviation of curvature are presented after 1/x transformation. Column n presents the total number of animals used for the calculation in each scale. The test was done across three laboratories in six inbred strains, with the BALB/cByJ excluded (see Fig. 5 for strain means).

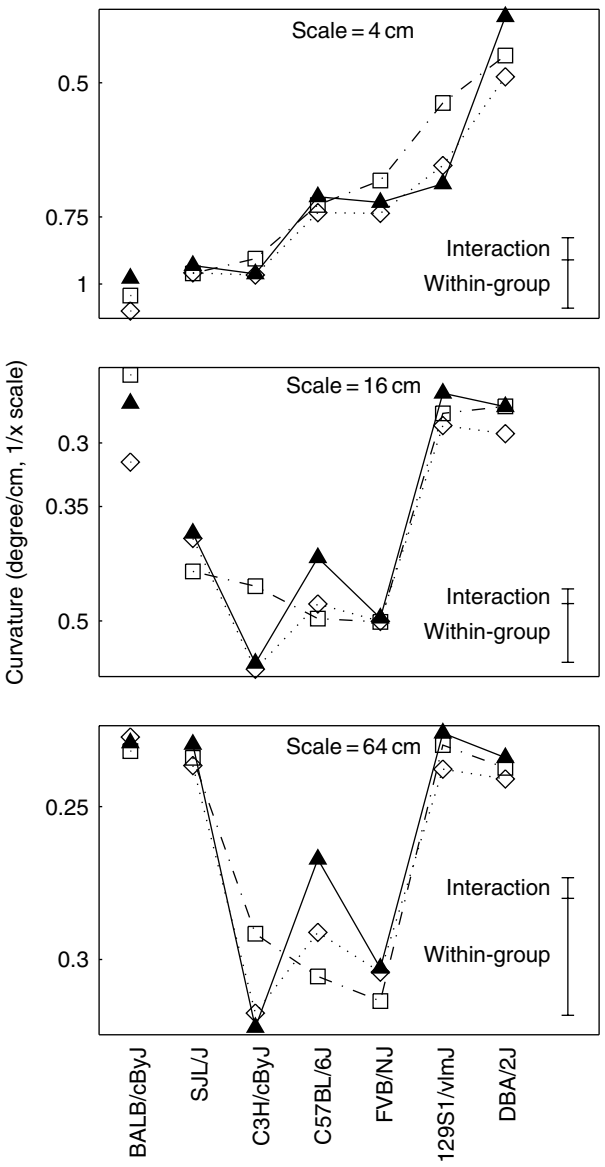


Figure 5: Replicability of path texture across laboratories. Strain means of curvature across the three laboratories (triangles: National Institute of Drug Abuse, diamonds: Maryland Psychiatric Research Center, squares: Tel Aviv University) for each of the scales of 4, 16 and 64 cm. Bars on the right represent the SD of within-group and strain × laboratory interaction as computed using mixed ANOVA (Table 1). Note that curvature is presented in 1/x scale, and the BALB/cByJ strain (isolated on the left side) was excluded from the mixed ANOVA statistics (see *Statistical methods* section). The strains are ranked according to their curvature in the 4-cm scale.

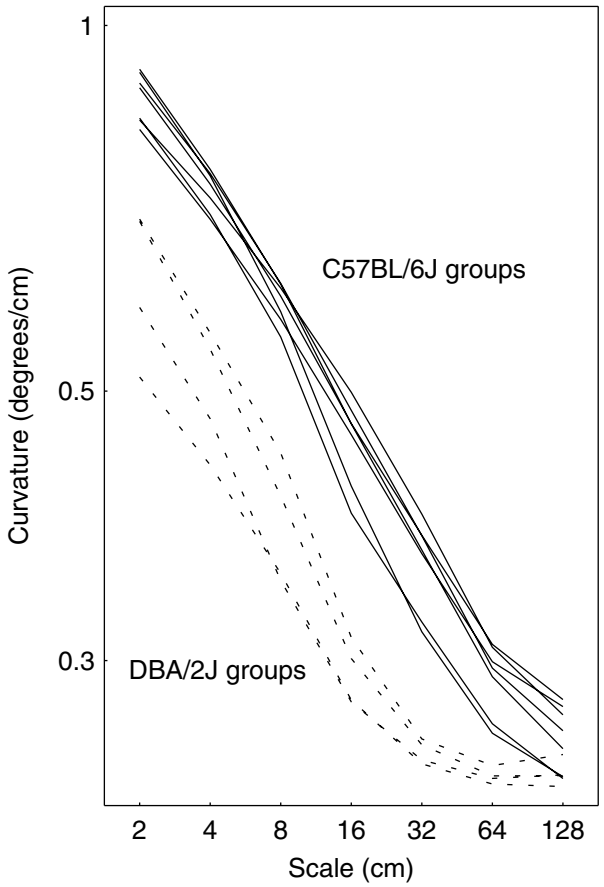
The BALB/cByJ strain was excluded from the mixed model statistics, because the formula for the effect size in mixed ANOVA assumes a balanced design, while several BALB/cByJ mice had to be excluded from the curvature computation, because they were inactive. The number of the excluded animals in each scale, however, was at most four out of 12 BALB/cByJ mice in each laboratory. Moreover, the significance of the strain differences and the SD of the interaction and within-group were similar with or without the BALB/cByJ. In addition, the effect sizes computed in the mixed ANOVA without the BALB/cByJ differed from the fixed ANOVA estimation (computed as in Kafkafi *et al.* 2003a), with or without the BALB/cByJ, by 4% at most. Therefore, we present the BALB/cByJ results in Figs 3 and 5 despite excluding them from the statistics.

The results from data set 2 and 3 were analyzed using dose × scale ANOVA for each of the drugs, with the scale as a repeated measure, because the curvatures at different scales were measured in the same animals.

Results

For statistical analysis purposes we consider, in the scope of the current study, only the simplest property of the path texture – the median of absolute curvature values in each scale, performed over data points pooled from all progression segments in the session. We term the absolute median curvature as a function of scale the 'texture profile'. Figures 3 and 6–8 display such texture profiles. Note that, using the absolute value of the curvature means, no differentiation is made between curving to the right and curving to the left.

The phenomenon of path curvature, however, seems to have many subtler properties that cannot be captured by the texture profile alone. Figure 9 displays the texture analysis of a single rather long progression segment of a C57BL/6J mouse. Each line in the texture plot on the right represents a single data point in the path plot on the left. This line connects the curvatures measured at this point in seven distance scales, computed as demonstrated in Fig. 2. The bold line connecting bold dots in the texture plot is the texture profile – the median absolute curvature in this single segment – but many other features of the path can be discerned, and quantifying them in future studies is straightforward. For example, the convergence of lines into a positive value at the largest scale (B) means that the general curvature of the segment was to the left. That is, many parts of the path, which in smaller scales meander either to the left or to the right, are all part of one large left curve in the largest scale. Similarly, the convergence of lines into a negative value (A) in a large but slightly smaller scale than the convergence of (B) means that, despite the general left curving of this segment, a large portion of it curves to the right. Note that the curvature is presented using a cube-root scale, in contrast with the log scale that is used in the subsequent analysis, in order to retain the distinction between positive and negative values (left and right turns). Note also that convergence of lines in the largest scale (B) is into a curvature value that is close to that of the arena wall (dashed bold line). This is because this progression segment generally follows the wall. The diverging of lines in smaller scales,



Strain	Data set	Lab	Treatment	Test phase	Tracking rate
C57BL/6J	1	NIDA	none	dark	30 Hz
C57BL/6J	1	MPRC	none	dark	30 Hz
C57BL/6J	1	TAU	none	dark	25 Hz
C57BL/6J	2	NIDA	vehicle	light	30 Hz
C57BL/6J	3	MPRC	vehicle	light	30 Hz
C57BL/6J	3	MPRC	vehicle	tigh	30 Hz
C57BL/6J	3	MPRC	vehicle	light	30 Hz
DBA/2J	1	NIDA	none	dark	30 Hz
DBA/2J	1	MPRC	none	dark	30 Hz
DBA/2J	1	TAU	none	dark	30 Hz
DBA/2J	2	NIDA	vehicle	light	25 Hz

Figure 6: Robustness of path texture. The mean texture profiles in all C57BL/6J groups (continuous lines) and DBA/2J groups (dashed lines) in the database that did not receive any drug. The graph is in log–log scale. The table above specified the differences in laboratories and conditions in these groups.

however, shows that even in such a segment the curvature in smaller scales is not overly constrained by the wall. Small and sharp meanderings in the path (C and D) are seen in the texture plot as lines that have high-positive or high-negative curvatures in small scales. Lines crisscrossing from positive to negative values imply smaller curves embedded within larger curves to the opposite direction. The slope of the line at the zero-crossing quantifies how meandered is this embedding, and it can be measured separately between each of two scales.

The texture plot thus retains a wealth of information regarding the animal's path in the level of a single progression segment and even small parts of a segment. Unlike the original path plot, however, the texture plot can be easily superimposed on and added to similar texture plots from other segments, animals and genotypes. In the discussion section we suggest some possibilities for a more sophisticated analysis of such representations, but as mentioned above, only the session texture profile will be presented and analyzed in the rest of this study.

Figure 4 displays four different segments, demonstrating how different path textures of the segment can be captured by the median absolute curvature in different scales. Segments B and C have similar curvatures across scales, but segments A and D have very different curvatures in different scales, with A having the higher curvature in the small scale and D having the higher curvature in the large scale. The existence of all four types of segments indicates that curvature should not be quantified independently of scale.

Figure 3 shows the session texture profile in seven inbred strains, each strain tested across three laboratories (*Data set 1*). For each animal, the texture profile (as in the bold line in Fig. 9, right) was computed over the whole session. The strain averages were calculated over these session texture profiles and pooled over the three laboratories. The strain standard errors in Fig. 3 therefore include also the laboratory effect and the laboratory × strain interaction, but as these errors are rather small, this does not confound the presentation of general strain differences. Note that hardly any two strains had similar curvatures across all scales. For example, C3H/HeJ and SJL/J had practically identical curvatures in the small scales (2 and 4 cm), but the C3H/HeJ had distinctly higher curvatures at medium (16 and 32 cm) and high (64 and 128 cm) scales. That is, the path of these two strains looks similar when zooming on very small details, but when looking at gross patterns, the path of the C3H/HeJ strain is much more curved. Comparing the example path plots of the C3H/HeJ mouse and the SJL/J mouse in Fig. 1 indeed confirms these properties. Figure 3 also shows that the SJL/J has a much higher curvature than 129S1/SvImJ in small and medium scales but similar curvature in high scale. This again agrees with the example path plots in Fig. 1, where the path of the SJL/J and 129S1/SvImJ mice look similar in the large scale, but when looking closely, the path of the SJL/J mouse is much more meandering. The only pair of genotypes that seems similar across all scales is C57BL/6J and FVB/NJ. It should be noted that, while these two genotypes could not be discriminated by their path texture, they are very different in the dynamics of movement. Specifically, FVB/NJ mice display much stronger accelerations (Golani *et al.* 2004).

The texture profiles in Fig. 3 were approximately linear in some strains, while clearly deviating from linearity in other strains (notably the BALB/cByJ). Certain pairs of strains, such as C57BL/6J and DBA/2J, had similar slopes over the linear range, but the absolute values were very different. It is

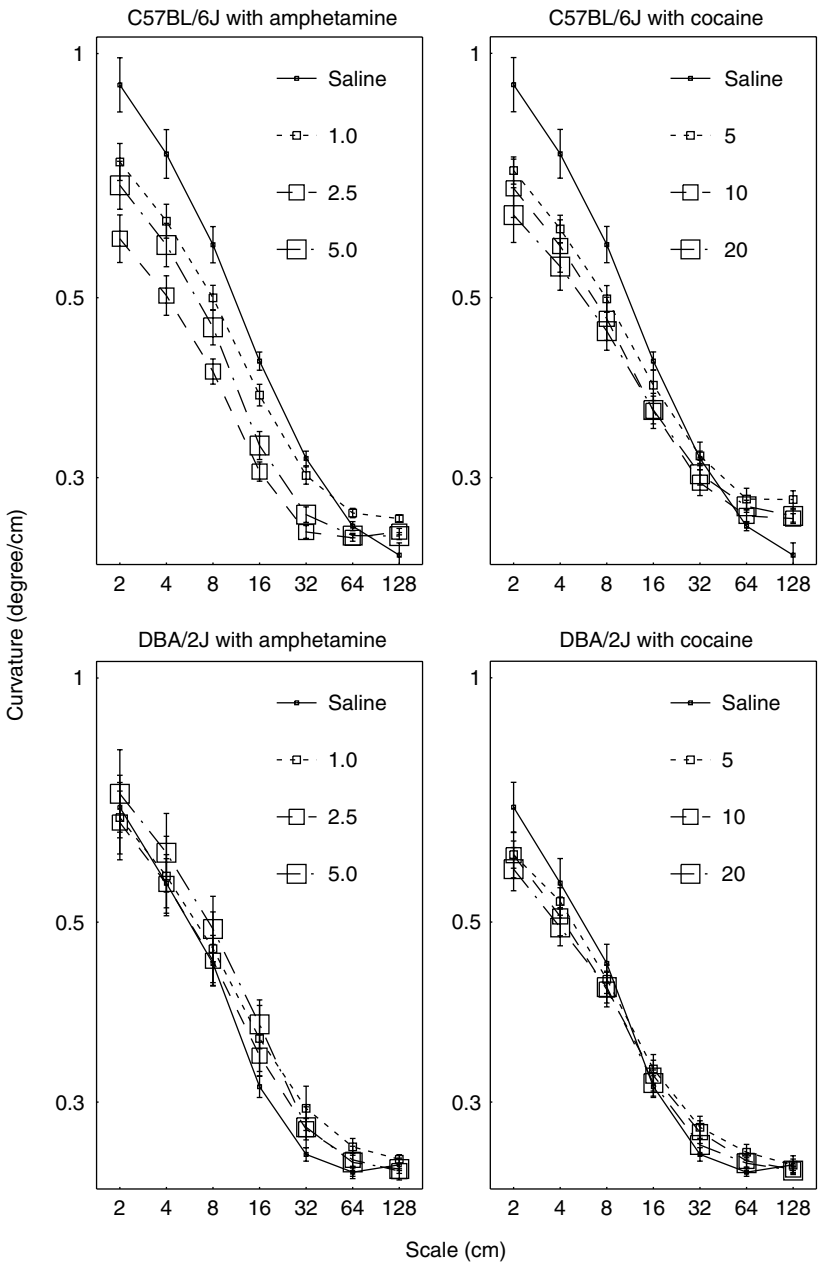


Figure 7: Amphetamine and cocaine effect on path texture. The dose effect of amphetamine (left column) and cocaine (right column) on the texture profile of C57BL/6J mice (top row) and DBA/2J mice (bottom row). All graphs are in log–log scale.

therefore clear that the median absolute curvature as a function of scale is highly specific to the genotype, but differences between genotypes are not easily captured by the slope or any other single parameter.

Estimation of strain differences and replicability across laboratories, using the mixed ANOVA framework (Kafkafi *et al.* 2005) is difficult when considering a series of numbers for each strain and is better performed with a single measurement per group. We thus chose three scales and compared the strains across laboratories separately for each scale. In Fig. 3 there seems to be a fair amount of correlation between neighboring scales. The extreme scales, 2 and 128 cm, are less credible because of boundary effects: the first approaches the tracking resolution and the second approaches the arena size and also suffers from smaller sample sizes, because some strains rarely perform such long segments. We therefore chose the scales of 4, 16 and 64 cm as representative scales of smaller than mouse size, about mouse size and larger than mouse size, respectively.

As Fig. 5 shows, the replicability across laboratories in the three scales was generally good, as the SD of the genotype × laboratory interaction is not large relative to the differences between the strain means (the more relevant statistics for such a comparison is actually the standard

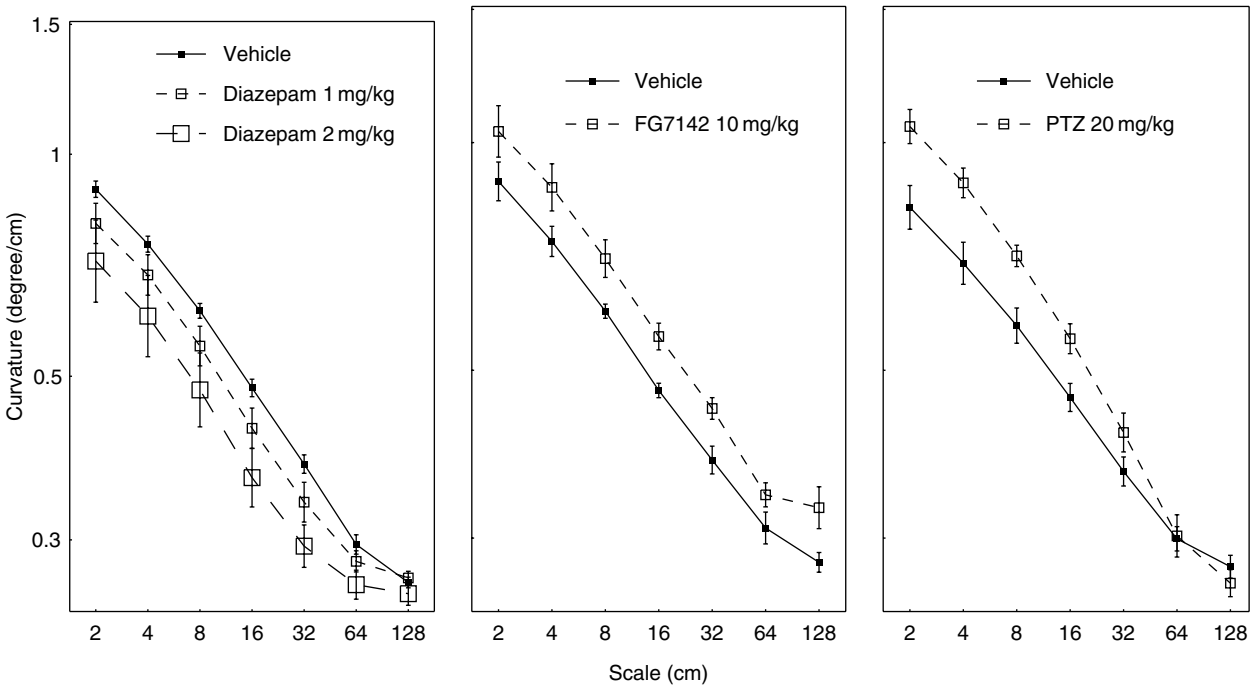


Figure 8: The effect of anxiety-related drugs on path texture. The effect of diazepam (two doses), FG-7142 and PTZ on the texture profiles in C57BL/6J mice (data set 3). All graphs are in log-log scale.

error, which is even smaller than the SD, but in this case could not be estimated, see *Statistical methods* section). The mixed ANOVA analysis (Table 1) shows that the difference between the genotypes was highly significant in all the three scales, and the percent of total variance that was attributed to the genotype, which is a relatively conservative estimation of the broad-sense heritability (Kafkafi *et al.* 2003a), was 43–71%, while the interaction contributed at most 6% and the laboratory at most 2%. Table 1 also summarizes the SD of laboratory, interaction and within-group effects. In the mixed model approach (Kafkafi *et al.* 2005), the SD of interaction can be used in any future experiment that will measure curvature in yet another laboratory in order to estimate whether its results are also replicable.

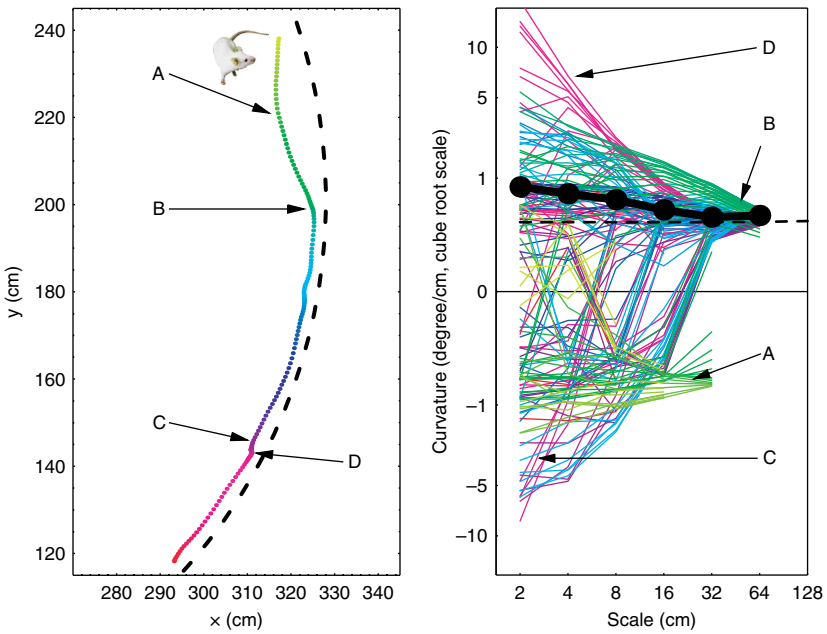


Figure 9: Path and texture plots. The path plot (left) and the texture plot (right) of a single progression segment of a C57BL/6J mouse. Each data point in the path plot is represented by a line of the same color in the texture plot, connecting the curvatures measured in several scales at this point. Letters A, B, C and D mark corresponding features in both plots (see text for details). The dashed bold line denotes the arena wall in the path plot and the curvature of the arena wall in the texture plot. A dark bold line in the texture plot connects the medians of absolute curvatures in each scale.

Figure 5 also demonstrates that the patterns of strain means in the scales of 4 and 16 cm were very different. This implied that the 16-cm scale contributes additional information that cannot be gleaned from the 4-cm scale alone. The pattern in the 64-cm scale (Fig. 5) was generally similar to the pattern in the 16-cm scale, but there is one clear exception: the SJL/J strain had intermediate curvature in the 16-cm scale in all the three laboratories, while having low curvature in the 64-cm scale, again in all the three laboratories. This means that the general correlation between intermediate and large scale is not categorical, and certain genotypes may be characterized by exemption from this trend.

Useful measures for behavioral phenotyping should preferably be robust not only to the laboratory but also to small changes in the experiment protocol. In order to get a notion of the robustness of texture, we present in a single graph (Fig. 6) the texture profiles of all C57BL/6J and DBA/2J groups in the database that did not receive any drug (i.e. both non-injected and vehicle-injected groups). As Fig. 6 shows, the texture discriminates these two inbred strains consistently despite testing in different laboratories, in different phases of the photoperiodic cycle, with different tracking rates and in non-injected vs. vehicle-injected animals. Note that these data cannot be used as a proper test for the effect of any of those factors, because these were different experiments, and theoretically the effect of repeating the experiment might have countered the effect of changing the factor so as to give an incorrect impression of robustness. With several different experiments, however, the chances for such a coincidence are small.

Amphetamine and cocaine (data set 2) dose-dependently decreased the curvature in C57BL/6J mice but mainly in the small and medium scales, while having little effect in large scales (Fig. 7). The main effect of amphetamine dose was very significant ($F_{3,48} = 21.7$, $P < 0.0001$), as well as the dose $\times$ scale interaction ($F_{6,48} = 5.1$, $P < 0.001$). The effect was bi-phasic, reaching the lowest curvature with 2.5 mg/kg and then slightly increasing again with 5 mg/kg. The dose effect of cocaine was not as significant ($F_{3,48} = 4.7$, $P < 0.01$), with the interaction still significant ($F_{6,48} = 2.8$, $P < 0.05$), but the dose effect was monotonic up to the highest dose used (20 mg/kg). Hence, cocaine made the path of C57BL/6J mice less curved only when looking at small path details, but under amphetamine it was less curved also when looking at gross patterns. In contrast, no significant effect of either amphetamine or cocaine on the curvature was found in DBA/2J mice, although their activity (distance traveled in cm) was increased threefold.

In data set 3, the median curvature was calculated for C57BL/6J mice in MPRC that were injected with the anxiolytic drug diazepam or either of the two anxiogenic drugs FG-7142 and PTZ (Fig. 8). Diazepam (1.0 and 2.0 mg/kg) significantly decreased the median curvature over scales 4, 16 and 64 cm in a dose-dependent manner ($F_{2,26} = 5.2$, $P < 0.05$). In contrast, the two anxiogenic compounds increased the median curvature across the scales 4, 16 and 64 cm: FG-7142 (10 mg/kg) ($F_{1,14} = 14.4$, $P < 0.01$) and PTZ (20 mg/kg) ($F_{1,14} = 7.5$, $P < 0.05$). The effect of PTZ also had a marginally significant dose $\times$ scale interaction ($F_{2,14} = 4.0$, $P = 0.067$). There were no significant differences between the three vehicle groups. Hence, the anxiolytic drug made the path straighter while the anxiogenic drugs made it more curved, when looking at smaller than mouse size, mouse size or larger than mouse size path details. It should be noted that PTZ, FG-7142 and 1.0 mg/kg diazepam did not have a significant effect on either the distance traveled or time spent in the center of the arena, while 2.0 mg/kg diazepam significantly decreased both distance traveled and center time. These results are not in line with Choleris *et al.* (2001) who found that 1.5 mg/kg diazepam slightly but significantly increased the time spent outside the wall area. This difference may be due to the difference in genotype (CF-1 vs. C57BL/6 in our study) and/or arena type (80 cm square vs. 250 cm circular in this study). Because the decrease in center time in the 2.0 mg/kg group was coupled with a decrease in the distance traveled, it was probably a result of the sedative effect of diazepam in higher doses.

Discussion

We showed that the texture of locomotor path, as measured by the path curvature over a wide range of distance scales, is a useful measure for phenotyping mouse behavior in the open field and possibly also for characterizing drug effects. The path texture is highly heritable, with each genotype having a unique texture profile. These genotype differences cannot be adequately characterized using only the slope of the log curvature as a function of log scale, or by any other single parameter. The curvature in all scales was replicable across laboratories, as determined using the approach of mixed model ANOVA, and was also relatively robust to the photoperiodic phase of testing and vehicle injections. In C57BL/6J mice, amphetamine and cocaine dose-dependently decreased the curvature but mainly in scales of mouse body length and smaller, and not in larger scales, while diazepam dose-dependently decreased the curvature across all scales. The anxiogenic drugs FG-7142 and PTZ increased the curvature in small and medium scales; FG-7142 did so as well in large scales.

Figures 3 and 9 show that in scales up to 32 cm, and even 64 cm in some strains, the curvature of the path is not constrained by the curvature of the arena wall (dashed line). This means that behavior in these scales may not be affected much by the shape of the arena. For example, the path along the straight wall of a square arena would probably have similar texture properties in these scales. In the corners of a square arena, however, the animal would probably be forced to increase path curvature even in smaller scales.

This study used the approach of mixed model ANOVA to test the replicability of results across laboratories (Kafkafi *et al.*

2005). According to this model, the laboratories in this study are used as a sample representing many possible phenotyping laboratories, and the strain differences are tested relative to the higher benchmark of the combined within-group and strain × laboratory interaction variabilities, as an added assurance that these strain differences would indeed be detected in yet an additional laboratory. Despite using this larger benchmark, the strain differences in path texture were very significant, and the laboratory and interaction effects together accounted for only 2–8% of the total variance, while the genotypic effect was 42–71%. According to the mixed model, the SD of the interaction reported in this study (Table 1) should be taken into account when testing differences of path texture in other strains and laboratories. Note that the significance of the strain × laboratory interaction is not tested in the mixed model, because it is assumed to be random, but most of the strain differences in Crabbe *et al.* (1999) are also significant using the mixed model (Kafkafi *et al.* 2005).

The texture plot (Fig. 9, right) captures several complex properties of a progression segment in a way that can easily be summed over many segments and animals. The present study has considered only the median curvature within each scale separately (bold line in Fig. 9) pooled over an entire segment or session. That is, it treated the texture plot in Fig. 9 as if it was a dot graph, practically ignoring the lines that join these dots and describe the real-time relations between scales. As this study has shown, even such a basic characterization was already enough to confirm the subjective impression that different genotypes display different qualities of path texture. This characterization, however, can be easily extended in future studies by straightforward analysis of line properties in the texture plot. Note that the same dot patterns in a texture plot (producing the same medians) may be connected in very different ways between scales. For example, an existence of lines that are far from zero curvature over the whole range of scales would indicate a tendency of the animal to break a segment into different sections in very different directions, divided by sharp turns. Lines crossing the horizontal axis of zero curvature (i.e. smaller curves embedded within larger curves to the other direction) would indicate a highly meandering path, which can be further classified according to the location of the zero crossing(s) on this axis. Texture-plot analysis thus has a potential of increasing the power to differentiate path style that characterize certain genotypes and treatments.

Additional obvious directions for analysis of curvature are its relation to the momentary distance from the arena wall and to the momentary velocity. The curvature in medium and large scales (but apparently not in small scales) is most probably constrained by the curvature of the arena wall when the mouse is moving near it (note in Fig. 3, how the wall's curvature seems to act as an asymptote with increasing scales). It is possible that genotypes that have similar path texture near the wall have different path textures during incursions into the arena or vice versa. This question can be investigated using the intrinsic categorization into wall progression and incursions as suggested by Lipkind *et al.* (2004). Regarding the momentary speed, for physical reasons, it is generally expected that increased speed will correlate with decreased curvature, but the exact parameters of this relation may again differ in different genotypes and treatments. In any case, studying each property or relation across several different scale of length (or possibly time, see for example Paulus & Geyer 1991; 1993a) is likely to separate different simultaneous processes that contribute to the final pattern of behavior.

Direction change in locomotor progression may result from responses to environmental stimuli and also may reflect innate tendencies even in the absence of a stimulus. In any case, a change in the direction implies a response or a decision taken by the animal, and the amount of direction change (resulting in the curvature of the path) is a gauge of the intensity of this response or decision. Higher median curvature thus suggests higher tendency to react to stimuli or to 'change one's mind'. Curvature in smaller scales, especially those smaller than a mouse body size, is likely to correspond to short-term, quick decisions that are affected mainly by the most proximal environment and are probably more sensory-motor in nature. In contrast, curvature in larger scales, especially those approaching the size of the large arena, implies more long-term decisions that are affected by more global properties of the environment and are related more to motivation and cognition. Our results suggest a degree of independence between the small-scale and large-scale decisions: genotypes with the same small-scale curvature can have markedly different large-scale curvature and vice versa. Note also that the variance attributed to the individual animal (within-group) in the 64-cm scale was twice as large as in the 4- and 16-cm scale (Table 1), possibly conforming to a notion that cognitive behavior is less heritable than motor behavior.

The fact that curvature was decreased by the anxiolytic drug diazepam while increased by the anxiogenic PTZ and FG-7142 suggests that increased curvature of the path is an expression of anxiety. This explanation, however, is not supported by amphetamine and cocaine decreasing the path curvature in C57BL/6J mice, because amphetamine (Kliethermes *et al.* 2003; Pellow *et al.* 1985; Simon *et al.* 1994) and cocaine (Rogerio & Takahashi 1992; Simon *et al.* 1994) are usually thought to have an anxiogenic effect in several behavioral paradigms including the open field. The decreasing curvature in the case of amphetamine and cocaine, however, was coupled with a marked increase of activity, while in the case of diazepam, it was coupled with no change or even a decrease (with the highest diazepam dose) of activity. This suggests that using both the activity of curvature, or possibly more refined analysis of texture, may prove useful for animal models of anxiety.

This study is an additional demonstration of the use of the exploratory data analysis approach to behavioral phenotyping

(Kafkafi *et al*. 2003b; Kafkafi *et al*. 2005; Lipkind *et al*. 2004). In this approach, raw behavioral data are treated as an information-reach source that can be stored in a database and reanalyzed. This database may include data from different experiments, laboratories, genotypes, treatments, arenas and conditions. By using an interactive programming environment that enables an easy access and analysis of any desired subsection of the data (Kafkafi 2003), this approach facilitates the characterization of complex behavior patterns and the derivation of increasingly more informative, discriminative and reliable measures of behavior.

References

Benjamini, Y., Drai, D., Elmer, G.I., Golani, I. & Kafkafi, N. (2001) Controlling the false discovery rate in behavior genetics research. *Behav Brain Res* **125**, 279–284.

Choleris, E., Thomas, A.W., Kavaleris, M. & Prato, F.S. (2001) A detailed ethological analysis of the mouse open-field test: effect of diazepam, chlodiazepoxide and an extremely low frequency pulsed magnetic field. *Neurosci Biobehav Rev* **25**, 235–260.

Cools, A.R., Ellenbroek, B.A., Gingras, M.A., Engbersen, A. & Heeren, D. (1997) Differences in vulnerability and susceptibility to dexamphetamine in Nijmegen high and low responders to novelty: a dose-effect analysis of spatio-temporal programming of behavior. *Psychopharmacology (Berl)* **132**, 181–187.

Crabbe, J.C., Wahlsten, D. & Dudek, B.C. (1999) Genetics of mouse behavior: interactions with lab environment. *Science* **284**, 1670–1672.

Crawley, J.N., Belknap, J.K., Collins, A., Crabbe, J.C., Frankel, W., Henderson, N., Hitzemann, R.J., Maxson, S.C., Miner, L.L., Silva, A.J., Wehner, J.M., Wynshaw-Boris, A. & Paylor, R. (1997) Behavioral phenotypes of inbred mouse strains. *Psychopharmacology (Berl)* **132**, 107–124.

Drai, D. & Golani, I. (2001) SEE: a tool for the visualization and analysis of rodent exploratory behavior. *Neurosci Biobehav Rev* **25**, 409–426.

Drai, D., Benjamini, Y. & Golani, I. (2000) Statistical discrimination of natural modes of motion in rat exploratory behavior. *J Neurosci Methods* **96**, 119–131.

Drai, D., Kafkafi, N., Benjamini, Y., Elmer, G.I. & Golani, I. (2001) Rats and mice share common ethologically relevant parameters of exploratory behavior. *Behav Brain Res* **125**, 133–140.

Eilam, D. & Golani, I. (1989) Home base behavior of rats (*Rattus norvegicus*) exploring a novel environment. *Behav Brain Res* **34**, 199–211.

Eilam, D., Golani, I. & Szechtman, H. (1989) D2 Agonist quinripole induces perseveration of routes and hyperactivity but no perseveration of movements. *Brain Res* **460**, 255–267.

Gingras, M.A. & Cools, A.R. (1997) Different behavioral effects of daily or intermittent dexamphetamine administration in Nijmegen high and low responders. *Psychopharmacology (Berl)* **132**, 188–194.

Golani, I., Benjamini, Y. & Eilam, D. (1993) Stopping behavior: constraints on exploration in rats (*Rattus norvegicus*). *Behav Brain Res* **53**, 21–33.

Golani, I., Elmer, G.I., Kafkafi, N. & Benjamini, Y. (2004) Exploratory behavior surveyed in three laboratories: data, submitted to the Mouse Phenome Database, http://aretha.jax.org/pub-cgi/phenome/mpdcgi?rtn=docs/home.

Hen, I., Sakov, A., Kafkafi, N., Golani, I. & Benjamini, Y. (2004) The dynamics of spatial behavior: how can robust smoothing techniques help? *J Neurosci Methods* **133**, 161–172.

Kafkafi, N. (2003) Extending SEE for large-scale phenotyping of mouse open-field behavior. *Behav Res Methods Instrum Comput* **35**, 294–301.

Kafkafi, N., Mayo, C.L., Drai, D., Golani, D. & Elmer, G.I. (2001) Natural segmentation of the locomotor behavior of drug-induced rats in a photobeam cage. *J Neurosci Methods* **109**, 111–121.

Kafkafi, N., Lipkind, D., Benjamini. Y., Mayo, C.L., Elmer, G.I. & Golani, I. (2003a) SEE locomotor behavior test discriminates C57BL/6J and DBA/2J mouse inbred strains across laboratories and protocol conditions. *Behav Neurosci* **117**, 464–477.

Kafkafi, N., Pagis, M., Lipkind, D., Mayo, C.L., Benjamini, Y., Elmer, G.I. & Golani, D. (2003b) Darting behavior: a quantitative movement pattern for discrimination and replicability in mouse locomotor behavior. *Behav Brain Res* **142**, 193–205.

Kafkafi, N., Benjamini, Y., Sakov, A., Elmer, G.I. & Golani, I. (2005) Genotype-environment interactions in mouse behavior: a way out of the problem. *Proc Nat Acad Sci USA* **102**, 4619–4627.

Kliethermes, C.L., Finn, D.A. & Crabbe, J.C. (2003) Validation of a modified mirrored chamber sensitive to anxiolytics and anxiogenics in mice. *Psychopharmacology (Berl)* **169**, 190–197.

Lipkind, D., Sakov, A., Kafkafi, N., Elmer, G., Benjamini, Y. & Golani, I. (2004) New replicable anxiety-related measures of wall versus center behavior of mice in the open field. *J Appl Physiol* **97**, 347–359.

Mandelbrot, M. (1982) *The Fractal Geometry of Nature*. Freeman and company, New York.

McCulloch, C.E. & Searle, S.R. (2001) *Generalized, Linear and Mixed Models*. Wiley, New York.

Paulus, M.P. & Geyer, M.A. (1991) A temporal and spatial scaling hypothesis for the behavioral effects of psychostimulants. *Psychopharmacology (Berl)* **104**, 6–16.

Paulus, M.P. & Geyer, M.A. (1993a) Three independent factors characterize spontaneous rat motor activity. *Behav Brain Res* **53**, 11–20.

Paulus, M.P. & Geyer, M.A. (1993b) Quantitative assessment of the microstructure of rat behavior: I, f(d), the extension of the scaling hypothesis. *Psychopharmacology (Berl)* **113**, 177–186.

Pellow, S., Chopin, P., File, S.E. & Briley, M. (1985) Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods* **14**, 149–167.

Ralph, R.J., Paulus, M.P. & Geyer, M.A. (2001) Strain-specific effects of amphetamine on prepulse inhibition and patterns of locomotor behavior in mice. *J Pharmacol Exp Ther* **298**, 148–155.

Ralph-Williams, R.J., Paulus, M.P., Zhuang, X., Hen, R. & Geyer, M.A. (2003) Valproate attenuates hyperactive and perseverative behaviors in mutant mice with a dysregulated dopamine system. *Biol Psychiatry* **53**, 352–359.

Rogerio, R. & Takahashi, R.N. (1992) Anxiogenic action of acute but not repeated cocaine administration in handling-habituated mice in the plus-maze test. *Braz J Med Biol Res* **25**, 713–716.

Rogers, D.C., Jones, D.N., Nelson, P.R., Jones, C.M., Quilter, C.A., Robinson, T.L. & Hagan, J.J. (1999) Use of SHIRPA and discriminant analysis to characterise marked differences in the behavioural phenotype of six inbred mouse strains. *Behav Brain Res* **105**, 207–201.

Simon, P., Dupuis, R. & Costentin, J. (1994) Thigmotaxis as an index of anxiety in mice. Influence of dopaminergic transmissions. *Behav Brain Res* **61**, 59–64.

Szechtman, H., Culver, K. & Eilam, D. (1999) Role of dopamine systems in obsessive-compulsive disorder (OCD): implications

from a novel psychostimulant-induced animal model. *Pol J Pharmacol* **51**, 55–61.
Tchernichovski, O. & Golani, I. (1995) A phase plane representation of rat exploratory behavior. *J Neurosci Methods* **62**, 21–27.
Tchernichovski, O., Benjamini, Y. & Golani, I. (1996) Constraints and the emergence of freedom in the ontogeny of rat exploratory behavior. *Behaviour* **133**, 519–539.
Tchernichovski, O., Benjamini, Y. & Golani, I. (1998) The dynamics of long term exploratory behavior in the rat, part I. *Biol Cybern* **78**, 423–432.
Wallace, D.G., Hines, D.J. & Whishaw, I.Q. (2002) Quantification of a single exploratory trip reveals hippocampal formation mediated dead reckoning. *J Neurosci Methods* **30**, 113.
Whishaw, I.Q., Cassel, J.C., Majchrzak, M., Cassel, S. & Will, B. (1994) 'Short-stops' in rats with fimbria-fornix lesions: evidence for change in the mobility gradient. *Hippocampus* **4**, 577–582.
Whishaw, I.Q., Hines, D.J. & Wallace, D.G. (2001) Dead reckoning (path integration) requires the hippocampal formation: evidence from spontaneous exploration and spatial learning tasks in light (allothetic) and dark (idiothetic) tests. *Behav Brain Res* **127**, 49–69.
Wolfram, S. (1999) *The Mathematica Book*, 4th edn. Wolfram Media and Cambridge University Press, NY, Cambridge (UK).

Acknowledgments

We thank Drs Ilan Golani and Yoav Benjamini for useful comments and Anat Sakov for computing the Mixed Model analysis. This study is part of the project 'Phenotyping mouse exploratory behavior' supported by NIH grant 1 R01 NS40234. The SEE software is available at http://www.tau.ac.il/~ilan99/see/help