

## Interactive report

**Behavioral organization is independent of locomotor activity in 129 and C57 mouse strains<sup>1</sup>**Martin P. Paulus<sup>a,c</sup>, Stephanie C. Dulawa<sup>b</sup>, Rebecca J. Ralph<sup>b</sup>, Mark A. Geyer<sup>a,b,c,\*</sup><sup>a</sup> *Laboratory of Biological Dynamics and Theoretical Medicine, School of Medicine, University of California, San Diego (0804), La Jolla, CA 92093-0804, USA*<sup>b</sup> *Department of Neuroscience, School of Medicine, University of California, San Diego (0804), La Jolla, CA 92093-0804, USA*<sup>c</sup> *Department of Psychiatry, School of Medicine, University of California, San Diego (0804), La Jolla, CA 92093-0804, USA*

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**Abstract**

Assessing locomotor behavior is a standard methodology to characterize the behavioral phenotype of a genetic manipulation. Typically, levels of locomotor activity are measured using various methods that are based on the frequency of photobeam breaks or distance traveled as assessed by video-tracking systems. Locomotor behavior, however, is multi-dimensional and reflects the combined influences of multiple processes. Here, we examine the number of independent dimensions of locomotor behavior in mice based on measures derived from a video-tracking system. In addition, we test the hypothesis that locomotor behavior varies substantially across mouse strains. 84 mice were tested for 30 min in a 41 × 41 cm enclosure. Based on previous investigations in rats, we also assessed the spatial and dynamical aspects of locomotor behavior using the spatial scaling exponent, *d*, and the dynamical entropy, *h*. A principal component analysis and a one-way repeated measure ANOVA were conducted. C57 mouse strains differ substantially from 129 mouse strains on almost all measures of locomotor behavior. The principal component analysis revealed that two independent factors influence this set of measures. The first factor reflects the amount or level of locomotor activity, the second factor quantifies the degree of spatial and dynamical organization of behavior. These strain differences and the existence of at least two independent dimensions when measuring locomotor behavior may help to parse the effects of gene manipulations relative to strain differences in mutant mice. © 1999 Elsevier Science B.V. All rights reserved.

*Keywords:* Behavioral organization; Locomotor activity; 129/SvJ mice; C57BL/6J mice

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**1. Introduction**

The assessment of rodent unconditioned locomotor behavior has become one of the most widely used behavioral paradigms to determine the effects of various experimental manipulations ranging from genetic changes, e.g. knockout mice, to pharmacological challenges, e.g. amphetamine-induced locomotor activity. This wide range of applications is based on the fact that unconditioned motor activity probes a variety of behaviors, can be recorded automatically, and can quickly generate an effect profile [8]. In rats, locomotor activity has been used to discriminate drug effects, to elucidate the functional roles of specific neurobiological systems, and to screen drugs for potential psychoactivity.

More recently, locomotor activity has been used as a critical assay to establish the phenotype for various genetic manipulations of mice. Nevertheless, as pointed out by others, the increased use of transgenic and null mutation techniques in the development of animal models of disorders underlines the importance of selecting the appropriate genetic background due to large strain-dependent differences in behavioral measures. For example, significant inter-strain differences have been demonstrated across twelve strains of inbred mice and seven F1 hybrids that were tested in multiple behavioral tasks including open-field locomotor activity, Y-maze activity, auditory and tactile startle reactivity, and prepulse inhibition of startle [14]. Similarly, large individual differences exist among mice in their behavioral responses to drugs of abuse. Comparing C57BL/6J and 129/SvJ, and their outcrossed F1 offspring using conditioned place preference, mice of the 129/SvJ strain were found to be hypoactive and very sensitive to the locomotor activating effects of cocaine

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Table 1  
Number, vendors, and strains of animals used in this investigation

Number of animals	Vendor	Strain
20	Jackson Laboratories, Bar Harbor, ME	C57BL/6 (x129)
14	Jackson Laboratories, Bar Harbor, ME	C57BL-6
20	Jackson Laboratories, Bar Harbor, ME	C57BL/6J
7	Harlan, Indianapolis, IN	C57DBA / B6D2F1
9	Taconic, Germantown, NY	129SvEv
15	Jackson Laboratories, Bar Harbor, ME	129SvEms – + <sup>Ter?</sup> /J
9	Jackson Laboratories, Bar Harbor, ME	129/SvJ
Total = 84		

[17]. Nevertheless, 129/SvJ did not develop cocaine-conditioned place preference under conditions that yielded significant place preference in C57BL/6J mice. Finally, these strain-dependent behavioral characteristics can be inherited in a non-additive manner. Such results emphasize the importance of investigating the underlying behavioral dimensions in different strains of mice and support the notion of using detailed assessment procedures to adequately quantify the strain-dependent behavioral phenotype.

While locomotor activity has been a widely used behavioral assay, its conceptual basis is complex. A variety of different concepts have been applied to the interpretation of aspects of unconditioned motor behavior of rodents in an open field, including arousal, novelty seeking, diversive and inspective exploration, anxiety, stereotypy, and perseveration [8]. Numerous investigators have recognized the necessity for analyses of multivariate profiles and/or spatio-temporal patterns of motor activity and proposed different approaches to quantify the various components of open field behavior [6,11,30]. Some of these approaches were based on observer ratings, while others have attempted to automate the entire measurement process. The measurement approaches developed in studies in rats are now being applied to phenotypic assessments of mice. As discussed elsewhere [8,10,23], multivariate characterizations

of rat locomotor activity, typically including measures of crossings, distance traveled, time in the center vs. the periphery, rearings, and sometimes holepokes, have many advantages over univariate assessments limited to measures of the amount of activity. Nevertheless, studies in rats also clearly demonstrate the additional utility of assessments of sequential patterns of locomotor activity in pharmacological and neurobiological studies [6,9,10,18,21], insofar as these measures of the organization of the behavior provide further information regarding the differential effects of various manipulations. The present report describes initial efforts to extend these measures of the organization of locomotor behavior, as developed and validated in studies of rats, to studies of the behavior of mice in an open field.

In extensive studies using rats, we have complemented the traditional assessments of the amount of locomotor activity by developing quantitative descriptions based on the application of new assessment techniques in order to derive constructs that assess independent dimensions of unconditioned motor behavior [10,23]. Specifically, we have developed techniques to quantify sequences of movements in order to assess patterns of locomotor activity [20,22,24]. This approach was based on the assumption

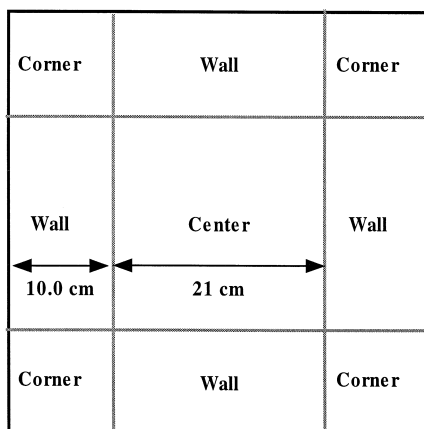


Fig. 1. Behavior in each of the  $41 \times 41$  cm open fields is assessed as transitions between and time spent in the arbitrary divisions shown here.

Movement Patterns with different spatial scaling exponents

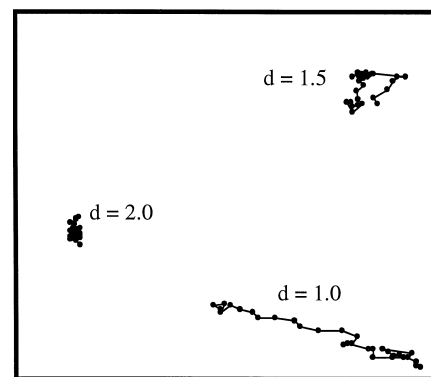


Fig. 2. Three different path patterns each consisting of 24 consecutive movements. The spatial scaling exponent,  $d$ , quantifies the degree to which the movements follow a straight line ( $d = 1.0$ ) or are located in a highly circumscribed area ( $d = 2.0$ ).

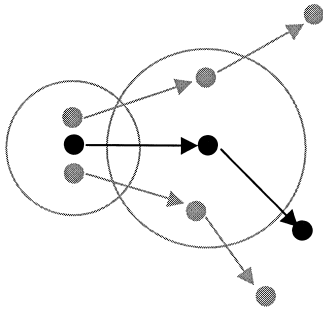


Fig. 3. Three difference schematic movement sequences beginning within a radius,  $r$ , of the enclosure. The dynamical entropy,  $h$ , corresponds to the growth rate of radius of these initially close movements. The higher the unpredictability the larger the growth rate.

that behavior consists of purposefully organized micro-events resulting in distinct patterns of activity. For example, exploratory activity consists of consecutive straight movements to reach a location in the environment combined with circumscribed movements to further process localized sensory stimuli. Previous investigations in rats have shown that this approach is both sensitive and relatively specific to the underlying neurobiological manipulations. First, the locomotor patterns as measured by their geometric and dynamical characteristics vary independently from the amount of locomotor activity observed [22]. Second, indirect dopaminergic drugs exhibit a characteristic overall motor pattern but can be distinguished based on individually different contributions to other neurotransmitter systems [19]. Third, the influence of the geometric characteristics on the locomotor patterns can be measured directly [25]. Fourth, developmental [18] and strain-related differences in locomotor patterns can be used as a more sensitive phenotypic marker than measures limited to the amount of locomotor activity.

This investigation evaluated two hypotheses. First, based on numerous reports of large variability in behavioral assays between different strains of mice, we hypothesized that the two strains of mice frequently used in our knock-

out experiments differ substantially across various measures of locomotor activity. Second, based on our previous work with rats, we hypothesized that detailed assessment of unconditioned motor behavior is able to quantify independent dimensions of this behavior in mice. In a corollary, we tested the hypothesis that variations in locomotor patterns are not simply detected by measuring horizontal activity, center versus perimeter distance, or time spent in different areas.

## 2. Methods

### 2.1. Animals

A total of 84 mice age 3 months were used for this study. Table 1 displays the different vendors and background strains. These animals correspond to control groups from various experiments involving knockout mice and other pharmacological manipulations. The animals were housed in groups on a reversed 12/12 h light/dark cycle and had ad libitum food access. As part of a standard acclimation phase, these mice were housed in the animal facilities for 1–2 weeks prior to testing. The locomotor activity experiment was the first exposure of the mice to the laboratory and to any behavioral testing.

### 2.2. Equipment

For all experiments with non-white mice, the Videotracker (VT) consists of 4 adjacent white Plexiglas  $41 \times 41 \times 34$  cm enclosures; for the experiment with white 129SvJ mice, four black enclosures were used. The enclosures are surrounded by a plastic drape to standardize outside visual stimulation. A video camera is mounted 158 cm above the four enclosures and provides the signal for the Polytracker software (San Diego Instruments, San Diego CA). The video camera signal is processed to obtain

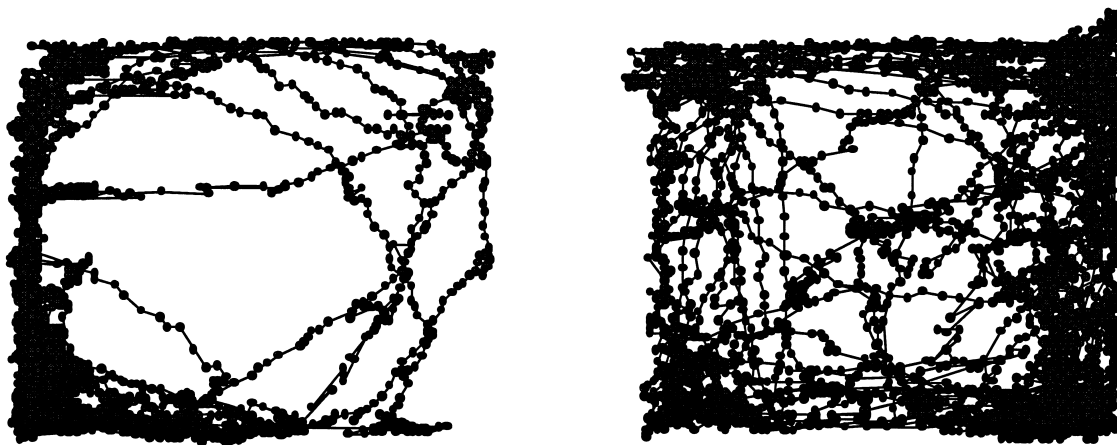


Fig. 4. Two representative movement patterns for 5 min of a 129 SvEv mouse (left) and a C57 B1/6 mouse (right).

the left-uppermost coordinate of up to four animals (each in separate enclosure) simultaneously. The signal is subsequently stored in a PC computer and is available for further off-line processing.

For the investigation, the (x,y) pixels of each animal sampled at a rate of 18 Hz were used to generate a (x,y,t) coordinate file consisting of the x-location, the y-location and the duration of time spent at that location. The pro-

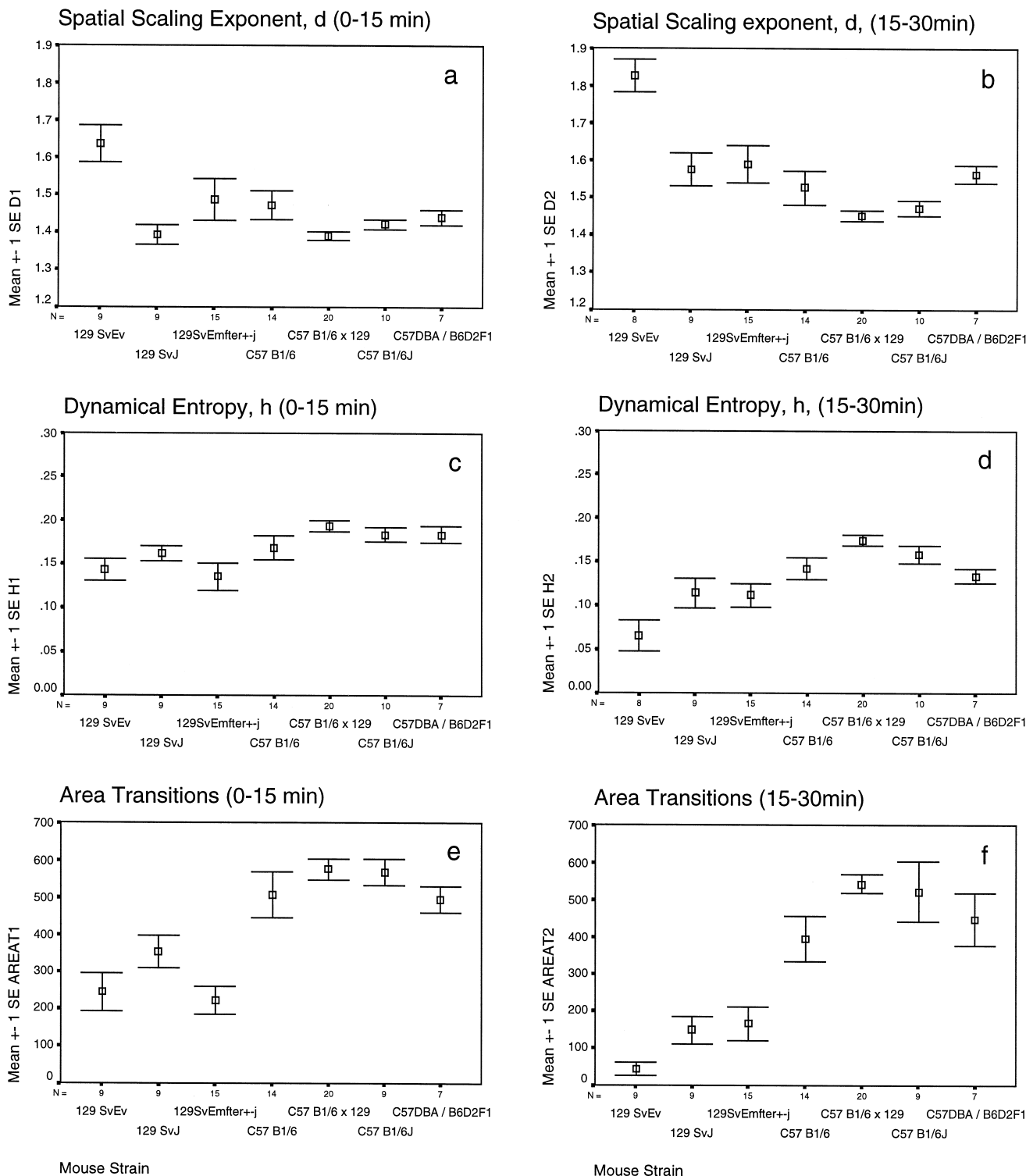


Fig. 5. (a)–(h) display the mean and SEM of the spatial scaling exponent: (a) and (b) the dynamical entropy, (c) and (d) the area transitions, (e) and (f) and the center time, and (g) and (h) the different mouse strains.

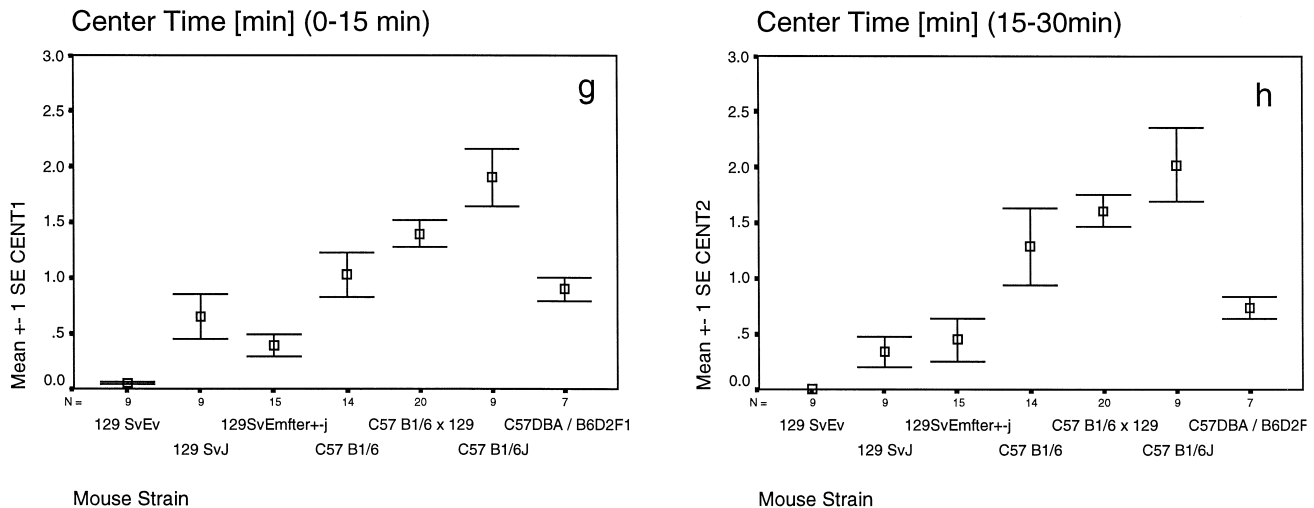


Fig. 5 (continued).

cessed image of all four enclosures is based on a spatial resolution of  $256 \times 256$  pixels for the entire set of four chambers. The individual chambers were traced by a reflecting object to obtain the pixel size for each chamber. Due to minor distortions, the number of pixels per 41 cm side length varied from 77–80 pixels for the different chambers, corresponding to a spatial resolution of approximately 0.53 cm/pixel. This coefficient was used to calculate an estimated distance traveled in cm. Thus, the minimum spatio-temporal resolution of one behavioral event or micro-event was (0.53 cm, 0.53 cm, 55 ms).

### 2.3. Measurement procedure

The animals were tested in groups of 4 per session, i.e. 4 open fields were used per session. Thus, 4 animals were tracked simultaneously within 4 adjacent open field environments. Each mouse was placed in the bottom left corner of the enclosure and activity was monitored for 30 min. After testing, the animals were returned to the animal facilities. Although these animals were used subsequently for other behavioral experiments, all data reported here were derived from the animals' first exposure to the VT.

### 2.4. Behavioral measures

Fig. 1 shows the division of the chamber into different areas. The four corner areas, each  $10.0 \text{ cm} \times 10.0 \text{ cm}$ , and the four wall areas, each  $10.0 \text{ cm} \times 21 \text{ cm}$ , were combined for the analysis of the distribution of movements. Three measures related to the distribution of time spent by the mouse were obtained: the amount of time spent in minutes in a corner, wall, or center area. In addition, the amount of locomotor activity was measured by the number of transitions from one area to another. This measure was used to assess ambulatory movements that are measured typically using coarse-grained photobeam systems. Conceptually,

the time (in minutes) spent in the center area was used to determine the extent to which animals explored an area that has been considered highly aversive for rodents.

The movement patterns were analyzed analogously to the scaling hypothesis that was advanced previously based on studies of rats [20] and which was derived from similar observations in physical and mathematical systems [15]. Briefly, sequences of movements observed with varying spatio-temporal resolutions may not yield a simple linear relationship between the (measured) distance traveled and the observational resolution. The resolution is determined by the number of micro-events that are considered for the calculation of the distance traveled. For example, straight movements along a wall of the enclosure or traversing the center are characterized by the fact that the distance traveled doubles if the number of micro-events used to calculate the distance is doubled. Therefore, there is a linear relationship between the number of micro-events and the distance traveled. However, meandering or circumscribed movements are not characterized by this simple linear relationship. Here, the distance traveled is less than double if the number of micro-events used to calculate the distance is doubled. Thus, the measured distance traveled as reported frequently in the literature is not an absolute metric, but depends upon the resolution used to obtain the measure and the interaction between the resolution and the pattern of behavior exhibited by the animal. The average spatial scaling exponent,  $d$ , quantifies the relationship between the number of micro-events,  $k$ , used to calculate the distance,  $L$ , and the change in resolution,  $a$ , via the following formula

$$L(ak) = a^{2-d}L(k)$$

Computationally, this number is obtained by plotting the distance traveled,  $L$ , versus the number of micro-events,  $k$ , on a double-logarithmic coordinate system and fitting a straight line using a least-squares algorithm. Here,  $k$  was

varied between 1 and 32. The average spatial scaling exponent,  $d$ , typically varies between 1 and 2 for straight and highly circumscribed movements, respectively (see Fig. 2 for three different examples).

The dynamical entropy measure,  $h$ , has been described in detail elsewhere [24]. Briefly, this measure quantifies the degree of uncertainty of predicting the next movement based on the sequence of preceding movements and is based on similar measures assessing the emergence of uncertainty in nonlinear dynamical systems [5].

The dynamical entropy is expressed in bits/step and is related directly to the number of different movement possibilities per step. Specifically, the number of different paths of length  $k$ ,  $N(k)$  is related to the length of the path via the following formula:

$$N(k) \approx e^{hk}$$

Thus, the more different paths are observed, the larger the value of  $h$  (see Fig. 3 for examples). The dynamical entropy approaches zero if the animal exhibits a highly repetitive path pattern. Conversely,  $h = 2$  indicates that the animal moves from any point in the chamber in all different directions with equal probability. Therefore, this measure quantifies the variety of different locomotor patterns. This measure was calculated based on the correlation integral [12] of movements in a coarse-grained grid of the enclosure with a spatial resolution of 5.3 cm. The estimated entropy for a given movement sequence is given by the decay of the number of similar sequences within a given resolution with increasing sequence length. Algorithmically, this measure is obtained by plotting the number of similar sequences versus the sequence length on a semi-logarithmic coordinate system and fitting a linear least squares fit to the scatter plot. The slope corresponds to the dynamical entropy,  $h$ .

Each of these measures was calculated for two time bins to determine changes of activity or patterns over time. Specifically, the spatial scaling exponent,  $d$ , the dynamical entropy,  $h$ , the transitions between different areas, and the time spent in the center area were obtained for the first and second 15 min time blocks.

## 2.5. Statistical analysis

A one-way ANOVA with strain/experiment as an independent measure was obtained separately for the first and second 15 min time block for all measures. A principal component analysis was conducted to determine whether measures of locomotor activity (transitions between different areas) and exploratory activity (center time) comprise a different dimension of behavior when compared to measures of the spatial organization of behavior (spatial scaling exponent, dynamical entropy). A minimum eigenvalue = 1.0 criterion was used to extract the factor solution and a Varimax rotation was conducted to maximize the interpretability of the factors.

## 3. Results

### 3.1. Visual pattern differences between strains

Fig. 4 shows representative mice from each of the two different types of strains used in these experiments (129 strain left, C57 strain right). Whereas the 129 mouse is found predominantly in one corner of the enclosure, the C57 mouse path patterns are distributed more evenly throughout the chamber. In particular, in contrast to the 129 mouse, the C57 mouse moves substantially more frequently through the center.

### 3.2. Strain differences in measures of locomotor activity

The one-way ANOVA revealed significant differences between strains for all measures for both time points. Specifically, the locomotor activity patterns for the first 15 min differed across strains for the spatial scaling exponent,  $d(F(6,83) = 4.68, p < 0.01)$ , the dynamical entropy,  $h(F(6,83) = 3.91, p < 0.01)$ , the number of area transitions ( $F(6,82) = 12.40, p < 0.01$ ), and the center time ( $F(6,83) = 13.969, p < 0.01$ ). Similarly, the spatial scaling exponent,  $d(F(6,82) = 8.08, p < 0.01)$ , the dynamical entropy,  $h(F(6,82) = 8.19, p < 0.01)$ , the number of area transitions ( $F(6,82) = 16.61, p < 0.01$ ), and the center time ( $F(6,82) = 9.75, p < 0.01$ ) differed significantly across strains for the second 15 min. As shown in Fig. 5 and supported by post-hoc analyses, 129SvEv mice exhibited significantly more circumscribed movement patterns during the first and second 15 min time bins, as confirmed by an increase in the spatial scaling exponent,  $d$ . Moreover, movement patterns of 129SvEms + Ter<sup>+</sup>/J mice were significantly more predictable during the first 15 min compared to those of C57BL/6 mice (Fig. 5c). In particular, all 129 strains tested showed significantly more predictable movement patterns during the second 15 min time bin when compared to the C57 strains (Fig. 5d). The locomotor activity as measured by the number of area transitions differed substantially across the different strains. As shown in Fig. 5e and f, all 129 strains exhibited significantly lower levels of locomotor activity when compared to all C57 strains. This difference was more pronounced during the second 15 min time bin. Similarly, the strains differed profoundly in the time spent in the center

Table 2  
Number of animals, means, and standard deviations for all variables included in the factor analysis

Measure	<i>n</i>	0–15 min		15–30 min	
		Mean	SD	Mean	SD
Spatial scaling exponent $d$	76	1.43	0.10	1.52	0.13
Dynamical entropy, $h$	76	0.17	0.04	0.14	0.04
Area transitions	76	460.8	187.5	373.9	233.5
Center time [min]	76	1.02	0.74	1.10	1.00

Table 3

Correlation coefficients of the extracted and rotated factors with the variables entered into the factor analysis as well as the percent explained variance

Time	Measures	Factor 1: locomotor activity	Factor 2: movement patterns
0–15 min	Spatial scaling exponent $d$		–0.873
	Dynamical entropy, $h$	0.361	0.825
	Area transitions	0.760	0.403
	Center time [min]	0.805	
15–30 min	Spatial scaling exponent $d$	–0.412	–0.766
	Dynamical entropy, $h$	0.447	0.762
	Area transitions	0.812	
	Center time [min]	0.814	
	Total % variance	38.35	37.44

area. Specifically, the 129 strains spent significantly less time in the center when compared to all C57 strains during the first and second 15 min time bins (Fig. 5g and h). Thus, to summarize, 129 strain mice exhibited more circumscribed movements, were more predictable, showed a reduced level of locomotor activity and spent less time in the center of the enclosure than C57 mice.

### 3.3. Principal component analysis of locomotor activity

76 animals with complete data sets (numbers and means are shown in Table 2) were entered into a principal component analysis. The analysis extracted two factors accounting for 75.8 percent of the variance. The correlations of the individual variables with the extracted and rotated factors are shown in Table 3. Specifically, the first factor correlates substantially with the time in center during the first and second 15 min time bins and with the number of area transitions during both time bins. In contrast, the second factor correlates strongly and negatively with the spatial scaling exponent,  $d$ , during both time bins and positively with the dynamical entropy,  $h$ , during both time bins. These results support the hypothesis that the first factor encapsulates the variance due to the amount of locomotor activity exhibited by the animals. In comparison, the second factor comprises the different geometrical and dynamical movement patterns exhibited by these animals, that is, the spatial and dynamical organization of the behavior. The negative correlation with the spatial scaling exponent,  $d$ , and the positive correlation with the dynamical entropy,  $h$ , supports the notion that mouse movement patterns are more unpredictable (higher  $h$ ) when engaging in particularly straight and directed movements (lower  $d$ ). To summarize, at least two independent factors related to the amount of locomotor activity and to the geometrical and dynamical path patterns govern locomotor behavior of mice in a novel environment.

## 4. Discussion

There are two main results of this investigation. First, the assessment of both the amount of locomotor activity and the spatial patterns of these movements confirms other

reports that mouse strains differ substantially in their locomotor behavior. Second, the detailed assessment of locomotor activity reveals that at least two independent dimensions characterize the behavior of a mouse in an open field: 1) the amount of locomotor activity; and 2) the geometrical and dynamical patterns of movements.

The current investigation supports our general hypothesis that locomotor behavior comprises a multidimensional construct that can be decomposed into independent dimensions. Moreover, the current extraction of two factors for mouse locomotor behavior parallels the factors obtained for unconditioned motor activity in rats [22]. We previously described these factors as assessing the amount of locomotor activity and the degree of behavioral organization [22]. The third factor identified in the similar studies of rat behavior primarily reflected exploratory activity as detected by investigatory holepokes and rearings. The absence of a third factor in the present study of mouse behavior is likely due to the fact that specific indices of exploratory behavior were not measured here. It should be noted, however, that such behaviors could be assessed using an elaboration of this paradigm, as has been reported in studies of serotonin receptor knockouts [31]. The limited studies to date using the combination of an open field and a holeboard in mice have confirmed that the exploratory component of mouse behavior is pharmacologically dissociable from the amount of activity in the same manner that it is in rats [9,31]. Thus, it appears that in both rats and mice, the unconditioned motor behavior in a novel open field is governed by three independent factors: the amount of activity; the amount of exploration; and the organization of behavior. Moreover, the corollary hypothesis that general measures of area distribution or wall-distance (data not shown here) are not sufficient to fully capture the complexity of locomotor behavior was also supported. Specifically, both center time and wall-distance were not significantly correlated with the Movement Pattern factor.

The concept of behavioral organization aims at quantifying the important temporal and sequential domain of behavior. Central to this concept is the quantitative analysis of how individual behavioral elements, i.e. movements of the animal in a novel environment, are arranged to form

characteristic patterns of exploration. Specifically, the values for the spatial scaling exponent and the dynamical entropy of mice in the VT support the notion that successive movements of these animals are not random but rather are organized to serve critical behavioral functions. Exploration of a novel environment has long been conceptualized as emerging from a conflict between approach (exploration) and avoidance (neophobia) [3]. Movements with different geometrical characteristics can be thought of as serving these conflicting processes. For example, successive movements along a straight line characterized by a spatial scaling exponent  $d \approx 1$  result in the exploration of different areas of the enclosure. In contrast, successive movements in a circumscribed area with a  $d \approx 2$  allow the animal to sample the current location in more detail. Based on the correlation patterns of the variables with the extracted factors, the straight movements are more unpredictable than circumscribed movements. This correlation supports the notion that the initiation of new exploratory bouts characterized by successive straight movements represents more unpredictable and thus more uncertain behavior. In the context of monitoring open field behavior, additional equipment is needed to assess exploratory behaviors such as holepokes and rearings. In contrast, the assessment of behavioral organization can be accomplished at no additional cost in either time or equipment, given only that the raw data describing the successive x,y positions of the animal are saved for post-hoc analyses. Such data can be obtained using either video-tracking systems, as used here, or photobeam systems, as used in other investigations [11,22,34].

The substantial and independent differences observed here in both the amount and organization of locomotor behavior between two background strains commonly used in the development of knockout mice substantiate an important aspect of mutant mouse research. Specifically, the phenotypic variation of background strain differences needs to be taken into account when interpreting the behavioral phenotype of mutants. In addition to more traditional measures of the amount of locomotor activity, the independent dimension of behavioral organization may provide crucial and novel information when assessing mouse mutants in general and dopaminergic knockout or transgenic mice in particular. The dopamine system has long been considered critical in the selection and sequencing of action [7,27,28] and the seemingly purposeless repetition of fragmented behavioral actions has been reported as a key behavioral indicator of an over-stimulated dopaminergic system [26]. In rats, combined assessments of both the amount and organization of locomotor activity have proven useful in differentiating the effects of a variety of dopaminergic and serotonergic agents [19,21]. The assessment of the organization of behavior in particular has enabled distinctions to be made between the effects of drugs within the category of indirect dopamine agonists [19] and between categories of drugs that all increase the

amount of locomotor activity, but via different mechanisms of action [10,11,22]. These distinctions were often impossible based only on the more commonly used measures of locomotor activity, such as distance traveled, time spent in different regions, rearings, etc. The present studies indicate that similar distinctions may be feasible in mice as in rats.

Nevertheless, recent studies of various dopamine receptor knockout mice have focused primarily on measures of the amount of locomotor activity. For example, in contrast to wild-type controls, repeated administration of amphetamine leads to an attenuated increase of locomotor activity in D1(-/-) mice [4]. Moreover, cocaine increased locomotor activity in D1(+ / +) and D1(+ / -) but not in D1(- / -) mice [16]. The D2(- / -) mice are akinetic and bradykinetic in behavioral tests [2] and were found to show approximately half of the locomotor activity when compared to drug-naive, strain-matched controls [13]. However, this group also noted significant inter-strain differences between wild-type, 129/SvEv, and C57BL/6 mice with functional D2 receptors and emphasized the importance of the interaction of multiple genetic factors in the analysis of complex behaviors in gene knockout mice. D3(- / -) mice are transiently more active than wild-type mice in a novel environment and exhibit increased behavioral sensitivity to combined injections of D1 and D2 class receptor agonists, cocaine, and amphetamine [33]. Other groups have shown that D3(- / -) mice exhibit hyperactivity in an exploratory test and display increased locomotor activity and rearing behavior [1]. Moreover, in the open field, D3(- / -) mice entered the center significantly more often than normal D3(+ / +) littermates [32]. Mice lacking the D4 receptor, D4(- / -), were found to be less active in open field tests but outperformed wild-type mice on the rotarod and displayed locomotor supersensitivity to ethanol, cocaine, and methamphetamine, suggesting that the D4 receptor modulates normal, coordinated, and drug-stimulated motor behaviors [29]. While these investigations have increased our understanding of the dopaminergic involvement in regulating the level of locomotor activity, future investigations may need to re-examine the roles of these receptors in modulating behavioral organization. In concert with many studies in rats [23], the current results confirm that the organization of behavior in mice also varies independently of the amount of locomotor activity. Therefore, the examination of these factors in these knockout mice may help to better differentiate the functional contributions of the different receptor subtypes. In order to gauge the effect of null-mutations and transgenic alterations, it is critical to quantify the changes in behavior along different dimensions relative to the characteristic behavioral pattern of the background strain. A database of independent behavioral dimensions will need to be established for a comprehensive group of mouse strains. Subsequently, the behavioral pattern of a mouse mutant can be quantified along differ-

ent behavioral dimensions. An axis of behavioral change can be defined to determine the degree to which a genetic manipulation changes the behavior in a direction similar to that of the background strain in reference to other mice strains. The direction of the axis due to the genetic manipulation can be compared quantitatively to the axis due to the mouse strain to partial the behavioral change into a background component and a genetic manipulation component.

Measuring unconditioned locomotor behavior in mutant mice provides critical information about the behavioral phenotype of the animal. As with other behaviors, however, behavioral differences between the background strains can be critical and should be considered when attributing observed differences to the deletion of the targeted gene. The advantages of measures of unconditioned locomotor behavior are that they are sensitive to perturbations in a variety of neurobiological systems, can be collected efficiently using automated procedures, and can provide quantitative indices of at least two independent dimensions of locomotor behavior. Assessment of behavioral organization may provide new insights into how the dopaminergic system regulates the geometrical and dynamical properties of locomotor behavior, that is how the animal interacts dynamically with the environment to generate characteristic spatio-temporal patterns.

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