Chapter 1

Longitudinal Assessment of Deliberate Mouse Behavior in the Home Cage and Attached Environments: Relevance to Anxiety and Mood Disorders

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Abstract

Understanding behavioral regulation can further progress by developing new approaches that allow refinement of behavioral phenotypes. The current availability of several thousand different mutant mice and of human candidate genes for emotional (affective) disorders challenges behavioral neuroscientists to extend their views and methodologies to dissect complex behaviors into behavioral phenotypes and subsequently to define gene–behavioral phenotype relationships. Here, we put forward multiday automated behavioral and physiological observations in carefully designed environments to assess evolutionary conserved behavioral strategies in mice. This offers the opportunity to design experimental setups that allow the animals themselves to regulate their own behavior, using representations of continuous kinematic variables, studying the dynamics of behavior (change across time or change across activity); i.e., growth or decay processes of behavior and concomitant physiological adjustments such as heart rate. The measures characterizing these processes should have discriminative power (across strains or treatments) and be replicable (across laboratories). Furthermore, cross species genetic studies for these neurobehavioral and physiological traits may provide a novel way toward identifying neurobiological mechanisms underlying core features of complex psychiatric disorders.

Key words: Genetics, Physiology, Replicability, Exploration, Psychiatry, Conditioned fear, Heart rate, Sequences of repeated motion, Dynamics of behavior, Behavioral growth, Arousal

1. Introduction

Behavioral testing in animals is a crucial feature of phenotyping in neuroscience research. In particular, animal models of human chronic diseases or behavioral symptoms, such as anxiety and mood disorders, should be tested in situations where long-lasting stable

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features ("chronic") of such behavior are manifested. It implies measuring the behavior for days rather than minutes. Testing animals in situations in which the animals respond to novel or transient stimuli is appropriate for studying "states," not "traits." Behavioral testing is usually based on measuring behavioral responses to environmental events that are induced by the experimenter. The frequently used open field test, e.g., is commonly employed to study general activity and fear-related behaviors in mice and rats (1, 2). In this test, movements of the animal are monitored up to 1 h after the animal has been placed in a novel open arena from which it cannot escape. Additional tests, such as the elevated plus maze (3) and the light–dark box (4), allow external validity of the observed open-field behaviors. However, these tests are short-lasting and depend on individual locomotor activity levels and novelty responsiveness of the animal and require human interference. This hampers their use for determining gene–behavioral phenotype relationships and stresses the need for new analytical procedures addressing the complex behaviors. Although some ideas for overcoming these problems have been put forward, such as improving currently available tests, using test batteries and increasing test information density (5, 6), behavioral complexity and gene–environment interactions require new methodologies in this field of research.

1.1. Interacting Physiological Processes

Behavior is triggered by internal and external motivational signals (such as hunger and food availability, respectively) and is guided by the ability of the animal to execute proper behavioral responses. For instance, a hungry mouse that searches for new food resources relies on an efficient exploration strategy in which finding the food resource in time and taking the risk of being exposed to predators need to be balanced. Furthermore, in the wild, mice face the risk of spending more energy to obtain food than this food gives them in return on any given day. Because exploration for food is influenced by different integrated physiological processes (e.g., energy balance, motor action, and fear), as well as by environmental factors (e.g., variations in ambient temperature, in food availability, and in photoperiod), the design of behavioral laboratory methods that dissociate the various behavioral components is a challenge and should focus on ethologically relevant behavior and appropriate environmental conditions for the species selected for the behavioral studies (7).

Conventional laboratory tests, such as the open-field test, touch upon different aspects of exploratory behavior, including locomotor activity and fear-related processes. However, during the relative short testing episode generally employed, it is impossible to discriminate between gene function in novelty-induced and baseline behaviors. For example, mice that lack the dopamine transporter gene have locomotor activity levels under baseline conditions that are comparable to those in wild-type animals, but they

exhibit a 12-fold increase of locomotion following placement in a novel environment. Although dissociation of novelty-induced and baseline locomotor behavior in this mutant was observed during a relative short-lasting testing procedure, the time of day that these tests are performed can highly influence the outcome of the observations (8–10). Furthermore, the response to novelty induces a dynamic transient response whose measurement provides variable results depending critically on the time interval measured and the size of the time window sampled. Thus, characterization of gene functions in exploratory behavior requires behavioral paradigms that allow dissection of this complex behavior into different components in view of circadian-induced variations of these components.

1.2. Interference and Order Effects

Executing multiple behavioral tests usually involves experimenter interference, such as handling or transport of animals (11), and cues from the experimenter that influences the behavioral performance of an animal (12). For example, measuring pain responses in mice revealed that experimenter effects account for more trait variability than genotype (13). In addition, problems of replicability across laboratories such as those reported in (14) could reflect the effects of forced testing. Mice exposed to a battery of various behavioral tests expressed significant lower levels of locomotor activity in the open field than mice that were naïve to behavioral testing (15). These order effects could even be amplified in animals with selective mutations in genes that are involved in physiological processes, such as coping strategies to changing environments. Circumvention of these interfering procedural aspects is required to reduce nonspecific environmental influences on the gene–behavioral phenotype relationship. Especially, the adverse impact of the experimenter as uncontrollable variable or even confounder of experiments should be taken into consideration.

1.3. Dissection of Behavioral Phenotypes

Studies in the field of biological rhythms have revealed that behavioral observations during several consecutive days or weeks in the home cage of an animal allow reliable assessment of stable behavioral circadian rhythms that are highly sensitive to environmental signals, such as light and human interference (16, 17). Because behavioral observations during several days can also dissociate novelty-induced and baseline behaviors at different phases of the light–dark cycle, behavioral monitoring in the home cage will significantly contribute to the refinement of behavioral phenotypes. In addition, by carefully designing a home cage environment, with or without additionally attached compartments or arenas that addresses different behavioral characteristics of interest, complex behaviors can be further dissected into behavioral phenotypes with minimum human interference. In this chapter, we would like to view recent developments in the fields of behavioral neuroscience

that uses the home cage and attached compartments as a basis for the assessment of behavioral exploration strategies in mice. Integration of longitudinal automated behavioral measures with physiological measures allows further refinement of these neurobehavioral traits. Furthermore, we provide an example on how interspecies trait genetics using home cage behavioral assessment in mice offers a basis for identifying novel neurobiological mechanisms underlying anxiety and mood disorders.

2. New Method Developments

In this chapter we introduce setups used in our own work, which attempt to separate state from trait anxiety by using side-by-side the home cage environment for long-term observation that might be more appropriate for longitudinal studies of trait, and environments attached to the home cage, which are most appropriate for the study of how mice manage deliberately novel input, but can also serve for long-term studies. In addition, we complement the type of information provided by the common assays and models with a large set of novel mouse-centered kinematic variables which imply active management of perceptual input. We suggest three requirements that should guide us in improving our choice of behavioral measures: measure kinematic variables that appear to be actively managed by the animal; demonstrate the discriminative power of these measures between strains and preparations; and demonstrate the replicability of these measures across laboratories (18–20). In what follows we briefly demonstrate what we do to fulfill these three requirements.

2.1. Segmenting Behavior into Animal-Centered Sequences of Repeated Approach-and-Avoid Motions

One way to obtain a view on the functional organization of exploratory behavior is to examine it in situations involving behavioral growth. To study and quantify this growth, we connect the mouse's home cage through a doorway to a large circular arena for an extended period of time, and allow the mouse to explore the arena at a self-regulated rate (Dimensionality Emergence assay, or DIEM assay; see (21)). In this setup the familiarity of the mouse with the environment increases gradually, allowing a correspondingly gradual, stretched out growth of behavior. This process exposes the elementary building blocks of behavior as they are progressively added to the animal's repertoire. The moment-to-moment developmental dynamics of exploratory behavior discloses its presumed function: a systematic active management of perceptual input acquired during the exploration of a novel environment, and active management of the arousal associated with the acquisition of that novel input (20, 21).

Having access to a technology that allows us to track and record a time series of locations occupied by a mouse during free exploration, and having developed analytical methods for quantifying continuous kinematic variables (https://www.tau.ac.il/~ilan99/see/help/), we segment the path, based on its intrinsic statistical and geometrical properties, into processes involving approach and avoidance: repetitive Peep and Hide motions from the home cage into the arena, repetitive Cross and Retreat motions performed across the doorway, repetitive Borderline round trips consisting of outbound–inbound movement along the wall, and repetitive incursions from the wall toward the center of the arena and back to the wall. All these are examples of what we term "sequences of repeated motion." The motions are performed in relation to specific reference values from which the motion commences and to which it returns: the inside of the home cage for Peep and Hide, the doorway for Cross and Retreat, the inside of the home cage plus the "garden," an area at the proximity of the doorway, for Borderline Roundtrips, and the Wall ring in the proximity of the arena wall for incursions. We further identify a growth of behavior that is manifested through a buildup in the extent of each of these motion types separately and an increase in complexity through the recruitment of additional sequences of repeated motion that are superimposed on previously emerged sequences of repeated motion. We finally quantify this process by computing the rates of growth in extent in each of the sequences of repeated motion, and by estimating the complexity of the sequence of sequences.

2.2. Management of Perceptual Input as Indicated by Buildup in Extent in Sequences of Borderline Roundtrips

A session of free exploration commences with peeping, where the mouse crosses the doorway into the arena, always leaving part of its body behind the doorway, and retreats back. The Peep and Hide sequence is followed by a Cross and Retreat sequence, Circle in Place, and Entry Head On, before commencing with the Borderline Roundtrip Motion sequence, which, in the BALB/c mice, commences strictly near and along the wall until the exhaustion of the borderline dimension (Fig. 2). The reference area near the doorway that we term garden is defined algorithmically by plotting a density of cumulative dwell time across the entire arena. This plot highlights a two-dimensional Gaussian located by the doorway, whose boundary defines the "garden" (21).

As illustrated in Fig. 1, Borderline movement builds up in maximal angular distance from home almost monotonically from one roundtrip to the next. This increase in borderline roundtrip amplitude is joined next by the option not to return all of the way into the home cage, as expressed by the emergence and subsequent proliferation of Cage skips and Home-related shuttles (blue dots in Figs. 1 and 2). The simple Borderline Roundtrips turn in this way into complex ones including one to several home-related shuttles. The buildup in the Borderline roundtrips in the other direction,

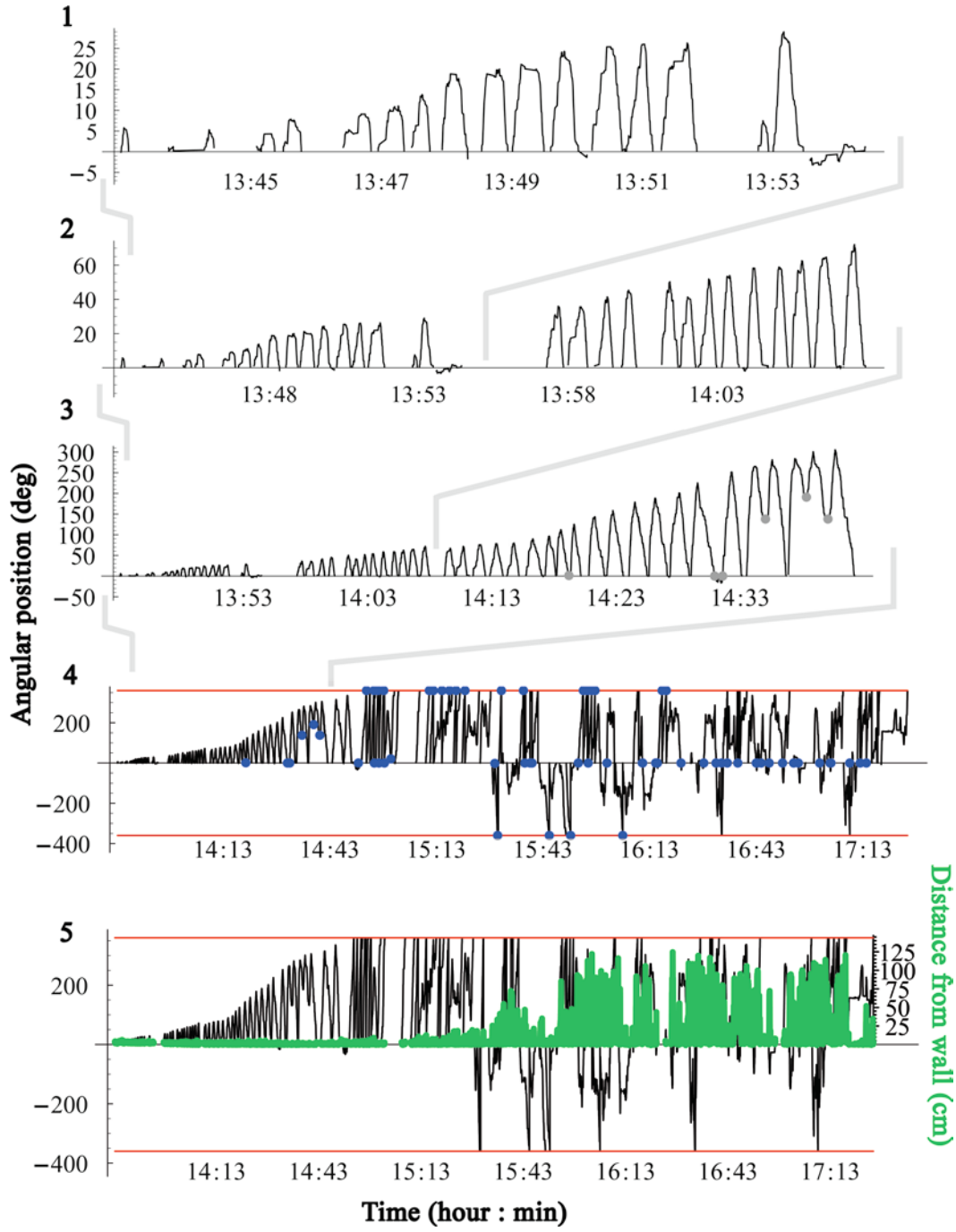

Fig. 1. The buildup of amplitude and complexity of movement in one-, and two-dimensions space in a free BALB/c mouse. (1–4): the buildup of angular positions reached across roundtrips. Note change of time scale from (1) through (4). *Black line* – borderline movements. *Blue* data points – cage skips. Positive values designate *right* and negative values *left* borderline directions. *Red lines*, angular positions of doorway at 360°. Graph lines between *X*-axis and *red line* represent full circles. All graphs start with the same initial roundtrip, progressively incorporating later roundtrips. (5): emergence and buildup of radial movement away from wall (in *green*), superimposed on the plot of angular positions (in *black*). Significant radial movements (incursions) are added only after 1.5 h.

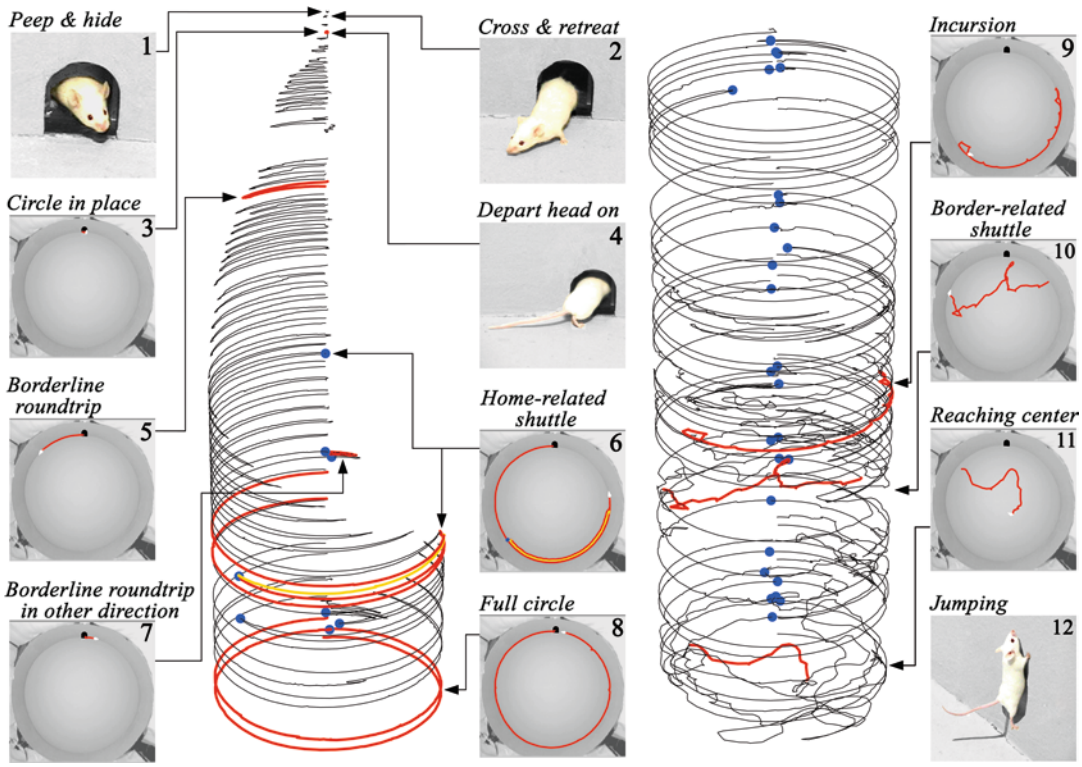

Fig. 2. The moment-to-moment developmental sequence of landmarks of free exploration of a selected BALB/c mouse-session performed across a 3-h period. The spiral proceeding from top to bottom, first in the left and then in the right column, presents the time series of two-dimension locations on the path traced by the mouse. The enumerated figure-inserts show the 12 landmarks described in the text, traced in *red* within the arena, and on the spiral. *Blue* dots indicate instances in which the mouse approached the cage doorway and did not enter the cage (cage-skips), or stopped short of returning all of the way home during a return (home-related shuttle). Absence of a blue dot implies departure into home cage. *Yellow* path stands for the return portion within a home-related shuttle.

which follows the extended sequence of one-sided roundtrips (Fig. 2, top part of left spiral), is steep in comparison to the corresponding buildup in the main direction (Fig. 1). One to several full circles in one or both directions herald the end of the one-dimensional stage and the emergence of the two-dimension movement stage (consisting of radial movement, plotted in green in Fig. 1).

2.3. Mouse Exploratory Behavior Is Composed of a Sequence of Sequences of Repeated Motion

Sequences of repeated motion are progressively added on top of each other, generating increasingly richer and more complex behavior, ultimately consisting of 13 types of sequences of repeated motion exposed so far (Figs. 2 and 3). The first occurrence of a new type of motion is a developmental landmark. It heralds the repeated performance of that type of motion in the immediate period that follows, and often across the rest of the session. The sequence of landmarks, the buildup in extent within sequences,

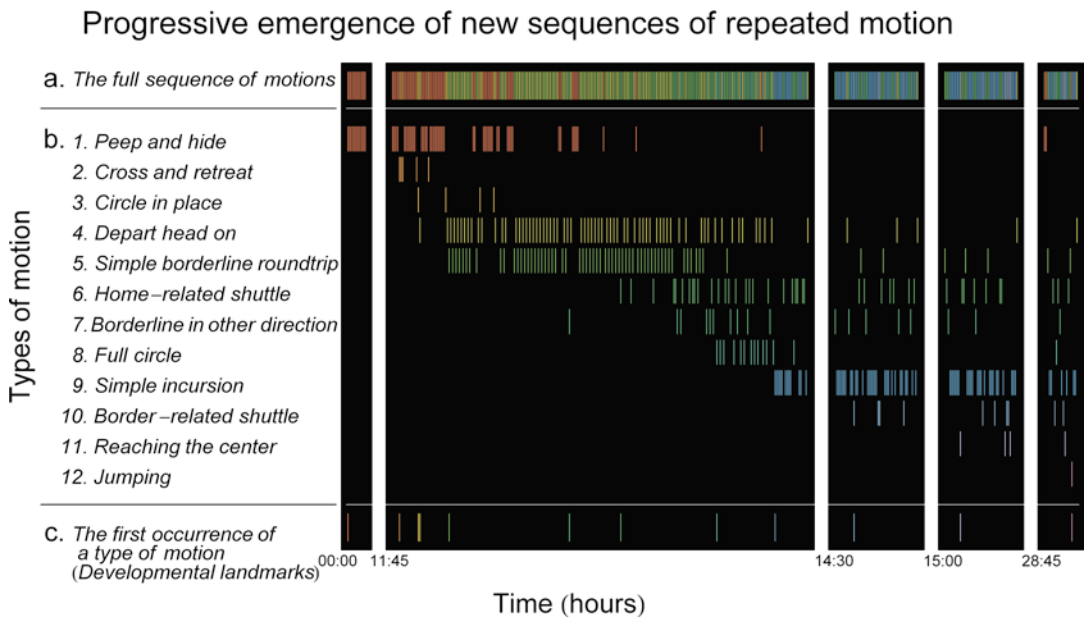

Fig. 3. Successive intervals of free exploratory behavior of a selected BALB/c mouse performed in a novel arena. The sequence of sequences is represented in the *bottom horizontal line* by the first performance of each of the motion types (developmental landmark) belonging to a sequence.

and the increase in dimensionality and freedom of movement across the session are all illustrated in Fig. 2. The growth of behavior proceeds across stages from staying-in place behavior (Fig. 2, developmental landmarks 1–4), to movement along one (Fig. 2, landmarks 5–8) then along two (landmarks 9–11), and then along three dimensions (landmark 12). It is accompanied by a buildup in amplitude and in complexity of the motions within and across the sequences of repeated motion. The regularity of the growth and the stability of the order within the BALB/c and C57BL/6 strains suggest *active management* of the measured kinematic variables (21).

The top horizontal line in Fig. 3 presents the original, "raw" sequence of motions of a selected BALB/c mouse. This sequence is algorithmically screened, yielding multiple sequences, each presented within an especially dedicated horizontal line in Fig. 3 (this particular BALB/c mouse performed only 12 sequences of repeated motion).

2.4. Quantifying the Buildup in Extent in Sequences of Repeated Motion

The quantification of the buildup in extent can be achieved in various ways. Figure 4a details one such way to quantify the buildup in the maximal arc reached during a Borderline Roundtrip, where the time to reach some threshold and the rate of growth at that threshold are calculated from the smoothed buildup curve.

2.5. Discriminative Power

The comparison between two different strains of the quantified buildup in Borderline Roundtrips demonstrated in Fig. 4 reveals that the differences in the times to reach the threshold and in the growth rates in the two strains are large and highly statistically significant (the two groups are almost entirely separated). Variations on the parameters estimated from this sequence of repeated motion, as well as the comparisons of measured buildup in the other sequences discussed, can all be assessed as to their discriminative

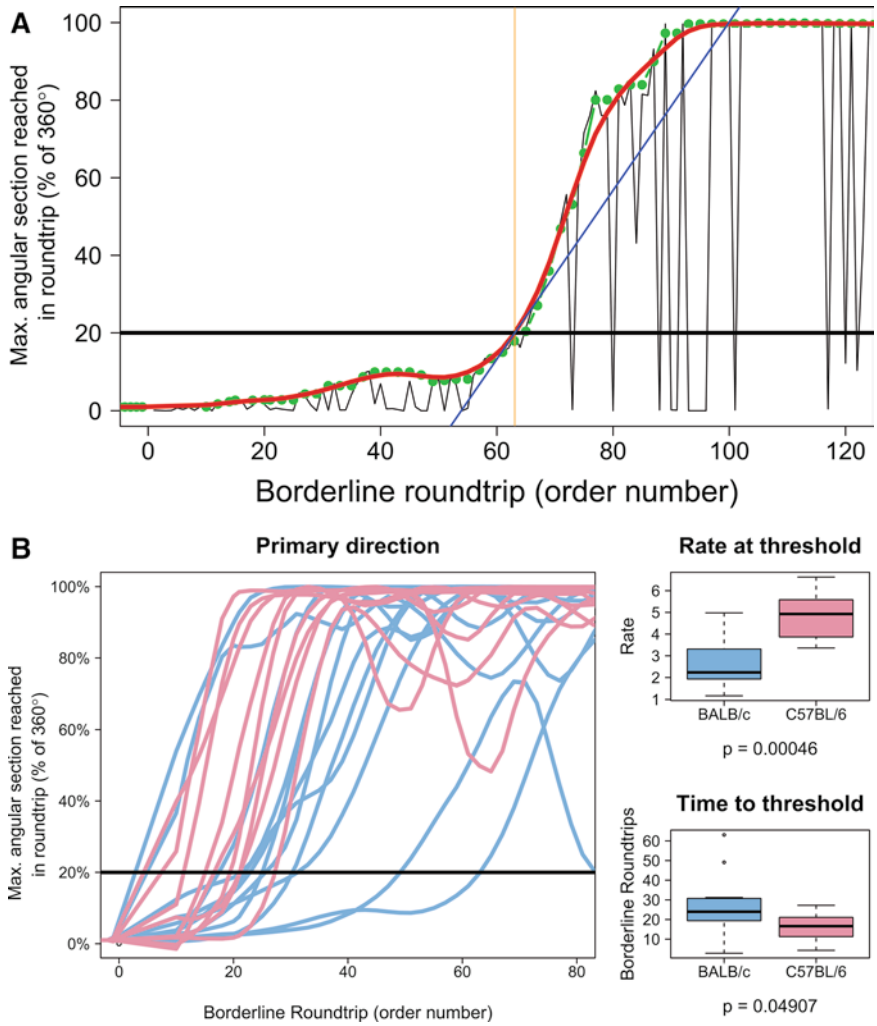

Fig. 4. (**a**) Quantifying the buildup in maximal arc during successive borderline roundtrips in the main direction of a mouse's exploratory session. (**b**) A quantitative comparison of the rate of growth of the maximal angular amplitude reached during successive borderline roundtrips in two strains. On the *left*, the smoothed percentile functions for all mice (*pink* for C57BL/6, *blue* for BALB/c) and the 20% threshold used (*horizontal line*). *Top right*: box plots comparing the growth rates of the mice in the two groups (rates are measured as additional percent of circle covered per roundtrip). *Bottom right*: box plots comparing the time to reach the threshold of the mice in the two groups (time is measured in terms of roundtrips performed). *P*-values indicate significant differences in magnitude between the two strains using Wilcoxon test.

power: comparing between strains (as was done here), treatments, and preparations. Measures with demonstrated high discriminative power should be chosen at this stage.

2.6. Replicability Across Laboratories

Having chosen measures of actively managed behavior enjoying discriminative power, the concern about replicability of results across laboratories should enter the decision which measures to adopt (14, 18, 19). In particular Kafkafi et al. (18) lay out a statistical approach for deciding whether a measure is replicable across laboratories, by utilizing the variation across laboratories in the construction of the yardstick for discrimination (mixed model analysis). Rather than entering the statistical details, we demonstrate the idea behind it with an example taken from a forced exploration experiment assessing inter-strain differences that was conducted in three laboratories (22).

For the number of incursions per session, the across laboratories analysis revealed that the strain difference was not significant ($p > 0.08$), which deemed this measure of little value. However, the aggregate of all incursions was identified to be a mixture consisting of three relatively distinct incursion types. Ignoring the "Near Wall" incursion type, for which the strain differences were found to be highly nonreplicable across laboratories, left us with two distinct incursion types, intermediate incursions and arena-crossing ones, and the inter-strain comparison of their numbers per session was found to be highly replicable across laboratories.

2.7. Implications for Phenotyping

This type of analysis thus illustrates our approach to the design of improved measures for quantified phenotyping of behavior. An aspect of behavior is worth phenotyping if its quantification supports the hypothesis that it is actively managed by the animal, indicating functionality in the animal's own *Umwelt* (operational environment) (23). A specific quantification is useful if it has discriminative power and if it is replicable across laboratories. Using these criteria jointly may require a search through many candidate measures, but it can guide the design of better ways to describe and quantify behavior.

2.8. Implications for the Study of Animal Models of Anxiety

The analysis presented by us provides new types of information regarding the common open field animal model of anxiety. It also casts some light on the reported failure to separate state from trait anxiety (24). At least two hypotheses that concern motivational and cognitive mechanisms stem from our descriptive model:

1. The periodic return to the home cage is suggestive of a perceptual input cutoff mechanism (25), whereby after being exposed to a given amount of novel environmental input the mouse appears to rush back home so as to cut off the novel input. Hence, the incremental growth between two successive round-trips reflects the amount of input the mouse can take in before

having to cut it off by returning home. It follows that a mouse having a low capacity for novel input, or, for that matter, lower information processing capacity, would cover smaller stretches of new terrain than a mouse with a higher capacity. It is therefore expected that low input capacity mice (e.g., mice that are highly aroused) would have gentler growth curve slopes than mice with high input capacity (e.g., mice that are experiencing low arousal).

2. Faced with the challenge of mapping a novel environment, periodic return to the home cage may reflect the need to parse environmental input into manageable chunks collected during a roundtrip. An animal with a lower information processing capacity would correspondingly be expected to parse the novel input into smaller chunks (such as BALB/c mice) than an animal with a higher information processing capacity (such as, perhaps, C57BL/6 mice).

Taken together, these two hypotheses expose two presumed functions indicated by our analysis of free mouse exploration: (1) active management of the arousal associated with the acquisition of the novel input, and (2) active management of dimension-specific perceptual input acquired during the exploration of a novel environment. Regardless of the validity of these two hypotheses, our results beg for experimental manipulations that would modify the magnitude of the incremental input managed by the animal.

3. Integrative Behavioral and Physiological Assessment of Emotional Behavior

The assessment of the emotional state of rodents and many animals is hampered by the fact that it cannot be measured directly. Instead the emotional state has to be inferred indirectly from, e.g., facial expressions (e.g., as in humans, wolf and dogs [canidae]) or other species-specific behavioral expressions based on postures (e.g., freezing in rats and mice). However, fear and anxiety are accompanied by a wide range of physiological adjustments mediated by the central and peripheral autonomic nervous system (26). Cardiovascular function is of major importance because heart rate and blood pressure are generally elevated during emotionally challenging conditions representing key symptoms of many affective disorders (e.g., posttraumatic stress and general anxiety disorder). Affective disorders such as depression show comorbidity with cardiovascular disease for various reasons (27). Particularly, reduced heart rate variability that is attributed to increased sympathetic and decreased parasympathetic tone is considered a risk factor in the clinical setting.

3.1. Physiological Adjustments by Aversive Emotional Challenge

A wide range of adjustments is elicited by an adverse emotional challenge ranging from fast startle response modulation, pupil reflex (dilation), sweating (e.g., in humans) and piloerection through heart rate, blood pressure, and body temperature increase, as well as endocrine responses such as corticosterone increase in rodents (28). All these adaptive response adjustments serve as additional independent physiological measures to support the interpretation of emotional states such as fear and anxiety (29). However, these responses show different response dynamics after the onset of an aversive stimulus. While pupil reflexes are very fast (in the ms range), other autonomic responses are considerably slower (e.g., body temperature and corticosterone elevations) with a lag of rise of more than 2 min after stimulus onset. In addition, the return of these responses to baseline conditions is generally slow, which may pose a problem for experimental conditions of repetitive stimulation at high frequency. Finally, the activation of many physiological parameters is not specific to aversive conditions. Appetitive conditions and physical activity also contribute to substantial elevations of many autonomic parameters (30). Therefore, the interpretation of observed physiological changes still has to be made with great care and should be guided by information on the responses range of, e.g., heart rate values observed throughout the circadian cycle when animals have been active. A rise of heart rate of mice by 70 beats per minute [bpm] from baseline values that is attributed to increased attention (31, 32) is unlikely to serve as index of a stressor. Relatively small autonomic changes such as a mild heart rate increase may be statistically significant but physiologically irrelevant unless persistent for long times. In general, the problem of nonstationarity and interdependence of heartbeat intervals in their temporal sequence confounds the use of linear statistics for the quantification of measures such as heart rate and blood pressure (see (33)).

3.2. Specificity of Physiological Responses

Unfortunately, the understanding of the consequences of behavioral actions such as grooming, running, or rearing on autonomic adjustments is very limited and detailed information on the temporal relations is lacking. Another major complication is the fact that traditional behavior experiments with rodents require handling. Handling is considered at least a mild stressor in these animals and will alter the baseline values of many, if not all, physiological measures although habituation to handling procedures leads to habituation, i.e., a reduction of the responses (34). Therefore, it is necessary to eliminate all unspecific interventions to be able to determine the proper responses. For that purpose, home cage-based behavioral assessment has been combined with physiological assessment using advanced telemetric methods to avoid any unspecific interference for optimal signal-to-noise ratio of, e.g., heart rate measures. The downside of this approach is that suppression of

ongoing behavior, a useful index of conditioned and unconditioned fear in specific environments, cannot be quantified in the home cage under habituated conditions, since basal activity is generally low. This leaves little room for the quantification of active suppression as commonly observed in the conditioning context. The positive side of this is that the impact of physical activity on the autonomic measures can be largely neglected in the home cage.

3.3. Fear-Induced Heart Rate Adjustments in Mice

A telemetric approach to determine heart rate and blood pressure dynamics has been used in mice during expression of fear conditioned to an auditory cue (reviewed in (35)). These experiments, partly combined with genetic and pharmacological interventions, indicated fear- and extinction-specific heart rate responses. Their dynamical features show initially fast acceleration of heart rate (half-time: ~3 s) after the onset of the aversively conditioned tone. When starting out from stress-free conditions, baseline heart rate is in the range of less than 600 bpm and heart rate variability is high (36, 37). Tone presentation drives heart rate to maximum levels close to 800 bpm with substantially reduced heart rate variability. Heart rate is maintained at that level for some time before it slowly recovers before the offset of the 180-s tone (38). The heart rate response contrasts with blood pressure adjustments that show slower rise (half-time of mean arterial blood pressure: ~50 s) to peak values of 130 mmHg and prolonged recovery (38). The maximum heart rate and blood pressure values measured during fear retention tests are not observed during circadian measurements under undisturbed conditions in the home cage in the absence of arousal and fear. Furthermore, mouse strains such as DBA/2 show low conditioned freezing levels and only mild conditioned tachycardia (39) indicating similar consequences of deficient fear learning on various readouts. Finally, repeated nonreinforced exposure to the tone causes a decline in the response magnitude indicative of extinction in C57BL/6J mice (29, 35).

While home cage measurements are well suited for phasic stimulation of animals by, e.g., auditory cues, the issue of handling complicates the assessment of heart rate responses during retention of conditioned contextual fear. A study with handling and novelty exposure in C57BL/6J mice indicated that all mice tested showed initially almost maximum heart rate (close to 800 bpm) irrespective of shock experience during training. Heart rate is generally inversely related to heart rate variability with minimal heart rate variability at maximum heart rate range. Starting out from maximum values, heart rate dropped faster in mice either not shocked or subjected to an immediate shock during training (which does not induce fear learning) despite substantially higher physical activity (exploration) than mice that received a late shock during training thereby being aversively conditioned to the conditioning context (40). The difference in heart rate emerged after 5 min and persisted for another 20 min. This indicates that all commonly used anxiety tests of short duration (≤5 min)

that require handling and novelty exposure are essentially unsuited for the assessment of autonomic functions such as heart rate. Therefore, new experimental approaches exploiting home cage-based deliberate behavior are crucial to determine the effect of anxiety and physical activity on basic physiological functions in mice and rats.

Based on these behavioral and autonomic constrains, a new experimental approach has been developed following concepts of deliberate novelty exploration starting out at home cage conditions (21). Following the approach of deliberate open field exploration described before, we investigated the effects of novelty exploration on heart rate in C57BL/6J mice. Preliminary experiments (Stiedl and Golani; unpublished results) indicated that heart rate of mice increases to maximum physiological levels during the first approach of the open field (Fig. 5) confirming the interpretation of high arousal or anxiety-like behavior as concluded from the behavioral performance.

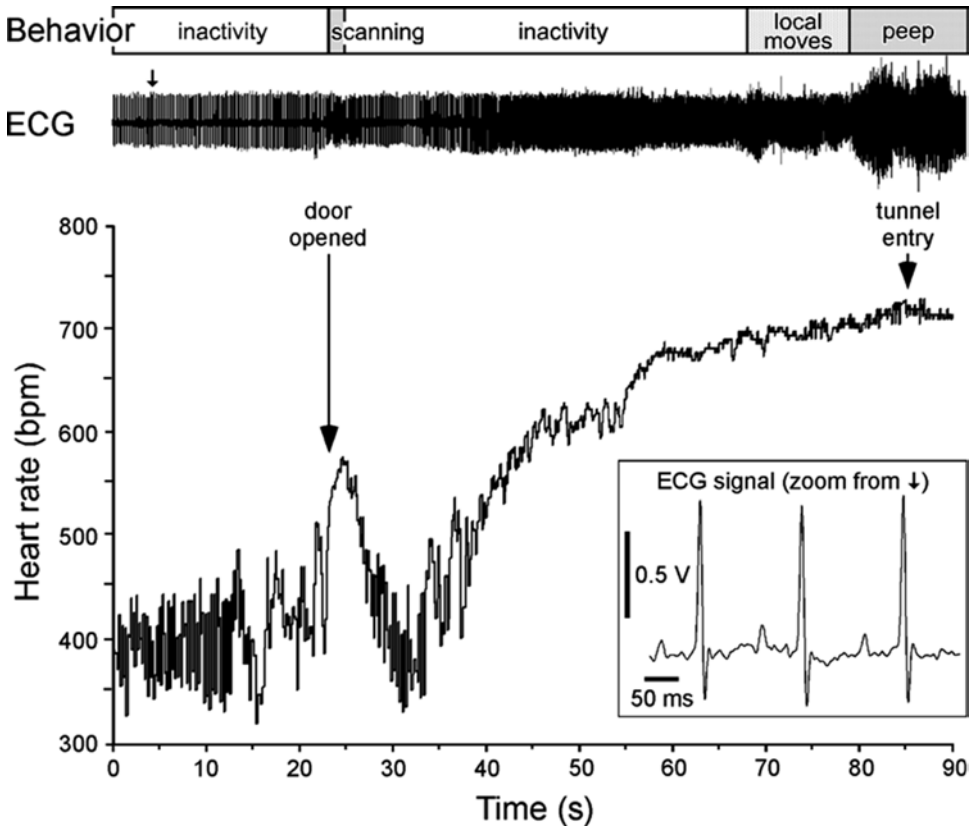

Fig. 5. Behavioral response, concomitant ECG pattern, and derived instantaneous heart rate of a male C57BL/6J mouse in its home cage before entering for the first peep and hide motion into a freely accessible open field. Interestingly, heart rate increased before the mouse moved toward the tunnel suggesting anticipatory arousal before the first physical activity (motion) by entering the tunnel and peeping into the open field. The zoom in the inset depicts a high resolution ECG signal from the location of the *arrow* above the ECG trace at the *top*. Local moves and peeping of the mouse lead to amplitude changes of the ECG signal due to changes of signal strength detected by the ECG receiver.

For the investigation of conditioned fear, another approach is required. Instead of connecting the home cage to a large open field, access is provided to a shock compartment via an automatically controlled door (41), similarly as used in passive avoidance experiments (42). Based on this design, deliberate exploration of the conditioning environment and contextual fear conditioning is investigated by exploiting two natural opposing incentives, fear vs. curiosity (novelty-seeking). Initial studies indicate that fear leads to avoidance of the shock compartment with stretch-attend postures and peeking into the shock compartment. Gradually, mice become more boldly, first entering the shock compartment partially only, before the first full body entry. Finally, mice explore the shock compartment at longer bouts and shorter intervals, a behavior that is indicative of fear extinction. It is important to note that this behavior is almost completely confined to the activity phase of the mice, i.e., the dark phase of the 12-h light-dark cycle. This experimental approach is ideal to quantify the progression of the behavioral responses from the first peep to full exploration and its concomitant heart rate responses in mice. Thereby, the progression of behavioral responses and concomitant heart rate changes can be monitored to better understand physiological states expressed during different phases of the test and determine potential pathological states such as delayed or impaired extinction as index of posttraumatic stress disorder-like behavior. Furthermore, this new approach serves as refined method to investigate the action of anxiolytic compounds with a deliberate choice of mice without experimenter influence on a long time scale of several days. Under these conditions, it is possible to investigate latent inhibition, the retardation of subsequent learning based on prior nonreinforced exposure, which indicates a schizophrenia-like phenotype when impaired.

3.4. Translational Value of Heart Rate Dynamics

An important feature of the heart rate dynamics is the highly dynamical beat-by-beat fluctuation that is essentially determined by the autonomic control originating from brain function. This has been shown by advanced nonlinear measures such as the *detrended fluctuation analysis* (33, 43) indicating persistent long-range correlation of heart beat intervals under baseline stress-free conditions. The long-range correlation is determined by tonic parasympathetic function that slows down heart rate similarly as observed in rats and humans. Blockade of this tonic parasympathetic function by atropine elevates heart rate similarly as functional denervation, as investigated in human heart transplant recipients (44). Both states compromise the control of the beat-by-beat fluctuation and lead to short-term correlation of the beat-by-beat fluctuation. Upon aversive stimulation, heart rate increases due to fast parasympathetic withdrawal and delayed sympathetic activation, thereby leading to reduced long-range correlation of

heartbeats in their temporal sequence. This state is also induced upon handling when combined with novelty exposure, indicating that the proper characterization of autonomic function in mice and rat models of physiological and pathological emotional states will have to be performed under home cage conditions in future experiments. The combination of these experimental conditions with nonlinear assessment of heart rate dynamics will be crucial for the qualitative assessment of physiological and pathological dynamics as observed in disease states (33, 43).

4. Cross Species Genetics of Neurobehavioral Traits; Relevance to Anxiety and Mood Disorders

Mood disorders have a major impact on the quality of life of many people, with a prevalence of 10–20% worldwide (45). Finding the mechanisms underlying these heterogeneous psychiatric disorders and obtaining valid animal models is essential for the development of selective pharmacological treatments (46). Interspecies genetic analysis of mood disorder endophenotypes is an important approach to the discovery of novel insights in causality and to identify translational preclinical models (47, 48). By using longitudinal automated home cage observations, we have recently focused on the genetic dissection of avoidance behavior in mice with the aim of finding more selective and effective pharmacological targets for behavioral disorders in humans.

The balance between approach and avoidance behavior is part of a behavioral strategy, which has been highly conserved across species, to obtain food or mediate social interactions while avoiding threatening situations. Such behavior is influenced by genetic variation, as shown by behavioral differences between inbred strains of mice and subsequent quantitative trait loci (QTL) analysis (49–52). Avoidance and motor activity levels are commonly studied in rodent species with traditional anxiety tests, such as the elevated plus maze and open field. However, the nature of these tests makes it difficult to differentiate between these two behavioral components, and experimenter effects can have a great impact on the behavioral outcome (53). Here, novel automated registration methods for longitudinal behavioral assessment in home cages are used to screen a panel of recently generated mouse chromosome substitution strains (CSSs) that are very powerful in QTL detection of complex traits (54). The automated home cage environment is designed to increase behavioral resolution by dissociating behavioral endophenotypes in mice (55). It assesses levels of avoidance behavior (sheltering) independent of motor activity levels (horizontal distance moved) and with minimal human interference. Longitudinal automated assessment of anxiety-related behaviors might also overcome inconsistent results, depending on subtle, short-term variations in the laboratory or test environment (56).

Furthermore, the avoidance behavior in the home cage is sensitive to benzodiazepines (55), providing predictive validity for this anxiety-related endophenotype that might relate, e.g., to mood disorders with anxious symptoms.

In contrast to the concepts of face and predictive validity, construct validity (similar to the underlying causes and mechanisms of the disease) is the most difficult to provide for animal models of psychiatric disorders, simply because of the lack of knowledge about the underlying etiological mechanisms of such complex disorders. Furthermore, for animal models of psychiatric disorders, it has been proven difficult to provide a 1:1 translation with respect to the face validity criteria. Currently, large-scale genome wide association studies (GWAS) are being performed and have in some cases revealed (unexpected) candidate genes that have low odds ratios and explain a small percentage of the variance. In light of this, genetic validity might provide a new entrance to the biology of psychiatric diseases with animal models (57). By integrating mouse and homologous human genetic mapping data, we identified a gene, adenylyl cyclase 8 (*ADCY8*), connecting mouse avoidance behavior obtained in automated home cage environments to human bipolar affective disorder (58). These findings point to novel mechanisms underlying bipolar affective disorders and open new roads for treatment and translational research of its psychiatric endophenotypes.

5. Summary

Animal models of human chronic diseases or behavioral symptoms, such as anxiety and mood disorders, should be tested in situations where long-lasting stable features ("chronic") of such behavior are manifested. Longitudinal automated home cage paradigms (or exploration from the home cage) are currently being designed as a behavioral laboratory method in order to dissociate various behavioral components. The field of behavioral neuroscience is challenged by these developments since it will allow studies on species-specific ethological relevant behavior and environmental conditions. Due to significant reduction in human interference, a major confounding factor in behavioral studies, with this method, increased sensitivity for detection of genetic or pharmacological interventions seems warranted and contributes to enhanced replication between laboratories. Integration of behavioral and physiological measures in freely behaving mice will serve as a powerful approach with diagnostic value to complement the interpretation of emotional state of rodents. Cross-species genetic studies have revealed novel translational findings that may open new roads for understanding neurobiological mechanisms underlying complex psychiatric disorders.

Novel phenotyping methods require large initial investments for development and validation time with a large contribution of descriptive analyses. Furthermore, automated acquisition of detailed behavioral observations over several days leads to large amounts of data with yet unknown depths of new information. Innovative approaches toward the analysis of these data in view of ethological species-specific relevance of behavioral components are necessary to handle, synchronize, and process these data sets efficiently. This holds especially true for scientists depending on high-throughput facilities for screening of large sets of genetically modified animals. Together, the development of these new behavioral paradigms and analysis methods as well as interpretation of the behavioral findings is expected to provide substantially improved clinical relevance for models of neurobehavioral disorders, mechanistic understanding, and novel therapeutic interventions. However, this new development will largely depend on the available critical pool of behavioral neuroscientists and their integrative view on behavior without ignoring its ecological relevance.

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