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| Abstract | Animal behavioral tests are useful tools for modeling complex human brain disorders. The Suok test (ST) is a relatively new behavioral paradigm that simultaneously examines anxiety and neurological/vestibular phenotypes in rodents. The novelty and instability of the ST apparatus induces anxiety-related behavior in mice, whereas the elevation of the horizontal rod allows for the assessment of motor and neurological phenotypes. This chapter discusses the utility of the ST in detecting mouse anxiety, habituation, exploration, motorisensory deficits, and the interplay between these domains. With a growing number of laboratories using this model, a detailed protocol for the ST behavioral analysis (with a focus on video-tracking tools and novel applications) is also provided. |



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| Key words: (separated by '-') | Mice - Behavioral models - Anxiety - Stress - Exploration - Ethological analysis - Vestibular phenotypes - Stress-evoked sensorimotor disintegration |

# Chapter 4

## Modeling Mouse Anxiety and Sensorimotor Integration: Phenotypes in the Suok Test

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### Abstract

Animal behavioral tests are useful tools for modeling complex human brain disorders. The Suok test (ST) is a relatively new behavioral paradigm that simultaneously examines anxiety and neurological/vestibular phenotypes in rodents. The novelty and instability of the ST apparatus induces anxiety-related behavior in mice, whereas the elevation of the horizontal rod allows for the assessment of motor and neurological phenotypes. This chapter discusses the utility of the ST in detecting mouse anxiety, habituation, exploration, motorisensory deficits, and the interplay between these domains. With a growing number of laboratories using this model, a detailed protocol for the ST behavioral analysis (with a focus on video-tracking tools and novel applications) is also provided.

**Key words:** Mice, Behavioral models, Anxiety, Stress, Exploration, Ethological analysis, Vestibular phenotypes, Stress-evoked sensorimotor disintegration

### 1. Introduction

Experimental animal models are widely used to improve our understanding of complex psychiatric disorders, and to screen the effects of various pharmacological, genetic, and behavioral manipulations (1–8). As will be shown in several chapters in this book, mice frequently display neurobehavioral similarities with humans. This supports the utility of murine models for anxiety research (9, 10), including both the improvements in existing tests and the establishment of new paradigms (11–13).

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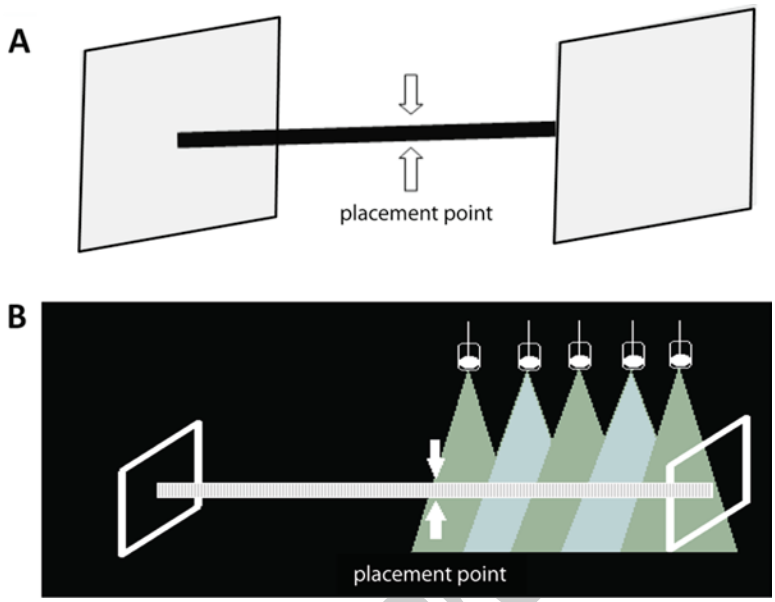


Fig. 1. Murine Suok test apparatus: the regular Suok test (**a**) and its light-dark version (**b**).

[AU1]

The Suok test (ST, Fig. 1) is a recently introduced behavioral model that applies ethological analysis to examine mouse and rat anxiety (5, 14, 15). The novelty and utility of this paradigm arise from its ability to simultaneously assess rodent anxiety, vestibular phenotypes, and motor performance, as well as their complex interplay, such as stress-evoked sensorimotor disintegration (SSD) (2, 16–19). Although SSD is a common clinical phenomenon, its pathogenesis remains largely unknown (17, 20). The ST's rationale and construct validity come from a well-known ability of unprotected, open, and elevated areas to evoke anxiety and panic (acrophobia) as well as vestibular symptoms (vertigo, dizziness) in both clinical patients (21–25) and in normal human subjects (26–29). The concept of SSD is further supported by anxiolytic drugs' ability to reduce vestibular deficits in humans (19, 30, 31) and by animal data on the comorbidity between vestibular and anxiety phenotypes (see (17) for a detailed review).

Compared to other anxiety tests, the ST enhances the dimensionality of mouse data, serving as a conceptual combination of the elevated plus maze, open field (OFT), and horizontal beam tests (32, 33). Representing a long, elevated horizontal rod with a Plexiglas wall on either end (Fig. 1a), the mouse ST simultaneously assesses lateral (e.g., horizontal locomotion) and vertical (e.g., head dipping, falls) behaviors (5, 15, 32–34). At the same time, the ST is a typical novelty-based paradigm, similar to the elevated plus maze and OFT, where anxiety is evoked and examined based on the classical approach-avoidance theory (35). While the ST

novelty couples with the instability of the apparatus to induce animal anxiety, the elevated testing surface is used to assess rodent balance and motor performance (similar to the traditional beam test (22, 36–38)) by the number of falls and hind leg slips (32, 33). The light-dark ST version (Fig. 1b), which utilizes animals' natural aversion to a novel and brightly lit environment, further enhances the model by adding an additional stressor (5).

Basic methodology of rodent ST behavioral testing and its validity has been discussed previously in detail (5, 15, 32–34). With a growing number of laboratories using the ST for different rodent applications (e.g., (5, 14, 39, 40)), this chapter aims to provide an update on this model and its utility for mouse behavioral phenotyping. We will specifically emphasize the ST ability to target multiple behavioral domains, and how this can be enhanced by the use of modern video-tracking technology. The latter not only enables the correction of manual observations but also generates additional indices reflecting velocity, immobility, high mobility, and distance traveled. The developing utility of the ST to study basic cognitive functions (e.g., habituation) as well as other aspects of mouse novelty-evoked responses (e.g., homebase behaviors) will also be discussed.

## 2. Equipment, Materials, and Setup

Various inbred, outbred, selectively bred, and genetically modified (mutant or transgenic) mice may be used in the ST to observe anxiety, motor function, and neurological phenotypes. When selecting a mouse model, the strain difference in activity and emotionality are important to consider. For example, BALB/cJ mice generally exhibit high anxiety, whereas C57BL/6J and NMRI have low baseline anxiety levels. Activity levels and novelty seeking also differ markedly between strains. For example, 129 S1/SvlmJ mice generally display low activity, the NMRI strain has moderate activity, while both BALB/cJ and C57BL/6J strains are usually highly active. Similarly, 129 S1/SvImJ and BALB/cJ mice are neophobic, and C57BL/6J mice show high novelty-seeking behavior (9, 41, 42). Factors such as age, weight, sex, estrous cycle stage, and husbandry should also be considered when designing ST experiments. In addition, the most updated and detailed nomenclature for mouse strains must be used (see Mouse Phenome Project for mouse strains: http://phenome.jax.org, and Mouse Genome Informatics for genetically modified mice: http://www.informatics.jax.org).

The equipment required for the regular or light-dark ST is simple, inexpensive, and sufficient to assemble the apparatus and collect data. The typical mouse ST apparatus is a 1–2-m aluminum

tube ~2 cm in diameter, elevated to a height of 20–25 cm above a cushioned test surface (Fig. 1a). The rods for both ST versions can easily be purchased from home utility stores, costing approximately $10 per rod. The rod is demarcated into 10-cm sectors to allow quantification of distance moved by the mouse. Two Plexiglas walls ($50 \times 50 \times 1$ cm) are fixed on either end of the aluminum tube to prevent mice from leaving the test apparatus, and paper towels or cloths placed directly underneath the rod act as protective cushions (to prevent injuries during falls and enable efficient clean up between subjects). Seventy percent Ethanol is required to clean the aluminum rod between sessions. To avoid the potentially confounding effects of bright lights (42), the experimental room must not be brightly illuminated (in our studies at Tulane University, 700–900 lux appears to be appropriate for mouse ST).

The light-dark ST apparatus, identical to the regular ST test, includes 4–6 light bulbs (60 W) fixed ~40–50 cm above one-half of the rod, providing the only light source in the dark experimental room (Fig. 1b). The few additional pieces of equipment for data collection are easily attainable, and include a manual observation template, timer, light meter, and video-recorder. The template generates a per-minute distribution of behavioral endpoints (see further) for the quick detection of temporal trends, such as habituation. For video-tracking mouse ST behavior, special software packages are required. For example, our laboratory uses Noldus Ethovision XT7 (Wageningen, the Netherlands) and Clever Sys LocoScan (Reston, VA).

The light meter (e.g., Sper Scientific, Scottsdale, AZ) is a handheld device that measures lighting of the ST apparatus. To ensure proper lighting (e.g., 700–900 lux) for the regular ST test, take 10–15 measures for three points on the ST apparatus (in the center and on either end). If necessary, adjust the light source or the ST apparatus location to ensure homogenous illumination.

## 3. Procedure

### 3.1. Acclimation

This period entails transporting mice from their holding room to the experimental room 1 h prior to behavioral testing, and leaving subjects undisturbed to minimize their transfer anxiety. If the mice are obtained from a commercial vendor or another laboratory, allow at least a 2–3-week acclimation period before testing, to reduce transportation stress.

### 3.2. Suok Test Procedure

Mice must be tested in the ST during their normal waking cycle, to avoid interference with circadian rhythms. When performing a battery of tests, consider how the effects of these prior tests may

confound the mouse ST performance and drug sensitivity. At the beginning of each trial and after each fall from the apparatus, place mice at the center of the rod (0 cm) with snout facing either end (or, in the light-dark modification, orient the animal facing the dark end). If necessary, subjects can be gently supported by hand during initial placement, to avoid falls caused by incorrect positioning. Note that if video-tracking is used, place mice back to the point where they fell off, to prevent artificial inflation of the endpoint "distance traveled" when the software analyzes the videos. To minimize detection problems, allow ~5 s to pass at the start of each recording before placing the subject into the test arena (see Troubleshooting 1).

### 3.3. Behavioral Testing and Analyses

While a typical ST experiment is a short 5–6-min trial, its duration can be altered at the discretion of the experimenter, depending on experimental needs (e.g., we recently applied an extended 20-min trial to examine mouse ST exploratory behavior in depth). A digital camera mounted in front (or on top) of the test apparatus, combined with video-tracking software, will enable the collection of accurate behavioral data. If video-tracking software is used, the camera should be positioned ~50 cm away from the apparatus. During the observational period, the experimenter usually sits and records mouse behavior ~2 m away from the apparatus. The observers must refrain from making noise or movement, as this may alter animal behavior. Also, intra- and inter-rater reliability should be assessed for consistency (desired level is ~0.85 or more) by Spearman rank correlation coefficient.

During each trial, the following behavioral measures are recorded manually or using video-tracking software: (a) horizontal exploration activity, which includes latency to leave central zone, number of segments visited (four paws), time spent moving, velocity, average inter-stop distance (distance traveled divided by number of stops) distance traveled, number of stops, time spent immobile; (b) vertical exploration (number of vertical rears and wall leanings); (c) directed exploration (number of head dips and side looks); (d) risk assessment behavior (stretch-attend postures); (e) vegetative responses (latency to defecate, number of fecal boli and urination spots); and (f) motor behavioral parameters (number of missteps or hind-leg slips and falls) (see Fig. 2 for details). Note that tail position may also be a useful index (usually elevated and erect if anxiety is high). The value of each "latency" endpoint will equate to total observation time if the animal does not show the respective behavior. At the end of each testing session, mice are returned to a holding room, and the ST apparatus should be wiped with 70% ethanol, to remove olfactory cues that may affect the behavior of sequential subjects.

### 3.4. Data Analysis

*Statistics*: The ST behavioral data can be analyzed with the Wilcoxon–Mann–Whitney U-test for comparing two groups (parametric Student's t-test may be used if data is normally distributed), or analysis of variance (ANOVA) for >2 groups, including one-way ANOVA with repeated measures (time), and n-way ANOVA for

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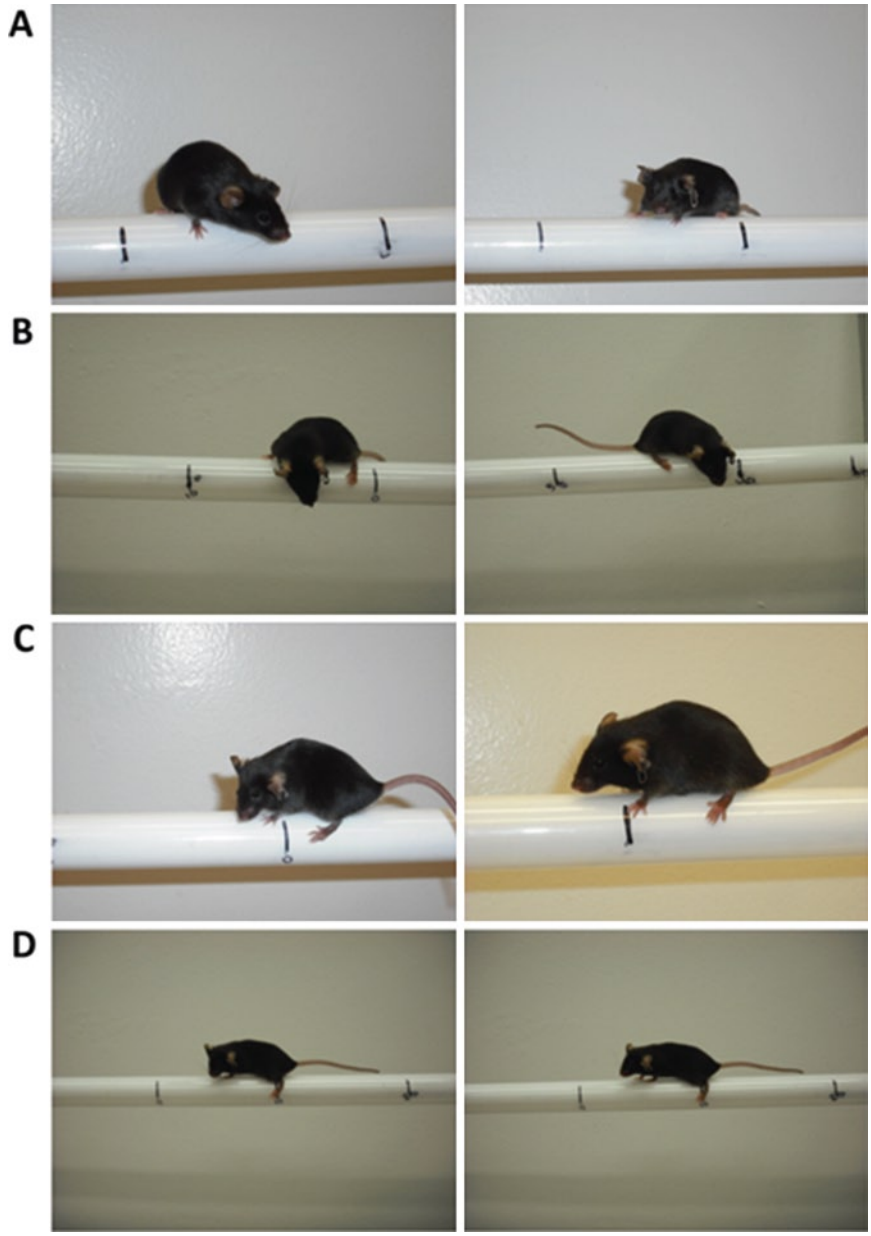


Fig. 2. Typical mouse behaviors observed in the Suok test: (**a**) side looks, (**b**) head dips, (**c**) freezing, (**d**) hind leg slips, (**e**) "anxious tail" position, (**f**) stretch-attend posture, (**g**) grooming behavior.

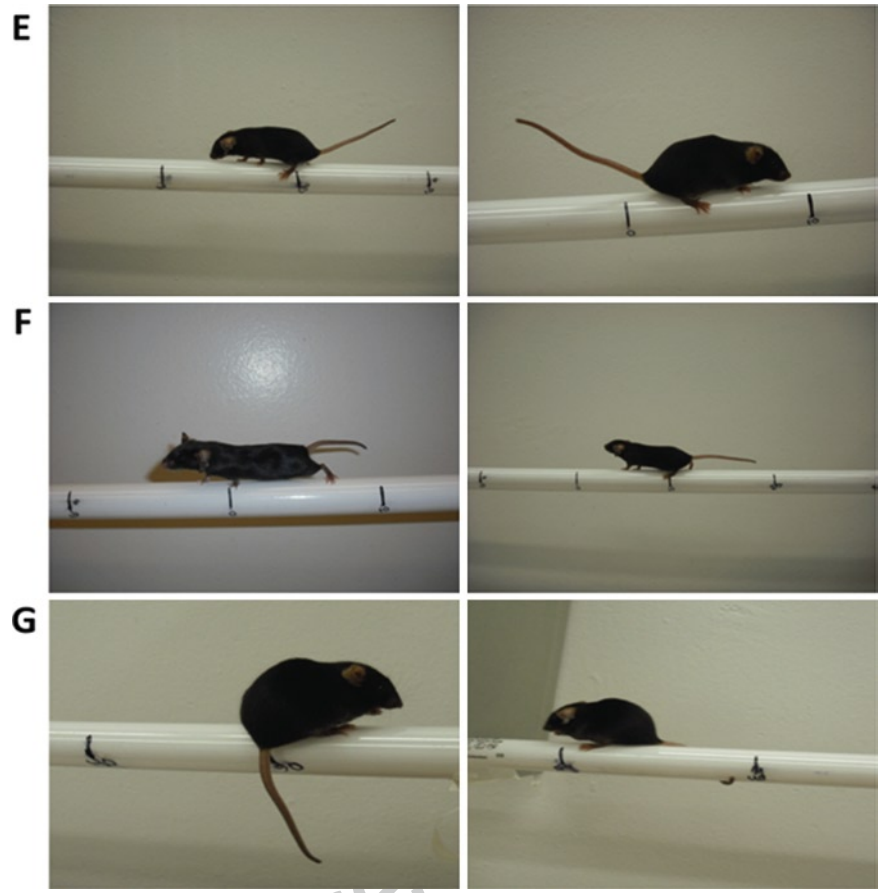


Fig. 2. (continued)



more complex studies (e.g., including treatment, genotype, sex, and/or stress), followed by an appropriate post-hoc test, such as Bonferroni adjustment, Dunn, Dunnett, or Tukey tests.

*Video analysis*: The ST videos can be analyzed and its endpoints (e.g., distance traveled, velocity, and time spent moving) calculated using an automated video-tracking system. Before analyzing videos, frames including the researcher must be removed to avoid skewing data. Generally, researchers stay out of camera sight, away from the ST apparatus during testing. However, at the beginning of each session or if the animal falls, they must be close to the apparatus and may briefly appear in the videos. If the frames are not removed from the video recording, researcher's body parts could be "detected" as mice (see Troubleshooting 2). A video-editing program, such as Windows Movie Maker, may be used to remove such frames.

After videos have been edited appropriately, they may be analyzed using video-tracking programs, such as Noldus Ethovision XT7. To properly acquire videos, first establish a rectangular arena for the experiment, with the boundaries of the arena formed by the bottom of the rod, including ~5 cm past each end (to include Plexiglas end walls), and a line ~10 cm above the rod. Limiting the size of the arena (by excluding the area between the test surface and the underside of the rod) ameliorates detection setting problems and reduces rogue endpoints. To determine which detection settings work best, evaluate the three detection settings, "Static Subtraction" "Differencing," and "Dynamic Subtraction," in concurrence with playing a video. When tracking using Noldus Ethovision XT7, yellow shading will cover the subject as it moves around the arena. On the Experiment Settings screen, set the program to track all morphological endpoints, including tail, center, and nose. These endpoints will appear as teal, *whatever* and *whatever* dots when the video is tracking correctly. After acquisition, remove any rogue detection points and interpolate missing data. If there are apparent errors, readjust detection settings and reacquire videos before exporting data for behavioral analyses.

The behavioral data generated by video-tracking complements the manual observation endpoints. Recommended indices to calculate include total distance moved, mean velocity, absolute and mean turn angle, turning rate (absolute and mean angular velocity), turning bias (relative and mean angular velocity), absolute and mean meandering, duration and frequency of movement, and duration and frequency of elongation. All of these behavioral endpoints reflect different aspects of the mouse ST performance and are common for many other behavioral paradigms and tests. Endpoints only attainable through video-tracking (e.g., velocity and movement) can quantify whether the subject moves in short, quick bouts or longer, more cautious movements. Calculations of turning rate and bias describe the nature of circular exploratory movement (turning movements with a higher velocity may represent potentially interesting phenotypes; see further).

### 3.5. Time Required

The acclimation period typically requires 1 h prior to the ST procedure. However, if the initial level of mouse anxiety is very high, using a longer acclimation time and/or handling each animal (e.g., for 5 min per day for 3–4 days prior to ST) may reduce potential anxiety related to experimental procedures. Animal testing in the ST requires approximately 9 min per animal (6 min of testing and 2–3 min of clean-up of apparatus). Depending on the amount of data collected, analysis for manual observations may take approximately 1 day, and an additional 2–4 days may be needed to analyze video-generated data.

## 4. Anticipated Results

In general, the ST is highly sensitive to behavioral differences in mouse anxiety. For example, the model correctly detects major differences between strains' behavioral phenotypes (e.g., anxiety and motor functioning) and state or trait behaviors (3, 5). A typical experiment examining baseline anxiety in BALB/cJ, NMRI, and C57BL/6J strains is shown in Fig. 3. Note that BALB/cJ mice, an



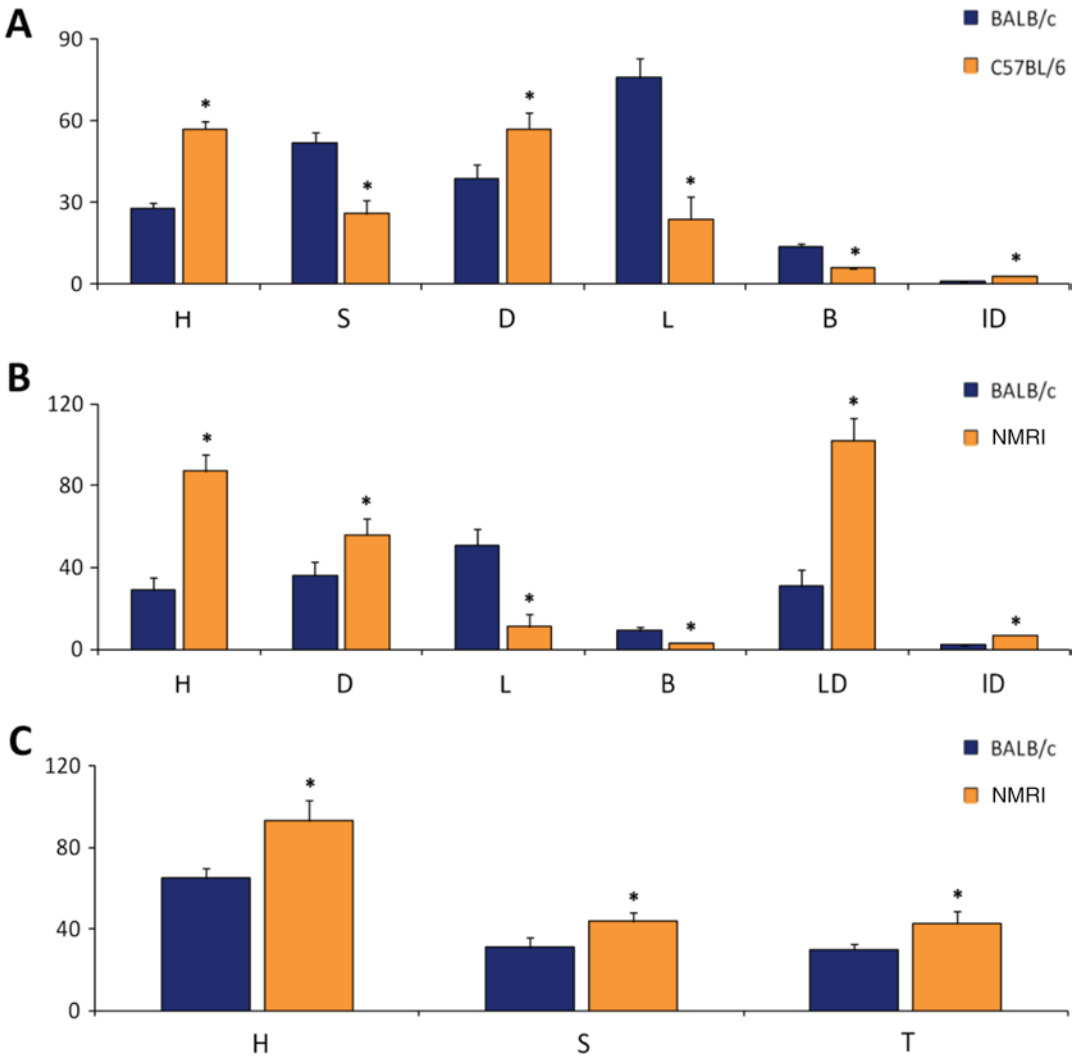


Fig. 3. Representative behavioral responses of male NMRI, BALB/cJ, and C57BL/6J mice in regular (**a**, **b**) or light-dark (**c**) Suok test for 5 min (graphs are based on data published previously by our group (5)). (**a**, **b**) *H* horizontal activity (segments); *S* stops; *D* head dips; *O* orientation (side-directed exploration); *L* latency to leave center; *B* defecation boli; *LD* latency to defecate; *ID* average inter-stop distance. (**c**) *H* horizontal activity in the light; *S* sectors visited in light; *T* time in light; values expressed as percentages. *$P < 0.05$ (U-test) between strains.

innately anxious strain, exhibit predictably more anxiety and less exploratory behavior than both NMRI and C57BL/6J strains. Increased anxiety was demonstrated by shorter inter-stop distance, increased stops and fecal boli, whereas exploratory behavior was signified by higher latencies to leave the center, less horizontal activity, and fewer head dips (Fig. 3). BALB/cJ mice show preference for the dark area of the light-dark ST, assessed by significantly fewer stops and less time spent in light, consistent with their higher trait anxiety (Fig. 3).

The ST sensitivity to evoked anxiety has been demonstrated in a recent experiment where C57BL/6J mice were roughly handled (ten strokes of backward petting) for 1 min (Fig. 4). The stressed mice displayed predictably higher anxiety, as indicated by more falls and decreased exploratory behavior (increased duration of stops and a lower total distance moved). Similar results were obtained using other psychological stressors in mice, such as pretest exposure to a rat, which is a strong stressor as rats are natural



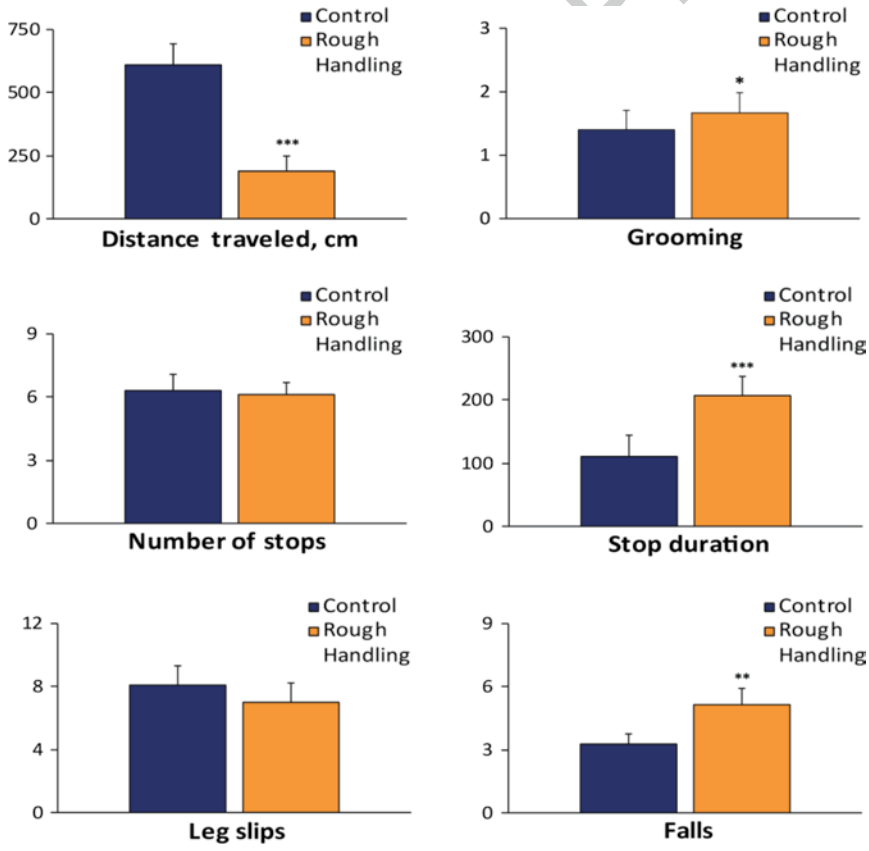


Fig. 4. Behavioral responses of control and roughly handled C57BL/6J male mice ($n=20$ in each group) tested in the regular Suok test. Handled mice exhibited a significantly higher number of falls, a longer stopping duration and a shorter distance traveled, suggesting their increased anxiety. *$P<0.05$, **$P<0.01$, ***$P<0.005$ (U-test).

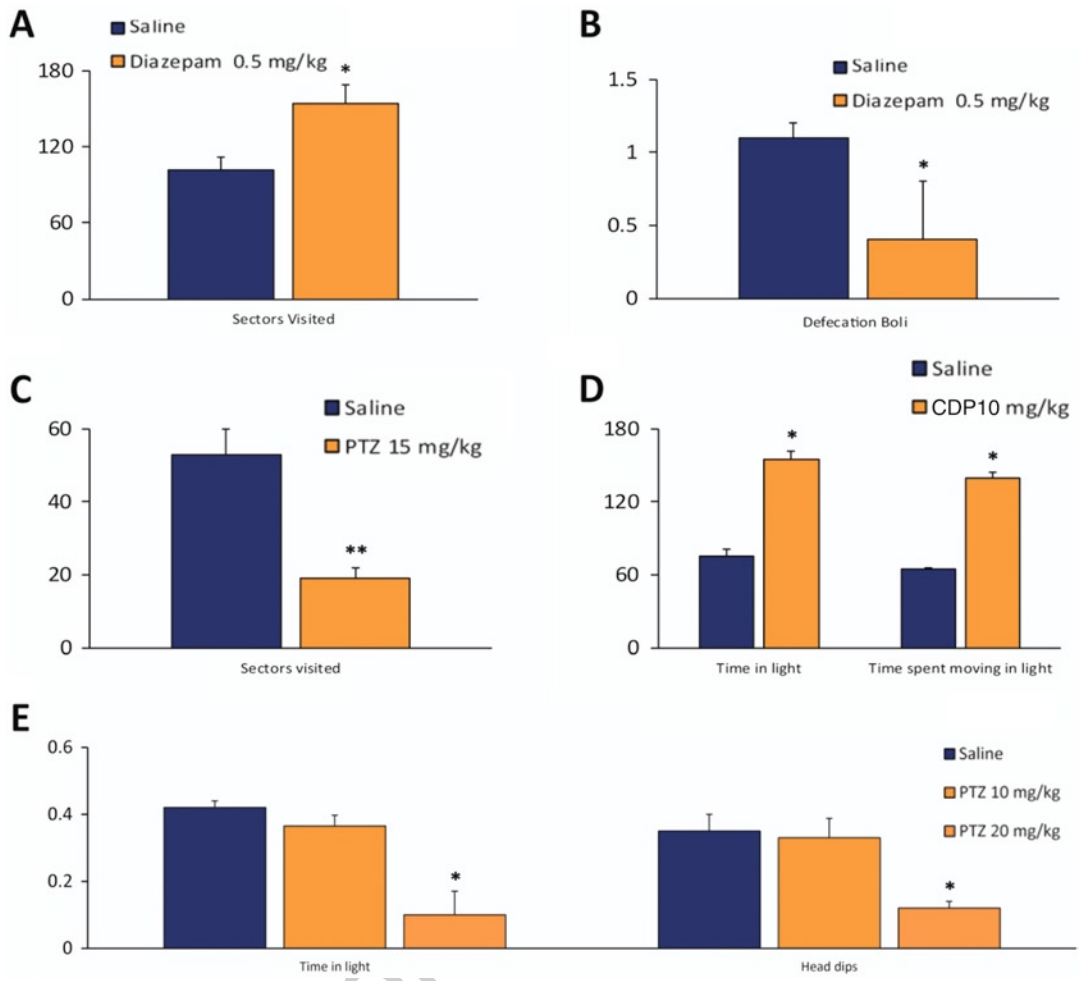


Fig. 5. Behavioral responses of male BALB/cJ mice to diazepam, chlordiazepoxide (CDP) and pentylenetetrazole (PTZ) in the regular (**a**–**c**) and light-dark (**d**–**e**) Suok tests. Diazepam increased exploration and lowered the number of defecation boli. PTZ increased anxiety in both tests by decreasing sectors visited, head dips and time spent in light, and showing decreased motor functioning by increasing the falls and misstep. CDP decreased anxiety by increasing time spent and movement in light. Graphs are based on data previously published by our group (5). *$P < 0.05$, **$P < 0.01$ (U-test).

predators of mice. Rat-exposed mice exhibited increased anxiety and impaired balance compared to a nonexposed control group (33).

In addition to genetic strain differences and experimental stressors, the ST is also sensitive to pharmacogenic anxiety (32). A typical experiment assessing the ST responses to various pharmacological agents is shown in Fig. 5. In this study, the anxiolytic drug diazepam increased exploration and lowered the number of fecal boli. In the light-dark ST version, the anxiolytic drug chlordiazepoxide (CDP) decreased anxiety by increasing time spent and movement in light. By contrast, the anxiogenic drug pentylenetetrazole (PTZ) increased anxiety in both the regular and light-dark ST (Fig. 5) and

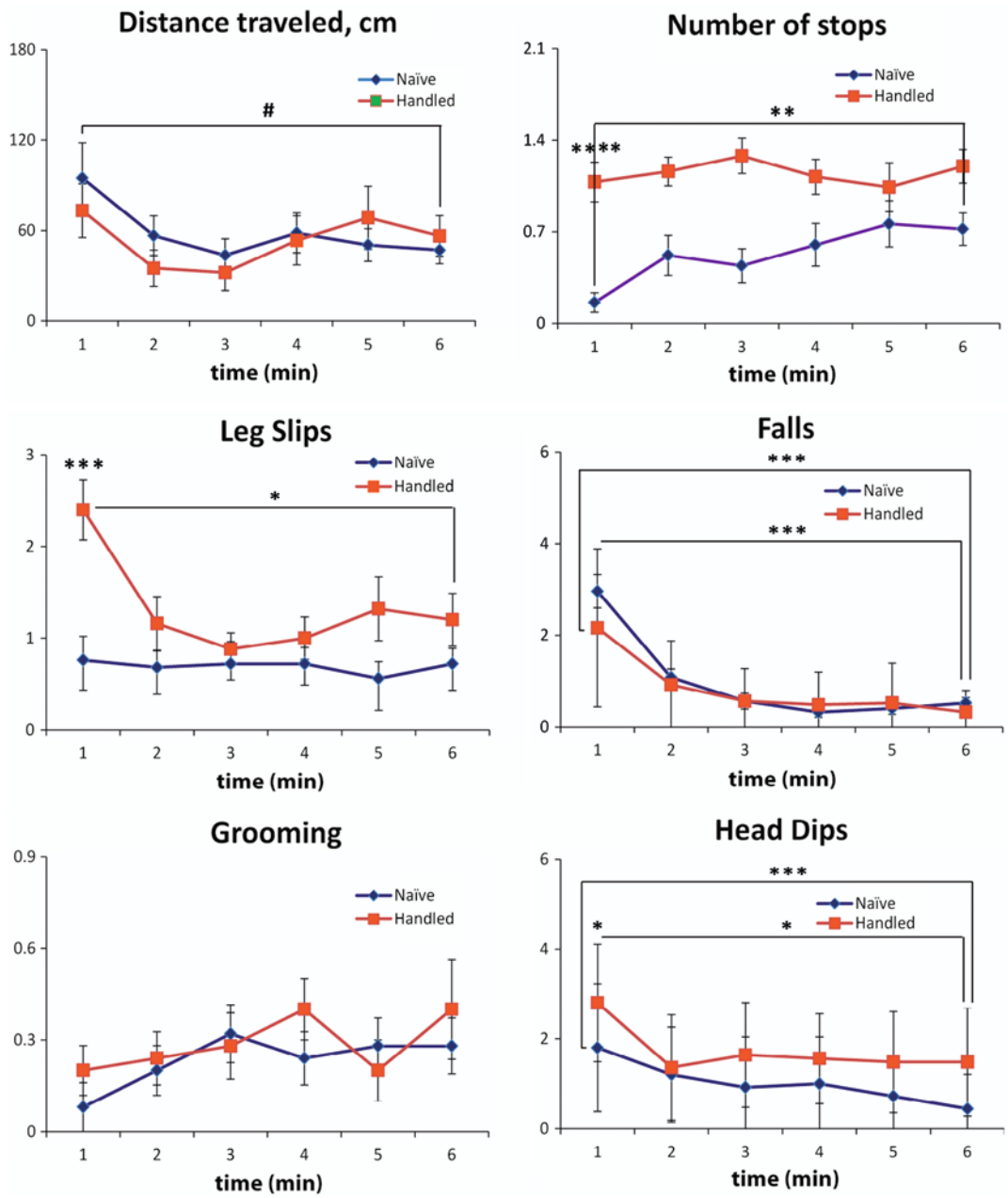


Fig. 6. Habituation of Suok test behaviors in male C57BL/6J mice. Control (naïve) mice traveled less distance over the course of the 6-min trial. Note that acutely stressed mice show slightly impaired habituation as compared to control mice, consistent with the known negative effect of acute stressors on rodent spatial working memory (57–59). Min 1 data between groups was compared using paired U-test. Min 1 vs. min 6 within each group was compared using unpaired U-test. *Asterisks* on *top* of *horizontal line* denote difference between respective min 1 and min 6. *Asterisks* on *top* of min 1 data denote difference between initial (min 1) anxiety in stressed (handled) vs. naïve control mice. *$P<0.05$, **$P<0.005$, ***$P<0.0005$, #$P=0.05$–$0.1$, trend (U-test).

also impaired mouse motor function by increasing the falls and missteps (43). Taken together, these findings support the utility of the ST for screening a wide spectrum of pharmacological agents in rodents.

In addition to producing quantifiable data, video-tracking software can provide an accurate and visual summary of murine ST traces (Fig. 6–9). Center-point tracking shows overall distance moved, as some subjects may never leave the center, show preference for certain areas of the rod, or utilize the entire apparatus. However, the tail and nose-point tracking, in our opinion, better detects exploratory behavior. For example, a head dip is represented in a side view trace by a nose-point line below the center-point trace. As shown in these traces, the nose and tail-traces often form circular patterns, indicating head dips and vertical explorations that occur in more of a sweeping manner. Top view traces can also be generated by positioning the video recorder above the test rod. Unlike side view traces, top view traces can visually represent and detect exploration on either side of the ST apparatus (Fig. 8), which appear as rotating or swiveling maneuvers.

Finally, video-tracking software can produce "density maps," which show the overall frequency of time spent over the length of the ST apparatus. As shown in Fig. 9, the density of behavior is not homogenous over the ST rod's length, as the mouse clearly prefers locations in the center (initial placement point) or close to the walls of the apparatus (thigmotaxis; see further).

## 5. Additional Potential Applications

Within-trial habituation is an important phenotype (observed in mouse behavioral tests), reflecting rodent spatial working memory (44–46). Our recent experiments reveal the ST's utility for examining mouse habituation. As shown in Fig. 6, roughly handled (stressed) mice demonstrate poorer habituation for distance traveled, head dips, and number of stops (vs. robust habituation curves in their controls). While control mice traveled less distance over the course of the trial, stressed mice traveled approximately the same distance each minute. Similarly, control mice performed less head dips per minute, while the stressed group had a less steeper decline (Fig. 6).

Although leg slips and falls are nonexploratory behaviors (and, therefore, do not reflect habituation), the negative slope of their graphs suggests the occurrence of some kind of aversive learning. An alternative explanation of these temporal phenotypes may also be due to reduced activity (e.g., an increased number of stops and decreased overall distance traveled, see Fig. 6) since if subjects

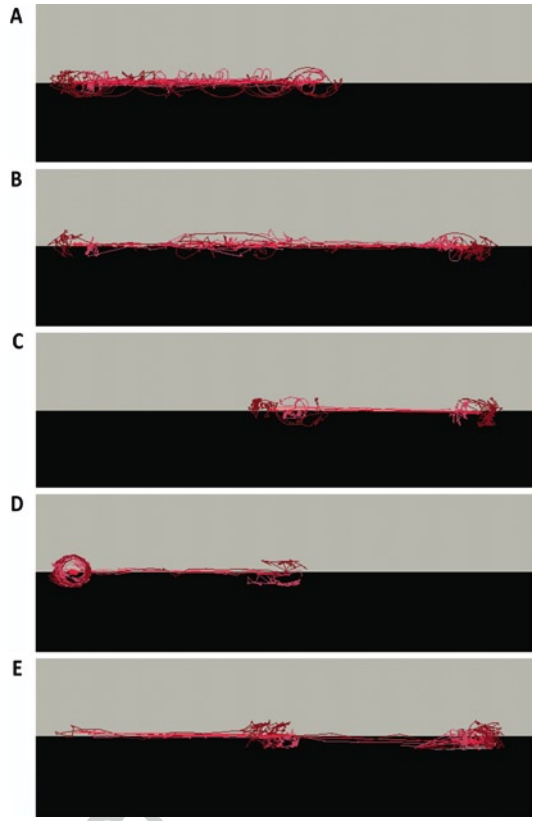


Fig. 7. Representative top-view Suok test traces generated using Noldus Ethovision XT7 video-tracking software. As explained in the text, Ethovision XT7 can track the nose, center, and tail points of subjects, to produce traces. The traces presented here were saved from the software and superimposed onto a *gray* and *black* background, to indicate the location of the test apparatus. (**a**) Trace in which the subject failed to leave the center, circular rings around the center point by the nose and tail points indicate that the mouse spun around to explore the novel environment; (**b**) traces in which the subject performed moderate exploratory behavior on one side only. This trace shows the mouse swiveled at regular intervals across the left side of the rod. (**c**, **d**) This mouse performed exploratory behavior on one side only, but most of the behavior was localized to the center and left endpoints. (**e**, **f**) These animals performed exploratory behavior over the entire rod. The lack of full circles in these traces shows that these mice did not perform as much swiveling behavior as in previous (**a**, **c**).



move less distance and stop more frequently, they are less likely to fall or slip. Whether this signifies altered habituation, different processing of sensory information, or both, it is an interesting direction for further studies (47, 48), also suggesting that the ST has the potential for screening various mnemotropic drugs.

While the behavioral effects of antidepressants have not been examined in the ST, the well-known ability of selective serotonin reuptake inhibitors' (SSRI) to improve balance and reduce anxiety

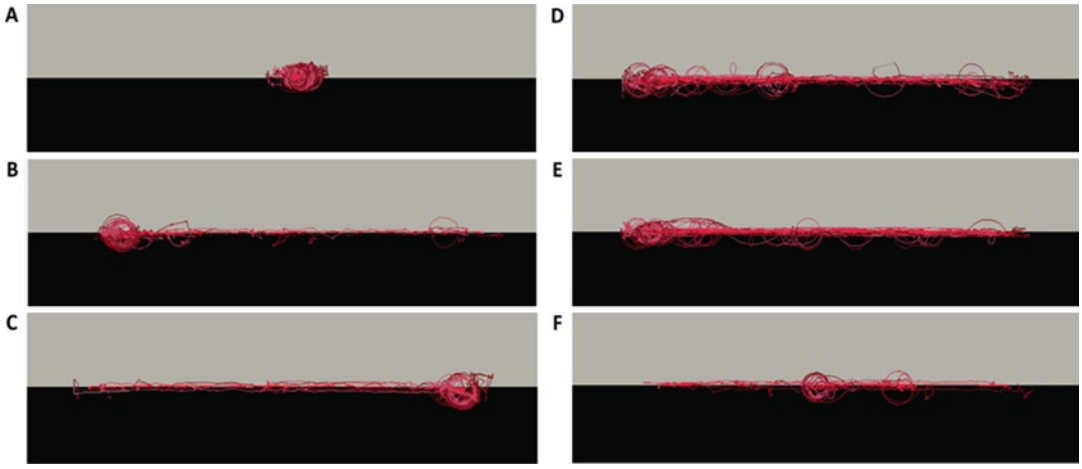




Fig. 8. Representative side-view Suok test traces generated using Noldus Ethovision XT7 video-tracking software. (**a**) subject failed to leave the center, showing extensive rotational exploratory behavior at the center point; (**b**) subject utilized the entirety of the test rod, spending more time on the left side of the test; (**c**) subject utilized the entirety of the apparatus, performing more consistent exploratory behaviors; (**d**, **e**) these mice utilized the entire of the apparatus, exhibiting vertical exploratory behaviors in certain nonregular intervals; (**f**) subject showed more horizontal exploratory behavior than vertical.

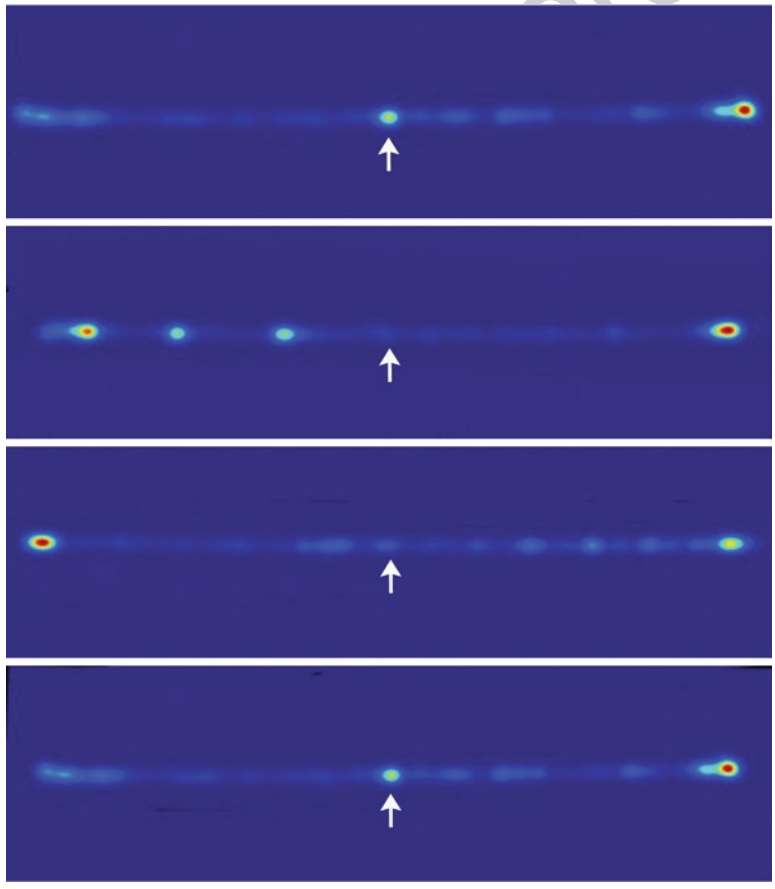



Fig. 9. Density maps of the mouse Souk test activity (*top* view) generated by Noldus Ethovision XT7 video-tracking software. Concentrated *red*/*yellow* color would indicate a large percentage of time spent in a particular zone on the apparatus (*white arrow* indicates the placement point).

in both humans and animals (48–50) implies the ST's potential sensitivity to these drugs. Furthermore, the ST is likely to be sensitive for novel drugs targeting the vestibular system, agents affecting SSD and anxiety, as well as some other drug classes, such as hallucinogens. For example, the sensitivity to a hallucinogenic lysergic acid diethylamide (LSD) has already been demonstrated in a mouse ST (4). Recent rodent studies from other laboratories have identified additional potential applications of the ST. For example, the test showed superior (vs. OFT) sensitivity to behavioral effects of long-term alcoholization (14), and sensitivity to behavioral effects of bioflavonoids' on stress-related behavioral activity (51) in rats, collectively suggesting that the rodent ST may also be applied to study a wide spectrum of drug abuse-related phenomena, such as long-term behavioral alteration, withdrawal-evoked anxiety and SSD.

Another potential novel application of the ST is the analysis of homebase formation. Homebase formation is an adaptive behavioral strategy used by rodents to facilitate spatial orientation and exploration (52–55). In a novel environment, animals establish one or two "safe" zones where they spend most of their time and frequently visit, while exploring their environment. Rodent homebases tend to be established near vertical surfaces and show higher grooming and rearing activity (56). Our observation of ST-induced behaviors presents an innovative opportunity for studying rodent homebase formation. For example, we observed the mouse ability to form preferred loci in the ST apparatus, (Fig. 9), demonstrating that mice spent considerably more time at 2–3 nonrandom locations, usually near the side walls or at the center drop point (Fig. 9).

## 6. Troubleshooting

Several practical recommendations, briefly summarized here, may enable more reliable and reproducible behavioral data in the mouse ST experiments.

1. When initially placing the mouse on the bar (or after a fall), orient the mouse with the snout facing either end. Support the animal during initial placement to avoid a fall due to poor positioning. If a mouse fell off the testing rod, place the animal back on the rod with minimal disturbance, to the same spot from where it fell (if the mouse is returned to a different location, a video-tracking program will artificially inflate total distance traveled by the mouse).
2. When using video-tracking software, minimize the amount of time researchers spend within camera range. For example, reduce the time spent in frames by having one individual

stationed near the ST apparatus to quickly return mice to the rod, and the other ready to pause the experiment timer. Alternatively, a careful editing of video files will help solve the problem. To edit videos using this program, open a new project file and import one video at a time. Remove all the video segments in which the mouse has fallen off the test apparatus or a researcher is in frame; alternating between various zoom settings may increase accuracy. Save the video as in DV-AVI format (the Windows Movie Maker version of AVI files supported by video-tracking software).

3. Setting the detection arena tightly around the testing rod can minimize confounds in the video tracking process. If raw points are still being detected, attempt to reduce the complexity of the entire screen shot. Try to buffer bright lighting with white paper and create a surface of white paper on the testing platform flush with the walls behind it to increase contrast for better detection.

[AU2]

4. Testing sessions around 5–6 min are usually sufficient for the ST. This testing time is desirable as it is sensitive to anxiety, yet long enough to produce significant habituation responses (Fig. 6). However, this amount of time may not be sufficient if mice with impaired motor or vestibular function are used. For example, several initial minutes may be lost from repeatedly returning the falling mouse to the rod. To retain experimental time, pause the experimental timer during each fall or run the experiment for a longer duration (e.g., 10–20 min). Pausing the experimental timer can also help synchronize manual observation data with edited tracking videos. Analysis of home-base-like behavior may require an even longer observation time, as suggested by early OFT studies investigating rodent homebase formation (56).

5. High levels of transfer anxiety may lead to poor initial retention on the testing apparatus. To prevent this problem, gently support the animals by hand for ~5 s to facilitate a better grip. If the animal continues to display high transfer anxiety, exclude it from the experiment (record, however, the % of such animals in each group). In addition, improved animal husbandry in the holding areas and the use of a dimly lit experimental room can reduce initial anxiety levels.

6. Depending on the overall motor ability of the experimental mice, the type of experimental rod can be altered. For mice with severely impaired vestibular function, masking tape along the surface of the rod, wider or wooden rods for a better grip, and (in extreme cases) a flattened surface similar to a narrow meter stick, can be used. In this case, the control mice would also fall and slip less, producing a habituation curve with less amplitude. If mice continue to struggle with balance or motor

abilities, assess motor and vestibular functions separately, as these behaviors may be due to a neuromuscular or motor coordination problem unrelated to vestibular deficits or anxiety.

7. Low motor or vertical activity may be a strain-specific phenotype. Less active mouse strains will produce lower activity overall, and may not be suitable for this model. Likewise, hyperactive strains generally display less nonhorizontal exploration and may have difficulties with balance. A narrower apparatus will encourage the animal to show its horizontal activity, enabling other behavioral responses.
8. Performance on the ST is strongly determined by physical factors, such as body size and weight (larger animals have predictably more difficulty). Only use animals of similar age, size, and weight to reduce possible confounds and accurately compare between groups.
9. If the study involves a battery of behavioral tests, consider the potential effects of test batteries on ST performance. For example, because the ST utilizes rather strong anxiety evoked by height and novelty, administer less stressful tests before subjecting animals to the ST. Acclimate animals for at least 7 days before or between STs to reduce habituation confounds. Likewise, this model may not be suitable for long-term follow-up studies, since mice quickly habituate to the apparatus (Fig. 6). However, the ST habituation itself may provide a readily testable mouse model with an additional (cognitive) dimension.

## 7. Conclusion

Overall, the ST simultaneously examines anxiety, vestibular, and neuromuscular deficits by combining an unstable, elevated rod with novelty. Anxiolytic or anxiogenic drugs predictably modulate mouse ST exploration, risk assessment, and vegetative behaviors. The model is also sensitive to anxiety-evoked vestibular/balancing deficits (such as SSD), as anxiogenic drugs increase the number of falls and missteps, while anxiolytic agents generally improve balance (4, 6). Some basic cognitive (e.g., habituation) phenotypes may easily be assessed in this model. A light-dark ST modification may also be employed to further examine these domains. The test combines an economical experimental apparatus (Fig. 1) with well-defined behavioral endpoints (Fig. 2). Representing a useful behavioral paradigm for mouse neurophenotyping, it can be strengthened by applying video-tracking and data-mining software.

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