The regular and light–dark Suok tests of anxiety and sensorimotor integration: utility for behavioral characterization in laboratory rodents

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Animal behavioral models are crucial for neurobiological research, allowing for the thorough investigation of brain pathogenesis to be performed. In both animals and humans, anxiety has long been linked to vestibular disorders. However, although there are many tests of anxiety and vestibular deficits, there are few protocols that address the interplay between these two domains. The Suok test and its light–dark modification presented here appear to be suitable for testing this pathogenetic link in laboratory rodents. This protocol adds a new dimension to previously used tests by assessing animal anxiety and balancing simultaneously, resulting in efficient, high-throughput screens for testing psychotropic drugs, phenotyping genetically modified animals, and modeling clusters of human disorders related to stress/anxiety and balancing.

INTRODUCTION

Animal models are indispensable tools for examining the effects of physiological, pharmacological, behavioral, and genetic manipulations, finding candidate genes for human brain disorders[1–3] and testing neurobiological hypotheses of brain pathogenesis[4,5]. Among the emerging important problems in this field, the pathogenetic link between anxiety and vestibular/balancing deficits is becoming particularly interesting[6]. Recent studies have revealed neural circuits (including the parabrachial nucleus, extended central amygdaloid nucleus, infralimbic cortex and hypothalamus) shared by pathways that mediate autonomic control, vestibulo-autonomic interactions and anxiety[7,8]. Modulated by the monoaminergic systems, these circuits provide a neurobiological substrate that directly links balance disorders with emotional dysregulation[7–9].

The growing recognition of this link in clinical settings[8–10] strengthens the need for animal models that target common aspects of this pathogenesis. However, despite well-known tests for animal anxiety or vestibular functions[1,11], until recently there were no animal models that specifically targeted anxiety-vestibular interplay. To tackle this problem, recent studies have used rotating beams and tunnels to compare emotionality and balance control in various rodent strains, also altering these phenotypes through anxiogenic, anxiolytic[12–15] and antidepressant (selective inhibitors of serotonin reuptake, SSRIs)[16] drugs. Confirming the anxiety-vestibular interplay in animals, these findings emphasize the importance of further experimental and translational research in this field.

Along these lines, we have recently introduced the Suok test for simultaneous profiling of rodent anxiety, activity and neurological/vestibular phenotypes[17–19] (**Figs. 1** and **2**). The vestibular construct of this model is based on extensive past research that traditionally used horizontal beams to assess balancing deficits in rodents[1,20–23] (see further for discussion on potential effects of motor/neuromuscular deficits). The anxiety construct of our model is built on the classical approach-avoidance theory[24,25] that determines animal exploration in all novelty-based tests (such as open field, elevated plus maze or holeboard tests)[1,26,27]. On the basis of rodent exploration and balancing on a long elevated beam, the Suok test evokes two threats—the fear of height (reinforced by the difficulty of retaining balance) and the fear of novelty. **Figure 2a** and **Supplementary Video 1** online demonstrate a rich spectrum of spontaneous exploratory behaviors in this test (in addition to motor/balancing performance) which, as will be shown further, are sensitive to various stressful manipulations. Moreover, comparing stressed and non-stressed animals, the Suok test targets stress-evoked sensorimotor disintegration (SSD)—an interesting, clinically relevant phenomenon of balance dysfunctions in stressed/anxious individuals[14].

In addition, we also developed the light–dark modification of this test with the brightly illuminated environment serving as an additional stressor (**Figs. 1b** and **2b**)[18,19]. Despite the originality of this model, one can argue that the Suok test is constructively similar to the stationary beam (balance beam, walking beam or horizontal rod) test[1,20,21,28,29]. However, it is not the shape of the apparatus, but rather the neurobiological rationale of the model that makes it different. Consider, for example, such properties of the paradigms as stability and novelty. Although previous studies have used the beam test as an *unstable* surface to assess motor coordination and balancing[21,28,29], its novelty has not been exploited in behavioral research. In contrast, we view the Suok test as both an *unstable* rod and *novel* arena (similar to the open field test) that the animals can explore. On the other hand, although many traditional anxiety models are based on novel arenas, they all utilize *stable* surfaces, and therefore are not suitable to study altered balancing (unless the problems with animal balancing are extremely severe). In contrast, the Suok test is an *unstable* arena, and therefore may be more sensitive to balancing deficits (in addition to being *novel* and thereby sensitive to anxiety). Therefore, while combining principles from several traditional behavioral models (e.g., beam, open field, elevated plus maze, light–dark box), the Suok tests differ from them qualitatively in the ability to evoke/assess anxiety and balancing phenotypes simultaneously[18,30].

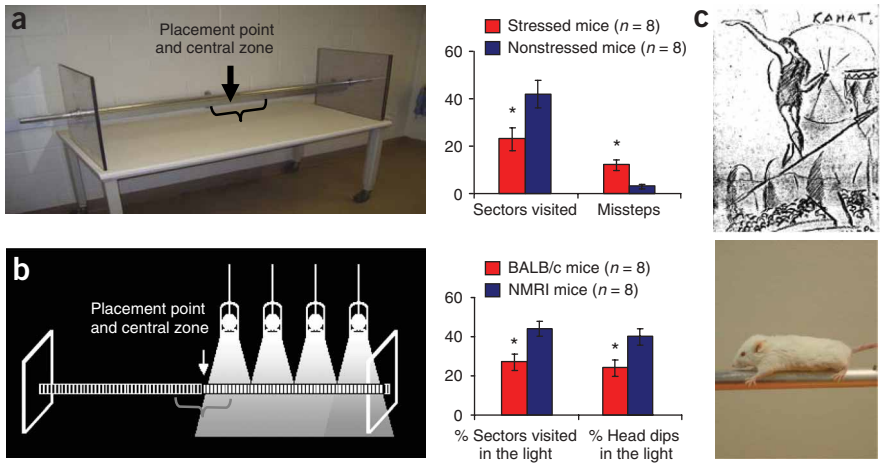

Figure 1 | The mouse Suok test. The model is based on innate rodent fears of heights and open novel unstable surfaces, and measures balance control (falls and missteps) and anxiety (assessed by reduced exploration). The test owes its peculiar name to a brave little ropewalker girl Suok in Yu. Olesha's 1927 novel, 'The Three Fat Men') and exists in two major modifications—the regular and light–dark tests. **Supplementary Video 1** shows the rich behavioral repertoire of CD1 mice in this test (note multiple exploration-related and risk assessment behaviors that go far beyond motor/neurological indices). (**a**) The regular test (apparatus constructed in the National Institute of Mental Health, NIH, USA): 129S1 mice stressed by a brief rat exposure show reduced exploration and poorer balancing (see more details and data for other strains in ref. 18), $*P < 0.05$, *U*-test. Their differences from the non-stressed group indicate anxiety and stress-evoked sensorimotor disintegration (rather than baseline neurological phenotype). (**b**) The light–dark test (apparatus used in Tampere University, Finland) adds an additional light–dark factor: half of the apparatus is brightly lit (aversive area) in the dark experimental room. Compared to non-anxious mice of nuclear magnetic resonance imaging strain, anxious mice of BALB/c strain display lower percentage of horizontal and directed exploration in the light part of the apparatus (% of total); see more details and data for other strains in ref. 18, $*P < 0.05$, *U*-test. (**c**) Note some possible behavioral analogy between a ropewalker (drawing by Olesha, ~1934) and a mouse tested on the Suok test (also see **Supplementary Video 1**).

Several factors seem to ensure the model's developing utility in biomedical research. Although behavioral research of animal stress is currently facing challenges to reduce animal number and suffering[31,32] and minimize the unwanted effects of test batteries[33], there is also a necessity for effective high-throughput screens and models[2,3]. The Suok test presented here assesses several domains (anxiety, motor coordination, balancing, SSD) simultaneously, and therefore reduces animal number needs. By targeting more domains and utilizing more behavioral endpoints per experiment, this model emerges as an efficient, high-throughput screen. The Suok test does not require pretraining of animals, and in most cases will display robust responses in Trial 1 (ref. 18,19). Finally, mounting evidence suggests the utility of the Suok test for modeling stress-evoked behavioral anomalies, screening potential anti-stress drugs and phenotyping genetically modified animals[17,18,34]. Since the introduction of the Suok test[18], we have accumulated a considerable amount of information that improves behavioral phenotyping and broadens the model's practical applications. This updated information and important methodological considerations are provided in this protocol.

MATERIALS

REAGENTS

- Laboratory mice or rats (see REAGENT SETUP)
- Drugs of choice: saline (used as a vehicle in most cases), anxiolytic or anxiogenic drugs, and antidepressants (see REAGENT SETUP)

EQUIPMENT

- Mouse regular Suok test apparatus (see EQUIPMENT SETUP)
- Mouse light–dark Suok test apparatus (see EQUIPMENT SETUP)
- Rat regular Suok test apparatus (see EQUIPMENT SETUP)
- Rat light–dark Suok test apparatus (see EQUIPMENT SETUP)
- Data collection (see EQUIPMENT SETUP)

REAGENT SETUP

Laboratory mice or rats Most mouse strains listed in the Mouse Phenome Project (http://www.jax.org/phenome) and many mutant mice listed in the Mouse Genome Informatics (http://www.informatics.jax.org) databases are suitable, although motor activity levels vary markedly between the strains, and may be confounded by neurological and other specific phenotypes.
! CAUTION Experiments must follow national and institutional guidelines for the care and use of laboratory animals (see ref. 35 for details on housing, husbandry and handling).
Drugs of choice The most frequent routes are i.p., i.m. or s.c. Pretreatment time varies depending on activity of the drugs and the route of administration.

EQUIPMENT SETUP

Mouse regular Suok test apparatus The apparatus is a long (ideally 2–3 m) aluminum tube or rod, 2–3 cm in diameter, elevated to a height of 20 cm from the floor (**Fig. 1a**). The diameter can be smaller for smaller/younger animals. The rod is separated into 10-cm segments by line drawings and fixed to two Plexiglas end walls (50 × 50 cm^2; 1–3-cm thick), with a 20-cm virtual 2-sector central zone around the placement point at the middle of the rod. **! CAUTION** Cushioning underneath the rod (using paper towels or linen) is necessary to avoid any harm to the animals caused by falling from the rod. The experimental room is dimly lit during this test.
Mouse light–dark Suok test apparatus This consists of the same aluminum rod, with four to six bulbs (45–60 W) fixed 50 cm above one-half of the rod to illuminate the 'light' part of the test, providing the only lighting in the experimental room (**Fig. 1b**). **! CAUTION** Make sure that the lamps do not heat the rod (avoid using dark materials for the apparatus). Use only *directed* light for this test.
Rat regular Suok test apparatus This exists in two different modifications. In the 'alley' version (**Fig. 2a**), the apparatus is a white aluminum alley (240 × 5 × 1 cm^3), separated into 16 sectors (15 cm each) by line drawings and elevated to a height of 20 cm from the cushioned floor by two vertical stands, with a 30-cm virtual 2-sector central zone around the placement point. The width of the apparatus can be reduced for smaller/younger animals. In the 'big rod' version (**Fig. 2b**), the apparatus is a sturdy rod (2–3 cm in diameter) constructed from a strong and durable material, such as metal or plastic, with an added layer of dense foam-like texturing (resulting in a final diameter of 5 cm) to enable the animal's firm grasp of the rod. The experimental room is dimly lit during the testing.
Rat light–dark Suok test apparatus This is as described above for the regular test, with four to six bulbs (directed light) fixed ≈50 cm above one-half of the apparatus to illuminate the 'light' half (**Fig. 2b**) in a way similar to the mouse light–dark Suok test.
Data collection Most measures can be easily assessed manually, emphasizing the procedural simplicity of the model. The use of an event recorder/timer or video recording may enable better detection and accuracy.

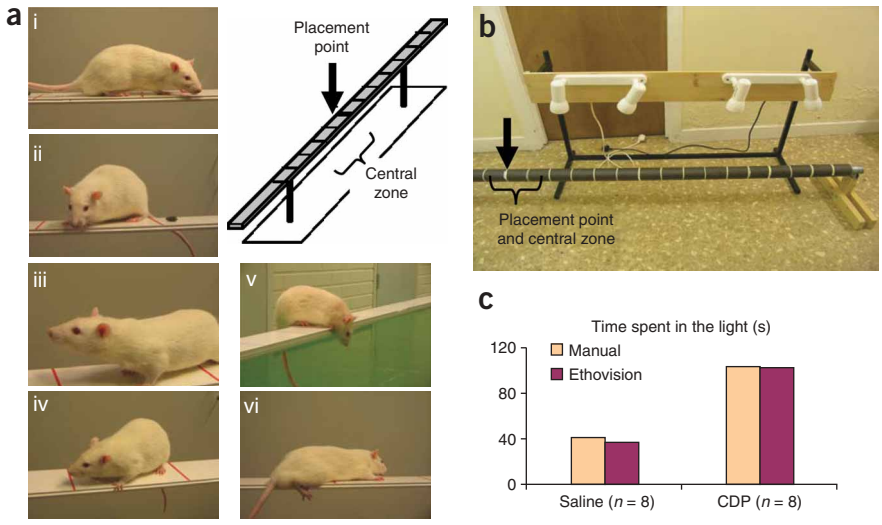

Figure 2 | The rat Suok test. The model exists in two modifications (regular and light–dark tests) and two versions of the apparatus (alley and big rod). (**a**) Regular test (alley version, used in Tampere University, Finland). Typical behaviors include (i–vi): horizontal locomotion, side-directed exploration, stretch-attend posture, head dip, freezing (stop) and misstep. (**b**) Light–dark test (big rod version, used in the National University of Ireland, Galway, Ireland; note that only the light half of the apparatus is shown). (**c**) Similar behavioral scores obtained in the rat light–dark test (**b**) between manual registration and automated registration by Ethovision video-tracking system (CDP, chlordiazepoxide, 10 mg kg^{-1}).

PROCEDURE

Acclimation

1| Transport animals from their holding room to the experimental room (for acclimation) 1 h before testing.

▲ CRITICAL STEP Perform all experiments in a blind manner, remaining unaware of the treatments.

Suok test

2| Place animals individually in the center of the regular (snout facing either end) or light–dark (snout facing the dark end) Suok test.

! CAUTION Support the animals by hand during their initial placement, if necessary, to avoid a fall due to incorrect positioning.

! CAUTION Although mice can be safely repositioned on the test by lifting them by their tails, lifting rats in this manner will result in skin being removed from the bone. Always lift the rat by the scruff of the neck to prevent this problem.

? TROUBLESHOOTING

Behavioral analyses

3| Observe the animal behaviors. During observations, sit and remain stationary 2 m away from the apparatus. In the light–dark Suok test, assess the animal's light and dark performance separately, and calculate the light:dark and light:total indices (similar to the light–dark box test[1]). Use a specially designed register to score behavioral measures (see examples in **Fig. 2a** and **Supplementary Video 1**) as follows: (i) horizontal exploration activity (latency to leave central zone, number of segments visited with four paws, distance traveled, time spent moving, velocity, the number of stops, time spent immobile, average inter-stop distance (distance traveled divided by the number of stops), the number of stops near the border separating the light–dark parts of the apparatus); (ii) vertical exploration (the number of vertical rears and wall leanings); (iii) directed exploration (head dips, side looks); (iv) risk assessment behavior (stretch-attend postures); (v) vegetative responses (latency to defecate, the number of fecal boli, urination spots); (vi) vestibular/motor indices (the number and latency of missteps (hind-leg slips) and falls from the rod). Note that distance traveled, velocity and time spent moving can only be calculated using automated video-tracking (e.g., Noldus Ethovision[36,37]) systems. In all experiments, the latency measures are reckoned as total observation time if the animals do not show the respective behaviors. As anxiety generally reduces animal exploration[1,38], the Suok test horizontal, vertical and directed exploration is lower in anxious animals. Risk assessment and vegetative behaviors, usually higher in anxious rodents[1,39,40], are increased in anxious animals in this test. Falls and missteps can reflect vestibular, motor coordination or neuromuscular deficits or SSD in this test.

! CAUTION If the animal falls, place it back on the rod in the same position.

? TROUBLESHOOTING

4| After testing, remove all animals and return them to their holding room.

5| Clean the apparatus thoroughly with 30% vol/vol ethanol to remove any olfactory cues or urine that may make the surface more slippery.

Data analysis

6| Use the Mann–Whitney *U*-test for comparing two groups (Student's *t*-test may be used for normally distributed data). For more than two groups, use an ANOVA, followed by a post-hoc test.

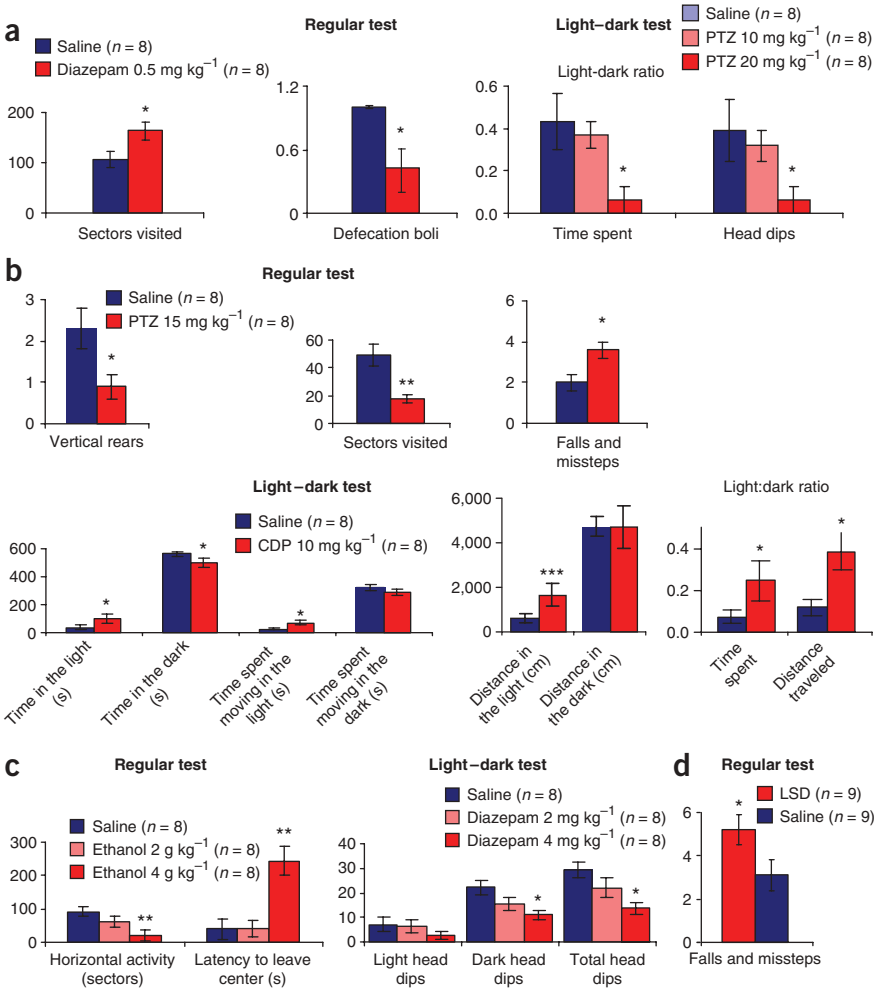

Figure 3 | Selected examples of pharmacogenic behavioral responses that may be evoked in the rodent Suok tests by various psychotropic drugs. (**a**) Behavioral effects produced in BALB/c mice by classical anxiolytic drug diazepam and anxiogenic drug pentylenetetrazole (PTZ): $*P < 0.05$ versus saline, *U*-test (see refs. 19,34,51 for details). (**b**) Behavioral effects produced in rats by classical anxiogenic drug PTZ[17] and anxiolytic drug chlordiazepoxide (CDP): $*P < 0.05$, $**P < 0.001$, $***P = 0.06$ (trend) versus saline, *U*-test. (**c**) Pharmacogenic ataxia/sedation produced in 129S1 mice by high doses of ethanol and diazepam: $*P < 0.01$; $**P < 0.001$ versus saline (for significant ANOVA data). (**d**) Impaired mouse balancing in the regular test by hallucinogenic drug lysergic acid diethylamide (LSD, 0.32 mg kg^{-1}), $*P = 0.05$ versus saline, *U*-test (CD1 mice; also see **Supplementary Video 2** for other LSD-evoked mouse behaviors in this test).

● TIMING

Step 1, acclimation: 1 h for cohort of experimental animals

Steps 2 and 3, testing: 5 or 10 min per animal

Steps 4 and 5, clean-up: 2–3 min per animal

Step 6, data analysis: 1–2 d per experiment

? TROUBLESHOOTING

Step 2: low motor activity

Abnormally low motor activity may occur in the test owing to baseline strain inactivity (re-assess if the strain is suitable), aberrant neurological/vestibular phenotype (consider additional behavioral testing for motor coordination and balancing[1,20,28]) or a strain-specific response to the Suok test situation (re-assess if the strain is suitable). We also noticed that in some inactive strains re-testing animals on Trial 2 the following day, may increase their overall behavioral performance (however, bear in mind that habituation may affect animal performance in this test). Calculation of Trial 1:Trial 2 ratios for animal behaviors may be a source of valuable information about mouse anxiety and spatial working memory (habituation) in this test.

Step 2: high transfer anxiety

Individual animals may display poor initial retention on the Suok test due to high transfer anxiety and/or SSD. To avoid this problem, gently support the animals by hand for ≈5 s, if necessary, to enable a firm grip. If, however, the individuals still display poor grip, exclude them from the experiments. Although abnormally high initial anxiety on beam tests may disappear on subsequent trials (e.g., performed on the next day[41,42]), bear in mind that habituation may affect animal performance in this test. The lighting conditions of both holding and experimental rooms must be carefully controlled, and using a dimly lit experimental room can reduce transfer anxiety. Note, however, that high transfer anxiety in horizontal beam situations may be an indicator of strain-specific neophobia[41], and therefore represents an interesting phenotype for further studies.

Step 3: low vertical activity

Vertical activity in the Suok test is generally much lower than the horizontal activity, and some strains may fail to show this behavior (particularly if they display strain-specific low vertical activity in other behavioral tests). Rats usually display some vertical activity in this test, and this exploratory behavior is predictably sensitive to anxiogenic factors (**Fig. 3b**). Although vertical activity is an optional parameter in the Suok tests, it may be a useful index to assess in some active rodent strains (also calculating horizontal:vertical activity ratios[43] to compare the two dimensions of animal exploration).

Step 3: high motor activity
Some animals may show abnormal hyperactivity in the Suok test that leads to lower non-horizontal exploration and/or less accurate balancing, and therefore may confound other behavioral measures in the Suok test. In some cases, this may represent overall strain hyperactivity (if so, re-assess if the strain is suitable) or a strain-specific response to the Suok test situation (re-assess if the strain is suitable; but may be interesting to examine). In some cases, using a narrower apparatus may lower the animal's horizontal activity, therefore enabling a better focus on other behavioral responses.

Step 3: high variability of responses
This situation is common in behavioral research. In some cases, this may be explained by different genetic influences[30] or overall stress in the animal facility (so improved husbandry[31,35,44] could normalize animal behavior). Observation room conditions (ventilation, temperature, humidity, soundproofing, lighting) have to be carefully controlled in the experiments.

Step 3: role of prior experience
As many studies currently involve batteries of tests[45], consider the potential effects of test batteries on the Suok test performance. Use less-stressful novelty tests before subjecting rodents to the Suok test. Acclimate animals for at least 7 d before the Suok test, to minimize potential confounds (such as habituation to novelty-based paradigms). For these same reasons, the model is not suitable for long-term follow-up studies using the Suok test. Also, avoid using the same animals in the regular and the light–dark Suok tests.

Step 3: physical factors
The Suok test performance is affected by physical factors (such as the body size and weight) and bigger animals have more difficulties performing on this test. Only use animals of similar body age/size/weight in this test to allow more accurate comparison between the groups.

Step 3: balancing and motor problems
Mice of most strains perform well on the aluminum Suok test rod (**Fig. 1**; **Supplementary Video 1**). However, testing some other strains (e.g., mutants with motor-vestibular or coordination anomalies; http://www.informatics.jax.org) may require a less slippery surface texture on the apparatus. Adding a layer of masking tape or using a more textured material (e.g., wooden rod) will increase traction. The diameter of the rod may also be increased for such animals to enable a better grip. Importantly, care is needed when interpreting poorer rod retention and missteps in this and other tests as an aberrant balancing phenotype. In some cases, this behavior may be due to neuromuscular or motor coordination problems unrelated to vestibular deficits or SSD[11,20,46–49], emphasizing the importance of not relying on a single test for interpreting animal phenotypes[1,26]. As a practical solution, assess motor and vestibular functions separately (using other tests specific for each domain[20,28,46–50]) to dissect motor/neuromuscular deficits from vestibular problems. Performing several such tests in addition to the Suok test will enable a more accurate interpretation of rodent phenotypes (note that if overt motor/neurological or balancing abnormalities were found, testing of animal anxiety or SSD may not be possible in the Suok test).

Step 3: strain and species differences
Our research has shown that mice from different strains can demonstrate differences in the Suok test anxiety, activity, SSD and balancing performance. Some strains (such as emotional but active BALB/c mice) show good responsivity to stress and bidirectional sensitivity to various drugs[18,34,51], and may be therefore used as 'reference' strains in the Suok test. In addition, there are some species-specific differences in the Suok test: mice show more motor and exploration activity, whereas rats display more information gathering and risk assessment[17]. Although our past research focused mostly on exploration and balancing indices, other behaviors may merit further scrutiny in this test. For example, stretch-attend postures are frequent in rats in this test (**Fig. 2a**), and may be easily measured and analyzed.

Step 3: testing time
Sessions lasting 5 min are usually long enough for testing mice from most strains. This testing time seems to be ideal, as it is sensitive to anxiety, yet allows for examination of within-trial habituation (per minute distribution of activity) as an additional index of animal exploratory strategies. However, extending testing time to 10 min may be necessary for some inactive or anxious strains as a way to reduce false negatives. Likewise, given lesser horizontal activity in rats, using 10-min testing sessions may be appropriate for the rat Suok test. If a 10-min testing time is used, calculation of first 5:last 5 min ratios for all rodent behaviors may serve as additional indices of habituation.

Step 3: freezing versus 'exploratory' stops
Owing to the complexity of factors influencing the animal's locomotion and immobility in this and many other behavioral tests, behavioral interpretation of stops (**Fig. 2a**) represents a methodological problem. With some stops being due to increased

anxiety/freezing, they may also have an exploratory nature (enabling information gathering and processing[52]) that clearly requires further studies. Assess the average distance between stops in the Suok test, as it is usually shorter in anxious animals[17] and may serve as an additional index of anxiety. Assess other (nonstopping) behaviors in parallel to gain a better understanding of the overall anxiety phenotype.

Step 3: stereotypic behaviors

We have noted that some animals display instances of stereotypic behaviors in the Suok test, including short bouts of rostral grooming[17] or characteristic repetitive turning in the areas close to the walls. This may be a displacement activity provoked by stress[53] or an obsessive-compulsive disorder-like phenotype that merits further scrutiny.

Step 3: method of registration

Although both manual and automatic registration may serve equally well to assess some Suok test endpoints (**Fig. 2c**), other behaviors (such as falls and missteps) cannot be measured by the Ethovision system, and have to be scored manually. Manual scoring requires high intra- and inter-rater reproducibility that may be achieved by training the observers and testing them for rater reliability. Video-tracking enables recording of a wider spectrum of parameters[36,37], their re-analysis, as well as re-play in slow motion. A combination of manual and automated registration may be an ideal solution, to enable more data to be generated. Note, however, that most video-tracking systems (such as Ethovision) detect objects based on the brightness contrast between the animal and the background. Because animals can fall from the Suok test (requiring repositioning on the apparatus), wear clothing that does not contrast with the background, so that the experimenter's movements are not falsely detected as the 'object'.

ANTICIPATED RESULTS

In general, the Suok test appears to be a useful new tool in behavioral research using laboratory rodents. For example, the method is sensitive to genetic differences in anxiety and balancing performance observed across popular rodent strains, where baseline anxiety (reduced horizontal and directed exploration) and poorer balancing were higher in several anxious (compared to less anxious) mouse strains[18] (see example in **Fig. 1b**). Likewise, our test is sensitive to *evoked* anxiety and SSD produced by different stressors, as stressed animals showed increased anxiety and impaired balancing[17,18] compared to nonstressed controls (see example in **Fig. 1a**). In addition, given its clear-cut rationale, the test may be a useful tool to examine and/or model other types of sensorimotor deficits beyond SSD.

Importantly, a common problem with almost every behavioral test is the differentiation of animal motor activity from emotionality (e.g., low activity from high anxiety)[1,54]. Our protocol seems to address this problem in several ways. For example, the use of mild stress[17,18] (unable to cause overt motor/muscular deficits) and testing of stressed versus nonstressed control animals allows for the dissection of affected anxiety-related behaviors from baseline motor phenotypes (**Fig. 1a**). Likewise, analyses of light–dark Suok test behaviors enable further dissociation of anxiety from changes in motor activity levels. For example, anxiety reduces the percentage of light activity in this test (**Fig. 1b**) in a fashion similar to the elevated plus maze test (where the ratios of open:total or open:closed arm entries were introduced to separate changes in anxiety from those in activity levels[38,55]). Although direct comparisons of the Suok test with other traditional anxiety tests (such as the elevated plus maze) have not yet been performed, this may be an interesting task for future studies using our protocol.

Mounting evidence suggests that our model also has practical applications in behavioral pharmacology. For example, we observed reduced exploration and poorer test retention in animals following pharmacogenic anxiety[17,34] (see examples in **Fig. 3a**,**b**). In contrast, Suok test exploration was higher in animals treated with anxiolytic drugs such as ethanol, diazepam[17,34,51], chlordiazepoxide (see examples in **Fig. 3a**,**b**) or quercetin[56], suggesting bidirectional sensitivity of Suok tests to anxiogenic and anxiolytic drugs. Pharmacological studies using the light–dark Suok test enable further dissociation of anxiety from changes in motor activity, as anxiolytics increase, and anxiogenics reduce, light:dark ratios (**Fig. 3a**,**b**) in a fashion similar to their effects on open:closed ratios in the elevated plus maze[38,55].

Although we did not study behavioral effects of antidepressants in the Suok test, the demonstrated ability of SSRIs to improve balancing and reduce anxiety in both humans[57] and animals[16] implies our model's potential sensitivity to these drugs. In this case, the Suok test may be a useful tool to target some additional related clinical phenomena (such as SSRI discontinuation syndrome[58,59]) that link anxiety and balancing. Moreover, the test's sensitivity to sedating effects of high doses of ethanol or diazepam (**Fig. 3c**) suggests its utility for measuring nonspecific drug effects (such as pharmacogenic ataxia[60]). It is also expected that the Suok test may be used to screen for drugs with specific actions on the vestibular system (i.e., vestibular suppressants) as well as hallucinogenic drugs (known to evoke sensorimotor disintegration and anxiety[61–63]); see **Fig. 3d** and **Supplementary Video 2** online for mouse Suok test responses to lysergic acid diethylamide. Collectively, this suggests the utility of the Suok test for high-throughput pharmacological screening of a wide spectrum of psychotropic drugs in laboratory rodents.

Finally, it is expected that the extensive use of video-tracking systems may enable further ethological dissection of rodent behaviors in this model. For example, meaningful ethologically derived parameters such as meandering, turning angle/velocity,

and heading may be calculated using Ethovision system, expanding the already rich spectrum of Suok test measures. Sophisticated software-based analytical tools (e.g., SEE[64,65]) may further increase this spectrum, focusing on complex behavioral characteristics such as path texture, darting behavior, relative phase and activity density[64–70]. Finally, microbehavior recognition-based systems (e.g., Clever System[71]) may also be useful, given their ability to detect not only most rodent anxiety and motor responses, but also some other relevant endpoints (body position or micro-movements such as missteps or tail angle[16]), automatic registration of which could increase the method's effectiveness.

Summary

As already mentioned, our model is not meant to replace the existing behavioral tests and screens that continue to generate valuable biobehavioral information. Instead, the Suok test may complement the existing behavioral protocols, further fostering high-throughput neurophenotyping research. The Suok test emerges as a useful protocol for behavioral phenotyping of rodents, including characterization of genetic differences, screening various psychotropic drugs, testing neurological and motor/balancing phenotypes, as well as studying animal anxiety, anxiety-balancing interplay and SSD. The test is based on animal spontaneous activity, can be automated, and produces results within a short (5–10 min) testing period. An additional strength is the simplicity of the apparatus and testing procedure, which would facilitate implementation of this protocol in different laboratories.

Note: Supplementary information is available via the HTML version of this article.

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