An effective textured Novel Object Recognition Test (tNORT) for repeated measure of whisker sensitivity of rodents

Behavioural Brain Research 472 (2024) 115153

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research article

An effective textured Novel Object Recognition Test (tNORT) for repeated measure of whisker sensitivity of rodents[1]

Yurie Hayashi [a], Najeeba Alamir [a], Guoyang Sun [a], Francesco Tamagnini [b,c], Yoshikatsu Hayashi [a], Claire Williams [c,d], Ying Zheng [a,c,*]

[a] School of Biological Sciences, Whiteknights, University of Reading, Reading RG6 7AY, UK
[b] School of Pharmacy, University of Reading, Whiteknights, Reading RG6 6LA, UK
[c] Centre for Integrative Neuroscience and Neurodynamics (CINN), University of Reading, Reading RG6 6AL, UK
[d] School of Psychology and Clinical Language Science, Whiteknights, University of Reading, Reading RG6 6AL, UK

ARTICLE INFO

Keywords:
Texture discrimination
Repeated novel object recognition
Whisker sensitivity threshold

ABSTRACT

Rodents use their whisker system to discriminate surface texture. Whisker-based texture discrimination tasks are often used to investigate the mechanisms encoding tactile sensation. One such task is the textured Novel Object Recognition Test (tNORT). It takes advantage of a tendency of rodents to explore novel objects more than familiar ones and assesses the sensitivity of whiskers in discriminating different textures of objects. It requires little training of the animals and the equipment involved is a simple arena with typically two objects placed inside. The success of the test relies on rodents spending sufficient time exploring these objects. Animals may lose interests in such tasks when performed repetitively within a limited time frame. However, such repeated tests may be crucial when establishing a sensitivity threshold of the whisker system. Here we present an adapted rodent tNORT protocol designed to maintain sustained interest in the objects even with repeated testing. We constructed complex objects from three simple-shaped objects. Different textures were provided by sandpapers of varying grit sizes. To minimise olfactory clues, we used the sandy and the laminar side of the same sandpaper as the familiar and novel textures assigned at random. We subsequently conducted repeated tNORTs on eight rats in order to identify a critical threshold of the sandpaper grit size below which rats would be unable to discriminate the sandy from the laminar side. With an inter-test-interval of seven days and after five tNORTs, the protocol enabled us to successfully identify the threshold. We suggest that the proposed tNORT is a useful tool for investigating the sensitivity threshold of the whisker system of rodent, and for testing the effectiveness of an intervention by comparing sensitivity threshold pre- and post-intervention.

1. Introduction

The whisker-mediated texture discrimination task has been used to investigate both cognitive function and neural mechanisms underlying the sensory networks of rodents [1–7]. The firing rate of neurons in the barrel cortex was shown to impact directly on the rodent's judgement of texture [8], with average firing rate increasing as the roughness of the texture increased. Furthermore, activity of layer V pyramidal neurons was shown to impact behavioural reaction time in a whisker-based texture discrimination task [9]. It was suggested that the activation of a specific inhibitory circuit underlay such control. A recent study used an array of neurophysiological methods and behavioural assessments including tNORT and demonstrated that increasing tonic inhibition in the thalamus enhanced tactile acuity through texture discrimination in mice [10]. Specifically, they conducted the tNORT using two textures with subtle differences and showed that the control group failed the test while the experimental group with enhanced tonic inhibition was able to complete the test successfully. These findings suggest that a measure of the threshold of whisker sensitivity to texture discrimination between groups of rodents, or a change of such measure within a group before and after an intervention, may reflect differences in the effectiveness of neural inhibition underlying sensory perception, with potential implications on altered balance between neural excitation and inhibition.

Historically, the whisker-mediated texture discrimination task

[1] NOR: Novel Object Recognition. tNORT: textured Novel Object Recognition Test. OSBT: Object Shape Bias Test. DI: Discrimination Index.

* Corresponding author at: School of Biological Sciences, Whiteknights, University of Reading, Reading RG6 7AY, UK.
E-mail address: ying.zheng@reading.ac.uk (Y. Zheng).

https://doi.org/10.1016/j.bbr.2024.115153
Received 20 March 2024; Received in revised form 12 July 2024; Accepted 15 July 2024

required a prolonged training period involving food/water rewards. More recently, a simpler texture discrimination paradigm was developed based on the novel object recognition (NOR) test [11]. The paradigm exploited an innate preference of rodents for novelty, i.e., a rodent will spend more time exploring novel rather than familiar objects [12–16]. Instead of using object shapes, the proposed paradigm used the surface texture of objects to distinguish a novel object from a familiar one. Due to this innate preference, the paradigm required no food/water deprivation and only limited training consisting of two 10-min sessions (one session per day) in order to habituate rodents to the test arena prior to the day of the test. Since its publication, the textured novel object recognition test (tNORT) has been used to investigate texture discrimination learning [17], cortical-hippocampal connectivity in somatosensory processing in mice displaying autistic-like behaviours vs controls [18,19] and other neural and genetic mechanisms underlying sensory information processing [10,20,21].

An important consideration when conducting a texture discrimination experiment in rodents is the selection of appropriate textures for the objects. If two textures are too similar, the discrimination task will be less likely to succeed. On the other hand, if they are too different and easy to discriminate, comparison or improvement of whisker sensitivities between or within groups will not be possible. When investigating the sensitivity threshold of the whisker system, iterative tNORT plays a pivotal role in identifying the minimum particle size difference that a specific group of rodents may be able to distinguish.

In many studies, sandpapers of varying grit sizes are used to cover the surface of objects in tNORT to provide a range of textures. The difference between the average particle diameter of a pair of sandpapers is used as an indicator of difficulty associated with the discrimination task [10,11, 18–20]. However, it is possible to select different pairs of sandpapers with the same difference in particle diameters, while the roughness of the sandpapers themselves is different. For example, the difference in average particle diameters (ϕ) between sandpapers P40 ($\phi = 35$ μm) and P60 ($\phi = 25.8$ μm) is the same as that between sandpapers P800 ($\phi = 21.8$ μm) and P1500 ($\phi = 12.6$ μm). It has been shown that the roughness of the sandpapers themselves plays a role in the discrimination task [4].

Another consideration associated with tNORT is the length of exploration time with respect to the objects used. If the animal is not interested in an object during in the task, possibly due to its size, the complexity of its shape, or its familiarity, the object will be ignored, or the exploration time will be very brief. Such events will need to be excluded from the study, resulting in reduced animal numbers and less robust calculations of the discrimination index. This issue was closely examined in several NOR studies. It has been shown that if a NOR test was conducted repeatedly with the same group of rodents within a short time frame (e.g., daily), the total exploration time decreased markedly [22]. However, if the NOR task was repeated about 1 week apart, total exploration time could be maintained [23]. Furthermore, total exploration time was shown to increase if more complex objects were used in NOR tasks [24,25]. Very limited studies using tNORT have examined the effect of repeated tests on the exploration time. We conducted a pilot study of tNORT (not published) which indicated that a tNORT repeated in three consecutive days markedly decreased the exploration time of rats. By the third test, over 50 % of the rats explored one of the objects less than 2 s within a 5 mins duration. Our pilot study suggested that in order to conduct repeated tNORTs to investigate whisker sensitivity to texture discrimination, careful consideration is needed in designing its protocol.

Here, we propose a modified tNORT paradigm which is robust to repeated tests. We increased the complexity of the object shapes by combining several simple objects and increased the inter-test-interval to one week. Instead of selecting two different sandpapers for each test, we used both sides (i.e., sandy and laminar) of the same sandpaper, with one side acting as the familiar texture, and the other as the novel texture, thus removing the need to select two different sandpapers for a tNORT while simultaneously eliminating possible olfactory cues. We suggest that the modified tNORT is sufficiently robust to repeated testing, allowing whisker sensitivity to texture to be investigated and compared across multiple groups of rodents.

2. Materials and methods

All experimental procedures were carried out in accordance with United Kingdom Home Office regulations (Animals (Scientific Procedures) Act, 1986) and approved by the Research Ethics Committee at the University of Reading, UK.

2.1. Animals

Eight male Lister hooded rats (weighing 309 ~ 329 g at purchase) were used. Rats were caged in pairs for three days to acclimatise to the environment after arrival, with ad libitum access to food and water and housed under controlled temperature (21°C), humidity (50 ± 10 %) and 12-hr light-dark cycle (lights off 6:00–18:00 hr). All procedures were conducted in the morning under dim red-light illumination to minimize stress and visual cues contributing to task performance [11]. All habituation and behaviour tests were conducted in the same room as the rats were housed.

2.2. Construction of complex objects

Eight complex objects were constructed from four simple shapes 3D printed in-house (Fig. 1A). Each complex object combined three simple objects (Fig. 1B) to create different shapes, with multiple copies made for each complex shape so that no single object was used more than once in a tNORT to eliminate any lingering olfactory cues.

2.3. Experimental protocols

Two sets of experiments were conducted. In the first study, we conducted object shape bias tests (OSBTs) to investigate if rats explored certain objects more than others among the eight complex objects we constructed. In the second study, we performed repeated tNORTs, with each test using a different complex object covered in sandpapers of different grit sizes chosen prior to the experiment. The repeated tNORT stopped when rats failed the texture discrimination task in two tests with sandpapers of finer particle sizes.

The overall schedule is shown in Fig. 2. Week 1 is defined as the week immediately after the acclimatisation period; and Day 1 refers to the first day of Week 1. Animals were handled by an experimenter for the first five consecutive days in Week 1 in order to familiarise them to the experimenter and the environment, thus minimising anxiety during behavioural tests [14]. The handling protocol was as follows. For Days 1 and 2, rats were handled in their cage for 10 mins. The experimenter first put their hands in the cage to allow rats to sniff them. After a few minutes, the experimenter scooped one rat with both hands at a time, allowing it to jump between hands. For the following two days, rats were handled in their cage in a similar manner for 5 mins before two rats housed from the same cage were transferred to the empty test arena and handled in that environment for 5 mins by allowing them to jump on and off the experimenter's hands placed inside the arena and to explore the arena itself. On the last day of handling, rats were individually transferred and handled in the test arena for 5 mins. On Day 8, 24 hrs prior to the first OSBT, rats were individually put in the empty test arena for 10 mins without any handling [14].

The test arena, made of clear acrylic, has a dimension 52x52x80 cm (WxDxH), with its base divided into a 16-square grids, each square being 13×13 cm (Fig. 3A). The grid was drawn on the outside base of the arena to ensure they were in clear view of the camera throughout the study. For all experiments describe here, two complex objects were placed at the base of the arena, secured with double-sided tapes, 26 cm apart and

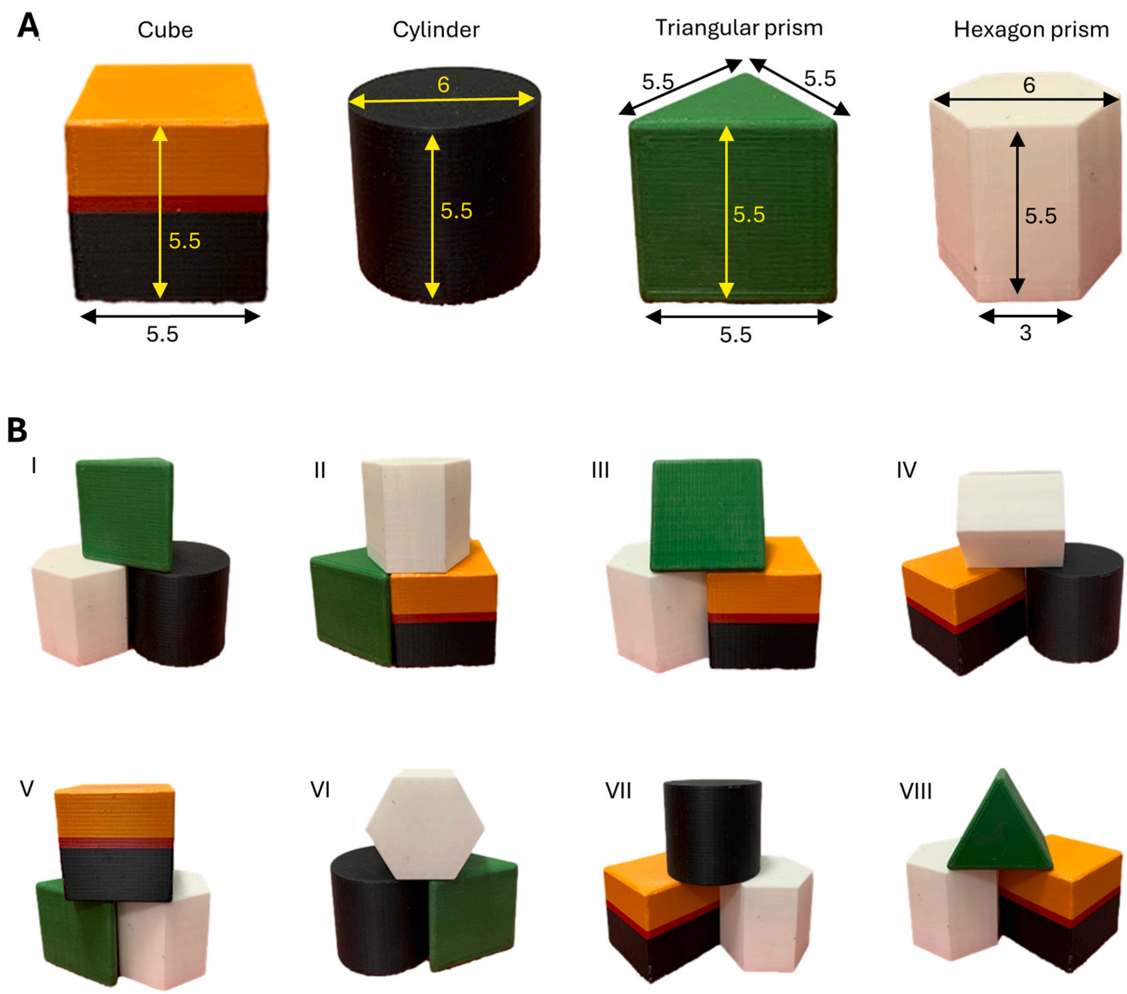

Fig. 1. Design of objects. (A) Photos and dimensions of four simple objects. (All dimensions are in cm.) (B) Photos of eight complex objects made from three simple objects. Note that the colour of each object is irrelevant to the task as all objects were covered in sandpaper during experiments.

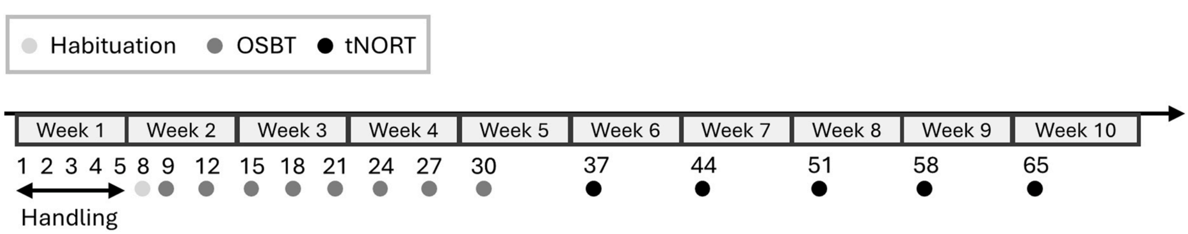

Fig. 2. Overall schedule from the first day of rat handling to the completion of the tNORT test. OSBT: Object Shape Bias Test. tNORT: textured Novel Object Recognition Test.

13 cm away from each adjacent wall (Fig. 3A and B). The walls of the arena were covered with cardboards to reduce the influence of any cues from the external environment during experiments. All experiments were conducted between 6 am and 8 am on each experimental day to minimise potential acoustic contamination from the background.

The test arena was lit by four red light bulbs attached to two floor lamps, with two bulbs in each lamp. The lamps were placed opposite of each other on two sides of the test arena. The positions and angles of the light bulbs were adjusted to provide as a uniform illumination as possible on the base of the arena, with luminance values $\leq$ 14 lux measured at the base. The position of the arena in the test room and the positions of the two lamps were kept consistent throughout all experiments presented here.

Prior to each experiment, the experimenter made sandpaper covers for all objects to be used. These covers were precisely measured, and the edges were fixed together by double-sided tapes. They were made to fit each object securely without using further tapes or glue so that they could be removed from the object quickly after each test. During an experiment, double-sided tapes were used to combine three simple objects, covered with sandpapers, together to create the desired complex object (Fig. 3C). Care was taken to ensure no tape was exposed on the outer surface of the objects. For all the tests presented here, no cover was removed or destroyed by the animals.

2.3.1. *Selection of object shapes based on animal preference*

OSBTs started on Day 9. All rats went through eight tests each separated by 72 hrs. For each test, two of the same shaped complex objects covered by the same grit-sized sandpaper P1200 ($\phi = 15.3$ μm)

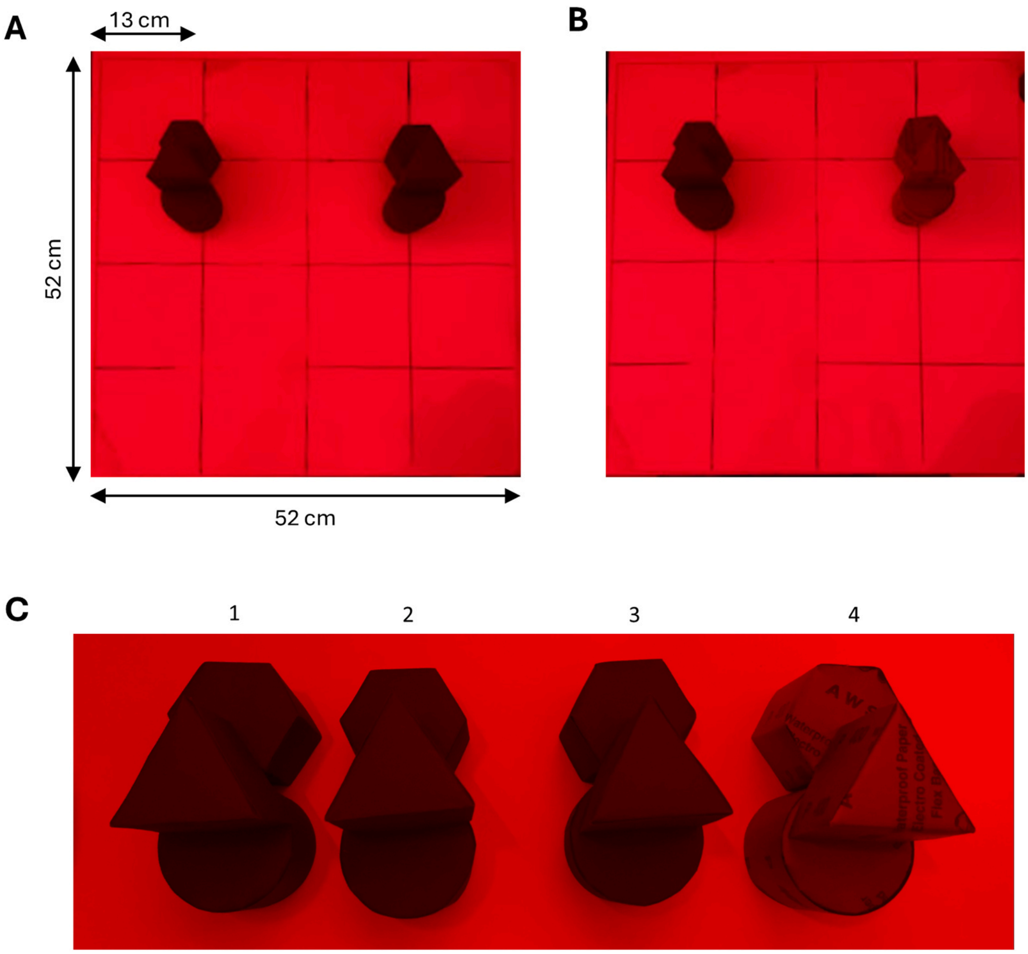

Fig. 3. Experimental set up for OSBT and tNORT under red light illumination. (A) Two objects having the same shape and cover were placed securely on the base of the test arena. This set up was used for OSBT as well as the sample phase of the tNORT. (B) Two objects having the same shape but different covers were placed securely on the base of the test arena. This set up was used for the test phase of the tNORT. (C) A close up of objects covered with sandpapers of the same grit using the sandy side (Objects 1, 2 and 3) and the laminar side (Object 4).

were secured in the test arena. This extra fine sandpaper was selected primarily based on two considerations. First, finer sandpapers were easier to fold to make object covers. Second, the sandpaper used for OSBT was not used for the subsequent tNORTs to avoid bias due to familiarisation. Once the two objects were positioned inside the arena, the rat was placed close to the midpoint of the wall opposite, facing away from the objects, and left to explore the objects and the arena for 5 mins. The rat was then returned to its home cage. Sandpaper covers were discarded after the test, and the objects and the test arena cleaned with 70 % ethanol to remove olfactory cues before objects with new sandpaper covers were placed in the arena for the next test. For each test day, all eight objects (Fig. 1B) were used, one for each rat (see Table 1 for the allocation of object shapes for all OSBTs). After eight test days, all rats had been exposed to all eight objects once. By Week 5, OSBTs were complete.

All tests were recorded with a video camera placed above the test arena. Exploration times were measured using the Observer software (Noldus Information Technology). The exploration time was defined as the time during which a rat's nose was less than 2 cm away from an object, excluding when the rat was resting, grooming, or playing with its tail near or on top of the objects [11,13,14]. Before using the Observer software, the experimenter was trained to code the rat's exploratory behaviour as follows. First, a video of a rat exploring two objects in the arena was randomly selected. Three types of exploratory behaviour were coded: no exploration, exploration of the left object, and exploration of the right object. The video was coded in real-time first by multiple users with an accuracy level set at 98 % for inter-rater reliability. Once this was reached, the video was coded again until an intra-rater reliability of

Table 1
Allocation of objects used for each rat of each experimental day.

Rat #	Day 9 (Tue)	Day 12 (Fri)	Day 15 (Mon)	Day 18 (Thu)	Day 21 (Sun)	Day 24 (Wed)	Day 27 (Sat)	Day 30 (Tue)
1	I	II	III	IV	V	VI	VII	VIII
2	II	V	I	VII	III	VIII	IV	VI
3	III	I	IV	II	VI	V	VIII	VII
4	IV	III	VII	I	VIII	II	VI	V
5	V	VI	II	VIII	I	VII	III	IV
6	VI	IV	VIII	III	VII	I	V	II
7	VII	VIII	V	VI	II	IV	I	III
8	VIII	VII	VI	V	IV	III	II	I

98 % was achieved. The experimental data was only coded when these criteria were met.

2.3.2. Repeated tNORT to investigate whisker sensitivity

Repeated tNORT started in Week 6, with one tNORT conducted per week for each rat. The tNORT consisted of two phases, the sample phase and the test phase. For each tNORT, four of the same shaped complex objects were prepared and covered with the appropriate sandpaper covers (Fig. 3C). In the sample phase, the two objects had the same sandpaper cover (Fig. 3A). In the test phase, one object, known as the familiar object, had an identical cover as that used in the sample phase (the cover was changed between sample and test phase to prevent use of olfactory cues), while the other, known as the novel object, was covered by sandpaper of a different texture (Fig. 3B). At the start of both phases, the rat was placed in the test arena equidistant to, and facing away from, the two objects and was allowed to explore for 4 mins. In between the two phases, the rat was moved to the holding cage for 2 mins [26], known as the delay period. We set the delay period as short as possible to minimise hippocampal-mediated learning [11]. During the delay period, both objects in the sample phase were removed, the sandpaper covers discarded, and the test arena cleaned with 70 % ethanol to remove olfactory cues. The two new objects prepared for the test phase were then placed in the arena. After each tNORT, all objects were cleaned with 70 % ethanol, and the apparatus was thoroughly cleaned.

2.4. Selection of familiar and novel textures

When selecting sandpapers for familiar and novel textures in the tNORT, instead of selecting two different sandpapers with different grit sizes, we used the same sandpaper but assigned at random the sandy and the laminar sides of the paper as familiar (used for both objects in the sample phase and one object in the test phase) and novel (one object in the test phase) textures, thus eliminating olfactory cues from using different types of sandpapers. The texture selection protocol was balanced across all eight rats used for tNORTs.

2.5. Whisker sensitivity test

To test whisker sensitivity to textures, we conducted a set of tNORTs using different grit size sandpapers with a criterion for stopping the experiment if rats failed the tNORT for two discrimination tests using sandpapers of finer particle diameters, an indication that a breakpoint was reached where the animals could no longer discriminate between two textures. Fig. 4 shows the five sandpapers used and the corresponding particle diameters. The sandpaper selection procedure was based on the statistical analysis of the tNORT data with respect to each selection and will be described in the Results section.

The position (left or right) and texture (sandy or laminar) of the novel object were pseudo-randomised for each rat and balanced throughout. Table 2 shows the sandpaper grit and object shape used for each tNORT in the whisker sensitivity study.

Table 2
Allocation of sandpaper grits and objects for the five tNORTs over five weeks[#].

	Day 37	Day 44	Day 51	Day 58	Day 65
Grit size	P80	P240	P120	P180	P400
Object	VII	I	III	II	VIII

[#] Note that both the sandy and laminar sides of sandpapers acted as the familiar and novel textures in a balanced manner. Thus, only one sandpaper type was selected for each tNORT.

2.6. Data analysis and statistics

All data analysis was performed in MATLAB (The MathsWork, Natick, MA, USA). To analyse the tNORT, the discrimination index (DI) d was calculated as follows [27]. In the sample phase,

$$d_s = \frac{T_L - T_R}{T_L + T_R} \tag{1}$$

where T_L and T_R were the exploration times for the left and right objects respectively; whilst for the test phase,

$$d_t = \frac{T_N - T_F}{T_N + T_F} \tag{2}$$

where T_N and T_F were the exploration times for the novel and familiar objects respectively. Rats with total exploration time (T_{tot}) less than 2 s were excluded from the analysis (Wu et al., 2013). From its definition, the value of DI would be 0 if the exploration times for the left and right objects, or the familiar and novel objects, were identical.

To determine if T_{tot} was significantly different across test days and between eight objects, all data were first processed to identify outliers (MATLAB function 'filloutliers'). They were then tested for normality using the Jarque-Bera goodness-of-fit test [28] (MATLAB function: 'jbtest'). As no outliers were identified and all data were normally distributed, a one-way repeated measure ANOVA was performed in each case (MATLAB function 'RMAOV1'; [29]). For pairwise multiple comparisons with Bonferroni corrections, the MATLAB function 'multcompare' was used.

DIs calculated from repeated tNORTs were also screened for outliers and tested for normality before one-sample t-tests were performed on both d_s and d_t against the chance level of 0. The null hypothesis was that rats were unable to discriminate between the left and the right objects (i.e., $d_s = 0$) and that they were also unable to discriminate between the familiar and the novel objects (i.e., $d_t = 0$). If the mean DI was significantly greater than zero under a condition, it was taken as an indication that rats had successfully discriminated between the two objects. A *p*-value of 0.05 or less was considered statistically significant.

3. Results

3.1. Total exploration time was maintained by increasing object complexity

Fig. 5A shows T_{tot} for the eight test days of OSBTs, each with an inter-

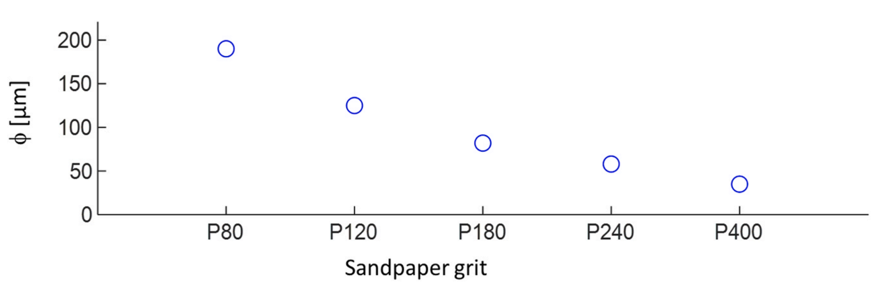

Fig. 4. Sandpaper average particle diameters ϕ used for the five tNORTs.

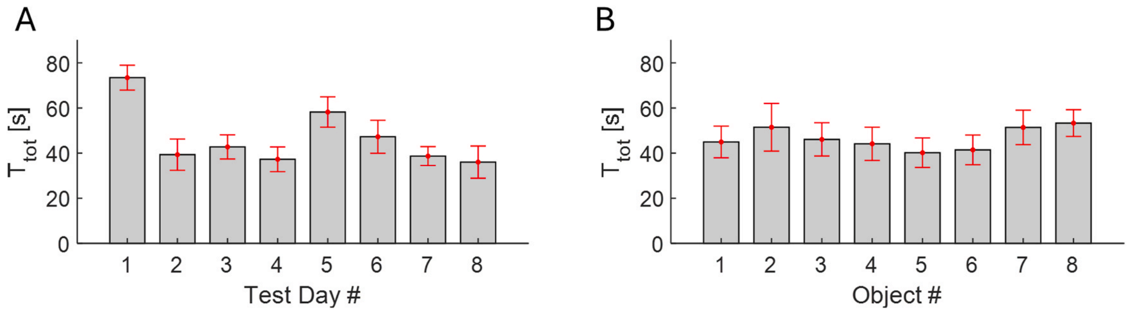

Fig. 5. Total exploration time in OSBT. (A) Total exploration time in each test day. (B) Total exploration time for each object. Error bars indicate the standard error of the mean.

test-interval of 72 hrs. We observed that T_{tot} for the first test day appeared to be longer than the other test days and repeated measures ANOVA using data from all eight test days showed a significant difference in T_{tot} ($p<0.001$).

When multiple comparison tests were conducted for pairwise comparison between the eight days with Bonferroni correction, it revealed that the significant difference was indeed the result of significantly longer exploration time on Day 1 compared with all other days, with the exception of Day 5.

We also noted that all eight tests had a mean $T_{tot} > 30$ s, indicating that increasing object complexity could maintain rats' interests in object exploration over 8 repeated tests with an inter-test-interval of three days, provided that different shaped objects with sufficient complexity were used. The range of exploration times found in this study was similar to other NOR studies in the literature [13,14,26,30–32] where only a single NOR test was conducted.

We subsequently investigated if rats had preference for certain object shapes over others by comparing T_{tot} across the eight complex objects (Fig. 5B). Again, all data were tested for normality before a repeated measure ANOVA was conducted. No significant difference was found ($p=0.814$), suggesting that rats had no preference to any of the objects. Nevertheless, for the subsequent repeated tNORTs, we chose five objects (I, II, III, VII, and VIII) which produced slightly higher T_{tot} across the eight objects.

3.2. Modified tNORT was robust for repeated measures

We performed five tNORTs using five sandpaper grit sizes, with an inter-test interval of seven days. The sandpaper selection process was as follows. The first tNORT used the rather coarse sandpaper P80 (ϕ= 201 μm) to ensure successful discrimination of the novel texture from the familiar one. A one-sample t-test on the DIs obtained from the test phase confirmed that the animals were indeed able to discriminate between the familiar and novel textures ($p=0.012$). For the second tNORT, a finer sandpaper with grit size P240 ($\phi = 58.5$ μm) was used. Statistical analysis showed that rats failed to detect the novel from the familiar texture ($p=0.149$). For the third and fourth tNORT, we used sandpapers P120 ($\phi = 125$ μm) and P180 ($\phi = 82$ μm) respectively and found that rats successfully detected the novel object during both tNORTs, with $p=0.008$ and $p=0.024$ respectively. For the fifth test, we further decreased the sandpaper particle size by using P400 ($\phi = 35$ μm) and found that rats were unable to detect the novel texture at this finer scale ($p=0.065$). Thus, the experiment was terminated.

Fig. 6A shows the exploration times for individual rats for the left and right objects in the sample phase (top) and familiar and novel objects in the test phase (bottom) respectively. The panels from left to right were arranged in the order of decreasing sandpaper particle diameters. We noted that all exploration times with respect to a single object were greater than 2 s, thus no data was excluded from the DI calculations. To examine if rats' interest in exploring the objects decreased significantly over the five tNORTs, we conducted repeated ANOVA for T_{tot} for the sample and test phases respectively over the five weeks (Fig. 6B). No significant difference was found in either the sample phase ($p=0.059$) or the test phase ($p=0.447$). In addition, the mean T_{tot} for the sample and test phases were > 30 s for all sessions, suggesting that rats' interests in exploring these complex objects were maintained across the five weeks of testing. Fig. 6C shows the DIs calculated for each tNORT for both the sample (top) and test (bottom) phases. It showed that during the sample phase, all DIs were not significantly different from zero, suggesting no significant bias in exploration time between the L and R objects. On the other hand, during the test phase, DIs were significantly different from zero for the three tests when the sandpapers were coarser (P80, P120, and P180). As the grit size increased (P240 and P400), or the particle size of the sandpaper decreased, the DIs became smaller and the group mean values showed no significant difference from zero, indicating that rats were unable to discriminate between the sandy and the laminar sides of sandpapers when the particle size of the sandpaper was less than 58.5 μm (P240). (For particle diameter information, see [4,33,34].)

4. Discussion

The tNORT is a valuable behaviour test to examine rodents' whisker system sensitivity with little training. In the present study, we extended the existing tNORT to facilitate its repeated use, and successfully identified, after five iterative tNORTs, a breakpoint where the animals could no longer discriminate between two textures. We showed that T_{tot} of the animals was consistently above 30 s in both the sample and the test phases across the five tNORTs with an inter-test-interval of seven days. In addition, exploration time with respect to individual objects for all animals was above the threshold of 2 s, thus no animal was excluded from the subsequent texture discrimination analysis. We suggest that the modified tNORT provides a viable protocol for the investigation of texture discrimination abilities of rodents at progressively finer spatial scale.

4.1. Effect of other sensory cues on tNORT performance

Although it has been shown that rodents could use their whisker system alone to perform texture discrimination [35,36], other sensory system may also contribute to the task [15]. This was examined carefully by Wu et al. in their original tNORT study [11]. To determine the extent to which visual cues may contribute to the tNORT due to different sandpapers having different visual appearances even under dim red light conditions, objects were covered with plastic transparent film to create 'texture-less' objects while maintaining visual differences between them. It was found that mice with intact whisker system were unable to discriminate between these objects. Furthermore, Wu et al. investigated if mice used their paws to aid their ability to discriminate textures. They grouped mice into those that used their paws and those that did not during the tNORT. Both groups were found to be able to distinguish between the familiar and novel textures that differed by 25 μm, suggesting that tactile sensation from paws was not a major contributor to

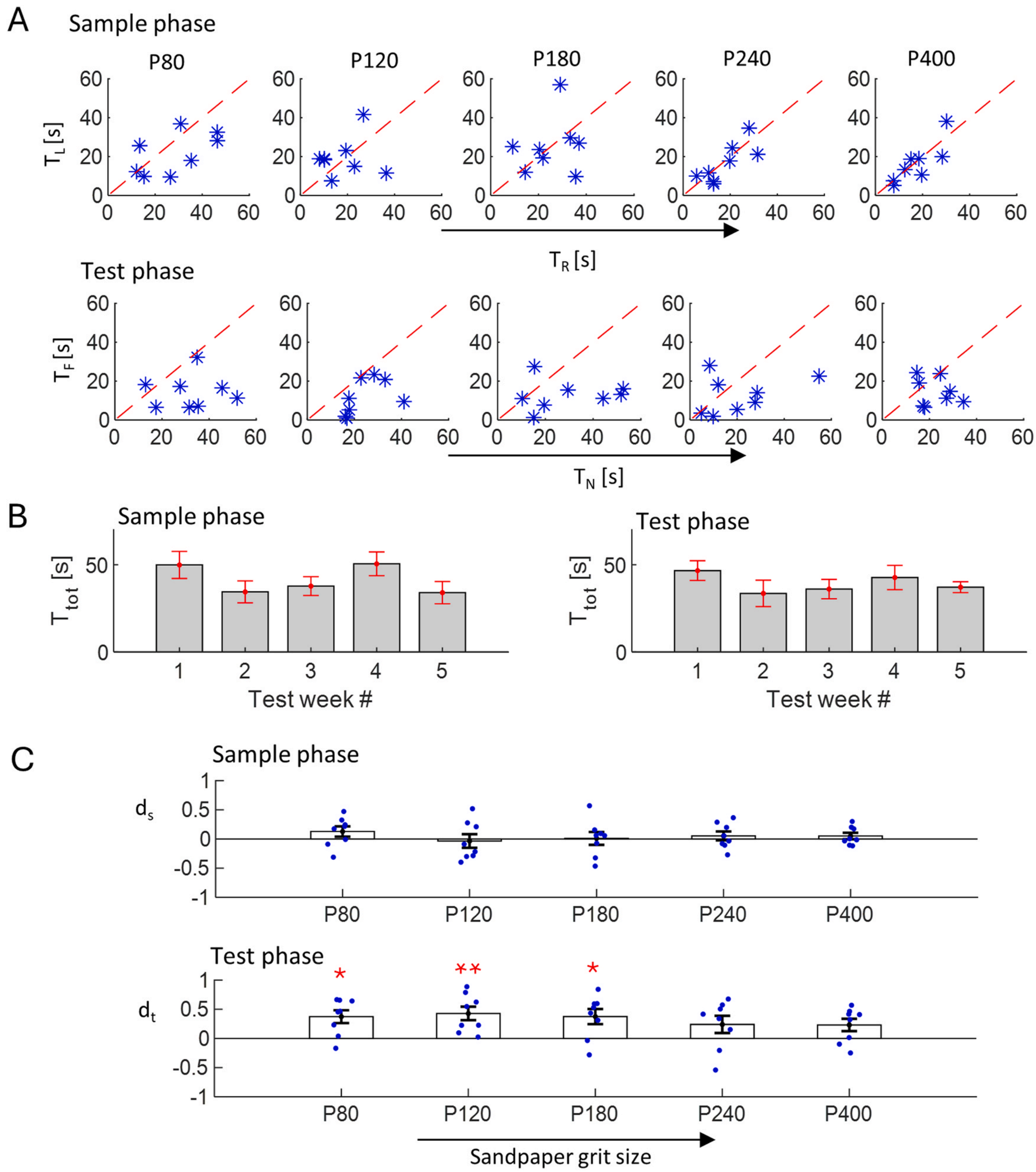

Fig. 6. Exploration time and DI in tNORT associated with complex objects. (A) Exploration times T_L vs T_R in the sample phase (top) and T_F vs T_N in the test phase (bottom) for individual rats using sandpaper of five grit sizes. The broken diagonal line represents equal exploration time for both objects. (B) Total exploration time over the five tNORTs for the sample phase (left) and the test phase (right). Error bars indicate the standard error of the mean. (C) Top: DI in each test during the sample phase. Bottom: DI in each test during the test phase. The DI for each rat was displayed as a blue dot. The height of each bar indicates the mean of IDs over all rats for each test, and error bars indicate the standard error of the mean. * and ** indicate $p < 0.05$ and $p < 0.01$ respectively.

texture discrimination. Finally, it was demonstrated that mice with their mystacial vibrissae removed bilaterally were unable to discriminate between novel and familiar textures, and that the removal did not predict the number of mice that used their paws.

Evidence that the sensory system responsible for texture discrimination was primarily the whisker system was also presented in a texture discrimination study of rats with prolonged training period (52–90 days) involving rewards [4]. It was shown that a whisker trimmed rat reduced its texture discrimination performance to chance level. The study also found that rats had better ability for texture discrimination with respect to a fine sandpaper (P1500) than to a coarse sandpaper (P150), adding another dimension to the complex neurophysiological mechanisms underlying texture discrimination.

The assumption that rodents were functionally blind under red-light illumination was challenged by a recent study [37]. Using a visual stimulus in the form of a black and white square-wave grating, the study examined the performance of rats in categorising the stimulus orientation under conditions of white light illumination and red-light illuminations (using two red light wavelengths 626 nm and 652 nm respectively) among other wavelengths. No significant difference in the average performance was found between the white light and two red-light illuminations, with success rates reaching 87 %, 84 % and 86 % respectively. However, the performance was achieved after a lengthy training period of 4–6 weeks, with one session per day, and with rewards.

Although the above study demonstrated the range of illumination wavelengths, including red light illumination, within which the rat's visual perception capacity was intact, there were distinct differences between the above study and the tNORT paradigm presented here. First of all, the object contrast used in the above study was much stronger than those used for common tNORTs. The visual appearance between different grit sandpapers and between the sandy and laminar sides of the same sandpaper have much less contrast compared to the white-black grating (Fig. 3). This difference in object contrast may affect the strength of the visual cues in the tNORT, thus significantly reduce the ability of the animal to utilise vision for the task [38]. Secondly, it is unclear, in the above study, if the level of performance of the rats under red light illumination was partly due to prolonged training. The tNORT protocol is a one-trial object recognition test. With little training, rodents are unlikely to discriminate colours of objects under dim red light illumination [15].

For our modified tNORT, we also noted that the smoothness of the laminar side of the sandpaper made it more reflective compared with the sandy side. To minimise this, we took extra care in positioning the red light bulbs so that they pointed away from the arena, and the red-light illumination was dim (<14 lux). Although we cannot be certain if visual cues contributed to the successful texture discrimination for the three tNORTs using rougher sandpapers, the consistent failure of tNORTs corresponding to the two finer sandpaper grits suggested that the rodent whisker system was the dominant sensory system for texture discrimination.

4.2. Key parameters influencing the performance of repeated tNORTs

As far as we are aware, most objects used in published tNORT studies were simple objects such as 2-dimentional rectangular boards [11,20,21], simple cubes [10], or simple cylinder-shaped objects [18,19]. This may be due to the fact that covering complex shaped objects using sandpaper was difficult. However, our own pilot study suggested that repeated tNORTs using simple shaped objects were likely to lead to significantly reduced object exploration time. To minimise exclusion of data due to short exploration time, we designed eight complex objects by combing three simple shaped objects to maintain the interest of rats in object exploration in repeated tNORTs. The OSBT indicated that the mean T_{tot} for all objects exceeded 30 s. Notably, after the first test when T_{tot} was the highest, the exploration time remained consistent statistically across the subsequent seven repetitions at 72-hr intervals. Furthermore, when these objects were used in iterative tNORTs five times with an inter-test-interval of seven days, the mean T_{tot} was maintained above 30 s. The sandpaper covers for the objects were not difficult to make but it was time consuming, as each tNORT involved 12 simple objects to be covered. One possible modification to our protocol would be to combine two instead of three simple shaped objects into a complex object, thus reducing the preparation time for conducting a tNORT. However, this simplification would need to be assessed to ensure sufficiently long and consistent T_{tot} after repeated tNORTs.

In addition to object shapes that can enhance the robustness of repeated tNORTs, the inter-test-interval also plays a key role. Repeated tNORTs separated by 24 hrs are likely to lead to significant reduction in T_{tot}, similar to that found in NOR studies [23]. Our OSBT had an inter-test-interval of three days, with each test consisting of a single exploration session with a 5 mins duration, whereas our repeated tNORTs had an inter-test-interval of seven days, with each test consisting of two exploration sessions. The seven-day interval was chosen to ensure that the rat's interest in the objects was maintained while experimental day did not fall to a weekend for practical reasons.

One feature of the repeated tNORT protocol presented here was that instead of using two different sandpapers in a tNORT, we used both sides of a sandpaper, one acting as a familiar texture, the other as a novel texture. This approach was adopted to minimise potential olfactory cues from two different grit sandpapers while simplifying the process of making object covers.

A potential source of bias in the tNORT protocol was associated with the manual classification of the rat's exploratory behaviour by watching videos recorded during the sample and test phases. There were situations when the rat's behaviour was ambiguous and difficult to judge. For example, if a rat was sitting on an object with its head and nose facing downwards towards the object, it would be difficult to judge the distance between its nose and the object. During the software training period, experimenters would agree a set of criteria when dealing with difficult situations. We found that inter-rater agreement (98 %) on exploration time was typically reached within one week, and the subsequent intra-rater agreement (98 %) reached within one day. The training does not imply the elimination of bias during behaviour coding. However, such bias should be statistically the same regardless of the object being familiar or novel. As the DI reflects the difference in exploration time between the two objects, such bias should not impact significantly on the value of DI. Instead of manual classification, an alternative for behavioural coding of rodent is to adopt automated software (e.g., EthoVision), with the advantage of consistency and faster analysis.

In conclusion, the tNORT protocol presented here is a cost-effective way of investigating whisker sensitivity of rodents based on their behaviour. It has been demonstrated to be robust to repeated tests, thus allowing a range of textures to be used to identify the sensitivity threshold of rodent whisker system for texture discrimination. As the whisker sensitivity of rodents has been shown to be altered by the manipulation of tonic inhibition, the repeated tNORT protocol may be used as a tool to examine whisker sensitivity difference between different groups of rodents, or within a single group before and after an intervention. Thus, the threshold for whisker-mediated texture discrimination may be used as a marker for shifted balance between neural excitation and inhibition.

Ethics approval

All experiments were carried out in accordance with the British Home Office regulations (Animals (Scientific Procedures) Act, 1986) and approved by the Research Ethics Committee at the University of Reading, UK.

Funding

This work was supported by the Biotechnology and Biological Sciences Research Council (BBSRC Grant number: BB/K010123/1), and the University of Reading regional PhD bursary scheme which funded the PhD fees for Yurie Hayashi.

CRediT authorship contribution statement

Yurie Hayashi: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Najeeba Alamir:** Writing – review & editing, Validation, Methodology. **Guoyang Sun:** Writing – review & editing, Validation. **Francesco Tamagnini:** Supervision. **Yoshikatsu Hayashi:** Supervision, Resources. **Claire Williams:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **Ying Zheng:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors have no competing interests to declare that are relevant to the content of this article.

Data availability

We have shared the link to our data/code within our manuscript.

Acknowledgement

We would like to thank the BioResource Unit at the University of Reading for their support, and Dr Sungmin Kang for providing some technical advice at the initial stage of the study.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

References

[1] G.E. Carvell, D.J. Simons, Biometric analyses of vibrissal tactile discrimination in the rat, J. Neurosci. 10 (1990) 2638–2648.
[2] M.E. Diamond, M. von Heimendahl, E. Arabzadeh, Whisker-mediated texture discrimination, PLOS Biol. 6 (2008) e220.
[3] J. Wolfe, D.N. Hill, S. Pahlavan, P.J. Drew, D. Kleinfeld, D.E. Feldman, Texture coding in the rat whisker system: slip-stick versus differential resonance, PLOS Biol. 6 (2008) e215.
[4] T. Morita, H. Kang, J. Wolfe, S.P. Jadhav, D.E. Feldman, Psychometric curve and behavioral strategies for whisker-based texture discrimination in rats, PLOS ONE 6 (2011) e20437.
[5] L. Garion, U. Dubin, Y. Rubin, M. Khateb, Y. Schiller, R. Azouz, J. Schiller, Texture coarseness responsive neurons and their mapping in layer 2–3 of the rat barrel cortex in vivo, Elife 3 (2014) e03405.
[6] J.L. Chen, D.J. Margolis, A. Stankov, L.T. Sumanovski, B.L. Schneider, F. Helmchen, Pathway-specific reorganization of projection neurons in somatosensory cortex during learning, Nat. Neurosci. 18 (2015) 1101–1108.
[7] R.A. Grant, V.G.A. Goss, What can whiskers tell us about mammalian evolution, behaviour, and ecology? Mammal. Rev. 52 (2022) 148–163.
[8] M. von Heimendahl, P.M. Itskov, E. Arabzadeh, M.E. Diamond, Neuronal activity in rat barrel cortex underlying texture discrimination, PLOS Biol. 5 (2007) e305.
[9] D. Vecchia, R. Beltramo, F. Vallone, R. Chéreau, A. Forli, M. Molano-Mazón, T. Bawa, N. Binini, C. Moretti, A. Holtmaat, S. Panzeri, T. Fellin, Temporal Sharpening of Sensory Responses by Layer V in the Mouse Primary Somatosensory Cortex, Curr. Biol. 30 (2020) 1589–1599, e1510.
[10] H. Kwak, W. Koh, S. Kim, K. Song, J.I. Shin, J.M. Lee, E.H. Lee, J.Y. Bae, G.E. Ha, J. E. Oh, Y.M. Park, S. Kim, J. Feng, S.E. Lee, J.W. Choi, K.H. Kim, Y.S. Kim, J. Woo, D. Lee, T. Son, S.W. Kwon, K.D. Park, B.E. Yoon, J. Lee, Y. Li, H. Lee, Y.C. Bae, C. J. Lee, E. Cheong, Astrocytes Control Sensory Acuity via Tonic Inhibition in the Thalamus, Neuron 108 (2020) 691–706, e610.
[11] H.P. Wu, J.C. Ioffe, M.M. Iverson, J.M. Boon, R.H. Dyck, Novel, whisker-dependent texture discrimination task for mice, Behav. Brain Res. 237 (2013) 238–242.
[12] D.E. Berlyne, Novelty and curiosity as determinants of exploratory behavior, Br. J. Psychol. 41 (1950) 68–80.
[13] A. Ennaceur, J. Delacour, A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data, Behav. Brain Res. 31 (1988) 47–59.
[14] R.A. Bevins, J. Besheer, Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study; 'recognition memory', Nat. Protoc. 1 (2006) 1306.
[15] A. Ennaceur, One-trial object recognition in rats and mice: methodological and theoretical issues, Behav. Brain Res. 215 (2010) 244–254.
[16] O.Y. Chao, S. Nikolaus, Y.M. Yang, J.P. Huston, Neuronal circuitry for recognition memory of object and place in rodent models, Neurosci. Biobehav. Rev. 141 (2022) 104855.
[17] N. Pacchiarini, R. Berkeley, K. Fox, R.C. Honey, Whisker-mediated texture discrimination learning in freely moving mice, J. Exp. Psychol. Anim. Learn. Cogn. 46 (2020) 40–46.
[18] L. Balasco, M. Pagani, L. Pangrazzi, G. Chelini, A.G. Ciancone Chama, E. Shlosman, L. Mattioni, A. Galbusera, G. Iurilli, G. Provenzano, A. Gozzi, Y. Bozzi, Abnormal Whisker-Dependent Behaviors and Altered Cortico-Hippocampal Connectivity in Shank3b-/- Mice, Cereb. Cortex 32 (2022) 3042–3056.
[19] L. Balasco, M. Pagani, L. Pangrazzi, G. Chelini, F. Viscido, A.G.C. Chama, A. Galbusera, G. Provenzano, A. Gozzi, Y. Bozzi, Somatosensory cortex hyperconnectivity and impaired whisker-dependent responses in Cntnap2(-/-) mice, Neurobiol. Dis. 169 (2022) 105742.
[20] M. Sabzalizadeh, M.R. Afarinesh, S. Esmaeili-Mahani, A. Farsinejad, A. Derakhshani, E. Arabzadeh, V. Sheibani, Transplantation of rat dental pulp stem cells facilities post-lesion recovery in the somatosensory whisker cortex of male Wistar rats, Brain Res. Bull. 173 (2021) 150–161.
[21] H. Kuang, T. Liu, C. Jiao, J. Wang, S. Wu, J. Wu, S. Peng, A.M. Davidson, S.X. Zeng, H. Lu, R. Mostany, Genetic Deficiency of p53 Leads to Structural, Functional, and Synaptic Deficits in Primary Somatosensory Cortical Neurons of Adult Mice, Front. Mol. Neurosci. 15 (2022) 871974.
[22] N.J. Broadbent, S. Gaskin, L.R. Squire, R.E. Clark, Object recognition memory and the rodent hippocampus, Learn. Mem. 17 (2010) 5–11.
[23] R. d'Isa, R. Brambilla, S. Fasano, Behavioral Methods for the Study of the Ras–ERK Pathway in Memory Formation and Consolidation: Passive Avoidance and Novel Object Recognition Tests, in: L. Trabalzini, S.F. Retta (Eds.), Ras Signaling: Methods and Protocols, Humana Press, Totowa, NJ, 2014, pp. 131–156.
[24] T. Aubele, R. Kaufman, F. Montalmant, M.F. Kritzer, Effects of gonadectomy and hormone replacement on a spontaneous novel object recognition task in adult male rats, Horm. Behav. 54 (2008) 244–252.
[25] A. Chrzanowska, K. Modlinska, K. Goncikowska, W. Pisula, Rat's response to a novelty and increased complexity of the environment resulting from the introduction of movable vs. stationary objects in the free exploration test, PLoS One 17 (2022) e0279006.
[26] G. Taglialatela, D. Hogan, W.R. Zhang, K.T. Dineley, Intermediate- and long-term recognition memory deficits in Tg2576 mice are reversed with acute calcineurin inhibition, Behav. Brain Res. 200 (2009) 95–99.
[27] M. Antunes, G. Biala, The novel object recognition memory: neurobiology, test procedure, and its modifications, Cogn. Process. 13 (2012) 93–110.
[28] C.M. Jarque, A.K. Bera, A Test for Normality of Observations and Regression Residuals, Int. Stat. Rev. 55 (1987) 163–172.
[29] A. Trujillo-Ortiz, R. Hernandez-Walls, R.A. Trujillo-Perez, RMAOV1: One-way repeated measures ANOVA. A MATLAB file., WWW document, DOI (2004).
[30] A. Ennaceur, K. Meliani, A new one-trial test for neurobiological studies of memory in rats. III. Spatial vs. non-spatial working memory, Behav. Brain Res. 51 (1992) 83–92.
[31] S.N. Burke, J.L. Wallace, S. Nematollahi, A.R. Uprety, C.A. Barnes, Pattern separation deficits may contribute to age-associated recognition impairments, Behav. Neurosci. 124 (2010) 559–573.
[32] M.R. Afarinesh, F. Shafiei, M. Sabzalizadeh, T. Haghpanah, M. Taheri, S. Parsania, F. Golshan, V. Sheibani, Effect of mild and chronic neonatal hypothyroidism on sensory information processing in a rodent model: A behavioral and electrophysiological study, Brain Res. Bull. 155 (2020) 29–36.
[33] E. Arabzadeh, E. Zorzin, M.E. Diamond, Neuronal Encoding of Texture in the Whisker Sensory Pathway, PLOS Biol. 3 (2005) e17.
[34] L.M. Montuori, R.C. Honey, Perceptual learning with tactile stimuli in rats: Changes in the processing of a dimension, J. Exp. Psychol. Anim. Learn. Cogn. 42 (2016) 281–289.
[35] E. Guić-Robles, C. Valdivieso, G. Guajardo, Rats can learn a roughness discrimination using only their vibrissal system, Behav. Brain Res. 31 (1989) 285–289.
[36] E. Guic-Robles, W.M. Jenkins, H. Bravo, Vibrissal roughness discrimination is barrelcortex-dependent, Behav. Brain Res. 48 (1992) 145–152.
[37] N. Nikbakht, M.E. Diamond, Conserved visual capacity of rats under red light, Elife 10 (2021).
[38] A.E. Schnell, K. Vinken, H.O. de Beeck, The importance of contrast features in rat vision, Sci. Rep. 13 (2023) 459.