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# Whisker-mediated texture discrimination learning in freely moving mice

Running head: Whisker-mediated learning

Nicole Pacchiarini, Rhiannon Berkeley, Kevin Fox, and R.C. Honey  
Cardiff University, UK

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Correspondence should be addressed to: R.C. Honey, School of Psychology, Cardiff University, Park Place, Cardiff, CF10 3AT, UK.

email: [honey@cardiff.ac.uk](mailto:honey@cardiff.ac.uk); telephone: +44(0)29 2087 5868; fax: +44(0)29 2087 4679

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## Abstract

Texture is often used as a convenient stimulus dimension to study aspects of discrimination learning in rodents. However, the basis of texture discrimination learning is often left untested: While learning could involve the whisker system, it could also be based on other senses (e.g., olfactory or visual). Here, we investigated whether or not mice use their whisker system to learn texture discriminations. Mice were placed in an apparatus illuminated with dim red light and the mice had to learn which of two sawdust-filled bowls contained a buried reward. The outer surfaces of the bowls were 3D printed with different textures (grooved or smooth). Within a 60-min session, mice learned to dig in one bowl (e.g., grooved) rather than the other (e.g., smooth) to gain the reward; this learning and an equivalent odor discrimination was retained overnight (Experiments 1 and 2); and whisker trimming disrupted learning based on the texture of the bowls, but not learning based on the odor of the sawdust in the bowls (Experiments 3 and 4). These results provide a secure basis upon which to investigate the behavioral and brain basis of texture learning in rodents.

*Keywords:* Texture learning, odor learning, rodent, whiskers

Humans mainly use their visual system to understand and interpret the world, which makes it difficult for us to imagine the world of touch in any detail or to understand its importance. The importance of touch becomes more apparent when, in the absence of light, we are feeling our way in the dark or searching for an object that is out of sight. Firefighters rely on their tactile sense when exploring the contents of smoke filled rooms; surgeons need to relearn how to feel and grasp objects when they wear surgical gloves, which alter the tactile experience. In these cases, the texture of a surface provides important information about an object's identity. In contrast, rodents are not known for their reliance on visual information, but they possess a variety of sensory systems that enable them to learn about a broad range of stimulus dimensions. Although this characteristic enables them to adapt effectively to their environments, it often complicates interpretation of even the simplest experiments. For example, if one is interested in the capacity of rodents to use their whisker system to learn about different textures then one might use different grades of sandpaper (e.g., Montuori & Honey, 2016) or different media in which rodents dig to find a reward (e.g., Birrell & Brown, 2000). However, these stimuli are also discriminable on the basis of other visual and/or olfactory cues (Botly & De Rosa, 2009; Garner, Thogerson, Würbel, Murray, & Mench, 2006; Huang et al., 2014). Isolating the sensory system that is used in the context of a given task is often a secondary concern to those who study learning and memory at a behavioral level. However, this is not the case for those interested in the neural mechanisms that underpin these processes, where the possibility that learning involving different sensory domains might involve different principles has greater importance. Given the considerable interest in investigating the behavioral and neural mechanisms that underpin whisker-based learning in rodents as a model system, it is important to establish that behavioral indices of learning are specific to the whisker system rather than involve, for example, the visual or olfactory systems.

Rodents investigate environments with their facial whiskers (also referred to as vibrissae; Vincent, 1912), which are tapered rods ( $\approx 30$  mm/ $3000\ \mu\text{m}$  in length) located on each side of their

faces. Whiskers serve as sensors for tactile information (Sofroniew, Cohen, Lee, & Svoboda, 2014). Rats and mice have two types of whiskers known as macrovibrissae and microvibrissae. Macrovibrissae comprise a matrix of about 25 mobile sensors on either side of the snout, and microvibrissae are shorter whiskers around the mouth, chin and nose (Deschenes, Moore, & Kleinfeld, 2012). The macrovibrissae, organised in a grid that is made up of 5 rows (see Diamond & Arabzadeh, 2013), are used to palpate objects through an active process known as ‘whisking’ involving fast, large-amplitude rhythmic sweeping movements (Carvell & Simons, 1990; Knutsen, Derdikman, & Ahissar, 2005). These movements result in the whiskers bending when they come into contact with an object or surface, which exerts forces on the follicle sinus at the base of each whisker (Sofroniew *et al.*, 2014). A pathway of three synapses links the primary afferents from the whisker follicle receptors on one side of the face to the neurones in the contralateral cortex with the final thalamocortical projection into layer IV producing the barrel pattern identifiable in horizontal section through this layer (Woolsey & Van der Loos, 1970). The position of each whisker is topologically related to a single barrel in the cortex. Neurones in the barrel cortex have multiwhisker receptive fields and are therefore capable of integrating information from several whiskers (Armstrong-James & Fox, 1987). This property could be useful for identifying different textures. However, understanding the role of the barrel cortex in texture learning (Diamond, von Heimendahl, Knutsen, Kleinfeld, & Ahissar, 2008; Stüttgen & Schwarz, 2017) has been limited by a paucity of behavioral assays involving freely moving mice (see Pacchiarini, Fox, & Honey, 2017). Several behavioral procedures have been designed for head-fixed mice; for example, to assess the role of vibrotactile frequency, whisker position, and the lateral distance from a wall (Hong, Lacefield, Rodgers, & Bruno, 2018; Mayrhofer *et al.*, 2013; O'Connor *et al.*, 2010; Sofroniew *et al.*, 2014). However, these reduced preparations are not necessarily representative of how rodents naturally use their whiskers, and the generality of the results might therefore be limited.

Here, we assessed long-term whisker-based learning in freely moving mice. The procedure is adapted from the digging task originally reported by Birrell and Brown (2000) in which rats learned to dig in one bowl rather than another in order to find a reward. This task resulted in rapid learning and was used to great effect to investigate, among other things, the neural mechanisms involved in attentional set shifting. They used textures as a convenient dimension that could be combined with others: The outer surfaces of the bowls could be covered with fine or coarse sandpaper, waxed or grain paper, and the front and reverse surfaces of velvet, which could be combined with odors and different digging media. The rats might well have used their whiskers to discriminate between each pair of textures (or their paws), but it is also possible that they were discriminable on the basis of their olfactory or visual properties. We conducted four experiments in order to establish that freely moving mice use their whiskers to acquire a discrimination between two textures. This issue is of behavioral significance, but it also paves the way for future analyses of the functional significance of the barrel cortex.

First, we assessed the ability of mice to learn and to retain overnight a tactile discrimination and an odor discrimination (Experiments 1 and 2). Tactile cues were provided by the texture printed on the outer surface of the bowls (grooved or smooth) that were made of the same material. Conducting the experiments in dim red light eliminated visual cues (Chalupa & Williams, 2008; Jacobs, Williams, & Fenwick, 2004). Odor cues (e.g., presence or absence of the Coco Pop dust) were distributed in the digging medium. In both experiments, mice in the control groups received the same discrimination on days 1 and 2 (e.g., grooved->reward and smooth->no reward), whereas for those in the reversal groups, the stimuli that signaled reward and no reward were reversed between days 1 and 2 (e.g., day 1: grooved->reward and smooth->no reward; and day 2: smooth->reward and grooved->no reward). If the mice retained overnight what they had learnt on the first day (i.e., for approximately 24 hours), then the control groups should perform more accurately than those in the reversal group on the second day. Reversing the rewarded texture provides an

additional means of ruling out the possibility that the mice were simply basing their choice to dig on the presence of the odor of the Coco Pop (though the odor was in any case present in both bowls). If the mice were using the odor as a cue there would be no reason to predict a difference in their behavior on the second day between control and reversal groups. We then assessed whether the whiskers were used selectively for the tactile discrimination (Experiment 3) and not the odor discrimination (Experiment 4) by trimming the whiskers in one group of mice but not another. The whiskers grow back in approximately two weeks, and discrimination learning took place within a single session. As we will show, these procedures have several appealing features: learning is rapid, long-term, and requires relatively few mice to produce reliable results.

## Method

### Subjects

Across Experiments 1-4 a total of 32 male C57BL/6J mice (Charles River Laboratory, Margate, Kent, UK; mean ad lib weight: 28.4g; range: 24.0g - 32.0g) were housed individually in  $25 \times 45 \times 15$  cm Plexiglas cages. Different groups of 8 naïve mice were used in each experiment. In Experiments 1 and 2, the anticipated discrimination learning effects with textures and odors were based on 8 mice. A power analysis showed that with an expected effect size of .90, 8 mice allows a one-sample *t* test to detect a difference with power of .74 using a one-tailed test: There is no good reason to expect hungry mice to dig consistently in an unbaited bowl (e.g., with a smooth outer texture) when they have only received reward in a baited bowl (e.g., with a grooved outer texture). However, as we will demonstrate, statistically significant results can be demonstrated consistently with  $N = 4$  in both texture discrimination learning and odor discrimination learning. In Experiments 3 and 4, the anticipated effects were based on groups of 4 mice. In these cases, the manipulation (whisker trimming) should make a texture discrimination based on whiskers impossible, but should have no effect on an odor discrimination. The mice were maintained on standard laboratory diet and water *ad libitum* and were kept in a humidity- and temperature-controlled environment. Experimental testing began at 8 weeks of age and was conducted in the light phase of a 12 hr

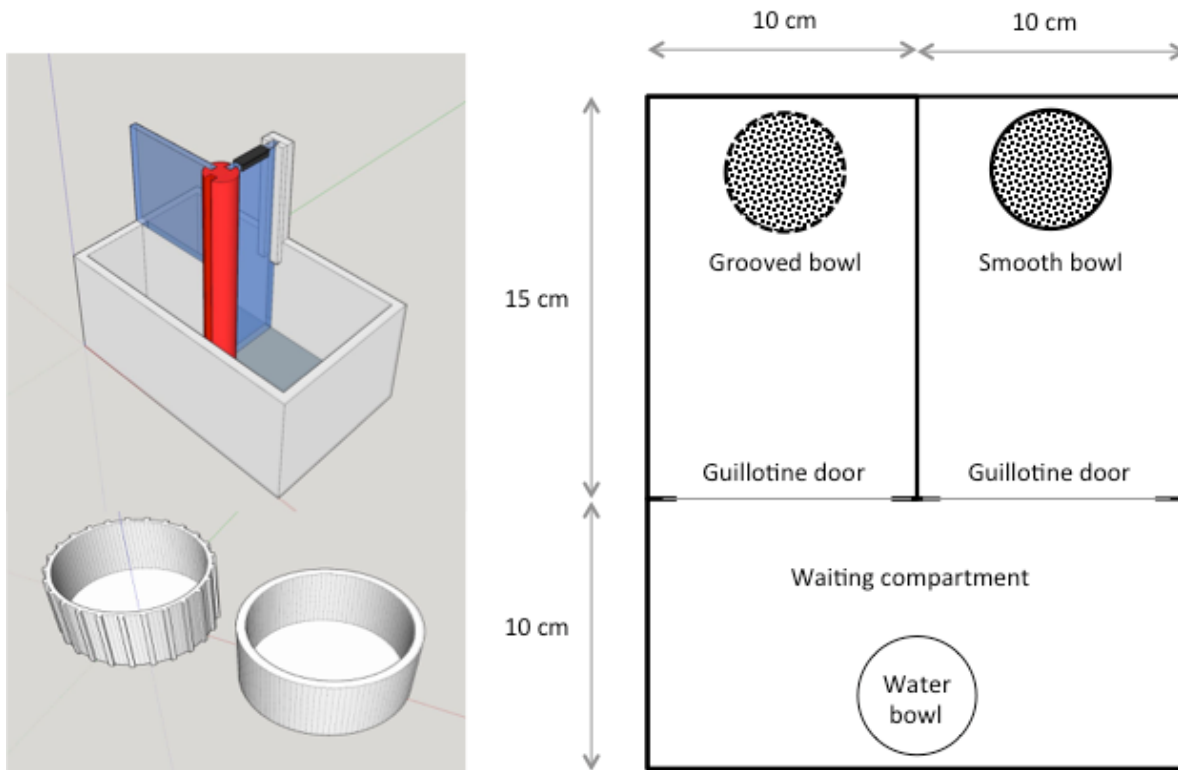
light/dark cycle (lights on at 7 A.M.). Mice were weighed before each training session and were maintained at 85% of their ad libitum weights by being given 3–4 g of food per day at the end of the day. Water freely available in the home cage. All procedures were approved by the ethical review committee at Cardiff University, UK, and were performed in accordance with the United Kingdom Animals (Scientific Procedures) Act (1986).

## Apparatus

The apparatus was based on that described in Huang *et al.* (2014) and is depicted in Figure 1. It was placed on a table in a small experimental room that was illuminated with dim red light. The arena was constructed from opaque acrylic, with an acrylic base and walls, and two transparent Plexiglas guillotine doors (25 x 20 x 15 cm). The apparatus included a waiting compartment (20 x 10 cm), which allowed access to two equally sized choice compartments via guillotine doors (15 x 10 cm). Cylindrical digging bowls (45 mm in diameter, 25 mm in height) were created using 3D printer technology (Ultimaker B.V., The Netherlands) and RS 3D Printer Filament Polylactic Acid (PLA; 2.85mm 1kg). A drinking bowl was also creating using Wood PLA, which was placed in the waiting compartment filled with water (the STL files for generating the bowls are freely available from the authors). One digging bowl was placed in each choice compartment. The bowls could be baited with a small piece of cereal (30 mg; Coco Pops, Kellogg; nutrient composition: whole white rice (59%), sugar, cocoa (3%), minerals (calcium carbonate, iron, zinc oxide), salt, flavours, dextrose, barley malt extract, vitamins (vitamin C, niacin, thiamin, riboflavin, folate). The cereal was then fully covered with sawdust (mixed with 2% Coco Pop cereal blended to form a dust) and mice were required to dig to retrieve the hidden food reward. In Experiments 1 and 3, the bowls were discriminated by their outer surface (smooth or grooved) and both contained cereal dust (to mask any odor generated by the reward), whereas in Experiments 2 and 4, the bowls had the same outer surface (smooth for half of the mice and grooved for the remainder) and the two were discriminated by their odor (either the presence or absence of 2% Coco Pop cereal dust in one set of



4 mice; or the presence of 0.5% ginger or 0.5% cinnamon for another set of 4 mice; cf. Grieves *et al.*, 2016; Davies *et al.*, 2013).



*Figure 1.* A schematic (left) and plan (right) of the two-choice discrimination apparatus, including two digging bowls one of which has a grooved outer surface (grooves: 0.9 mm deep  $\times$  1.5 mm across, with 1.6 mm spaces) and the other has a smooth outer surface. These two bowls were filled with sawdust, and one (e.g., the grooved bowl) was baited with a small piece of cereal irrespective of whether it was placed in the left or right choice compartments. There was a third bowl in the waiting compartment that was made of a different material and filled with water.

## Procedure

*Habituation and pretraining.* The general details of the procedure were modified from previous studies (Birrell & Brown, 2000; Colacicco, Welzl, Lipp, & Würbel, 2002; Garner, Thogerson, Würbel, Murray, & Mench, 2006; Huang *et al.*, 2014). One week prior to testing, mice were gradually accustomed to the experimenters through regular handling. Before each training session, mice were weighed and transferred to the testing room for a 30-min period to acclimatise them to the room. In order to habituate the mice to the diggings bowls and to train them to retrieve

the reward by digging in the bowls, a baited digging bowl, filled with sawdust, was left in their home cage overnight for two nights. On the two days before the start of training, mice were given 10 minutes to explore the empty arena. Immediately after the 10-minute periods, mice received two consecutive trials with free access to two (baited) bowls until both rewards were consumed. For half of the mice the baited bowl was grooved and for the remainder it was smooth; the identity of the bowl that was baited during pretraining was the same as during day 1 of discrimination training. This protocol ensured that the mice would dig in the bowls on the subsequent training days.

*Experiments 1 and 2: Discrimination learning and reversal.* A trial was initiated by lifting the guillotine doors, thereby allowing the mice access to the two choice compartments containing the bowls (see Figure 1). The identities of the bowls that were baited or unbaited (Experiments 1 and 3; grooved or smooth) and of the odors that were baited or unbaited (Experiment 2: presence or absence of Coco Pop dust; and ginger or cinnamon; Experiment 4: presence or absence of Coco Pop dust) were counterbalanced. The first day of training began with 4 exploratory trials (trials 1-4) in which mice were allowed to dig in both bowls and to self-correct if an incorrect choice was made. On trials 5-24 they were only allowed to dig in one bowl per trial. If a mouse began to dig in the baited bowl (e.g., grooved) then they were allowed to retrieve and consume the reward before returning to the waiting compartment, whereas if they began to dig in the unbaited bowl (e.g., smooth), an error was recorded and the next trial was initiated by returning them to the waiting compartment. Digging was defined as moving the sawdust with the paws or nose; climbing over the bowl was not included as digging. Each of these training sessions lasted approximately 1 hour for each mouse. Fixed pseudo-random sequences were generated to counter-balance the number of times the baited bowl was in the left or right compartment. These sequences were mirror images of one another and had the constraint that there were no more than 3 trials in succession where the baited bowl was in a given compartment (Fellows, 1967). Each of the textured bowls was regularly substituted throughout a training session to ensure that mice did not use cues attached to one

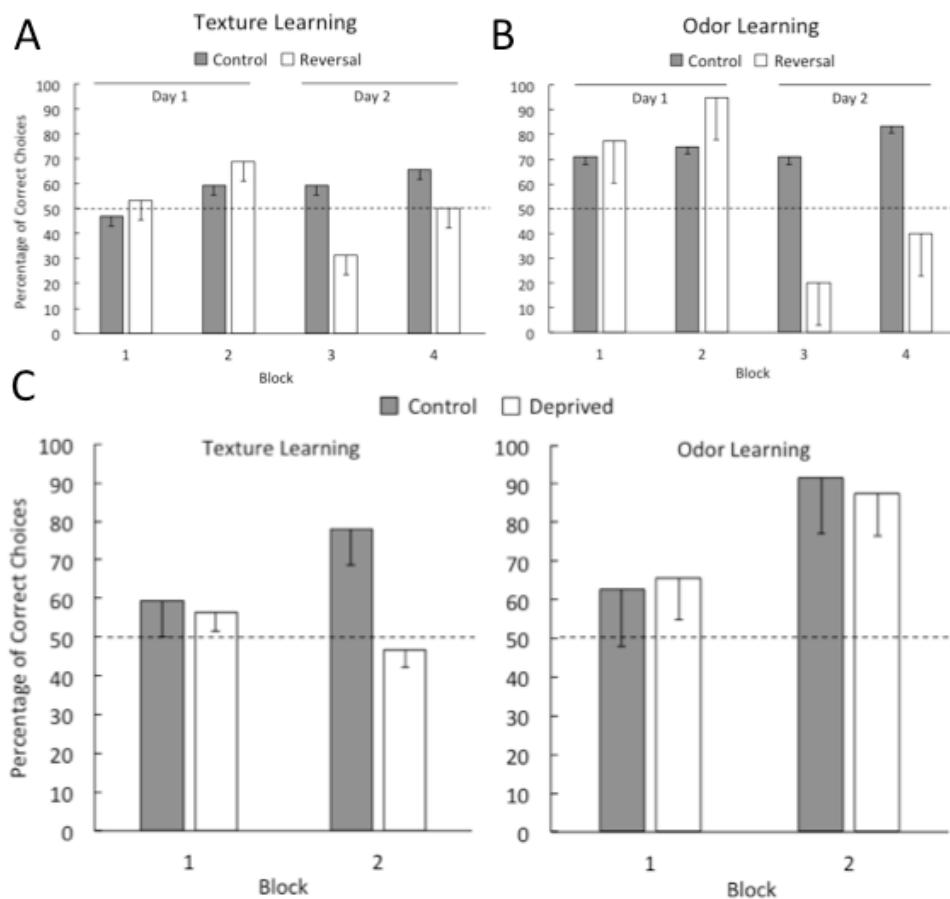
specific bowl. Furthermore, any possible olfactory cues left by the animal was removed by cleaning the bowls with 70% alcohol. Once testing was complete the mice were returned to their home cages and given their daily food quotient. On the second day of training, the 4 exploratory trials were omitted and the mice received 20 standard training trials. For mice in the control group, the contingencies on the second day were the same training as on the first day of training (e.g., grooved->reward and smooth->no reward), whereas for those in the reversal group the previously baited bowl (e.g., grooved) became unbaited and the previous unbaited bowl (e.g., smooth) became baited. If performance on day 2 was determined solely by the effects of training on day 1 (at least initially), then all 8 mice in Experiments 1 and 2 should continue to dig in the bowl that was baited on day 1. This could be assessed by coding the accuracy of the choices of both groups of mice on day 2 with respect to the bowl that was correct on day 1, and using one-sample *t* test to assess the difference from chance levels (i.e., 50%). Similarly, the accuracy of all mice could be coded with respect to the contingencies that were in force on day 2 and compared between the control groups and the reversal groups. In this case, the clear prediction is that those in the control groups should perform more accurately than those in the reversal groups. This was the approach taken in both Experiments 1 and 2, which allows the effects of texture and odor learning on day 1 to be assessed with  $N_s = 8$ , and for the critical differences between the control and reversal groups on day 2 to be replicated with different stimulus modalities (textures and odors). In fact, the results from the control group in Experiment 1 were also replicated in a supplementary experiment, which like the corresponding control group in Experiment 1, employed 4 mice. This experiment was conducted as part of a large-scale project involving the neural substrates of whisker-based learning. The behavioral treatments that the mice received was identical to the control group in Experiment 1, with the exception that they had received an IP injection (10 uL/g of CNO in saline) administered an hour before testing on days 1 and 2

*Experiments 3 and 4: Whisker trimming.* The behavioral procedures used in Experiments 3 and 4 were the same as for day 1 of Experiments 1 and 2, respectively. For the deprived group, all macrovibrissae were trimmed bilaterally to the base of the snout ( $< 1\text{mm}$ ), whereas for the control group the whiskers were lightly stimulated by brushing against them for two minutes each side to simulate the stimulation produced by the trimming procedure but without the trimming. Both groups of mice were lightly anesthetized with isoflurane (1.5 % isoflurane in O<sub>2</sub>, 0.75 L/min) after the two habituation days and 24 hours before behavioral training, but only mice in the deprived groups had their whiskers trimmed.

## Results and Discussion

The results of Experiment 1, involving texture learning, are depicted in Figure 2A (DVD files of task behavior are available from the authors). The percentage of correct choices are binned into two consecutive blocks on day 1 (blocks 1 and 2), and day 2 (blocks 3 and 4). All mice completed at least 16 trials on both days, and on both days the blocks contained the first and second sets of 8 completed trials. Trials on which mice abstained tended to be towards the end of the session on both days, and presumably reflected satiation and/or fatigue. Inspection of Figure 2A shows that the percentages of correct choices increased between the first and second block of training on the first day, and that this increase was similar in both groups, which was to be expected as they had received the same training up to this point. On the second day, the performance of mice in the control group was more accurate than those in the reversal group. Analysis of the overall percentages of correct choices on the first day confirmed that the groups did not differ ( $t(6) = -.928$ ,  $p > .05$ ); and a one-sample  $t$  test confirmed that the percentages of correct choices on day 1 were significantly higher than chance (i.e., 50.00;  $M = 57.03$ ,  $SEM = 4.16$ ,  $t(7) = 13.57$ ,  $p < .001$ ,  $d = 0.59$ ). Analysis of performance on the second day confirmed that the percentage of correct choices was significantly higher in the control group ( $M = 62.50$ ,  $SEM = 4.41$ ) than in the group for which the rewarded and nonrewarded textures were reversed ( $M = 40.62$ ,  $SEM = 4.03$ ),  $t(6) = 3.65$ ,  $p < .05$ ,

$d = 0.83$ ). This difference is important for two reasons. First, it shows that the mice were not simply using the odor of the Coco Pop to guide digging; had they been doing so then there should have been no difference between groups control and reversal. Second, it demonstrates that what was learned on the first day was retained overnight. The observation that differences in performance are evident with group sizes as small as 4 suggests that the training procedure used on day 1 is very effective. This finding was replicated in a supplementary experiment with 4 mice that were treated in the same way as the control group in Experiment 1. The percentages of correct choices in these mice was significantly higher than chance for both day 1 ( $M = 68.75$ ,  $SEM = 5.70$ ,  $t(3) = 11.96$ ,  $p < .01$ ,  $d = 1.64$ ), and day 2 ( $M = 71.87$ ,  $SEM = 4.03$ ,  $t(3) = 17.69$ ,  $p < .001$ ,  $d = 2.71$ ).



**Figure 2.** Mean percentages of correct choices (-SEM). Discrimination learning involving textures in Experiment 1 (panel A) and odors in Experiment 2 (panel B). In the control groups (grey bars), the rewarded stimulus and nonrewarded stimulus was the same on days 1 and 2, whereas in the

reversal groups, the rewarded and nonrewarded stimuli were reversed between days 1 and 2 (white bars). Panel C shows the effect of whisker trimming on discrimination learning involving textures (Experiment 3) and odors (Experiment 4). The dotted lines indicate chance level (i.e., 50%).

The results from Experiment 2 are shown in Panel 2B pooled across the odor pairs that were used (i.e., presence and absence of Coco Pop dust; ginger and cinnamon) because the pattern of results was not affected by this difference. Comparison of the results from Experiment 2 with those from Experiment 1 suggests that the discrimination involving odors was acquired somewhat more readily than the discrimination involving textures. On the first day, the mice in Experiment 2 rapidly learned to dig in the bowl with the reward, there being some tendency for those in group reversal to perform somewhat better than those in the control group; in spite of the fact that on this day both received the same training. On the second day, the mice in group control performed at above chance level (i.e., 50%), while those in group reversal performed at below chance level. Analysis of the percentages of correct scores on the first day revealed no difference between the groups ( $t(6) = -2.11, p > .05, d = 0.65$ ); and a one-sample  $t$  test confirmed that the percentages of correct choices were significantly higher than chance (i.e., 50.00;  $M = 81.25, SEM = 3.73, t(7) = 21.61, p < .001, d = 2.95$ ). Analysis of performance on the second day confirmed that the scores in the control group ( $M = 77.08, SEM = 9.08$ ) were significantly higher than those in the group that received the reversal of the reward contingencies ( $M = 30.00, SEM = 11.42, t(6) = 2.83, p < .05, d = 0.75$ ). The difference in performance between the control and reversal groups on the second day confirms that the mice were not simply basing their choice to dig on the presence of the odor of the Coco Pop reward, and that what had been learned on the first day was retained overnight. The pattern of results from Experiment 1 involving textures was replicated in Experiment 2 using odors.

The effects of whisker trimming on texture discrimination learning (Experiment 3) and odor discrimination learning (Experiment 4) are depicted in Figure 2C. It is clear that while whisker trimming had a dramatic effect on the acquisition of the texture discrimination (left-hand panel) it had no effect on the odor discrimination (right-hand panel). In Experiment 3, there was a marked

increase in the percentage of correct choices between blocks 1 and 2 in the control group, but not in the group deprived of their whiskers. In fact, the increase was numerically greater than in Experiment 1 (pooled over the groups that were equivalently trained on Day 1; block 1 = 50.00% and block 2 = 64.06%). The basis for this difference is not clear. More importantly, in Experiment 4 there was an equivalent increase in the percentages of correct choices between blocks 1 and 2 in the control and whisker deprived mice. ANOVA conducted on the results from Experiment 3 confirmed that there was a significant effect of group,  $F(1, 6) = 8.44, p < .05, \eta_p^2 = .58$ , no effect of block,  $F < 1$ , and a significant interaction between these factors,  $F(1, 6) = 5.17, p < .05, \eta_p^2 = .46$ . Additional analyses confirmed that the groups did not differ on block 1,  $t(6) = .45, p > .05, d = 0.15$ , but differed significantly on block 2,  $t(6) = 3.16, p < .05, d = 0.79$ . In fact, training in Experiment 3 continued for a second day, with the same reward contingencies in place, and in the same way as for group control in Experiment 1. On this second day, the mice in the deprived group continued to perform at chance levels on both block 1 ( $M = 46.87\%, SEM = 3.12$ ) and block 2 ( $M = 53.12\%, SEM = 3.12$ ), whereas the scores in the control group were above chance on both block 1 ( $M = 62.50\%, SEM = 5.10$ ) and block 2 ( $M = 71.87\%, SEM = 3.12$ ). ANOVA confirmed that there was an effect of group,  $F(1, 6) = 33.00, p < .01, \eta_p^2 = .85$ , no significant effect of block,  $F(1, 6) = 3.26, p > .10, \eta_p^2 = .35$ , and no interaction between these factors,  $F_s < 1$ . Turning now to Experiment 4, ANOVA revealed that there was no significant effect of group,  $F < 1$ , a significant effect of block,  $F(1, 6) = 22.23, p < .01, \eta_p^2 = .78$ , and no interaction between these factors,  $F < 1$ . Further analyses revealed that the percentages of correct choices on block 1 ( $M = 64.06, SEM = 3.68$ ) and block 2 ( $M = 90.62, SEM = 3.91$ ) were significantly higher than chance,  $t(7) = 17.23, p < .001, d = 0.98$ , and  $t(7) = 23.00, p < .001, d = 0.99$ , respectively.

Mice tended to acquire the texture discriminations (in Experiments 1 and 3) less rapidly than the odor discriminations (in Experiments 2 and 4). This leaves open the possibility that the different effects of whisker trimming on the two types of discrimination might have reflected a

scaling effect. This possibility is undermined by two observations. First, on the second block of training in Experiment 3 the mice were performing at approximately the same overall level of accuracy as the mice in Experiment 4 achieved on the first block of training; and yet there was an effect of whisker trimming in Experiment 3, but not in Experiment 4. Second, in Experiment 4 there was no sign of an effect of whisker trimming when the mice were performing at quite different levels of performance that were both above chance. Finally, it is worth noting the fact that we have observed the same dissociation between texture and odor discrimination learning when the barrel cortex of the mice is temporarily inactivated during training: discrimination learning involving textures, but not odors, is disrupted (Pacchiarini, Honey & Fox, 2018). The results that we have presented using small-*N*-designs are highly reliable and the dissociations are clear-cut. This reliability should not come as a surprise, because discrimination learning is consistently observed across the animal kingdom, and having designed the experiments to tax specific sensory systems should allow clear dissociations to be observed.

## General Discussion

The study of learning and memory in rodents often requires considerable ingenuity in generating stimuli that they can both learn about readily and can be combined in such a way that the perception of stimuli from one dimension is not affected by the presence of stimuli from another dimension. The procedures described by Birrell and Brown (2000) represent a good example of such ingenuity. However, it is often the case that the way in which animals are processing the stimulus dimensions within studies of learning and memory is a secondary consideration. This fact can limit further analysis of the requisite behavioral and brain mechanisms. We adapted the procedure developed by Birrell and Brown (2000) to investigate whether freely moving mice use their whisker system to learn a texture discrimination.

The discriminations took place in dim red light and the textures were made of the same material (3D printed PLA). The mice learned within a single session which of the two textured



bowls contained a reward and this learning was evident the next day (Experiment 1). The development of an equivalent procedure in which the bowl that contained the reward was signalled by the odor of the digging medium (Experiment 2), allowed us to show that whisker trimming had an impact on discrimination learning involving the texture of the bowls but not the odor of the digging medium (Experiments 3 and 4). The latter observations directly implicate the whisker system in the discrimination of texture in the digging paradigm developed by Birrell and Brown (2000), which might operate in a synergistic fashion with stimuli from other senses (e.g., information from the paws) when both are available. The fact that the bowls were 3D printed provides a ready means of changing the discriminability of the textures (e.g., the spatial frequency of the grooves). This will enable future research to manipulate the difficulty of the task; and for processes including perceptual learning to be studied under circumstances where the sensory basis for the discrimination is known (cf. Montuori & Honey, 2016).

A final important feature of Experiments 1-4 is that robust and reliable learning effects were observed with a small number of mice; in fact, the minimum number of mice (i.e., 4) that is necessary to conduct an experiment that counterbalances the nature of the rewarded stimulus (e.g., grooved or smooth) and the side of the arena on which it was first positioned during training (left or right; cf. Pacchiarini *et al.*, 2017). The development of procedure that produces rapid learning, which is retained overnight, will allow future research to investigate the neural systems that underpin texture learning and memory in freely moving mice (cf. Diamond *et al.*, 2008; Stüttgen & Schwarz, 2017); complementing research using head-fixed mice where the sensory features of the stimuli can be manipulated (e.g., Hong *et al.*, 2018). The fact that our procedures result in reproducible effects with small-*N*-designs (Smith & Little, 2018) is consistent with the 3Rs agenda (e.g., [nc3rs.org.uk](http://nc3rs.org.uk)) and also enables the efficient assessment of mice with rare or costly mutations.

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