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Function and Acuity of the Rat Vibrissa System during Texture Discrimination

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The Thesis of Takeshi Morita is approved and it is acceptable in quality and form for publication on microfilm and electronically:

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DEDICATION

This work is dedicated to my mother Hiromi, my father Hiroshi, and my sister Aimi for their constant support. To all my relatives in Japan, especially aunt Yukari and uncle Itaru for being my valuable advisors. I would not be here writing this thesis if it wasn't for them.

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ABSTRACT OF THE THESIS

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There has been a strong presumption that the rodent vibrissae are fine tactile feature detectors, sensing position, shape, and texture of objects. However, how rat vibrissae extract fine surface features, and their quantitative acuity during texture discrimination remain unknown. The goal of this thesis is to elucidate the functions of the rat vibrissa system during fine texture discrimination at the behavioral level.

The first goal of this thesis is to detail training strategies developed specifically for rodent vibrissa-dependent texture discrimination tasks. In Behavioral Setup 1, rats were trained to discriminate smooth vs. grooved aluminum surfaces, and to palpate across a moderate gap for water reward. In Behavioral Setup 2, rats were trained to discriminate between sandpaper of two different roughnesses, presented across a gap. Results showed that rats could learn texture discrimination in 8 weeks (Setup 1) and 2–10 weeks (Setup 2) of training.

The second goal of the thesis was to examine the relative functional roles of the micro/macrovibrissa system during texture discrimination. The specific roles of the two systems are not known. We tested whether macrovibrissae are sufficient for fine texture discrimination by removing the microvibrissae. Microvibrissa trimming did not decrease performance indicating that macrovibrissae alone can support texture discrimination. We also investigated the acuity of the vibrissa system in identifying texture differences. Sandpapers were varied to measure the psychophysical limit of texture discrimination. Though results were only obtained in one rat, these observations suggest that rats can discriminate sandpapers with finer resolution than previously known.

Chapter 1: Introduction to somatosensory psychophysics

1.1 Introduction

The sense of touch is composed of many attributes. By stimulating the skin surface, one can simultaneously experience pain, temperature, proprioception and vibrotactile sensation, demonstrating the complexity of the perception which we simply refer to as touch. Tactile perception has been investigated using psychophysical studies in humans and monkeys, elucidating how activation of skin receptors may lead to perception and decision making in higher brain areas (Romo et al., 2002). However, the complexity of receptors embedded between the skin layers has made it difficult to isolate and study individual touch channels one at a time.

The rat whisker system has emerged as a primary model system for studying tactile sensation. Highly motile and dynamic rodent whiskers are known for their superb ability to extract fine feature and spatial properties within their environment. Its acuity has even been suggested to be comparable to human fingertips (Carvell and Simons, 1990). Unlike human and monkey skin surface containing multiple receptors for all touch channels, vibrissa follicles are selective for vibrotactile sensation , making this system an attractive model for studying this isolated somatosensory attribute. In spite of the simplicity and usefulness of the rodent whisker system, questions regarding the psychophysics have not been explored as thoroughly as in monkeys and humans, and their functional capacities remain unclear. In this thesis, I investigate at the behavioral level, in how rats utilize their whiskers to extract texture properties.

1.2 The rodent vibrissa system

The vibrissae have been recognized as an important tactile sensory organ for rodents since original studies conducted by Vincent in 1912. Because rats are nocturnal creatures, these large and elaborate facial hairs have been thought to act as fine tactile detectors for navigating through closed environments where rats naturally live.

Vibrissae are aligned in a bilaterally symmetrical, stereotypical array on the mystacial pads on the sides of the snout (figure 1.1A). This whisker array is distributed in five well-defined rows that are lettered from dorsal to ventral as A to E, and are also arranged in columns numbered 1 to 7 in caudal-to- rostral direction. The four most caudal whiskers, called the "straddlers" or the "greeks" are designated as α, β, γ, δ, in the dorsal-to-ventral direction (Vincent, 1913). The vibrissae are further divided into two subclasses: large moveable macrovibrissae and small stationary microvibrissae, which are discussed below in greater detail (figure 1.1B).

During exploration, rats often exhibit a rapid, rhythmic whisker movement termed "whisking". Whisking consists of repeated cycles of protraction (forward whisker movement) and retraction (backward whisker movement) at frequencies between 5 – 25 Hz (Berg and Kleinfeld, 2003; Hill et al., 2008; Welker, 1964). Rats modulate their whisking cycles according to their behavioral states. High amplitude, low frequency whisking cycles (5 – 15 Hz) are seen during exploratory whisking, whereas low-amplitude high frequency whisking cycles (15 – 25 Hz) are associated with a distinct pattern of whisking called foveal whisking, in which rats extend their whiskers forward and whisk across objects directly in front of them (Berg and Kleinfeld, 2003; Carvell and Simons, 1990). Even though individual vibrissa can be controlled independently, the

entire vibrissa array moves coherently during whisking (Bermejo et al., 2002). Protraction is mediated by intrinsic muscles that pivot the whiskers forward, while retraction is driven by a combination of passive re-coil and contraction of extrinsic muscles that shifts the attachment point of the follicles backward on the face (Berg and Kleinfeld, 2003). This forward-backward movement allows whiskers to sample an extended region of space parallel and in front of the face (Bermejo et al., 2002). Whisking cycles are also highly correlated with head movement and sniffing cycles (Mitchinson et al., 2007; Welker, 1964).

When whiskers contact an object, whisker vibrations are induced that reach the follicle, where sensory endings of primary afferent neurons are located. These axons ascend the infraorbital branch of the trigeminal nerve to the trigeminal nuclei in the brainstem, mainly the trigeminal nucleus principalis. Axons from the trigeminal nuclei project to the contralateral thalamus, innervating primarily the ventral posterior nucleus (VPm). Cells in the VPm send their axons to layer IV of the primary somatosensory cortex (S1), in turn projects to higher brain areas, such as secondary somatosensory cortex (SII) and the prefrontal cortex (PFC) where tactile perception is thought to occur (Romo et al., 2002). Anatomical maps of the whiskers exist at each stage of the relay, up through S1, in the form of cell clusters called “barrelettes” in the brainstem (Ma, 1991), “barreloids” in the thalamus, and “barrels” in layer IV in S1 (Woolsey and Van der Loos, 1970). Anatomical and electrophysiological studies have shown a one-to-one relationship between each whisker and a corresponding barrelette-barreloid-barrel, making the vibrissa system an attractive model for studying neurocircuitry, plasticity, and sensory processing (Petersen, 2007).

In contrast to the depth of investigation that focuses on plasticity and neurocircuitry using the rodent vibrissae as a model system, much less is known about how the system is utilized ethologically. The functional role of the vibrissa system in guiding rat behavior was first studied by Vincent (Vincent, 1912). Rats were placed on an elevated platform maze, and time required to reach the goal was measured. Rats were also placed on a texture discrimination task, which required rats to choose the rippled hallway baited with food. In both experimental setups, increases in performance were observed across trials. By trimming off all whiskers, both locomotion and texture discrimination were impaired, demonstrating the function of whiskers as spatial/tactile sensors. However, in the texture discrimination task, the lack of a behavior monitoring device made the interpretation impossible to dissociate whether the discriminatory behavior was due to paw, nose, or vibrissae contact.

Since this initial study by Vincent, numerous studies had demonstrated and confirmed the multifunctional role of the vibrissa system in guiding tactile dependent behaviors in rodents. Studies have shown that rats actively use their whiskers for distance detection (Hutson and Masterton, 1986; Jenkinson and Glickstein, 2000), aperture width discrimination (Krupa et al., 2001), object orientation (Polley et al., 2005), and angular discrimination (Knutsen et al., 2006; Mehta et al., 2007). The rat whisker system is also used for fine feature detection tasks, including object shape recognition (Brecht et al., 1997; Harvey et al., 2001), and texture discrimination (Guic-Robles et al., 1989; Carvell & Simons, 1990; Prigg et al., 2002). In these tasks, tactile sensitivity of whiskers often rivaled the sensitivity of human fingertips. All these were found to be whisker-dependent tasks, in which experimenters saw dramatic deficits in performance after removal of all

whiskers, and in which performance was recovered after whisker re-growth (Guic-Robles et al., 1989). Furthermore, many of these active whisker behaviors are barrel cortex dependent, as shown by cortical lesion studies (Guic-Robles et al., 1992; Hutson and Masterton, 1986; Krupa et al., 2001).

Rats can also discriminate passively stimulated whisker inputs, that is, in tasks that do not involve voluntarily whisking. For example, rats can learn to recognize whether left or right whiskers were deflected, and in which order (Wada et al., 2005) and to discriminate the direction of whisker deflection (dorsal/ventral, rostral/caudal) (Narumi et al., 2007). Rats can also discriminate different whisker deflection frequencies (Hutson and Masterton, 1986). Compared to the active whisker behaviors, these passive whisker behaviors are not generally barrel cortex dependent (Hutson and Masterton, 1986).

1.3 Vibrissa-dependent texture discrimination

This thesis will focus on whisker-dependent texture discrimination. Numerous studies have demonstrated the rat's superb ability to utilize its vibrissae for fine texture discrimination. The first study was conducted by Guic-Robles et al. in which rats were trained to discriminate two different sandpapers – one with mean grain diameter ~0.4 mm and the other with coarse grain, 2.0 mm. Rats successfully learned to discriminate this difference. Whisker trimming led to chance-level performance, and behavioral performance recovered after whisker re-growth, indicating the requirement of whiskers for texture discrimination (Guic-Robles et al., 1992). Performance dropped to chance-level after bilateral lesion of the cortical barrel field, indicating that this behavior is barrel cortex-dependent (Guic-Robles et al., 1992; Guic-Robles et al., 1989).

Further studies demonstrated that rats can discriminate finer sandpaper gratings in micrometer resolution. In the finest sandpaper discrimination to date, rats were trained to discriminate sandpapers with mean grit diameter of 201 μm vs 100 μm. (Aggestam and Cahusac, 2007). Mice can also successfully complete such a task, involving 190 μm vs 50 μm sandpaper discrimination (Cybulska-Klosowicz and Kossut, 2001). One study failed to demonstrate sandpaper discrimination when rats were trained using a head-fixed preparation, suggesting that rats require active head movement along with vibrissae palpation for fine texture discrimination (Harvey et al., 2001).

Additional studies confirmed that rats not only use their whiskers for fine sandpaper discrimination, but can also discriminate fine grooved surfaces. These studies looked mainly at the biometrics of whisker contact during discrimination of grooved plastic cylinders. Rats discriminated grooved surfaces with resolution as fine as 50 μm, between a 50 μm grooved cylinder versus a smooth cylinder. Furthermore, this study found that rats modulated their whisking pattern depending on different texture gratings (Carvell and Simons, 1990). Additional studies demonstrated that rats can discriminate fine textures by using only a single whisker. Macrogeometric texture features were more difficult to discriminate compared to the microgeometric differences (i.e. discrimination between two coarse features (in mm scale) was more difficult than discrimination between two fine features (in μm scale)) (Carvell and Simons, 1995, 1996).

These behavior findings initiated a new wave of interest in trying to decipher how texture information was converted into whisker mechanics and were represented in the brain. An initial attempt was made to measure neuronal firing rates in S1 of awake

behaving rats to identify a neural correlate of texture discrimination, which they failed to see any difference in firing rates between smooth and grooved surface (Prigg et al., 2002). A recent study successfully recorded in awake behaving animals during discrimination between a plate mold of P100 sandpaper and a smooth plastic plate.

Results showed that overall firing rate was modest but significantly higher when the animal made contact with the rough surface compared to the smooth surface, suggesting that neuronal firing rate could be a key parameter in texture coding (von Heimendahl et al., 2007). Two recent unpublished studies from the Feldman lab found that SI neurons encode rapid slip-stick motion events during palpation on sandpapers and that these events may encode texture (Wolfe et. al., submitted; Jadhav et al., in preparation). Other study confirms the existence of these slip-stick events on textures (Ritt et al., 2008).

1.4 Functions of macrovibrissae vs. microvibrissae in texture discrimination

The rat vibrissae system appears to be a single tactile sense organ, but evidence suggests that two separate morphological subsystems exist within the rodent whisker array, which may have separate functions (Brecht et al., 1997). First, is the macrovibrissa system, which is defined as all A and B row whiskers, as well as caudal C, D, and E row whiskers that are longer than 4 mm. These are the longest whiskers on the face, and have relatively large distances between neighboring follicles, resulting in a sparse distribution across the mystacial pad (figure 1.1B, highlighted red). Macrovibrissae are highly motile during exploratory whisking, and their movement is directly controlled by intrinsic muscles around each follicle.

Second is the microvibrissa system, which is defined as the shorter, more rostral whiskers. The C – E rows, as well as the entire F – J rows, and additional small hairs located on the lower lip. Microvibirissae are shorter than macrovibrissae (< 7 mm in length), and follicles are spaced close together, resulting in a high density distribution, about 40 fold higher than the macrovibrissa system (figure 1.1B, highlighted blue). Unlike macrovibrissae, microvibrissae are largely stationary and are used mostly when head movements bring objects close to the face. These two whisker subsystems are present not only in rodents, but also in other species such as shrews, moles, and hedgehogs (Anjum et al., 2006; Brecht, 2007; Catania, 2005; Haidarliu and Ahissar, 1997).

In addition to the morphological differences between macro and microvibrissae described above, functional differences are observed as well. Brecht et al. trained rats on a shape discrimination task by requiring rats to find a single sweet triangular-shaped cookie out of 11 bitter square-shaped cookies in a 4 x 4 cookie array in the dark. Catch trials confirmed the cookies smelled the same, suggesting that rats used shape information from the whiskers to make the discrimination. When training was complete, either microvibrissae or macrovibrissae were trimmed. Macrovibrissa trimmed rats did not exhibit deficits in shape recognition but were heavily impaired in spatial navigation. Conversely, microvibrissa trimmed rats displayed only minor deficits in spatial navigation, but shape recognition was completely abolished. Therefore, Brecht et al. hypothesized that the longer caudally located macrovibrissae are used as spatial detectors, whereas the shorter rostrally located microvibrissae function as fine feature detectors for shape discrimination (Brecht et al., 1997).

Other studies had proposed an alternate function to the two vibrissa subsystems. Carvell and Simons trained rats to discriminate different grooved cylinders over a gap to force vibrissa use, and demonstrated that during discrimination, microvibrissae were kept in contact with the surface while macrovibrissae were repeatedly tapped against the surface throughout the sampling period (Carvell and Simons, 1990). Further analysis showed that rats modulated their macrovibrissa whisking pattern with different groove size, and this was used to argue that macrovibrissae are fine feature detectors for texture discrimination, while microvibrissae act as "spacers" that are used to keep the nose at a specific distance from the surface (Carvell and Simons, 1995, 1996). A separate study demonstrated that rats discriminate differently shaped objects relying solely on the macrovibrissae. In this study, head fixed rats learned to discriminate different shaped objects presented specifically to the macrovibrissae. In agreement with Carvell and Simons, the author concluded that rat macrovibrissae function as fine feature detectors, in this case for shape (Harvey et al., 2001). Texture discrimination was not examined in this study, and contribution of microvibrissae was not examined explicitly. Neither of these studies directly tested the roles of microvibrissae versus macrovibrissae by trimming those whiskers and assessing effects on discrimination ability.

1.5 Open questions

The literature analysis above indicates that two major questions exist on the function of the whiskers in texture discrimination. First, the relative functions of the two vibrissa subsystems are unclear. As mentioned above, there are two hypotheses regarding this question. The experiment that supported the first hypothesis used an object shape

recognition task and not textured surfaces. Therefore, it is still unclear whether the functional role of the microvibrissae as fine feature detectors extends to textured surfaces. While the presumption is that macrovibrissae are used for fine texture discrimination in the second hypothesis, this has not been directly demonstrated because microvibrissae were not trimmed in prior texture studies. Thus, microvibrissae could have been used to perform the task, consistent with the idea that these are a higher-resolution tactile system than the macrovibrissae (Brecht et al., 1997).

A second remaining question is that while rats can detect fine texture differences with whiskers, the quantitative resolution across different textures is not known. Carvell and Simons demonstrated that rats can discriminate a series of fine grooved cylinders (~15 μm versus 500, 250, 200, 150, 100, 75, 50 μm) with resolution as small as 50 μm, but all other texture discrimination experiments failed to show this psychophysical performance, none using sandpapers. The finest texture discrimination done to date using sandpapers was between P80 versus P150 (201 μm versus 100 μm mean grit diameter), and rats were able to perform the task (Aggestam and Cahusac, 2007). Therefore, the upper limit of vibrissa acuity in sandpaper discrimination is not known. Psychophysical performance on sandpaper discrimination is important to correlate with neural recordings on fine sandpapers (Jadhav, et al., in preparation) and with whisker motion signatures across different sandpapers (Wolfe et al., submitted). In addition, psychophysical limits on tactile system in humans and primates have been intensely studied and characterized (LaMotte and Mountcastle, 1975; Mountcastle et al., 1972; Mountcastle et al., 1990), and it will be interesting to learn how these limits compare to rodent whisker-based tactile sensation.

The goal of this thesis is to elucidate the functional properties of the rat vibrissae system for fine texture discrimination at the behavioral level. First, I tested which whisker subsystem – micro or macrovibrissa - rats employ to detect and discriminate textures. Second, I quantitatively analyzed how well rats discriminate a series of sandpaper textures in order to construct a psychophysical curve for texture discrimination.

1.6 References

Aggestam, F., and Cahusac, P.M. (2007). Behavioural lateralization of tactile performance in the rat. Physiol Behav *91*, 335-339.

Anjum, F., Turni, H., Mulder, P.G., van der Burg, J., and Brecht, M. (2006). Tactile guidance of prey capture in Etruscan shrews. Proc Natl Acad Sci U S A *103*, 16544-16549.

Berg, R.W., and Kleinfeld, D. (2003). Rhythmic whisking by rat: retraction as well as protraction of the vibrissae is under active muscular control. J Neurophysiol *89*, 104-117.

Bermejo, R., Vyas, A., and Zeigler, H.P. (2002). Topography of rodent whisking--I. Two-dimensional monitoring of whisker movements. Somatosens Mot Res *19*, 341-346.

Brecht, M. (2007). Barrel cortex and whisker-mediated behaviors. Curr Opin Neurobiol *17*, 408-416.

Brecht, M., Preilowski, B., and Merzenich, M.M. (1997). Functional architecture of the mystacial vibrissae. Behav Brain Res *84*, 81-97.

Carvell, G.E., and Simons, D.J. (1990). Biometric analyses of vibrissal tactile discrimination in the rat. J Neurosci *10*, 2638-2648.

Carvell, G.E., and Simons, D.J. (1995). Task- and subject-related differences in sensorimotor behavior during active touch. Somatosens Mot Res *12*, 1-9.

Carvell, G.E., and Simons, D.J. (1996). Abnormal tactile experience early in life disrupts active touch. J Neurosci *16*, 2750-2757.

Catania, K.C. (2005). Evolution of sensory specializations in insectivores. Anat Rec A Discov Mol Cell Evol Biol *287*, 1038-1050.

Cybulska-Klosowicz, A., and Kossut, M. (2001). Mice can learn roughness discrimination with vibrissae in a jump stand apparatus. Acta Neurobiol Exp (Wars) *61*, 73-76.

Guic-Robles, E., Jenkins, W.M., and Bravo, H. (1992). Vibrissal roughness discrimination is barrelcortex-dependent. Behav Brain Res *48*, 145-152.

Guic-Robles, E., Valdivieso, C., and Guajardo, G. (1989). Rats can learn a roughness discrimination using only their vibrissal system. Behav Brain Res *31*, 285-289.

Haidarliu, S., and Ahissar, E. (1997). Spatial organization of facial vibrissae and cortical barrels in the guinea pig and golden hamster. J Comp Neurol *385*, 515-527.

Harvey, M.A., Bermejo, R., and Zeigler, H.P. (2001). Discriminative whisking in the head-fixed rat: optoelectronic monitoring during tactile detection and discrimination tasks. Somatosens Mot Res *18*, 211-222.

Hill, D.N., Bermejo, R., Zeigler, H.P., and Kleinfeld, D. (2008). Biomechanics of the vibrissa motor plant in rat: rhythmic whisking consists of triphasic neuromuscular activity. J Neurosci *28*, 3438-3455.

Hutson, K.A., and Masterton, R.B. (1986). The sensory contribution of a single vibrissa's cortical barrel. J Neurophysiol *56*, 1196-1223.

Jadhav, S.P., Wolfe J.H., Feldman D.E., untitiled.

Jenkinson, E.W., and Glickstein, M. (2000). Whiskers, barrels, and cortical efferent pathways in gap crossing by rats. J Neurophysiol *84*, 1781-1789.

Knutsen, P.M., Pietr, M., and Ahissar, E. (2006). Haptic object localization in the vibrissal system: behavior and performance. J Neurosci *26*, 8451-8464.

Krupa, D.J., Matell, M.S., Brisben, A.J., Oliveira, L.M., and Nicolelis, M.A. (2001). Behavioral properties of the trigeminal somatosensory system in rats performing whisker-dependent tactile discriminations. J Neurosci *21*, 5752-5763.

LaMotte, R.H., and Mountcastle, V.B. (1975). Capacities of humans and monkeys to discriminate vibratory stimuli of different frequency and amplitude: a correlation between neural events and psychological measurements. J Neurophysiol *38*, 539-559.

Ma, P.M. (1991). The barrelettes--architectonic vibrissal representations in the brainstem trigeminal complex of the mouse. I. Normal structural organization. J Comp Neurol *309*, 161-199.

Mehta, S.B., Whitmer, D., Figueroa, R., Williams, B.A., and Kleinfeld, D. (2007). Active spatial perception in the vibrissa scanning sensorimotor system. PLoS Biol *5*, e15.

Mitchinson, B., Martin, C.J., Grant, R.A., and Prescott, T.J. (2007). Feedback control in active sensing: rat exploratory whisking is modulated by environmental contact. Proc Biol Sci *274*, 1035-1041.

Mountcastle, V.B., LaMotte, R.H., and Carli, G. (1972). Detection thresholds for stimuli in humans and monkeys: comparison with threshold events in mechanoreceptive afferent nerve fibers innervating the monkey hand. J Neurophysiol *35*, 122-136.

Mountcastle, V.B., Steinmetz, M.A., and Romo, R. (1990). Frequency discrimination in the sense of flutter: psychophysical measurements correlated with postcentral events in behaving monkeys. J Neurosci *10*, 3032-3044.

Narumi, T., Nakamura, S., Takashima, I., Kakei, S., Tsutsui, K., and Iijima, T. (2007). Impairment of the discrimination of the direction of single-whisker stimulation induced by the lemniscal pathway lesion. Neurosci Res *57*, 579-586.

Petersen, C.C. (2007). The functional organization of the barrel cortex. Neuron *56*, 339-355.

Polley, D.B., Rickert, J.L., and Frostig, R.D. (2005). Whisker-based discrimination of object orientation determined with a rapid training paradigm. Neurobiol Learn Mem *83*, 134-142.

Prigg, T., Goldreich, D., Carvell, G.E., and Simons, D.J. (2002). Texture discrimination and unit recordings in the rat whisker/barrel system. Physiol Behav *77*, 671-675.

Ritt, J.T., Andermann, M.L., and Moore, C.I. (2008). Embodied information processing: vibrissa mechanics and texture features shape micromotions in actively sensing rats. Neuron *57*, 599-613.

Romo, R., Hernandez, A., Salinas, E., Brody, C.D., Zainos, A., Lemus, L., de Lafuente, V., and Luna, R. (2002). From sensation to action. Behav Brain Res *135*, 105-118.

Vincent (1912). The function of the vibrissae in the behavior of the white rat. Behav Mono *1*, 82.

Vincent, S.B. (1913). The tactile hair of the white rat. The Journal of Comparative Neurology *23*, 1-36.

von Heimendahl, M., Itskov, P.M., Arabzadeh, E., and Diamond, M.E. (2007). Neuronal activity in rat barrel cortex underlying texture discrimination. PLoS Biol *5*, e305.

Wada, M., Moizumi, S., and Kitazawa, S. (2005). Temporal order judgment in mice. Behav Brain Res *157*, 167-175.

Welker (1964). Analysis of sniffing in the albino rat. Behavior *22*, 21.

Wolfe J.H., Pahlavan S., Hill D., Kleinfeld D., Feldman D.E., untitiled.

Woolsey, T.A., and Van der Loos, H. (1970). The structural organization of layer IV in the somatosensory region (SI) of mouse cerebral cortex. The description of a cortical field composed of discrete cytoarchitectonic units. Brain Res *17*, 205-242.

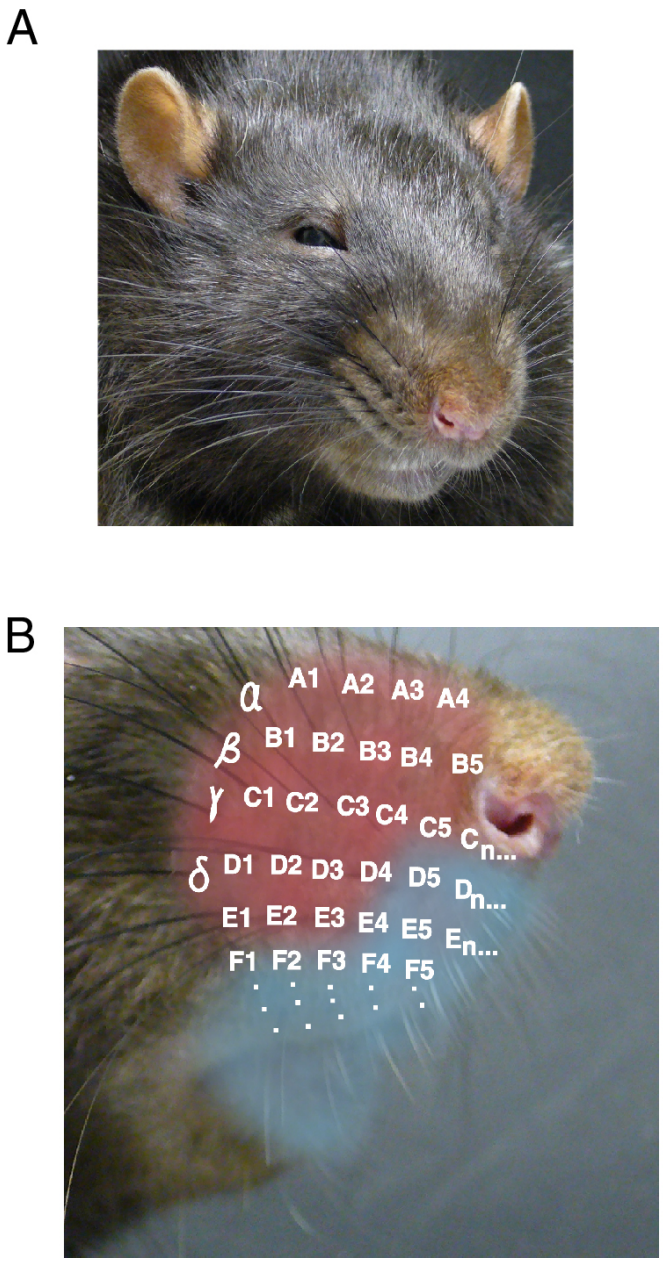


Fig 1.1. Rat Vibrissa System. A) Long vibrissae extend laterally from the mystacial pad. B) Rat vibrissa array is composed from longer, sparsely distributed macrovibrissae (highlighted red), and shorter, densely distributed microvibrissae (highlighted blue). Whiskers are aligned in rows, labeled A – E… in dorsal-to-ventral direction and in columns, numbered 1-6… in caudal-to-rostral direction.

Chapter 2: Development of two texture discrimination tasks in rats

2.1 Introduction to behavior training

The goal of this study was to train rats on vibrissa-dependent texture discrimination tasks in order to measure the functional roles of the two vibrissa subsystems (macro- and microvibrissa), and to measure rats' limit in discriminating fine texture differences with their whiskers. In previous studies, rats have been trained to discriminate textures by reaching with the whiskers across a gap, and then jumping across the gap to a target platform containing one specific texture ("gap crossing") (Aggestam and Cahusac, 2007; Carvell and Simons, 1990, 1995, 1996; Cybulska-Klosowicz and Kossut, 2001; Guic-Robles et al., 1992; Guic-Robles et al., 1989; von Heimendahl et al., 2007). In these studies, rats performed only 20–30 trails per day with training requiring intense manual labor. Therefore, we designed a computer-controlled training setup expecting to achieve a performance of ~100 trials per day.

We developed two different operant conditioning tasks. In the first task ("Behavioral Setup 1"), rats were trained to discriminate between an aluminum surface milled with 2 mm-spaced grooves from a smooth aluminum surface. These surfaces were placed next to each other, with the left-right position of the two surfaces varying randomly between trials. Rats freely whisked across a moderate gap to palpate the surfaces. Rats were rewarded for drinking from a water reward port located near the grooved surface. In the second task ("Behavioral Setup 2"), rats were trained to discriminate a rough sandpaper from a smooth sandpaper. The two sandpapers were

again presented next to each other, with left-right position varying randomly between trials. Rats whisked across a gap to palpate the surfaces, and jumped across the gap to the target platform associated with the rougher surface to receive a water reward. After initial training of texture discrimination on both setups, micro- and/or macrovibrissae were trimmed to assess the role of each whisker subsystem in texture discrimination. In addition, a psychophysical curve for texture discrimination was measured in Behavioral Setup 2 by varying the relative roughness of the two sandpapers.

Careful design of the behavior apparatus and training protocol was necessary for successful behavioral training. In this chapter, I will present the detailed training strategies developed for these tasks and report the learning curves and discrimination performance for the trained rats. Results of micro/macrovibrissa trimming and the psychophysical curve for texture discrimination are presented in Chapter 3.

2.2 Texture discrimination training strategies

Subjects

Female Long-Evans rats with initial weight approximately 150 g were housed in pairs or in threes, and maintained on a 12hr/12hr light/dark cycle. Food was available *ad libitum*, while water was given only as reward for correct behavior during daily training sessions and in a 30-60 min free access period after every training session. Training was conducted daily usually 5 days per week (sometimes 7 days per week). Rats continued to gain weight and displayed normal behavior throughout the entire training period (which lasted 3 – 4 months)

Behavioral Setup 1: Outline

In Behavioral Setup 1, rats were trained to perform a two-alternate forced choice discrimination using the training cage illustrated in Figure 2.1. The behavior apparatus was 50 cm by 35 cm, surrounded by walls 35 cm high. The floor was made from 1/4" plexiglass and walls were made from 1/16" aluminum sheet. The cage was elevated 25 cm above a base platform containing electronics. Trials started when rats were placed in the time-out chamber. A door dividing the time-out chamber and the discrimination chamber was opened manually, giving access to the discrimination chamber. Each rat was required to sample two aluminum textured surfaces (one grooved termed the S+ and one smooth termed the S-, see below) placed across a moderate gap (9.0 – 9.5 cm) before making a behavioral response by poking its nose to either the left or the right reward port. Reward ports contained an infrared (IR) LED sensor that detected nose entry. Correct nose pokes to the S+ water port were rewarded with 20 second access to 50 µl of water, whereas incorrect choices resulted with presentation of a distinct tone (default tone, beep.vi LabView subprogram). Once rats received either a water reward or a tone, they were gently and manually ushered back to the time-out chamber to prepare for the next trial. Rats were initially trained in a light room, but switched to a light-proof room to control for visual cues. To determine whether rats used olfactory cues in decision making, textures were wiped with ethanol during half of the trials in each training session. A video camera was placed below the gap to capture whiskers and head motion while textures were sampled. Each session was continued while motivation remained high, which lasted from 30 – 45 min. The training cage was operated using custom routines in

LabView and National Instruments DAQ boards and performance was further analyzed with MatLab.

Behavioral Setup 1: Stimuli

The texture stimulus was composed of two squares, 6 cm^2 aluminum plates – one plate was milled with 2 mm spaced grooves while the other was not milled and was smooth (Figure 2.3A). The grooved surface was considered S+ stimulus, while the smooth surface was S-. The two plates were positioned side-by-side with one texture on the right and the other on the left, and both were mounted on the axle of a rotating stepper motor. The right-left position of the two textures was randomly interchanged between trials by rotating the plates via the stepper motor. Rotation was performed in two 90º steps to eliminate possible auditory cues (to equalize duration of motor rotation). For example, to keep the same texture orientation for an upcoming trial, the motor was rotated 90º in one direction and 90º in the opposite direction. If the texture orientation was opposite for an upcoming trial, then the motor was rotated 90º twice in the same direction.

Behavioral Setup 1: Training Stages

Behavioral training was divided into 5 stages. In stage 1, rats were handled gently for ~10 min per day and acclimated to the training cage for a period of one week.

Stage 2 was designed to associate the reward port with water reward. During this stage, rats were placed in the discrimination chamber with a passage to the time-out chamber blocked. A single reward port was placed at the middle of the discrimination

chamber and water was manually dispensed whenever rats were in close proximity to the reward port. Rats usually noticed and drank from the reward port on the first day. This stage was continued for 1 – 2 additional days.

Stage 3 was designed to teach rats to voluntarily nose poke in the reward port to obtain water reward. This stage had the same cage configuration as stage 2, except reward delivery was triggered automatically whenever the nose was inserted into the reward port. Once a rat learned to nose poke voluntarily, they were placed in the time-out chamber at the beginning of each trial. Trials were initiated when the door was opened, allowing access to the reward port in the discrimination chamber. Rats were ushered back to the time-out chamber after each water reward, and training continued as long as motivation was high (typically ~50-70 trials in ~30-45 minutes). This stage lasted 2 – 3 days.

Full contingency was introduced in stage 4. Textures were placed at the center edge of the discrimination platform with no gap, and two reward ports were placed on either side of the textures. Rats were placed in the time-out chamber at beginning of each trial, and the door was opened to allow access to the discrimination chamber. Rats only received rewards for choosing the reward port on the side of the S+ texture. Stage 4 was continued until rats learned the contingency, which lasted 1 – 2 months. Stage 5 was identical to stage 4, except the gap was widened to a final distance of 9.0 – 9.5 cm in order to promote exploration of the textures only with the macrovibrissae. Video analysis of each trial showed that at 9.0 – 9.5 cm gap distance, rats positioned their nose < 2 mm from the texture in 50-80% of trials (these were trials in which nose or microvibrissa contact could have occurred), while in 20-50% of trials, the nose remained ≥ 2 mm from

the texture, with contact made by macrovibrissae (see below for more details). Training was switched to dark when rats learned the contingency. Trials were monitored manually using IR goggles.

Behavioral Setup 2: Outline

In Behavioral Setup 2, rats were trained on a two-alternative forced choice gap crossing task using a modified apparatus based on the Guic-Robles et al (Guic-Robles et al., 1989) (figure 2.2). This apparatus consisted of a figure eight-like platform maze, constructed of plexiglass and with low (1-cm) plexiglass walls in all locations except the jumping edge of the launch platform and the landing edge of the reward platform. The maze was elevated 30 cm above a base platform containing control electronics and infrared video elements. Training was conducted in a light-proof room from the very beginning.

Rats were placed on top of the launch platform at the beginning of each training session. Rats initiated each trial by activating a floor sensor on the launch platform (Floor sensor was made from a set of IR LED (λ = 890 nm) and phototransistor pointed upwards. Phototransistors were activated by capturing the reflected IR light whenever rats passed over the LEDs). Rats were required to lean across the gap to palpate two sandpaper textures (a rough sandpaper which served as S+ and a smoother sandpaper that served as S-, see below) placed on the ledge of the reward platform. Textures were raised 0 – 1.0 cm above the reward platform. The gap was incorporated to ensure that only the macrovibrissae contacted the surfaces during texture discrimination and to eliminate possible nose or paw contact. Once textures were sampled, rats made a choice by

jumping onto either the left or the right half of the reward platform, which were separated by a plexiglass wall. The rat's choice was detected by landing sensors on the reward platform. Reward was only given when the rat jumped to the side that the S+ texture was presented. A correct choice was rewarded with 100 µl of water, whereas an incorrect choice was presented with a discrete tone (default tone, beep.vi LabView subprogram). Rats were then required to run around the left or the right arm to return back to the launch platform to initiate the next trial. Textures were interchanged automatically between trials, while the rat was running back down the left or right return arm (in response to the rat passing over a floor sensor in each return arm). Trials continued as long as rats were motivated. Training sessions usually lasted 30-45 min and with ~80-120 trials. A video camera was placed below the gap to record whisker and head motion as the rat sampled the textures. The cage was operated using custom routines in LabView, and National Instruments DAQ boards. Data was analyzed offline with MATLAB.

Behavioral Setup 2: Stimuli

Textures were made from two different 6 cm x 18 cm strips of commercial sandpaper (3M, wetordry) taped side-by-side on a 12 cm x 18 cm acrylic plate. Texture combination of P120 versus plastic was used initially. Texture combination was changed to P150 versus P800 at later stages. Additionally, this plate was attached perpendicular to the rod of the rotating stepper motor allowing the textures to be randomly interchanged between every trial (figure 2.3B). The textures were interchanged in the same way as described for Behavior Setup 1.

Behavioral Setup 2: Training Stages

Training was divided into 5 stages. During stage 1, rats were handled for ~10 min daily and acclimated to the training cage for a period of 1 week.

Stage 2 was used to train the rats to associate reward ports with water reward. Rats were trained in the dark starting this stage. Trials were monitored manually using IR goggles. During this stage, passageways to both left and right arms and to the reward platform were blocked, and a single reward port was placed at the end of the launch platform. Rats were placed on the launch platform and water was dispensed manually when approaching the reward port. Rats noticed and drank form the reward port on the first day, and this stage lasted 1 – 2 additional sessions.

Stage 3 was designed to teach rats to voluntarily nose poke in the reward port to receive a water reward. This stage had the same cage configuration as stage 2, except the reward port was triggered automatically when the nose was inserted into the reward port. Water reward was triggered once (60 msec, 100 μl) for every nose poke. To obtain additional water reward, rats were required to pull their nose out and poke with their noses again. Each session was repeated until rats were no longer motivated. Stage 3 lasted 1 – 2 additional sessions for most rats. 3 out of 15 rats failed to consistently drink from the water port and were removed from training.

In stage 4, full contingency was introduced, using P120 sandpaper versus smooth plastic film, and rats had to both self-initiate trials and jump to the S+ reward platform to receive a reward. Doors that blocked the passageway to the left and right arms and the reward platform were removed. In initial training, we found that many rats failed to learn to run back down the return arms after receiving a water reward; thus, rats were manually

lifted from the reward platform after making their jump (either correctly or incorrectly), and were manually placed on top of the launch platform to start each trial. Rats required 2 weeks – 1 month to learn the task, and 6 out of the 12 remaining rats failed to learn and were removed from training. After rats were performing significantly above chance, the gap was widened to force whisker contact during texture sampling.

Video analysis

In both cages 1 and 2, a camera was placed below the gap to visualize whisker and nose movements during texture sampling. Videos were played using Windows Movie Maker and analyzed frame-by-frame. Any trials when the nose approached < 2 mm from the surface were considered nose/microvibrissa contact trials (figure 2.4A), whereas trials in which the nose remained ≥ 2 mm from the texture were considered macrovibrissa contact trials (figure 2.4B). Macrovibrissae could be clearly seen in the videos and any trials in which rats did not make any whisker contact with the textures were discarded from the analysis. Behavior performance for both behavior strategies was calculated independently.

2.3 Results

Performance in Behavioral Setup 1

Two rats, G5R1 and G5R2, were trained to discriminate between smooth versus 2 mm grooved aluminum plates using Behavior Setup 1 (Figure 2.5A, G5R1; Figure 2.5B, G5R2). Rats were initially trained in stages 1 – 4. Discrimination training began under dim light (Session 1 was defined as 1st day of stage 5 discrimination training).

Behavioral performance gradually improved over the course of training, reaching criterion of three consecutive daily performances above 0.80 (fraction of correct choices) (G5R1, session 30; G5R2, session 27), with a final gap distance of 9.5 cm for G5R1 and 9.0 cm for G5R2. Further training (25 sessions) produced no further improvements in performance. To eliminate possible visual cues, rats were trained in complete darkness starting at session 58. Performance immediately plunged to chance level, but rats regained criterion at faster rates compared to initial training under dim light. Performance eventually stabilized around 0.80 for both rats.

To test whether rats performed the discrimination using olfactory rather than tactile cues, texture surfaces were wiped with ethanol in roughly half of the trials in each daily session. Ethanol wipe was intended to reduce or eliminate olfactory cues that could have been deposited by nose contact, licking, urine, etc. Behavioral performance was calculated separately for ethanol wipe and non-wipe trials (Figure 2.6A top panel, G5R1; Figure 2.6A lower panel, G5R2). Ethanol wipe had no effect on performance for either G5R1 or G5R2 (G5R1: Fig 2.6B upper panel, fraction correct trials = 0.80 ± 0.05, $n = 9$ sessions; non-wipe trials: 0.84 ± 0.05, $n = 9$ sessions, $p > 0.5$; G5R2: Fig 2.6B lower panel, wipe trials: 0.82 ± 0.02, $n = 10$ sessions; non-wipe trials: 0.73 ± 0.06, $n = 10$ sessions, $p > 0.1$). This suggests that discrimination was not based on olfactory cues.

Since the goal was to train rats on a vibrissa-dependent texture discrimination paradigm, trials in which rats made bodily contact, such as with the nose and paws, were distinguished from trials in which rats made pure whisker contact. Trials were videotaped from below the gap to determine how close rats approached the textures. Trials in which the nose reached < 2 mm from surface were considered nose contact trials, while trials in

which the nose was ≥ 2mm away were considered whisker contact trials. Nose contact trials could include nose, lips, jaw or microvibrissa contact. Trials with paw contact were rare and excluded from analysis. Both rats performed significantly better during nose contact trials compared to whisker contact trials (G5R1: 0.89 ± 0.03, $n = 9$ sessions; whisker contact trials: 0.76 ± 0.05, $n = 7$ sessions, $p < 0.03$; G5R1: nose contact trials: 0.88 ± 0.03, $n = 10$ sessions; whisker contact trials: 0.70 ± 0.04, $n = 10$ sessions, $p < 0.002$, Fig 2.7). However, performance during whisker contact trials was significantly above chance for both rats (G5R1: $p < 0.002$; G5R2: $p < 0.0005$, single group t-test). Thus, rats can discriminate textures by solely employing their whiskers (whisker contact trials), though their performance is better when tactile contact with nose or microvibrissae occur (nose contact trials).

Performance in Behavioral Setup 2

Even though both rats learned the task in Behavioral Setup 1, the semi-automated training strategy required intense manual interaction and monitoring. To increase efficiency, Behavioral Setup 2 was developed. This setup involved a gap-crossing task on a narrow launch platform to constrain the rat's approach to the textures. Rats were required to make a gap-crossing response by jumping onto the correct platform. Rats could then return to the launch platform to initialize a new trial. Texture combination of P120 versus plastic film was used during the initial training stages and switched to P150 versus P800. Rats were initially trained on blocks of trials in which S+ was consistently located to the right (or left) for 5-10 trials. This block training was incorporated to break the habit of rats jumping to only one side of the platform. Once rats started jumping to

both sides equally (i.e., recognizing and following the block structure), texture stimuli were changed randomly with each trial for the remainder of training. Gap width was constantly monitored to limit nose contact trials. For most rats, a gap was not introduced on the first session of stage 4 (except G9R1 = 2.0 cm, B1R1 = 7.5, B1R2 = 6.0 cm, B1R4 = 8.0 cm, B1R6 = 7.5, gaps were introduced in earlier stages for these rats). Final gap width differed according to size and motivational level of individual rats (G9R1 = 13.0 cm, G11R1 = 12.5 cm, G11R3 = 9.0 cm, B1R4 = 10.0 cm, B2R2 = 12.0 cm, B2R3 = 12.0 cm) (Figure 2.8 and 2.9, red traces).

A total of 15 rats were trained using Behavioral Setup 2. Six out of 15 rats reached criterion of three consecutive daily performances above 0.80 with final gap width described above (Figure 2.8, G9R1 = session 15; G11R1 = session 10; G11R2 = 10 session; B1R4 = 13 session; B2R2 = 65 session, B2R3 = 39 session). Video analysis confirmed that rats were mainly using their whiskers with occasional nose contact, except for G11R1 and G11R3 which relied on their paws during discrimination. Two types of learning were observed – one group displayed rapid learning, while other group showed long gradual improvement over many sessions. Behavioral performance fluctuated as gap width was changed (Figure 2.8 red traces). Daily trial numbers remained stable, ranging between 50 – 100 trials a day, depending on the motivational level of individual rats (Figure 2.8 green traces).

The six out of the nine remaining rats never learned to discriminate with their whiskers (Fig. 2.9). The reasons varied: 1) G11R2 performed at criterion, but was using its paws during discrimination and was removed from training. 2) B1R1, B1R6, B2R1, and B3R4 were too hesitant to cross the gap, which can be seen from low trial numbers

per session. Gap width was decreased to minimize the rat's fear of gap crossing, yet it was still unsuccessful. 3) B1R2 completed trials without displaying fear, but never reached performance above chance because this rat only jumped to one side. This habit was not corrected even on block trials. 4) Three additional rats never learned the basic behavior strategy required for stage 4 when full contingency was introduced (Data not shown). These rats learned training in stages 1 – 3, but not 4.

2.4 Discussion

Behavioral Setup 1

Results from Behavioral Setup 1 suggest that whisker information was significantly contributing to texture discrimination. Behavior performance was collected when rats reached training stage 4. Training was initially conducted under dim light with performance slowly but eventually reaching criterion performance of three consecutive days above 0.80 correct. Lights were turned off to prevent use of visual cues. Performance dropped immediately to chance level, suggesting these rats were using visual information in dim light (Figure 2.5). However, these rats relearned the task by reaching criterion performance at faster rates compared to initial training. Steeper learning rates may suggest that these rats can translate previously learned visual information to tactile information. Faster learning rates may also be due to retention of required motor behavior, which may have already been hard-wired to a behavior pattern. Therefore, visual information was not used during this task.

Olfaction is arguably the most important cue that must be assessed, since there remains a possibility that rats were using scent traces (saliva, nasal fluid, urine, etc.) on

texture surfaces. Olfaction was controlled by wiping the textures with ethanol during half of each training session. Behavioral performance did not change between ethanol wipe and non-wipe trials for both rats, reducing the possibility of olfactory cues influencing discriminatory performance.

Auditory cues were controlled by moving the motor in two 90º steps. This procedure was taken to equalize the duration of motor rotation when textures were switched between trials. Since rats learned the task, it is clear that motor rotation was not contributing to performance. However, spectral analysis was not conducted to carefully analyze whether there was any significant difference between motor rotations during clockwise or counter-clockwise directions. Rats may have caught some slight differences in the auditory patterns and may have used it during discrimination. A remaining possibility is that rats can detect texture by sounds induced by whisker motion across surfaces. Such whisker-induced auditory discrimination has not been reported in literature, though it may be possible. This possible cue could have been controlled by training rats in white noise to mask sounds, although this was not carried out in our experiments.

To determine whether rats can discriminate textures solely using their vibrissae, trials were videotaped to determine how close rats approached the textures. The distance from the nose to the textures was measured to determine whether rats made nose or pure whisker contact. Performance was calculated individually, which revealed that nose contact trials were significantly better compared to trials with whisker contact alone. This suggested that the combination of whiskers and skin contact can extract texture information with higher resolution compared to whisker contact alone. Even though nose

contact trials were better than pure whisker contact trials, whisker contact trials were significantly above chance performance, confirming results of other studies that rats can rely on whiskers for texture discrimination (Carvell and Simons, 1990, 1995, 1996; Guic-Robles et al., 1992; Guic-Robles et al., 1989).

Even though rats learned to discriminate textures using Behavioral Setup 1, several problems were encountered throughout training. One problem stemmed from the fact that the water ports were readily available adjacent to the textures. As a result, rats occasionally approached water ports directly without sampling the textures, apparently accepting the 50% random chance of getting the trial correct. Second, even though a gap separated the platform and the textures, rats were still able to make contact with their nose. When the gap distance was made larger (which would have reduced the number of nose contact trials), rats tended to ignore the texture and went straight to the reward port. Finally, this training cage required direct monitoring and interaction with the rats, which made the training laborious for the experimenter. These problems made the training procedure difficult to conduct and analysis troublesome, which became the main motivation for developing Behavioral Setup 2.

Behavioral Setup 2

Behavioral Setup 2 was aimed to automate training and to increase efficiency in presenting the stimuli by incorporating a gap-crossing task. This cage was designed after Guic-Robles et al. because of three predicted advantages (Guic-Robles et al., 1992; Guic-Robles et al., 1989). First, textures were placed directly in front of the opposing ledge so the gap-crossing task forced rats to encounter the textures before making a behavioral

response, which was thought to resolve the problem of allowing direct access to the reward port. Second, gap-crossing tasks have been shown to encourage high amplitude whisking cycles to locate the opposite platform and was predicted that forced whisking would facilitate texture detection. Third, since the motivation was in developing a vibrissae dependent texture discrimination paradigm, adjustment of the gap width would ensure that only vibrissae contacted the textures on most trials.

Behavioral performance was collected once rats reached training stage 4. Two types of learning were observed from rats that reached criterion performance – rapid learning (G9R1, G11R1, G11R3, and B1R4), and slow learning (B2R2, B2R3) (Figure 2.8). Rapid learners required 13 – 16 sessions to reach criterion, while slow learners required 39 and 66 sessions. Fast learners were comparable to the previous reported sandpaper discrimination studies, which required 9 – 30 sessions to reach criterion performance (Cybulska-Klosowicz and Kossut, 2001; Guic-Robles et al., 1992; Guic-Robles et al., 1989). Slow learners required additional sessions to reach criterion performance, demonstrating that learning varies across rats. Additionally, two learning trends were observed during the course of training as previously reported (Cybulska-Klosowicz and Kossut, 2001). Some rats exhibited progressive improvement over several sessions until reaching criterion (G11R1, G11R3, B2R2, and B2R3), while others fluctuated around near chance performance until rapid learning occurred (G9R1, B1R4). Notably, grooved cylinder discrimination studies had reported that rats only required an average of 4.5 sessions to reach criterion (Carvell and Simons, 1990, 1995), which was shorter than any other sandpaper discrimination studies including ours.

Gap width for these rats was increased as training progressed, and final gap distance differed for each rat. Two factors determined the final gap width during training. First was rat size – larger rats required wider gaps while smaller rats required narrower gaps to isolate whisker contact during discrimination. Rats were not able to palpate the texture if the gap was too wide and frequently made nose contact if the gap was too narrow. The second factor was their motivational level. Highly motivated rats completed trials with wider gaps while narrower gaps were required for unmotivated rats. Highly motivated rats will even cross at distances that can barely be crossed or reached with their whiskers. In contrast, unmotivated rats were too cautious and never crossed with gaps too wide. These rats simply stepped over the gap instead of attempting to jump. The combination of these two factors made the gap difficult to perfect for individual rats. Previous gap crossing tasks using the rat vibrissae system have reported 15 – 18 cm wide gaps (Hutson and Masterton, 1986; Jenkinson and Glickstein, 2000), which was wider than those in our current study (10 – 13 cm). This discrepancy may be due to older and larger rats used in previous reports. Food, instead of water regulation may have also lead to higher motivation for crossing with wider gaps. G9R1, B1R4, B2R2, and B2R3 performed mainly using whiskers and occasionally made nose contact. From videography and manual observations, G11R1 and G11R3 were suspected to be using their paws, possibly because the gap was too small. It is therefore questionable whether the data from these two rats reflects real vibrissa-dependent performance.

The remaining rats never reached criterion performance, mainly due to fear and hesitant behaviors of navigating through the cage. Some rats displayed slow motor patterns with low trial numbers (B1R2, B3R4), while some stopped behaving completely

(B1R2, B1R6, and B2R1). Several rats did not learn in stages 1 – 3, even before behavior performances were measured (no data, since rats did not reach stage 4). The major problem for these rats was again, unsuccessful completion of the gap- crossing task. Rats quickly became hesitant to approach the gap when the gap was too wide. Occasional falls from the elevated platform further intensified fear. Gap width was narrowed to ease the fear of crossing, in which case rats performed trials slowly and gradually if the gap was small enough. However, their hesitant behavior reemerged as gap width widened again. Fear contributed heavily to their motivational states, which was not reversible. Once hesitant, rats only crossed the gap if distance was narrow enough so they could place their forepaws to confirm the existence of the platform. Previously reported-gap crossing tasks did not report these difficulties associated during gap-crossing, such as falling and refusing to complete trails. Since the majority of rats from the previous studies completed and learned the task, it is of interest why half of the rats could not learn the task.

Training rats on vibrissa-dependent tactile discrimination training has been challenging compared to discrimination studies in other sensory systems. Since our stimuli are physical objects, it cannot be applied passively to the animals. This training cage required animals to engage and explore to actively locate the stimuli. Furthermore, animals have to recognize slight difference in texture properties and make a risky behavior response. By using the current setup, we were able to increase the total daily trial numbers by factors of 2 to 3, but had to sacrifice success rates of rats accomplishing the task. Therefore, further improvements are needed to increase consistency in training rats to discriminate textures.

2.5 References

Aggestam, F., and Cahusac, P.M. (2007). Behavioural lateralization of tactile performance in the rat. Physiol Behav *91*, 335-339.

Carvell, G.E., and Simons, D.J. (1990). Biometric analyses of vibrissal tactile discrimination in the rat. J Neurosci *10*, 2638-2648.

Carvell, G.E., and Simons, D.J. (1995). Task- and subject-related differences in sensorimotor behavior during active touch. Somatosens Mot Res *12*, 1-9.

Carvell, G.E., and Simons, D.J. (1996). Abnormal tactile experience early in life disrupts active touch. J Neurosci *16*, 2750-2757.

Cybulska-Klosowicz, A., and Kossut, M. (2001). Mice can learn roughness discrimination with vibrissae in a jump stand apparatus. Acta Neurobiol Exp (Wars) *61*, 73-76.

Guic-Robles, E., Jenkins, W.M., and Bravo, H. (1992). Vibrissal roughness discrimination is barrelcortex-dependent. Behav Brain Res *48*, 145-152.

Guic-Robles, E., Valdivieso, C., and Guajardo, G. (1989). Rats can learn a roughness discrimination using only their vibrissal system. Behav Brain Res *31*, 285-289.

Hutson, K.A., and Masterton, R.B. (1986). The sensory contribution of a single vibrissa's cortical barrel. J Neurophysiol *56*, 1196-1223.

Jenkinson, E.W., and Glickstein, M. (2000). Whiskers, barrels, and cortical efferent pathways in gap crossing by rats. J Neurophysiol *84*, 1781-1789.

von Heimendahl, M., Itskov, P.M., Arabzadeh, E., and Diamond, M.E. (2007). Neuronal activity in rat barrel cortex underlying texture discrimination. PLoS Biol *5*, e305.

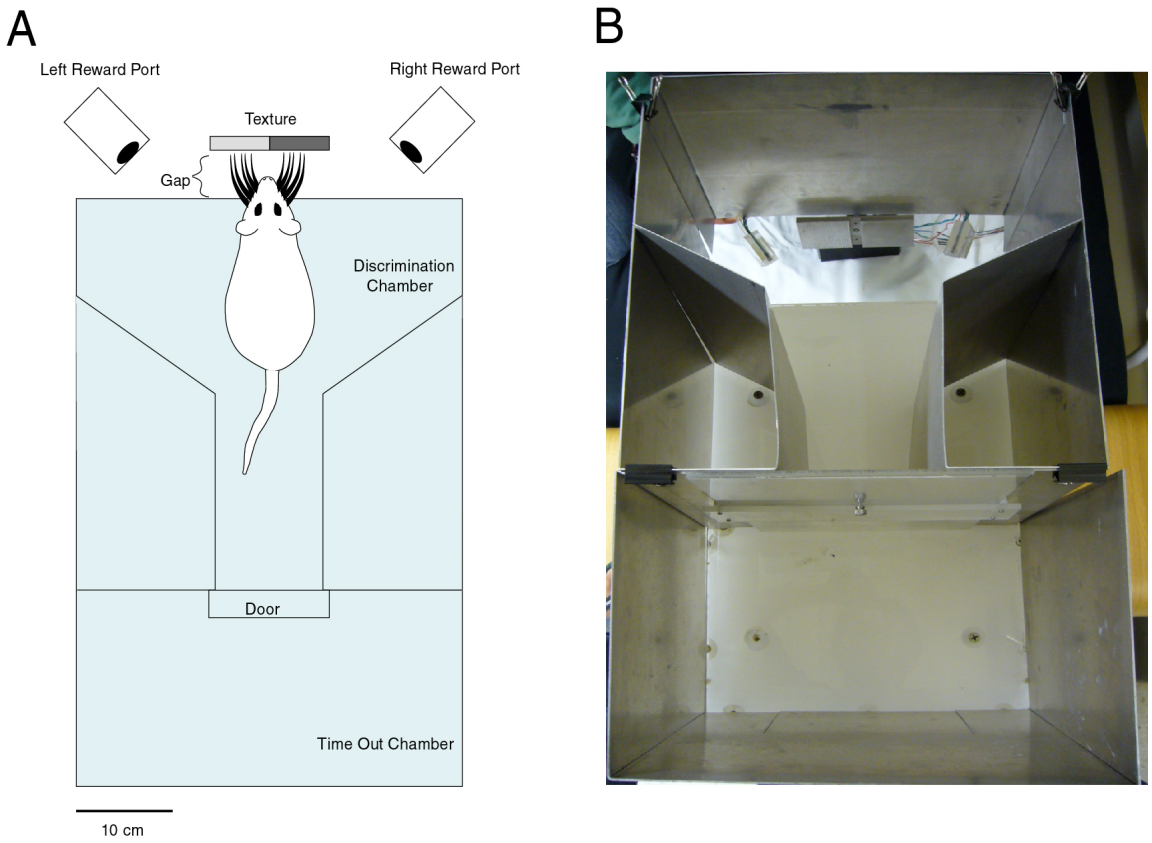


Fig 2.1. Behavioral Setup 1. A) Schematic diagram of the training cage used for Behavioral Setup 1. B) Actual training cage.

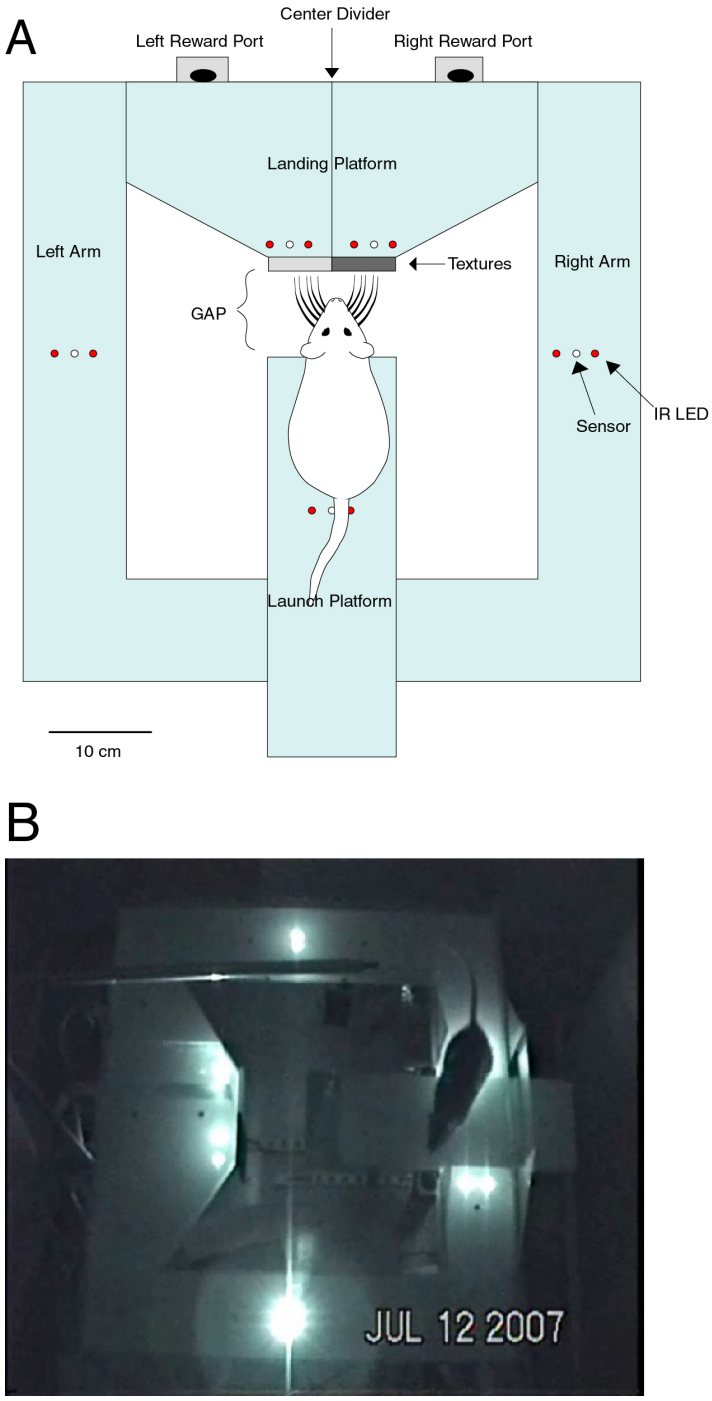
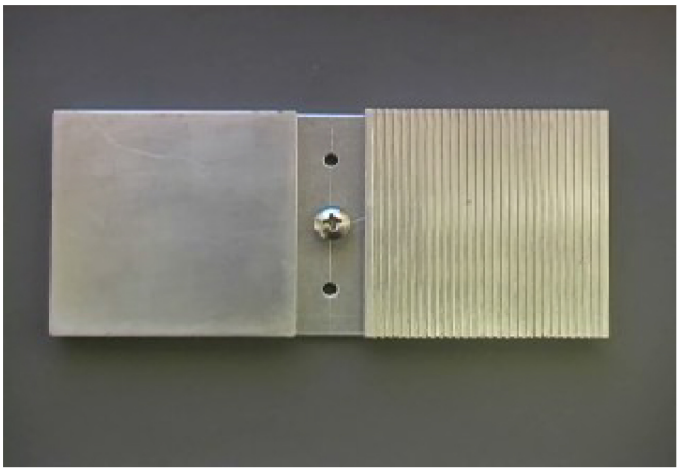


Fig 2.2. Behavioral Setup 2. A) Schematic diagram of the training cage used for Behavioral Setup 2. B) Actual training cage.

A



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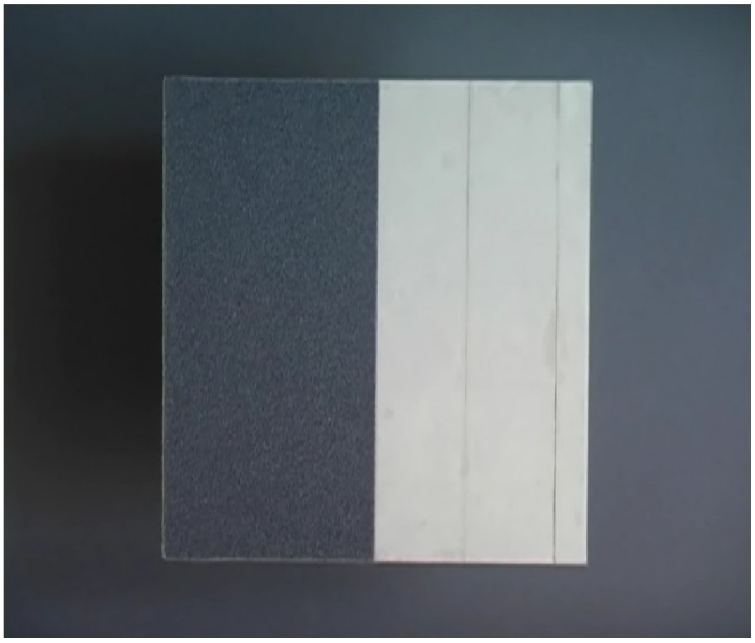

Fig 2.3. Textures. A) Textures used in Behavioral Setup 1. 2 mm grooved versus smooth aluminum surfaces were used. Grooved surface served as S+, while smooth surface was S-. B) Textures used in Behavioral Setup 2. Rough versus smooth sandpapers were used. Rough sandpaper was S+, while smooth sandpaper was S- (P120 versus plastic film shown).

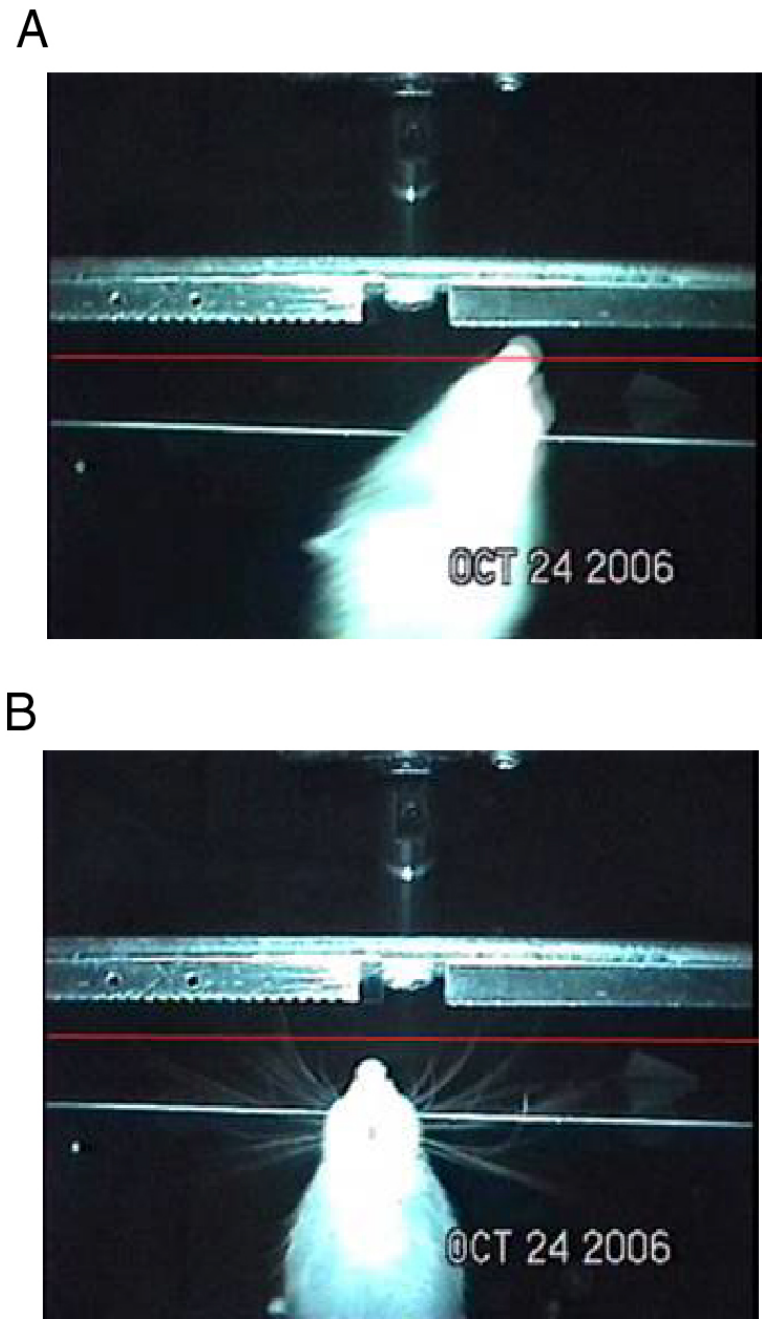

Fig 2.4. Nose versus pure whisker contact trials. A) Trials when the nose approached < 2 mm (red line) from the surface were considered nose/microvibrissa contact trials. B) Trials in which the nose remained ≥ 2 mm from the texture were considered macrovibrissa contact trials.

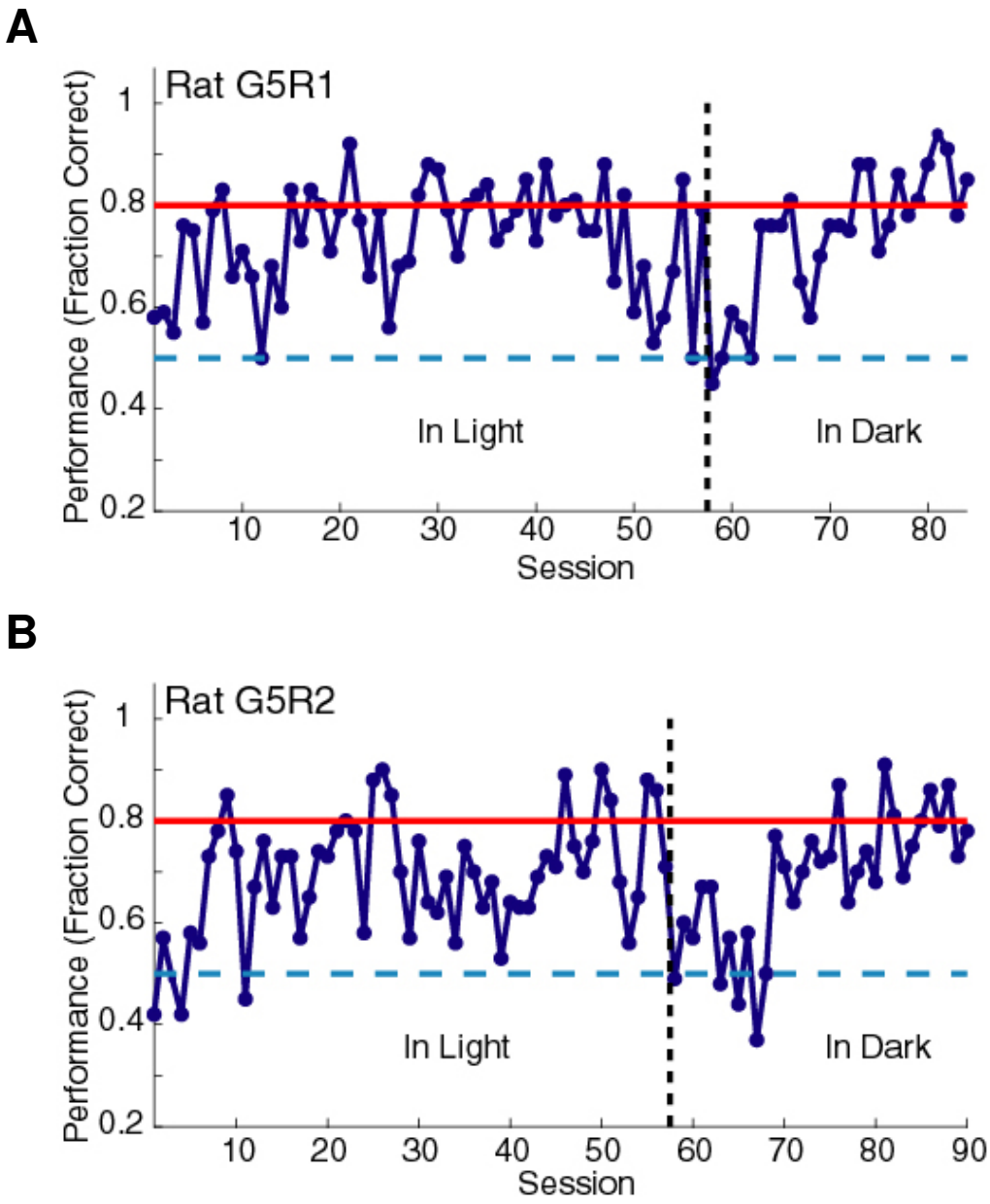

Fig 2.5. Learning curves for rats in Behavioral Setup 1. Rats were initially trained under dim light, and the training was changed to dark starting session 58. Session 1 was defined as the first training session of stage 4. Dotted line at 0.5 indicates chance performance.

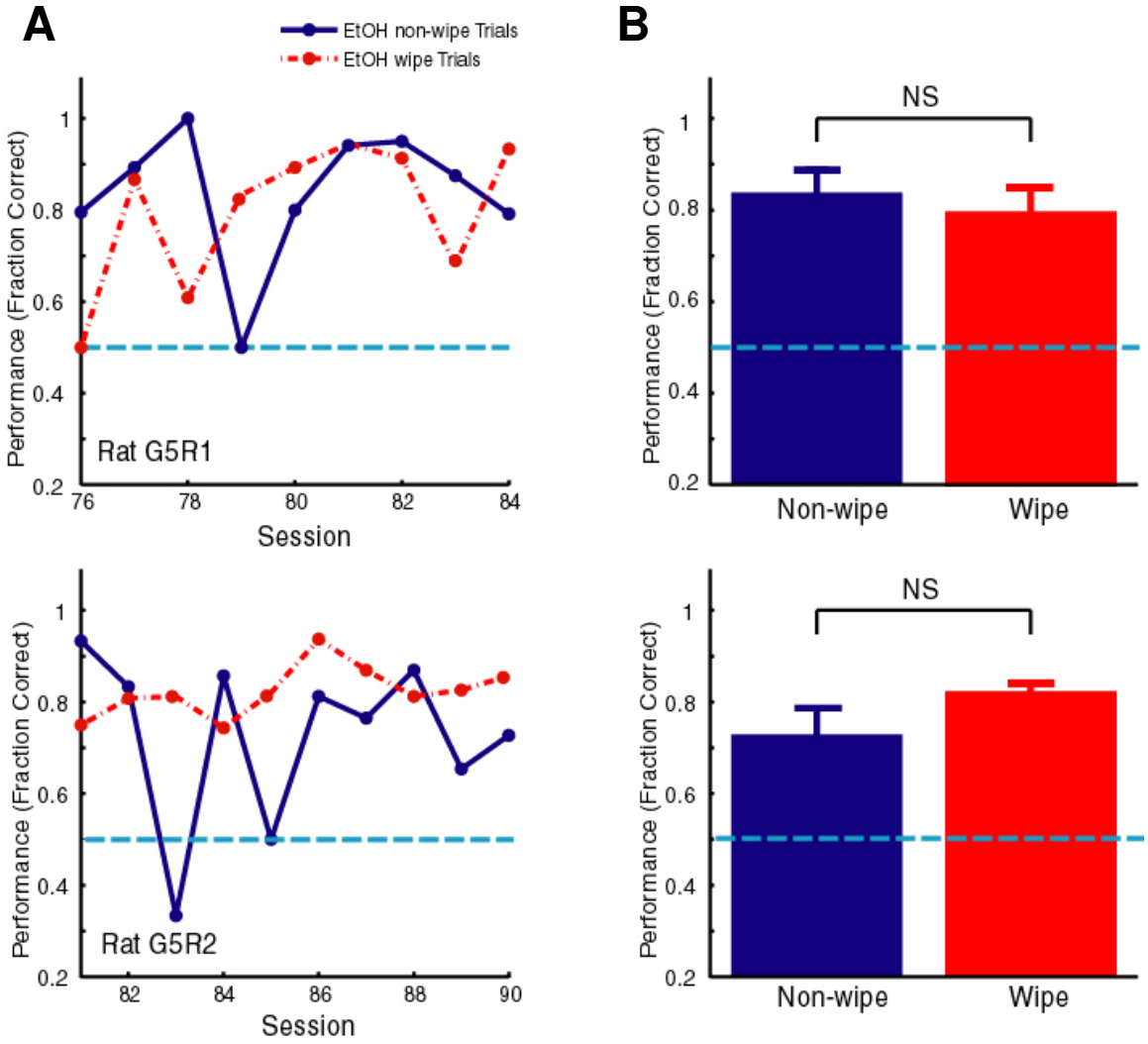

Fig 2.6. Olfactory control. Olfaction was controlled for G5R1 and G5R2 on Behavioral Setup 2. Performance was calculated separately for ethanol wipe and non-wipe trials. A) Raw learning curve for both ethanol wipe and non-wipe trials (G5R1: top panel, G5R2: bottom panel). B) Quantified mean performance for both ethanol wipe and non-wipe trials (G5R1: top panel, G5R2: bottom panel). Error bars are SEM.

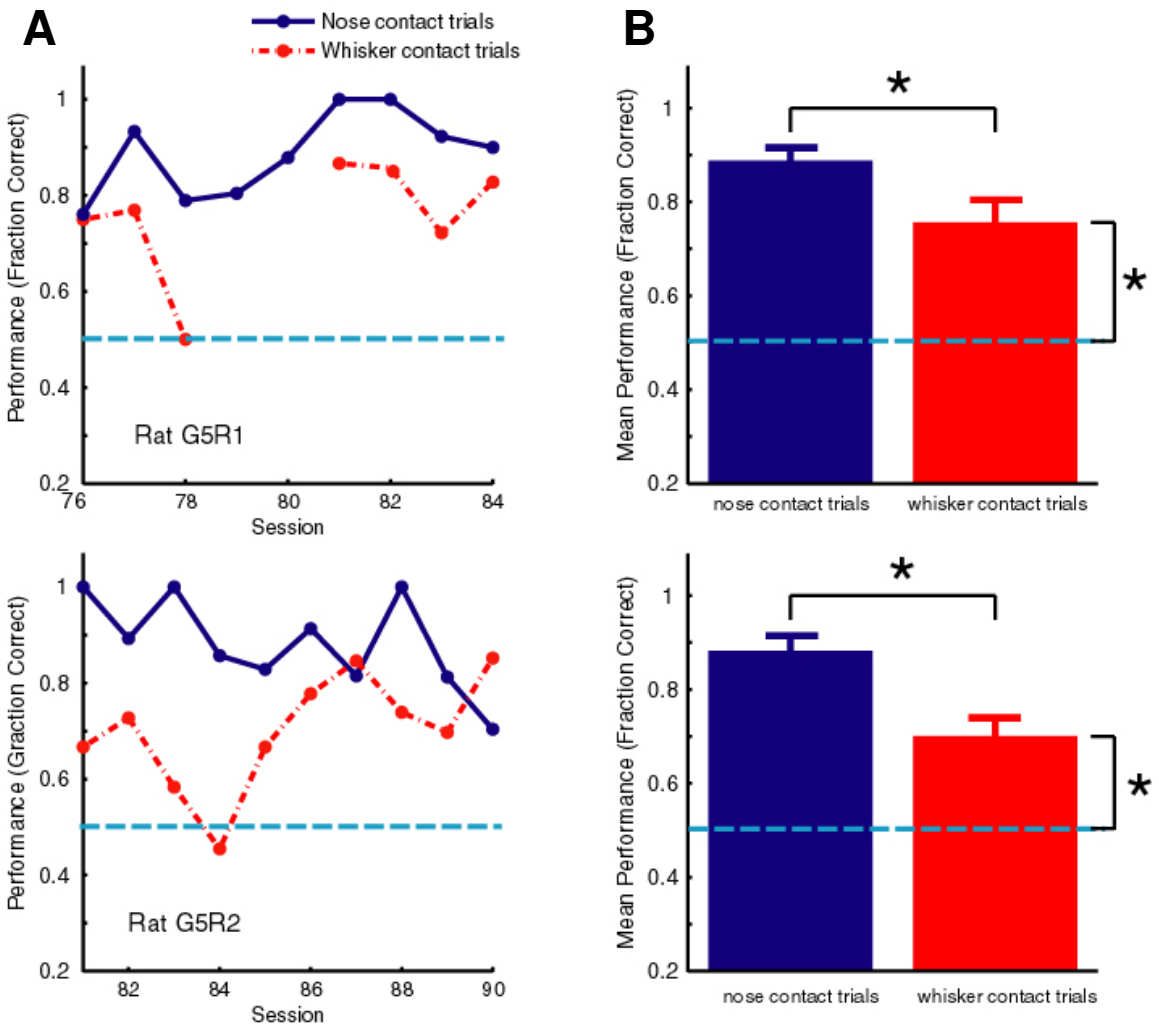

Fig 2.7. Behavioral performance during nose and whisker contact trials. Performance was calculated separately for nose and whisker contact trials. A) Learning curves for G5R1 (top panel) and G5R2 (bottom panel). Sessions 79 and 80 from G5R2 are missing because whisker contact trials were not seen. B) Quantified mean performance for both nose and whisker contact trials (G5R1: top panel, G5R2: bottom panel).

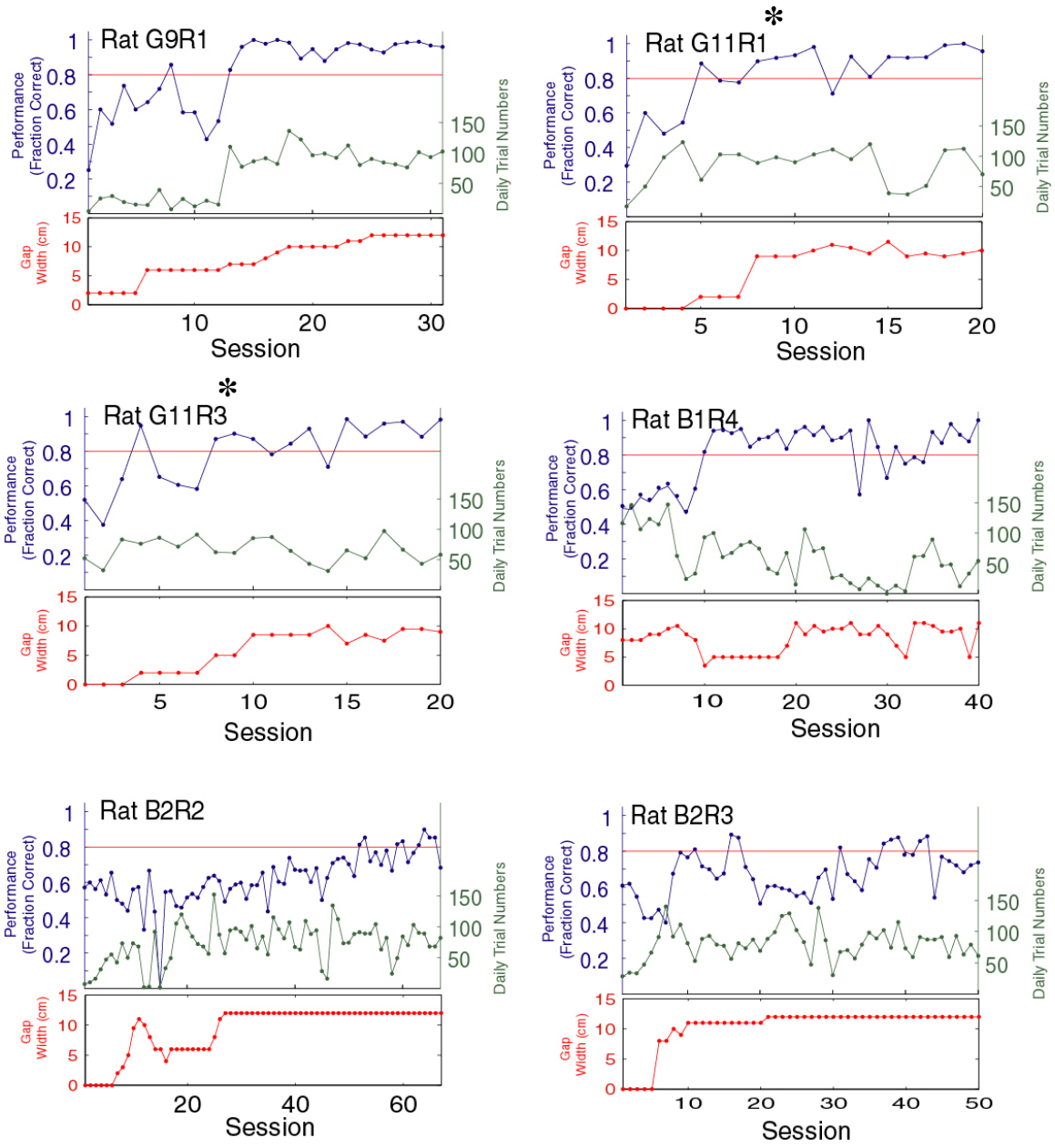

Fig 2.8. Learning curves for successful rats in Behavioral Setup 2. Texture combination of P120 versus plastic film was used for all rats during initial training. Session 1 is defined as the first day of stage 5, when all contingency was introduced. G11R1 and G11R3 performed trials mainly using their paws (*), while others were discriminating with nose/whiskers.

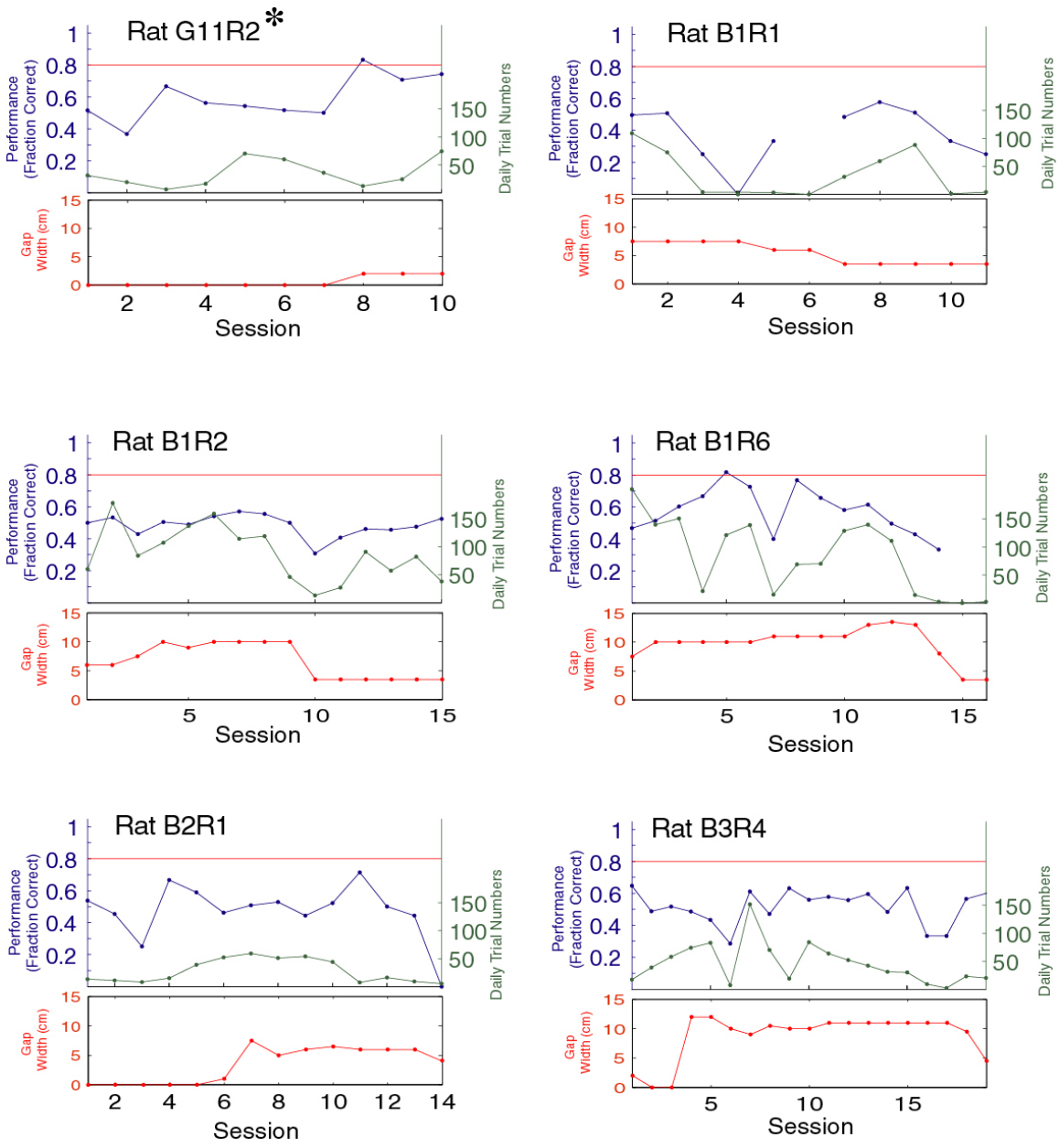

Fig 2.9. Learning curves for unsuccessful rats in Behavioral Setup 2. Texture combination of P120 versus plastic film was used for all rats during initial training. Session 1 is defined as the first day of stage 5, when all contingency was introduced. G11R2 performed at near criterion, but was found by video analysis to be using its paws (*). Session 6 for B1R1 and sessions 15 and 16 for B1R6 are missing because rats did not complete any trials.

Chapter 3: Acuity and role of micro vs. macrovibrissae in texture discrimination

3.1 Introduction

The highly sensitive and motile vibrissa system has intrigued many researchers in its ethological roles since the seminal study by Vincent in 1912. Numerous studies have demonstrated the rodent vibrissa system as superb tactile sensors for texture discrimination. However, as reviewed in Chapter 1 of this thesis, two major questions about the function of the whiskers remain.

The first question is the functional role of the two distinct vibrissa subsystems, the macrovibrissa and the microvibrissa. A first hypothesis suggested that microvibrissae detect fine features of objects, while macrovibrissae detect gross spatial location of objects (Brecht et al., 1997). In contrast, a second hypothesis posits that the microvibrissae are "spacers" that are used to position the face at a close, stereotyped distance from an object, while the macrovibrissae detect fine object features including texture (Carvell and Simons, 1990). The study supporting the first hypothesis, though highly controlled and ethologically realistic, focused on the role of microvibrissae in detecting object shape, but did not test whether microvibrissae also sensed surface texture. In the studies supporting the second hypothesis, microvibrissae were not trimmed, and therefore it is still unknown whether macrovibrissae alone can perform fine texture discrimination.

The second unanswered question is the acuity of the vibrissa system in discriminating fine textures. Carvell and Simons demonstrated that rats can discriminate a series of fine grooved cylinders (~15 μm vs 500, 250, 200, 150, 100, 75, 50 μm) with

resolution as small as 50 µm (Carvell and Simons, 1990, 1995). However, psychophysical discrimination limits for sandpapers, which are extensively used for texture discrimination behavior, have not been measured. Here I addressed these questions using rats trained on the texture discrimination tasks presented in Chapter 2. The first question was approached by systematically removing the microvibrissae to determine whether macrovibrissae alone are sufficient to support fine texture discrimination. The second question was approached by testing discrimination of varying pair of sandpapers, with the rat trained to distinguish the rougher from the smoother sandpaper. This allowed measurement of the psychophysical limit of texture discrimination using sandpapers.

3.2 Materials and methods

Behavior Training

Data was obtained from rats trained on texture discrimination in Behavioral Setups 1 and 2, as described in Chapter 2. (Chapter 2 described the initial training of these rats; the present chapter describes subsequent experiments, in the same animals, to test the involvement of macrovibrissae versus microvibrissae in texture discrimination, and the psychophysical curve for sandpaper texture discrimination).

Whisker Trimming

Microvibrissa trimming was performed by trimming all vibrissae except the macrovibrissae (defined here as alpha through delta, and arcs 1 – 4 from rows A – E) on both sides. Macrovibrissa trimming was performed after microvibrissa trimming. For rats

G5R1 and G5R2, all macrovibrissae were trimmed at once. For rat G9R1, subsets of macrovibrissae were trimmed sequentially: first trimming all but the C-row of whiskers, then trimming all but C3 on each side, then trimming all but C3 on the right side, and then trimming the final C3 whisker so that no whiskers were left intact. Throughout these trimming stages, microvibrissae were trimmed every 2 – 3 days to prevent their regrowth.

Textures

In Behavioral Setup 1, 2 mm grooved versus smooth aluminum plates were used as described in Chapter 1. In Behavioral Setup 2, two 6 cm x 18 cm sandpapers with different mean grit diameter were taped onto a 12 cm x 18 cm acrylic plate using double-sided tape (Fig 2.3B). Several texture combinations were use to assess the psychophysical limit for texture discrimination (from roughest to smoothest difference) – P120 versus smooth plastic film, P120 versus P1500, P150 versus P1500, P150 versus P800, P150 versus P400, P150 versus P150.

3.3 Results

Requirements of micro versus macrovibrissae in texture discrimination

To test whether microvibrissae are necessary for texture discrimination, we trimmed the microvibrissae in rats performing texture discrimination in both behavioral setups. For Behavioral Setup 1, 2 rats, G5R1 (Figure 3.1A) and G5R2 (Figure 3.1B) were trained to discriminate grooved versus smooth aluminum plates. Microvibrissae were trimmed from both rats after behavior performances stabilized around 0.80 with all whiskers intact (Figure 3.1A, G5R1 = session 85; Figure 3.1B, G5R2 = session 90).

Videography was used to identify trials in which the nose remained ≥ 2 mm from the texture throughout the trial, which were considered macrovibrissa contact trials, and trials with the nose < 2 mm from the texture surface, which were considered nose and/or microvibrissa contact trials. Performance was calculated individually for each types of trial. Trials were removed from the analysis if rats directly approached the reward port without sampling the textures.

Behavior performances from all trials, including both nose and whisker contact did not change after microvibrissa trim in either G5R1 (Fig 3.2A, 0.79 ± 0.03, $n = 7$ sessions; pre-trim controls: 0.85 ± 0.03, $n = 7$ sessions, $p > 0.2$), or G5R2 (Fig 3.2A, 0.76 ± 0.02, $n = 10$; pre-trim controls: 0.81 ± 0.02, $n = 9$, $p > 0.1$). In nose contact trials, microvibrissa trim did not affect performance in rat G5R1 (Fig 3.2B, 0.85 ± 0.02, $n = 7$ sessions; pre-trim controls: 0.90 ± 0.04, $n = 7$sessions, $p > 0.2$), while performance significantly dropped for G5R2 (Fig 3.2B, 0.76 ± 0.03, $n = 10$ sessions; pre-trim controls: 0.90 ± 0.03, $n = 9$ sessions, $p < 0.02$). In whisker contact trials, post-microvibrissa trim performance was unaltered for G5R1 (Fig 3.2C, 0.76 ± 0.04, $n = 7$ sessions; pre-trim controls: 0.76 ± 0.05, $n = 7$, $p > 0.9$), and significantly improved for G5R2 (Fig 3.2C, 0.77 ± 0.02, $n = 10$ sessions; pre-trim controls: 0.68 ± 0.04, $n = 9$ sessions, $p < 0.03$). For both rats, performance in whisker contact trials after microvibrissa trim was significantly above chance (Fig 3.2 C, G5R1, $p < 0.0003$; G5R2, $p < 1.6 \times 10^{-12}$, single-group t-test versus 0.5), indicating that rats can discriminate texture differences using only the macrovibrissae. Therefore, microvibrissae are not necessary during texture discrimination, but their contribution varies across rats and sampling strategies.

In one rat (G5R1), we further confirmed that texture discrimination was being performed using the macrovibrissae by measuring behavioral performance before and after macrovibrissa trim. This experiment was performed after microvibrissa trim, when performance was stable around 0.80. The entire macrovibrissa array was trimmed (Figure 3.1A, macrovibrissa trimmed at session 92). G5R1 used a combination of nose and whisker contact trials. On presumed whisker contact trials (distance from nose to texture ≥ 2 mm), macrovibrissa trim caused performance to drop significantly, as expected if the rat had been using the macrovibrissae to discriminate texture on these trials (Fig 3.3C, 0.63 ± 0.04, $n = 9$; pre-trim controls: 0.76 ± 0.04, $n = 7$, $p < 0.03$). In contrast, performance was unaltered in presumed nose contact trials (nose to texture distance < 2 mm) (Fig 3.3B, 0.85 ± 0.02, $n = 9$; pre-trim controls: 0.85 ± 0.02, $n = 7$, $p > 0.9$). Overall performance across all trials did not change because the rat increased its performance during nose contact trials (Fig 3.3A, 0.71 ± 0.03, $n = 9$; pre-trim controls: 0.79 ± 0.03, $n = 7$, $p > 0.08$). These findings indicate that macrovibrissae are not necessary for texture discrimination performed by direct nose contact, but are required for texture discrimination at whisker-length distances. Surprisingly, single group t-test revealed that the post-macrovibrissa trim performance on trials with nose to texture distance ≥ 2 mm, though disrupted, was still significantly above chance (Fig 3.3C, $p < 0.005$). This suggests the possibility that other sensory cues contributed to this discriminatory performance. G5R2 was not used for this experiment because it did not show stable discrimination performance after microvibrissa trim.

The micro- and macrovibrissa trim experiment was also performed in one rat, (G9R1) performing P150 versus P800 sandpaper discrimination using Behavioral Setup 2

(Fig 3.4). G9R1 performed the texture discrimination task at 0.96 ± 0.01 correct with all whiskers intact. Unlike rats in Behavioral Setup 1, nose contact trials were rarely observed (Fig 3.4A, red trace). Microvibrissa trim did not alter performance (0.984 ± 0.01, n = 5 sessions). Subsequently, all but the C-row of macrovibrissae were trimmed, followed by trimming all but C3 whiskers on both sides. Then all but right C3 whisker was removed. None of these manipulations decreased performance. Finally, all macrovibrissae were trimmed; after this, the rat performed only 5 trials on a single day, and no trials on 1 other day. However, all those 5 trials were correct. Thus, total loss of macrovibrissae interfered with some aspect of performance on this task, but because of the low trial number it is difficult to know whether this rat was using other sensory cues for texture discrimination, or whether performance was high by chance. Statistical analysis of behavioral performance across all macrovibrissa trim manipulations in this rat failed to find any significant effect of whisker trimming on performance suggesting again the possibilities of other sensory cues contributing to the discriminatory behavior (Fig 3.4B, $F = 1.63$, $p > 0.1$, standard 1-way ANOVA).

Psychophysical limit in vibrissa- dependent texture discrimination

As reviewed in Chapter 1 of this thesis, no studies have ever measured a psychophysical curve for sandpaper discrimination. In one rat, we measured the psychophysical limit of vibrissa-dependent texture discrimination by gradually varying the relative roughness of the two sandpapers. B2R3 was trained to discriminate two different sandpapers using Behavioral Setup 2 (Figure 3.5). Initial training was on P120 sandpaper versus smooth plastic film, to provide strong contrast to train texture

discrimination. After criterion performance was attained, the relative roughness of the two surfaces was successively decreased across sessions, using the following pairs of stimuli: P120 versus plastic film (● in figure 3.5A; 125 μm mean grain diameter versus ~ 0 μm), P120 versus P1500 (*; 125 μm versus 12.6 μm), P150 versus P1500 (x; 100 μm versus 12.6 μm), P150 versus P1200 (■; 100 μm versus 15.3 μm), P150 versus P800 (♦; 100 μm versus 21.8 μm), P150 versus P400 (▲; 100 μm versus 35.0 μm), P150 versus P150 (●; 100 μm versus 100 μm). Discrimination for each pair is shown in Fig 3.5 A and B. Behavior performance remained roughly stable up to P150 versus P800. Performance dropped at P150 versus P400, and reaching chance level at P150 versus P150. 1-way ANOVA confirmed an effect of texture on performances (Fig 3.5B, $F = 15.62$, $p < 2.45 \times 10^{-11}$, standard 1-way ANOVA). *Post hoc* analysis using Tukey test showed that discrimination of P150 versus P1500, and P150 versus P800, were significantly different from P120 versus plastic film. Performance on P150 versus P400 and P150 versus P150 were also significantly worse than all other texture combinations. Performance on all texture combinations except P150 versus P150 was significantly above chance (P120 versus plastic film, $p < 6.0 \times 10^{-5}$; P120 versus P1500, $p < 2.0 \times 10^{-6}$; P150 versus P1500, $p < 3.0 \times 10^{-12}$; P150 versus P1200, $p < 2.0 \times 10^{-9}$; P150 versus P800, $p < 2.0 \times 10^{-10}$; P150 versus P400, $p < 0.01$; P150 versus P150, $p > 0.7$; single-group t-test versus 0.5).

These results show that rats can discriminate very similar sandpapers, as close as P150 versus P400 (mean grain size 100 μm versus 35 μm), with near maximal discrimination performance for P150 versus P800. The gradual decrease in discrimination performance with increasing texture similarity suggests that tactile roughness cues were being used for this discrimination.

3.4 Discussion

Role of Microvibrissae versus Macrovibrissae during Fine Texture Discrimination

It has been proposed that microvibrissae act as fine feature tactile detectors, while macrovibrissae function as spatial sensors (Brecht et al., 1997). Studies comparing the function of these two subsystems have rarely been conducted, so their functional roles remain unclear. Moreover, no published study has removed the microvibrissae to test their role in texture discrimination. To elucidate the functional contribution of microvibrissae during fine texture discrimination, we systematically removed these whiskers and measured the effects on discrimination performance.

Under our behavior setups, we have demonstrated that macrovibrisssae alone can support texture discrimination. Microvibrissa removal did not alter performance in any rats. Vibrissa-dependence was further confirmed using videography. With one rat, B2R3, the behavioral performance dropped as difference between the mean grit diameters was gradually reduced, suggesting that the textures play a factor during discrimination, with controlled vision, audition, and olfactory cues. Our results also identified that rats can discriminate textures using only one macrovibrissa, which agrees with previous findings that single macrovibrissa is sufficient to solve tasks that involve active whisking strategies (Carvell and Simons, 1995; Celikel and Sakmann, 2007; Knutsen et al., 2006; Mehta et al., 2007).

Even though microvibrissae were not required during our tasks, it may play some role during specific behavioral strategies. Behavior effect was seen with G5R2 in Behavioral Setup 1, in which performance during nose contact trials dropped after

microvibrissa trim. However, behavioral effect was not seen with G5R1 tested under the same behavior setup, suggesting that the use of microvibrissae may vary across animals.

Macrovibrissa trim on the other hand produced an ambiguous result. When all macrovibrissae were trimmed off of G9R1, the animal stopped attempting to complete trials and had trouble navigating the behavioral cage. This finding agrees with the notion that macrovibrissae are heavily involved in spatial navigation as previously reported (Brecht et al., 1997; Guic-Robles et al., 1989; Jenkinson and Glickstein, 2000; Vincent, 1912). G9R1 performed only five trials on one day that were all correct on the first post-macrovibrissa trim session. Thus, macrovibrissae were playing some role in the overall behavioral performance, but whether those five correct trials were a statistical fluke due to low trial numbers, or indicate that the animal was using other cues, cannot be determined from our data. It is notable that G9R1 performed much better on the behavior than any other animal, suggesting it may have solved the discrimination using other sensory cues.

Thus, our study shows that 1) microvibrissae are not essential for texture discrimination, and 2) macrovibrissae alone can support texture discrimination with a single whisker intact. Our results also agreed that macrovibrissae are highly involved for spatial navigation. Although we found some evidence that other cues might have contributed to above chance performance with no whiskers intact, microvibrissae are not required for texture discrimination. Microvibrissae can be used during discrimination, but its contribution on performance varies across rats and behavioral strategies employed during the tasks.

Psychophysical Limit in Tactile Acuity

Psychophysical studies have shown in rat vibrissae system that rats can discriminate fine feature differences as small as 50 µm using finely grooved plastic cylinders (Carvell and Simons, 1990, 1995). This high acuity sensor has been considered to possess equivalent discriminatory capacity as human fingertips. However, psychophysical studies have not been tested using textured surfaces, especially with sandpapers, and its functional acuity remains unknown.

The psychophysical limit of rat vibrissae to discriminate fine sandpaper differences were studied using B2R3. Behavior deficits were not observed until differences of P150 versus P400 (100 µm versus 35 µm mean grit diameter) sandpaper combination, but performance was still statistically above chance level. Discrimination ability was completely inhibited when two identical sandpaper combination were used. Discriminatory ability was at chance performance. It is unlikely that any other extrinsic cues were contributing to the discriminatory performance during texture discrimination, since the only parameter that was changed throughout training was the texture combination.

Results from our study suggest that rats can discriminate absolute sandpaper grit size difference with finer resolution compared to what was previously known (101 µm) (Aggestam and Cahusac, 2007). This experiment demonstrated the rat vibrissa's ability to discriminate absolute mean grit diameter difference of 65 µm. However, since this measure was only taken from one rat, we do not know whether acuity of this rat was representative of the larger population. Testing more subjects is necessary to confirm whether real acuity of the vibrissa is similar to the range obtained from the current animal.

In order to compare the results in the context of traditional psychophysical studies, we used Weber's law to accurately estimate the just noticeable difference between the two applied stimuli. Using this measure, the just notable difference did not differ (Weber's fraction = 0.65 of the baseline stimuli) with Aggestam et al.'s findings (0.59), suggesting that the discriminatory behavior was quite similar in both studies. However, all texture combinations were not tested. By sampling more baseline sandpapers, we might obtain a closer estimate of the psychophysical limit of rat vibrissae. It is possible that rats can discriminate even finer differences.

3.5 References

Aggestam, F., and Cahusac, P.M. (2007). Behavioural lateralization of tactile performance in the rat. Physiol Behav *91*, 335-339.

Brecht, M., Preilowski, B., and Merzenich, M.M. (1997). Functional architecture of the mystacial vibrissae. Behav Brain Res *84*, 81-97.

Carvell, G.E., and Simons, D.J. (1990). Biometric analyses of vibrissal tactile discrimination in the rat. J Neurosci *10*, 2638-2648.

Carvell, G.E., and Simons, D.J. (1995). Task- and subject-related differences in sensorimotor behavior during active touch. Somatosens Mot Res *12*, 1-9.

Carvell, G.E., and Simons, D.J. (1996). Abnormal tactile experience early in life disrupts active touch. J Neurosci *16*, 2750-2757.

Celikel, T., and Sakmann, B. (2007). Sensory integration across space and in time for decision making in the somatosensory system of rodents. Proc Natl Acad Sci U S A *104*, 1395-1400.

Guic-Robles, E., Jenkins, W.M., and Bravo, H. (1992). Vibrissal roughness discrimination is barrelcortex-dependent. Behav Brain Res *48*, 145-152.

Guic-Robles, E., Valdivieso, C., and Guajardo, G. (1989). Rats can learn a roughness discrimination using only their vibrissal system. Behav Brain Res *31*, 285-289.

Jenkinson, E.W., and Glickstein, M. (2000). Whiskers, barrels, and cortical efferent pathways in gap crossing by rats. J Neurophysiol *84*, 1781-1789.

Knutsen, P.M., Pietr, M., and Ahissar, E. (2006). Haptic object localization in the vibrissal system: behavior and performance. J Neurosci *26*, 8451-8464.

Mehta, S.B., Whitmer, D., Figueroa, R., Williams, B.A., and Kleinfeld, D. (2007). Active spatial perception in the vibrissa scanning sensorimotor system. PLoS Biol *5*, e15.

Vincent (1912). The function of the vibrissae in the behavior of the white rat. Behav Mono *1*, 82.

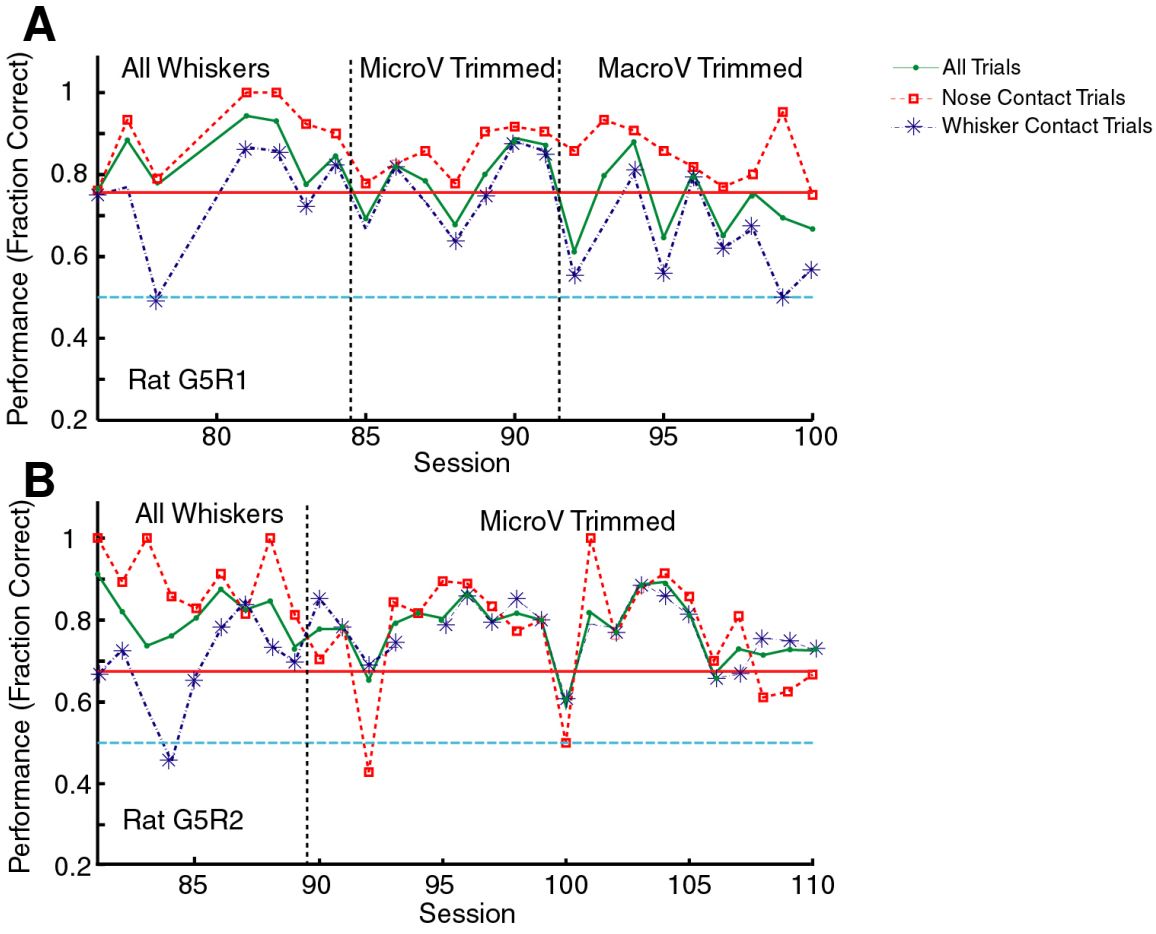

Fig 3.1. Behavioral effects of vibrissa trimming in Behavioral Setup 1. Performance during nose contact, whisker contact, and all trials are plotted separately. A) Rat G5R1 had both of its microvibrissae (session 85) and macrovibrissae trimmed (session 92). B) Rat G5R2 only had its microvibrissae trimmed (session 90). Mean whisker contact performance with all whiskers intact (G5R1 = 0.76, G5R2 = 0.68), and chance-level performance (0.5) are plotted for reference.

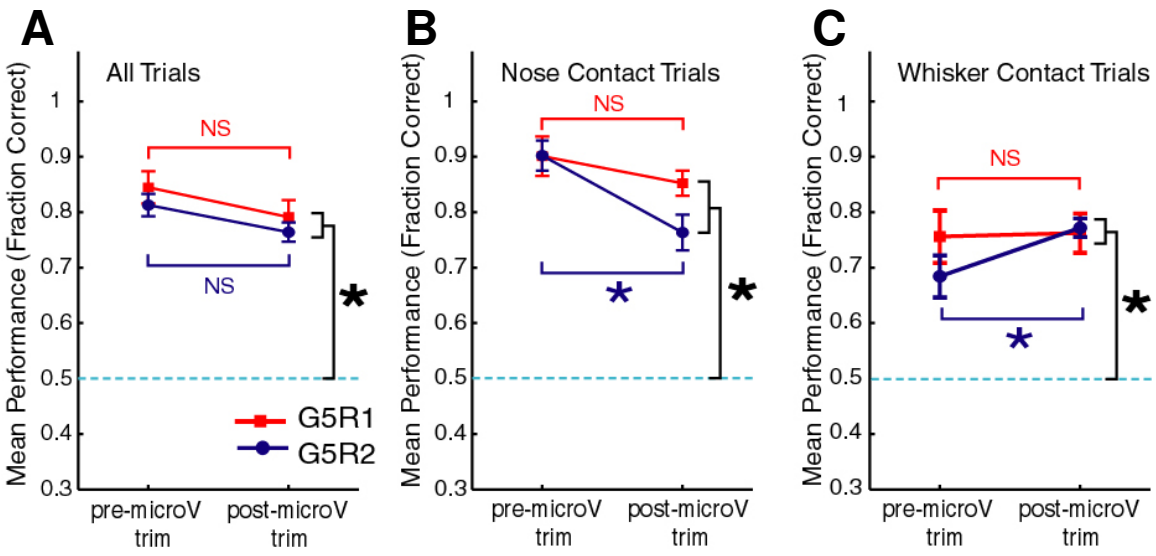

Fig 3.2. Microvibrissa trim effect on behavioral performance. Pre and post microvibrissa trim performances were quantified for G5R1 and G5R2. A) Mean pre-trim, post-trim performances for all trials, B) nose contact trials, C) and whisker contact trials are plotted separately.

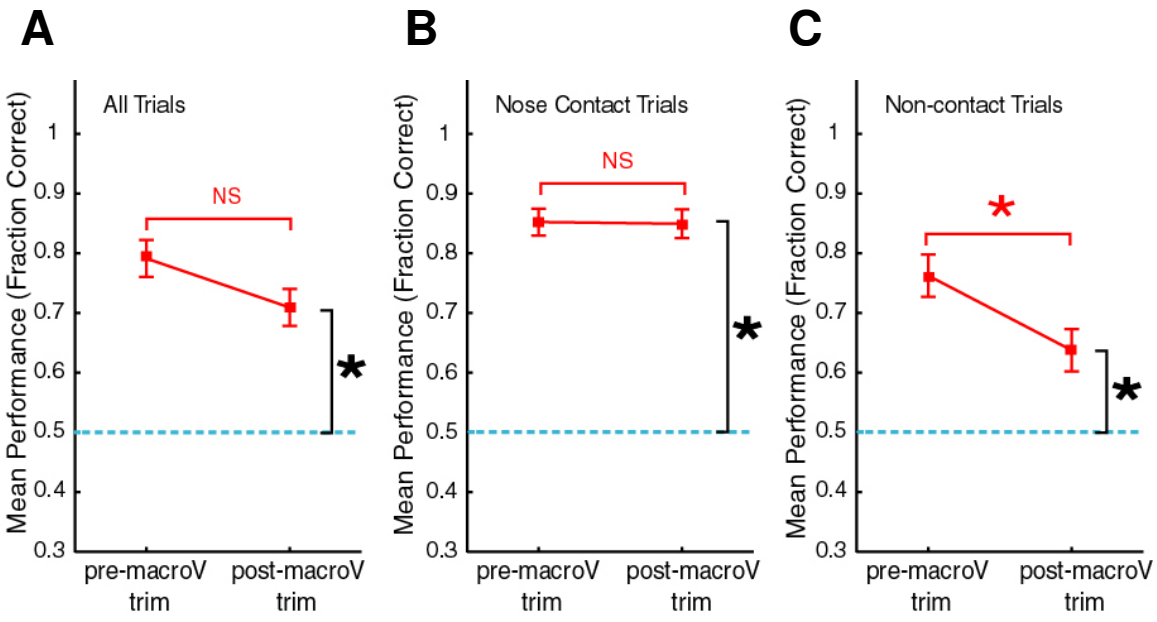

Fig 3.3. Macrovibrissa trim effect on behavioral performance. Pre and post macrovibrissa trim performances were quantified for G5R1. Mean pre-trim, post-trim performances for A) all trials, B) nose contact trials, C) and whisker contact trials are plotted separately.

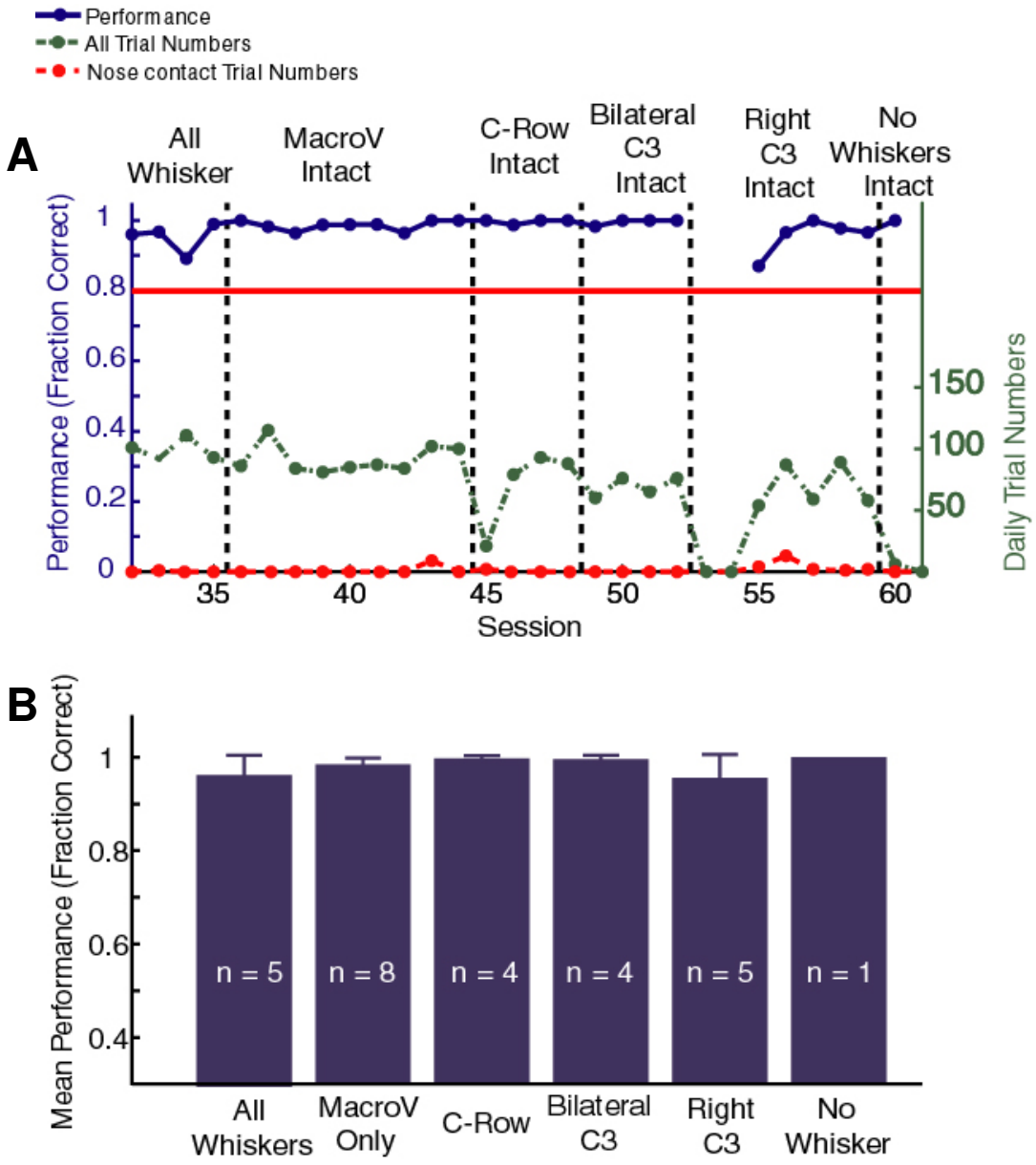

Fig 3.4. Behavioral performance after each successive whisker trim. A) Raw learning curve during the course of whisker trim (blue trace), numbers of daily trails (green trace), and nose contact trials (red trace) are plotted across each session for G9R1. B) Quantified mean daily performances during all whisker conditions ($F = 1.63$, $p > 0.1$ standard 1-way ANOVA).

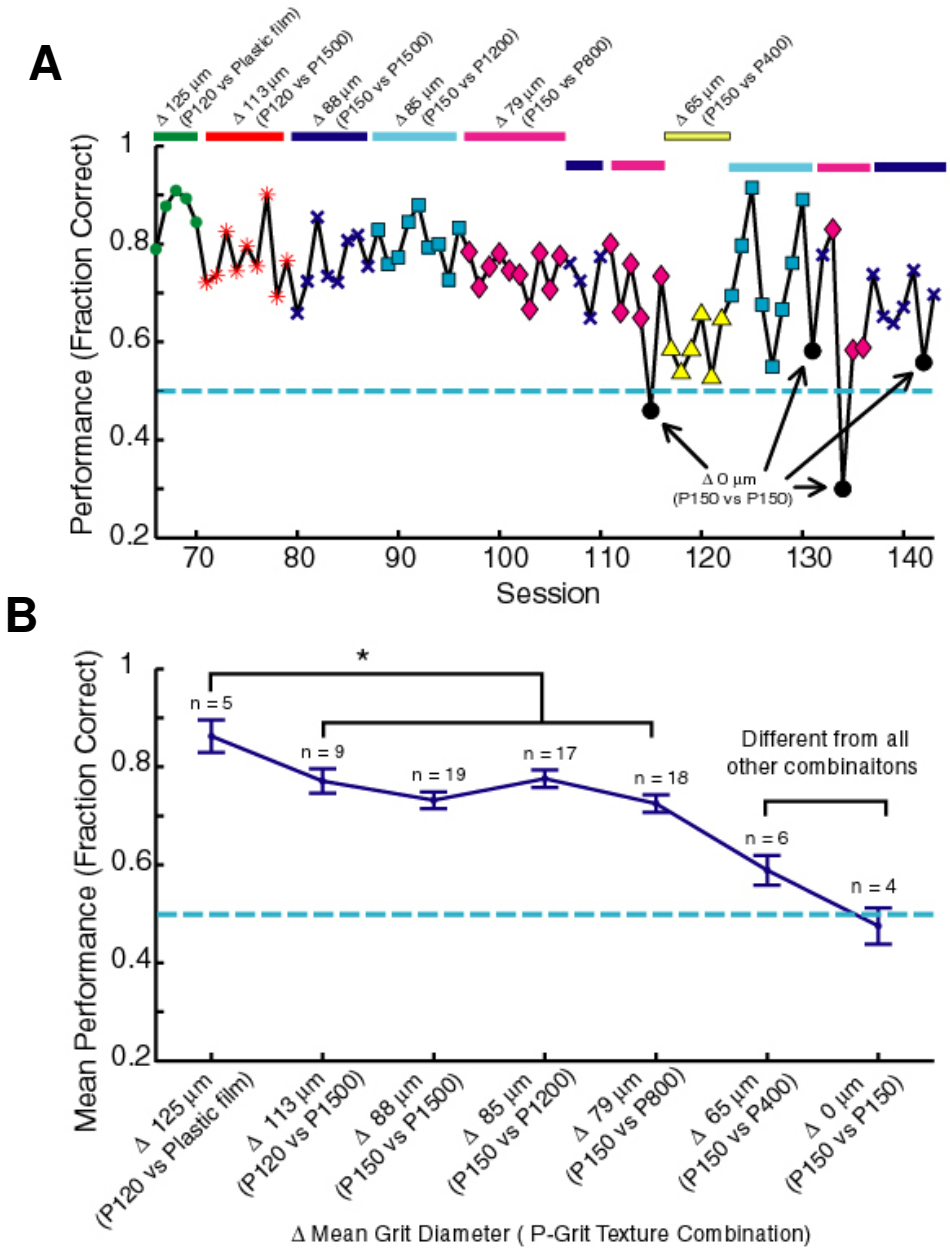

Fig 3.5. Psychophysical curve for texture discrimination. A) Raw learning curve during the course of training. Performance were measured for each texture combinations – P120 vs plastic film (●), P120 vs P1500 (*), P150 vs P1500 (x), P150 vs P1200 (■), P150 vs P800 (♦), P150 vs P400 (▲), P150 vs P150 (●). B) Quantified mean performance during each texture combination.

Chapter 4: Conclusion

4.1 Concluding remarks

Current study attempted to elucidate the functional roles of micro and macrovibrissae and the acuity of the rat vibrissae in discriminating fine textured surfaces. Two behavior paradigms were developed to optimize training, with half of the trained rats reaching criterion performance level. Vibrissa-dependence was demonstrated by controlling vision, olfaction, and audition. Videography also revealed that rats can discriminate textures without nose or paw contact. However, success rate of rats learning the task had to be sacrificed in order to increase obtainable daily trial numbers using our behavior setups. Further improvements are necessary in order to increase consistency in training rats to discriminate textures.

Our study demonstrated that rats can discriminate fine texture difference by utilizing only their macrovibrissae, which is consistent with the hypothesis that macrovibrissae function as fine feature sensors (Aggestam and Cahusac, 2007; Bermejo et al., 2002; Carvell and Simons, 1990, 1995, 1996). However, our results also suggested that microvibrissae may be important for some animals during specific behavior strategies, such as when making close encounter with the textures. This result agrees with the hypothesis stating microvibrissae function as fine feature detectors (Brecht et al., 1997). Therefore, both macrovibrissae and microvibrissae can be used during texture discrimination, varying across animals and employed behavior strategies. We further investigated the psychophysical limit of the rat vibrissa system in discriminating fine textured surfaces. Our results demonstrated that rats can discriminate finer absolute

sandpaper differences compared to what was known to date (Aggestam and Cahusac, 2007).

Even though our current study confirmed that rats can solve texture discrimination by solely utilizing their whiskers, it remains questionable whether whiskers function the same way in the natural environment. Results from Behavioral Setup 1 had identified that rats discriminated textures better using combination of nose and whiskers compared to whiskers alone. In addition, observations from Behavioral Setup 2 demonstrated that nose contact trials occurred quite often and some rats depended heavily on paws rather than whisker contact. If the whiskers function as the primary tactile sensors to extract fine texture information, then nose/paw contact should not have been seen during our training. Therefore, one interpretation may be that rats can 'learn' to use their whiskers during fine texture discrimination, but whiskers do not function ethologically as the primary feature detector.

Since humans don't possess a dynamic and elaborate vibrissa system, it is difficult to imagine the real functions that the system is capable of solving. There has been a strong presumption that the rodent vibrissa system functions as a fine feature detector that is comparable to human fingertips. Many studies have moved on to look at the neural codes driving vibrissa dependent texture discrimination, while lacking full understanding of its functional capacities (Prigg et al., 2002; von Heimendahl et al., 2007). Therefore, further controlled behavioral studies have to be developed in order to understand the true rodent vibrissa functions.

4.2 References

Aggestam, F., and Cahusac, P.M. (2007). Behavioural lateralization of tactile performance in the rat. Physiol Behav *91*, 335-339.

Bermejo, R., Vyas, A., and Zeigler, H.P. (2002). Topography of rodent whisking--I. Two-dimensional monitoring of whisker movements. Somatosens Mot Res *19*, 341-346.

Brecht, M., Preilowski, B., and Merzenich, M.M. (1997). Functional architecture of the mystacial vibrissae. Behav Brain Res *84*, 81-97.

Carvell, G.E., and Simons, D.J. (1990). Biometric analyses of vibrissal tactile discrimination in the rat. J Neurosci *10*, 2638-2648.

Carvell, G.E., and Simons, D.J. (1995). Task- and subject-related differences in sensorimotor behavior during active touch. Somatosens Mot Res *12*, 1-9.

Carvell, G.E., and Simons, D.J. (1996). Abnormal tactile experience early in life disrupts active touch. J Neurosci *16*, 2750-2757.

Prigg, T., Goldreich, D., Carvell, G.E., and Simons, D.J. (2002). Texture discrimination and unit recordings in the rat whisker/barrel system. Physiol Behav *77*, 671-675.

von Heimendahl, M., Itskov, P.M., Arabzadeh, E., and Diamond, M.E. (2007). Neuronal activity in rat barrel cortex underlying texture discrimination. PLoS Biol *5*, e305.