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A novel vibrotactile system for stimulating the glabrous skin of awake freely behaving rats during operant conditioning

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HIGHLIGHTS

- A novel vibrotactile system was developed for freely behaving rats.
- Vibrotactile stimuli can be applied on the glabrous skin regardless of head and body position.
- Rats were trained to perform a yes/no task by stimulating their fore- and hindpaws.
- Detection accuracy was lower compared to whisker stimulation reported previously.

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ABSTRACT

Background: Rat skin is innervated by mechanoreceptive fibers similar to those in other mammals. Tactile experiments with behaving rats mostly focus on the vibrissal system which does not exist in humans. The aim of this study was to design and implement a novel vibrotactile system to stimulate the glabrous skin of behaving rats during operant conditioning.
New method: A computer-controlled vibrotactile system was developed for various tasks in which the volar surface of unrestrained rats' fore- and hindpaws was stimulated in an operant chamber.
Results: The operant chamber was built from off-the-shelf components. A highly accurate electrodynamic shaker with a novel multi-probe design was used for generating mechanical displacements. Twenty-five rats were trained for four sequential tasks: (A) middle-lever (trial start signal) press, (B) side-lever press with an associated visual cue, (C) similar to (B) with the addition of an auditory/tactile stimulus, (D) auditory/tactile detection (yes/no) task. Out of 9 rats which could complete the tactile version of this training schedule, 5 had over 70% accuracy in the tactile version of the detection task.
Comparison with existing methods: Unlike actuators for stimulating whiskers, this system does not require a particular head/body alignment and can be used with freely behaving animals.
Conclusions: The vibrotactile system was found to be effective for conditioning freely behaving rats based on stimuli applied on the glabrous skin. However, detection accuracies were lower compared to those in tasks involving whisker stimulation reported previously, probably due to differences in cortical processing.

1. Introduction

Rodents can be trained for behavioral tasks based on sensory inputs from different modalities, such as olfaction (Rajan et al., 2006), vision (Horner et al., 2013), hearing (Talwar and Gerstein, 1999) and touch (Adibi and Arabzadeh, 2011; Harris et al., 1999; Tahon et al., 2011; Walker et al., 2011; Wiest et al., 2010). They can also be conditioned by electrical microstimulation in the cortex (Xu et al., 2004) and drug self-administration (Ikemoto and Sharpe, 2001). The novel vibrotactile system presented in this article is suitable for stimulating the glabrous skin of freely behaving rodents in an operant chamber.

The sense of touch is conveyed by the mechanoreceptive afferents innervating the skin. The type and distribution of cutaneous mechanoreceptors and their associated fibers depend on the skin location and species. Four types of mechanoreceptors

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are found in the mammalian glabrous skin: Meissner corpuscles, Merkel's discs, Pacinian corpuscles and Ruffini endings (Gardner and Johnson, 2012; Greenspan and Bolanowski, 1996; Güçlü et al., 2003; Halata, 1990; Willis and Coggeshall, 2004). In the hairy skin, there are touch domes, Pacinian corpuscles, Ruffini endings, afferents associated with hairs, field units, and C-mechanoreceptive fibers (Halata, 1990; Lechner and Lewin, 2013; Lumpkin et al., 2010; Willis and Coggeshall, 2004). Mechanoreceptive fibers are classified according to their innervation densities, receptive-field sizes, adequate stimuli (e.g. vertical skin displacement and its derivatives, horizontal skin stretch, stroking), response properties such as mechanical threshold, adaptation, entrainment, spontaneous rate, afterdischarge, tuning frequency, and conduction speed (Bolanowski and Zwislocki, 1984; Devecioğlu and Güçlü, 2013; Güçlü and Bolanowski, 2003, 2004; Iggo and Muir, 1969; Johansson, 1978; Johnson, 2001; Koltzenburg et al., 1997; Leem et al., 1993a,b; Lynn and Carpenter, 1982; Vallbo et al., 1995).

It was shown that rat skin contains all the mechanoreceptive fibers mentioned above. (Devecioğlu and Güçlü, 2013; Diamond et al., 2008; Leem et al., 1993a,b; Lynn and Carpenter, 1982; Sanders and Zimmermann, 1986). Additionally, rats have sinus hairs, especially the mystacial vibrissae on the snout, which do not exist in humans (Halata, 1990; Willis and Coggeshall, 2004). Sinus hairs have complex innervation with multiple slowly adapting and rapidly adapting fibers. Moreover, the sinus hair follicle includes striated muscle fibers which enable voluntary movement. Due to the highly magnified cortical representation in rats, the mechanoreceptive fibers and the associated pathways for the vibrissae have been extensively studied in the literature (Adibi and Arabzadeh, 2011; Diamond et al., 2008; Harris et al., 1999; Prigg et al., 2002; Tahon et al., 2011; Walker et al., 2011; Wiest et al., 2010). Additionally, because the afferent and efferent innervation form a closed loop of the sensorimotor pathways, rat vibrissal system is an ideal model for studying active touch (Ahissar, 2008; Kleinfeld et al., 2006). However, accurate stimulation of whiskers requires head restraining or a particular moment of a stationary head position during movement. Since humans mostly use mechanoreceptors in the glabrous skin for tactile exploration, we aimed to design a novel vibrotactile system for specifically targeting identical mechanoreceptor types in the glabrous skin of freely behaving rats.

According to our knowledge, all of the operant chambers reported in the somatosensory literature include tactile stimulators targeting the whiskers. For example, Tahon et al. (2011) constructed an operant chamber for a reaction task based on stimulating whiskers only on one side. They used a drum with a vertical axis placed just outside an opening near the lever which started a trial. The drum was controlled by a motor and an adhesive tape was placed on its surface. The rats were trained to respond when the whiskers touched the tape as the drum turned. In this setup, the rats learned to hold their heads close to the drum. Therefore, the conditioning was performed at a very small space within the chamber which may prevent designing tasks involving more complex stimuli (e.g. stimulating individual whiskers). Wiest et al. (2010) used operant chambers with two compartments (stimulus and reward). The stimulus chamber was equipped with a nose-poke hole and a sliding bar on each side of this hole. Distance between these bars was controlled by step motors, so that rats were presented different aperture sizes. During the nose-poke gesture the rats explored the aperture by active whisking. Because the stimulus consisted of a predefined aperture size before each trial, the rats could easily explore the aperture while starting the trial with a nose poke. However, this setup did not allow presentation of dynamically changing stimuli. Adibi and Arabzadeh (2011) trained rats by applying vibration to plates which the rats explored by active whisking or passive contact. The vibrations were generated by piezoelectric actuators driving the plates. Here, the plates were placed at either side close to the nose-poke sensor. Since a head restraint was not used in the above studies, additional checks were performed to verify stimulus contact (e.g. video recording). This is important because the response should be contingent on the stimulus when it is applied.

On the other hand, many other studies used head restraining for accurate stimulation of whiskers by piezoelectric motors (Gerdjikov et al., 2010) or air puffs (Martin et al., 2002; Sachdev et al., 2000). For example, Gerdjikov et al. (2010) fixed a whisker in a glass capillary which was moved sinusoidally by a piezoelectric bender. Sachdev et al. (2000) applied air puffs similarly in a head-restrained rat. However, they had to observe whiskers by a dissection microscope to verify the deflection of a single whisker.

In this study, we built a computer-controlled vibrotactile system for various tasks in which the glabrous skin of freely behaving rats was stimulated. It was specifically used to activate the mechanoreceptors in the volar surface of the hindpaw. A particular novelty in the design was a multi-probe tip adaptor for the mechanical shaker. This provided accurate stimulation of the skin surface regardless of the animal's position in the chamber. We also present a detailed training schedule suitable for this design. Our results show that response accuracies based on tactile inputs from the glabrous skin are somewhat lower than those obtained by whisker stimulation reported in the literature.

2. Materials and methods

2.1. Animals

25 adult Wistar rats (6 females, weight: 140–190 g; 19 males, weight: 300–350 g) were used in this study (Rats 1–25) (Table 1). They were housed in standard cages as pairs. Day–night cycle was 12:12 h. Rats were deprived of water 24 h before the first training session. Their weights were kept at >80% of their normal weights. Water was only available during the training in each daily session. If the rats could not get adequate water in a given training day (i.e. ∼300 correct responses), additional water was provided afterwards. Food was available ad libitum in both home cages and in the operant chamber. The experiments were approved by the Boğaziçi University Institutional Ethics Committee for the Local Use of Animals in Experiments.

2.2. Vibrotactile stimulator and the operant chamber

The custom-built operant chamber used in this study is shown in Fig. 1. Each component is indicated by a letter label (a–v). The chamber walls (e) are made of Plexiglas (length: 25 cm, width: 25 cm, height: 30 cm, thickness: 3 mm) and elevated 24.5 cm from the table top by an aluminum frame (o). The ground plate (r) of the chamber is made of aluminum (thickness: 3 mm) and has precisely spaced 230 holes (4 mm diameter, center-to-center distance: 6 mm) in a hexagonal pattern (∼10 cm diameter) (q) to allow movement of the probes (s) of the multi-probe tip adaptor (j). The pattern of holes covered a convenient location such that the rat skin always touched several probes simultaneously in each trial. A battery driven custom-made electrical circuit (k) was constructed to monitor skin contact with the probes. This circuit simply consists of a battery (9 V) and a resistor (1 kΩ) connected in series with the probes (s) touching the skin surface. When the rat touches the probes, the voltage across the resistor changes proportional to the contact surface. Additionally, a single chip accelerometer (k) (ADXL05; Analog Devices, USA) is used to monitor the mechanical vibrations generated by the shaker. There is also a holder for the camera (a generic webcam) (a), white-noise speakers (generic PC speakers) (b), reward buzzer (Digi-Key part #: 102-1124-ND;