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Improvement of Mechanical Stability for Single Unit Recording Based on Skull Cap in Living Chinchilla*

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Summary: Three-point head fixation was constructed to provide mechanical stability for single unit recording (SUR) on vestibular sensory system in living chinchilla previously. However, it is no more qualified to this work when the stimulation intensity becomes large because of frequent unit losing and neuron damage, which strongly implies that the mechanical stability has been broken during the stimulation. Here, we constructed a novel head fixation (skull cap assistant head fixation) provided by skull cap on the basis of three-point head fixation in order to improve the mechanical stability for SUR under the stimulation with large magnitude. The large area bone connection is the feature and advantage of this improved method, which directly fixes the tested local nervous tissue and microelectrode in an intact stable system through skull cap except two ear bars and a tube face mask. Our data exhibited that skull cap assistant head fixation could significantly improve the success rate of neural response activity recording in the population of semicircular canal neurons under the stimulation with large intensity (amplitude ≥100 deg/s). Based on the analysis of neural response activity and noise base-line during stimulation, our data further indicated that this method could significantly improve the mechanical stability for SUR during high-speed motion stimulation on vestibular system in living chinchilla. Skull cap assistant head fixation extends the application of SUR on vestibular neuron in linear response range and provides a solid foundation for electrophysiological research on vestibular sensory system in further studies.
Key words: single unit recording; skull cap; mechanical stability; improvement; semicircular canal neurons

Single unit recording (SUR) technique provides a method of measuring the electrophysiological responses of neurons and brain through a microelectrode system to study the behaviors and functions of animals and humans[1, 2]. Therefore, it is widely used in neuroscience, especially in cognitive science such as perception, memory, language, emotions and motor control[3]. However, SUR in living animals is a demanding technique and any of a variety of causes will result in failure to obtain successful recordings. Besides a good understanding of the technique and equipment required, the most important concern is ideal mechanical stability, which must make sure that there is no vibration and movement at the recording location and the tip of microelectrode is at a stable position in relationship to the nervous tissue while still allowing animal respiration and circulation. Thus, the ideal situation for SUR should be thought of as an intact stable system in which the tested local nervous tissue and microelectrode are directly fixed. In previous studies, generally there was the stereotaxic chair with two ear bars inserting into the left and right external auditory canal and a tube face mask holding the mouth to steadily fix the animal head (three-point head fixation, fig. 1) aiming to provide mechanical stability for SUR on semicircular canal neurons (SCNs) in chinchilla[4–6]. This fixation system was pretty good for response activity recording of SCNs as the stimulation

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intensity was small (peak head angular velocity <100 deg/s)[4–6]. However, it gradually could not be qualified to this work because of the extremely high failure rate of neural response activity recording as the stimulation intensity kept increasing, especially when the angular velocity was >180 deg/s in our previously study[7], which implied that the ideal mechanical stability was broken during the high-speed motion stimulation.

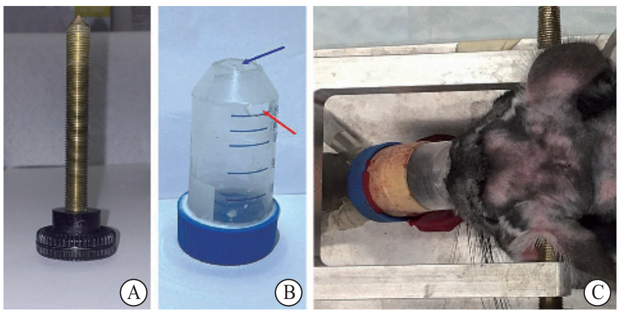

Fig. 1 Three-point head fixation
A: an ear bar that was used to insert into left or right external auditory canal for ear fixation. B: tube face mask for animal mouth fixation and anesthesia maintenance. The nasal bone and incisive bone of the mouth were pushed into the hole in the top (blue arrow) and then the incisor teeth came out of the hole in the side wall (red arrow). C: three-point head fixation on living chinchilla head

In order to extend our understanding to the response property of SCNs in wider nonlinear neural response range (head angular velocity >120 deg/s)[8, 9], it is necessary to improve the mechanical stability for SUR. Here, we designed a skull cap directly established on the skull not only to make a direct large area bone fixation but also to produce a rigid bridge from animal skull to stereotaxic chair, aiming to provide more ideal mechanical stability for SUR during the stimulation with large magnitude.

1 MATERIALS AND METHODS

Six adult female chinchillas (C. laniger) weighing 480–550 g were used in the present study. All the animal surgeries and SUR procedures were approved by the Johns Hopkins University Animal Care and Use Committee, and in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

1.1 Procedure for Skull Exposure

Animals were anesthetized with isoflurane, which was induced by an air-proof box with direct administration of 5% isoflurane and afterward by switching to a tube face mask with 1%–3% isoflurane for maintenance[10]. Additional local anesthesia (xylocaine, 0.5%) was applied to the local soft tissues of the operation field just on the top of skull. After successful anesthesia, the skin and tissues on the top of parietal bones and the latter half of frontal bone were all removed carefully with the aid of a scalpel, and any bleeding in the exposed field was stopped strictly (fig. 2A). The core body temperature of the chinchilla was maintained at 36°C–38°C[5] by an external servomechanism-DC temperature controller (FHC, model 40-90-8B) before and during surgery and SUR procedure.

1.2 Establishment of Skull Cap

After completely well exposure, the top surfaces of parietal bones and the latter half of the frontal bone were dried as much as possible firstly, and then a layer of universal dental adhesive (light-cure universal dental adhesive, model 35108, OptiBond XTR self-etch, USA) enhancing marginal integrity and reducing microleakage for long-lasting restorations was evenly covered on the surface of these bones (fig. 2B). The UV curing light (Maxima LED curing light, RU1200, USA) was used for about 60–80 s to solidify the dental adhesive (fig. 2C). When the layer of dental adhesive strongly gripped the skull, a dental resin skull cap was established on it. The grip cement powder (CAULK/DENTSPLY, 675571, USA) was well mixed with grip cement bulk liquid (CAULK/DENTSPLY, 675572, USA) firstly, and then the sticky fluid compound was straightly applied on the adhesive to establish a skull cap (fig. 2D). The curing time for the dental resin skull cap was about 10 min. A wooden rod of about 8 mm in diameter was imbedded in the skull cap before curing (fig. 2D), producing a rigid bridge from skull to stereotaxic chair not only to provide stable head fixation but also to avoid any phase difference between movement of rotational stimulation device and animal head.

1.3 Animal Fixation for Ideal Mechanical Stability

The animal was fixed in a stereotaxic chair where the head was immovably held and kept. Then the head of the fixed animal was adjusted to match that of unfixed animal. For each animal, there were two ear bars inserting into the left and right external auditory canal and a tube face mask holding mouth for primary head fixation (fig. 1), which was consistent with previous studies[4–6]. An assistant fixation frame was designed on the stereotaxic chair around the animal head to grip the wooden rod imbedded in dental resin skull cap (fig. 2E). Therefore, the frame and the gripped wooden rob established a rigid bridge between animal skull and chair. In this situation, the animal head was additionally fixed on the stereotaxic chair through direct bone connection skull cap on the basis of three-point head fixation (fig. 2E). If the assistant fixation frame disconnected the wooden rob, the animal head was only fixed by the basic fixation of ear bars and face mask (fig. 2F). In order to address the advantage of this improvement, SUR on SCNs was conducted in each

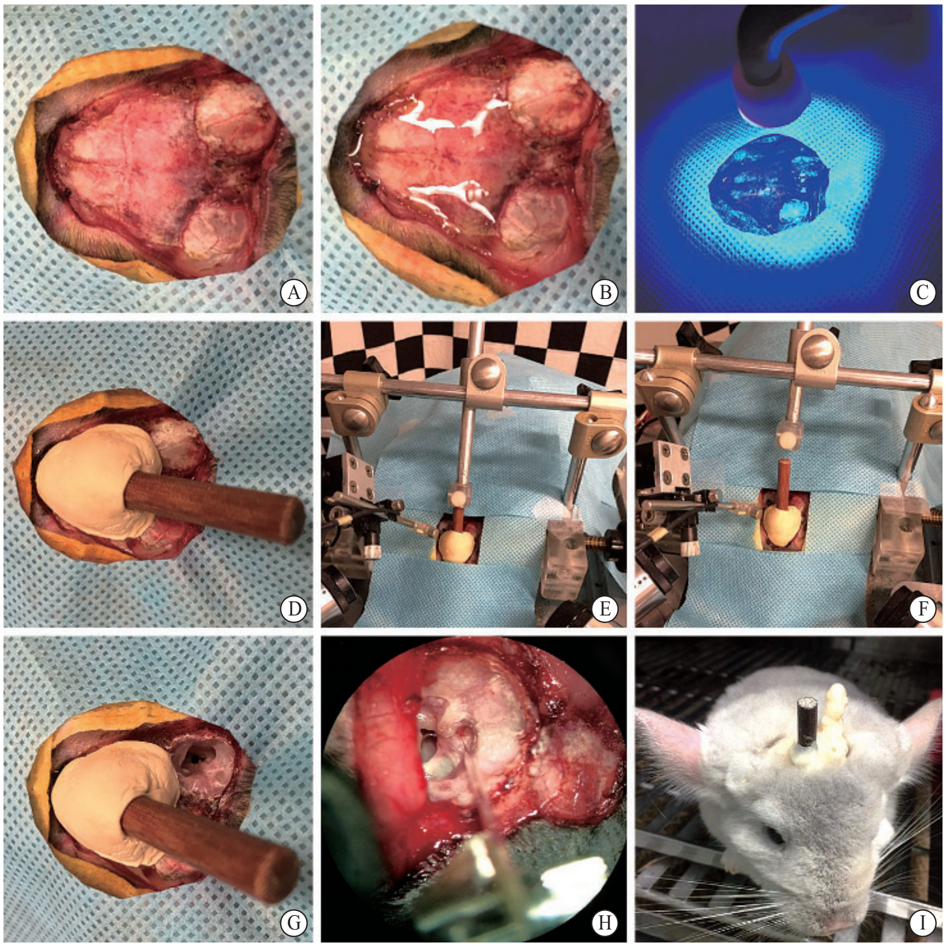

Fig. 2 Skull cap assistant head fixation

A: exposure for the top of parietal bones and the latter half of the frontal bone in living chinchilla; B: universal dental adhesive on the exposed chinchilla skull; C: The dental adhesive was solidified by UV curing light. D: skull cap established on the top of the chinchilla skull, where the wooden rod for connection was imbedded; E: the fixation frame bolted on the stereotaxic chair for skull cap assistant head fixation: The frame straightly gripped the wooden rod imbedded in skull cap, so that the tested nervous tissue and microelectrode were directly fixed on an intact stable system (stereotaxic chair) on the basis of three-point head fixation. F: basic head fixation provided by three-point head fixation: the frame disconnected the rod, thus there was no skull cap assistant fixation. G: the extracranial surgical approach through the opened mastoid bulla; H: the position on the superior wall of internal auditory canal for vestibular nerve exposure; I: the skull cap on the living chinchilla over 3 months for chronic experiment

animal alternately with the support of the three-point head fixation (fig. 2F) and skull cap assistant head fixation (fig. 2E). Thus, the neural response activity data acquired in the present study were classified into three-point fixation group and skull cap assistant fixation group.

1.4 Vestibular Nerve Exposure and SUR in Chinchilla

The extracranial surgical approach was chosen as described previously[5, 6]. Animal middle ear was entered by opening the top of the mastoid bulla (fig. 2G). Then an otologic drill was used to access vestibular neurons. A small fenestra (0.5 mm×0.5 mm) was made in the superior wall of internal auditory canal. The approximate location of the fenestra was 0.5–1.0 mm anterior to the facial nerve canal and 1.0–2.0 mm medial to the superior ampulla (fig. 2H). Thus, vestibular nerves and Scarpa's ganglion were exposed and remained integrally without any interference with brain structures.

To record neural activity, glass micropipette electrode (WPI, model M1B100F-4, United Kingdom) was pulled and afterward filled with 3 mol/L NaCl solution to achieve 20–40 mΩ impedance. Then, it was held in position on the exposed vestibular nerve using a three-dimensional manipulator (You, model US-3F, Japan) fixed on a hydraulic Microdrive (Narishige International USA, models MO-22, USA). Further, the glass electrode driven by Microdrive was carefully inserted into the vestibular nerve. Then the Microdrive was slowly advanced until glass electrode isolated a single nerve fiber or neuron cell body and identified extra axonal activity of neuron (spikes). Signals from neuron were passed through the headstage amplifier (resistance 1.1 to 10 K) first and then inputted into the extracellular amplifier (Dagan, model 2400A, USA) at the gains from 500 to 5000 and the band-pass filter from 300 to 3000 Hz. Potential uncoupling in the mechanical linkages of animal head was assessed using an accelerometer (InvenSense, MPU-9250A, USA) attached on the projecting position of animal head on the stereotaxic chair. Finally, the neural activities were recorded by the CED Spike2 neural signal acquisition software.

For animal position on stereotaxic chair, the longitudinal axis of animal lay through the axis of rotation, the head line connecting centers of left and right external auditory canal (along two ear bar inserting into left and right external auditory canals for head fixation) also run through the axis of rotation. The stereotaxic chair was placed on a gimbaled superstructural platform atop a servo-controlled rate rotational motor table (Kollmorgen goldline direct drive rotary servo motor, model D083M-22-1310, USA) programmed to supply rotation stimuli. Therefore, the orientation of the animal head could be freely adjusted. Once a neuron classified by normalized coefficient of variation (CV*)[4] was well isolated, a combination of stimuli consisting of yaw and pitch head rotation by hand were performed, and afterwards the innervating semicircular canal was immediately identified through monitoring the response activity during rotations[5]. Then the gimbaled platform was adjusted to make sure that the plane of the identified semicircular canal was brought into the rotation plane according to Hullar's measure[11]. Therefore, the anterior, horizontal and posterior semicircular canal neurons and their corresponding canal planes were all tested within horizontal plane in three-dimensional space (under the same situation). The constant frequency (0.2 Hz) sinusoidal rotational stimulation (SRS) with sequentially increased amplitudes (60, 80, 100, 120, 150, 180, 250, 300 deg/s) was used to test SCNs and repeated 5 cycles at each amplitude. The neural response activities under different stimulation intensities were continuously recorded. For an amplitude, neuron was deemed to pass only if its response activity was successfully recorded for five complete cycles under this amplitude. All the passed amplitudes for a neuron were recorded and used to calculate the successful rate in neural population.

2 RESULTS

Thirty-three and 31 robust SCNs were recorded in three-point fixation group and skull cap assistant fixation group, separately. All of those neurons were tested by constant frequency (0.2 Hz) sinusoidal rotations with sequentially increased amplitude (peak head velocity, 60 to 300 deg/s).

Our data exhibited that it was easier to hold a unit and keep going to record during the stimulation with larger amplitude if there was an additional head fixation provided by skull cap, although the time for experiment preparation and animal surgery before recording increased about 30 min. Fig. 3 shows the SUR examples for the dynamic response activity (instantaneous fire rate) of an anterior SCN (medium, CV*=0.118) and a horizontal SCN (bottom, CV*=0.338) tested by SRS. The recording for the anterior SCN was failed as peak head velocity increased over 180 deg/s when the animal head was fixed only by ear bars and the tube face mask. However, the recording for the horizontal SCN was successfully conducted under the stimulation with peak head velocity as high as 300 deg/s when the skull cap provided assistant head fixation.

Further, the success rate of SUR in SCNs population under SRS with different amplitudes increasing from 60 to 300 deg/s demonstrated the advantage of skull cap assistant head fixation. Table 1 shows the detail. Although the intensity of stimulation (amplitude magnitude) had a negative effect on success rate in these two groups, statistical analysis confirmed that the success rates in skull cap assistant fixation group were significantly increased when the intensity of SRS was increased higher than 100 deg/s (Chi-square test, P<0.001 under 100, 120, 150, 180, 250 and 300 deg/s). However, there was no significant difference in the success rate between three-point fixation group

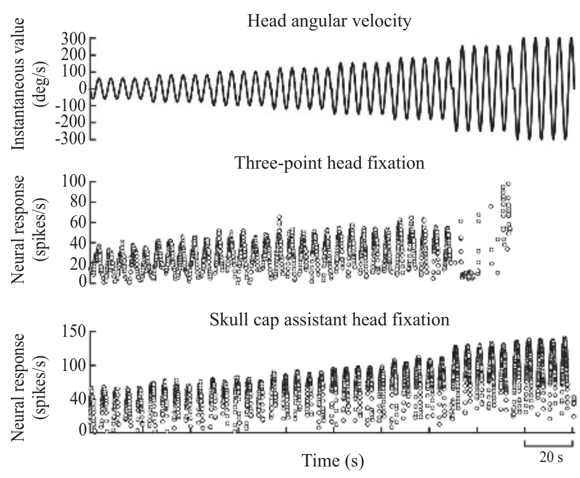

Fig. 3 SUR for the dynamic neural response activities under different head fixations

The top sinusoidal curves in plots indicate dynamic head angular velocity. The circles in medium and bottom show the instantaneous response activities (instantaneous discharge rate) of an anterior SCN (medium, CV*=0.118) and a horizontal SCN (bottom, CV*=0.338), separately. These two neurons come from one animal but under different situations of head fixation. The anterior SCN only had three-point head fixation, and the horizontal SCN was supported by skull cap assistant head fixation on the basis of three-point head fixation.

Table 1 Success rate of SUR in the population of SCNs under different magnitudes in the two groups

Groups	Success rate (%) under different magnitudes (deg/s)							
	60	80	100	120	150	180	250	300
Three-point fixation	87.88	84.85	60.61	42.42	27.27	15.15	3.03	0
Skull cap assistant fixation	96.77	96.77	96.77**	83.87**	77.42**	74.19**	54.84**	35.48**

*P<0.05, **P<0.001 *vs*. three-point fixation group (Chi-square test)

and skull cap assistant fixation group as the intensity stimulation was small (Chi-square test, P=0.19 under 60 deg/s, and P=0.10 under 80 deg/s). These results indicated that skull cap assistant head fixation had absolutely significant advantage for SUR under high-speed motion stimulation.

In order to address whether the advantage of this improved method benefited from mechanical stability, the detail of neural response activities (spikes) and noise base-lines, being directly related to mechanical stability during SUR, under different intensities of rotational stimulation in three-point head fixation group and skull cap assistant head fixation group were analyzed separately. Fig. 4 shows the two detail analysis examples of anterior SCNs in three-point group (fig. 4A) and horizontal SCNs in skull cap group (fig. 4B) under different intensities of SRS (100 and 300 deg/s were chosen as examples and exhibited in top and bottom traces respectively in fig. 4A and 4B). The neural response activities of these two SCNs were recorded pretty well (top in fig. 4A and 4B) when magnitude was small (100 deg/s). However, the neural response activity of the anterior SCN was lost and replaced by huge noise when the magnitude was increased to 300 deg/s in three-point head fixation group (bottom in fig. 4A). Much better results were acquired for SUR under skull cap assistant head fixation, and the neural response activity was successfully recorded although the amplitude was as high as 300 deg/s (bottom in fig. 4B). Additionally, the noise during the stimulation with the amplitude of 300 deg/s was kept as low and stable as that of 100 deg/s in fig. 4B. In SCNs population, the noise base-line change rate under high-speed motion stimulation (≥100 deg/s) was 75.25% in three-point head fixation group, which was significantly higher than 29.57% in skull cap assistant head fixation group (Chi-square test, P<0.001). Based on these detail analyses, it was strongly confirmed that the skull cap through direct large area bone fixation could conspicuously improve the mechanical stability for SUR on vestibular neurons during high-speed motion stimulation (≥ 100 deg/s) in living chinchilla.

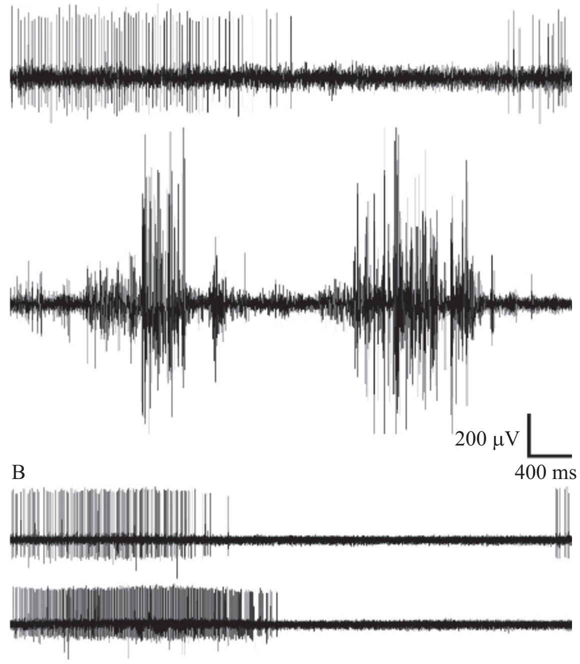

Fig. 4 Neural response activity (spikes) and noise base-line during a complete periodic sinusoidal rotation with different magnitudes

A: the neural response activity of the anterior SCN (CV*=0.118) and noise base-line during stimulation in the situation of three-point head fixation: the top trace shows the pretty well response activity and quite low and stable noise during stimulation with amplitude of 100 deg/s, and the bottom trace displays the response activity was lost and replaced by huge noise when the magnitude was increased to 300 deg/s. B: the neural response activity of the horizontal SCN (CV*=0.338) and noise base-line during stimulation in the situation of skull cap assistant head fixation: the top and bottom traces show the well recorded neural response activities and constantly low and stable noises under the amplitude of 100 and 300 deg/s, respectively.

3 DISCUSSION

Although within linear neural response range (amplitude <80 deg/s), vestibular neurons have been characterized in terms of dynamic response property from relatively low to extremely high frequency[4–6, 12], neural response detection threshold[13, 14], preferred rotation plane in three-dimension space[12, 15], and linear information encoding for upstream neurons or brain[5, 16–20], we still know little about the neural response property in the broader nonlinear neural response range. One of the most important reasons is that it is hard to acquire neural response activity data during the stimulation with larger amplitude in linear neural response range. Further, it is generally accepted that SUR is a fully demanding technique, especially on living animals. It can't be guaranteed that SUR can be conducted successfully or has a high success rate based on our current understanding of SUR[21]. Until now, even the biggest obstacle for SUR on vestibular neurons is still the frequent losing unit and complete recording failure during the stimulation of high-speed motion although a vestibular fiber or cell body is successfully isolated before stimulation, which sometimes no clue available can be traced for. Therefore, any improvement for SUR techniques, helping to hold the unit and improve the success rate during the stimulation in nonlinear response range, is urgently needed.

The present study provided an improved method using the direct large area bone connection skull cap for the stability of mechanics during SUR through a simple surgery. This skull cap assistant head fixation is an improvement of assistant technique for SUR, aiming to resolve the difficulties and problems we met in the previous research[7]. During our experiment, we observed that this improved method could significantly increase the success rate of SUR on SCNs during the stimulation with intensity larger than 80 deg/s in living chinchilla, although the advantage was not significant when the intensity of stimulation was less than 80 deg/s. Thus, this improved method can help us to acquire neural response data and further explore the response property in an extended neural response range. Furthermore, we didn't observe any negative effects of this method on the healthy condition of animal, including breath, heart rate and body temperature. Additionally, this method also can be used for chronic experiment. The fig. 2I displays a skull cap constructed on chinchilla head for 3 months. There was no exposure of subcutaneous tissue, bleeding and infection, and animal also did not express any uncomfortable and unpleasant signs such as crying, grimaces or scratching skull cap. Therefore, the direct large area bone connection skull cap for assistant head fixation is a pretty well improved method for SUR on vestibular neurons and other electrophysiological experiments in living chinchilla. This improved method breaks out of the restriction before and extends the application of SUR on vestibular neurons in linear response range, and provides a solid foundation for electrophysiological research on vestibular sensory system in our further study.

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Conflict of Interest Statement

The authors of this paper declare they have no conflict of interest.

REFERENCES

1 Boulton AA, Baker GB, Vanderwolf CH. Neurophysiological techniques: applications to neural systems. 1st edition. Clifton, New Jersey: Humana Press, 1990.

2 Boraud T, Bezard E, Bioulac B, *et al*. From single extracellular unit recording in experimental and human Parkinsonism to the development of a functional concept of the role played by the basal ganglia in motor control. Prog Neurobiol, 2002,66(4):265-283

3 Mukamel R, Fried I. Human intracranial recordings and cognitive neuroscience. Annu Rev Psychol, 2012,63(1):511-537

4 Baird RA, Desmadryl G, Fernández C, *et al*. The vestibular nerve of the chinchilla. II. Relation between afferent response properties and peripheral innervation patterns in the semicircular canals. J Neurophysiol, 1988,60(1):182-203

5 Hullar TE, Minor LB. High-frequency dynamics of regularly discharging canal afferents provide a linear signal for angular vestibuloocular reflexes. J Neurophysiol, 1999,82(4):2000-2005

6 Hullar TE, Della Santina CC, Hirvonen T, *et al*. Responses of irregularly discharging chinchilla semicircular canal vestibular-nerve afferents during high-frequency head rotations. J Neurophysiol, 2005,93(5):2000-2005

7 Ren P, Li B, Dong S, *et al*. The reliability of nonlinear least-squares algorithm for data analysis of neural response activity during sinusoidal rotational stimulation in semicircular canal neurons. PLoS One, 2018,13(1):e0190596

8 Della Santina CC, Migliaccio AA, Patel AH. A multichannel semicircular canal neural prosthesis using electrical stimulation to restore 3-d vestibular sensation. IEEE Trans Biomed Eng, 2007,54(6 Pt 1):1016-1030

9 Sadeghi SG, Minor LB, Cullen KE. Response of vestibular-nerve afferents to active and passive rotations under normal conditions and after unilateral labyrinthectomy. J Neurophysiol, 2007,97(2):1503-1514

10 Linde A, Summerfield NJ, Johnston M, *et al*. Echocardiography in the Chinchilla. J Vet Intern Med, 2004,18(5):772-774

11 Hullar TE, Williams CD. Geometry of the semicircular canals of the chinchilla. Hear Res, 2006,213(1-2):17-24

12 Hartmann R, Klinke R. Discharge properties of afferent fibres of the goldfish semicircular canal with high frequency stimulation. Pflugers Arch, 1980,388(2):111-121

13 Yu XJ, Dickman JD, Angelaki DE. Detection thresholds of macaque otolith afferents. J Neurosci, 2012,32(24):8306-8316

14 Yu XJ, Thomassen JS, Dickman JD, *et al*. Long-term deficits in motion detection thresholds and spike count variability after unilateral vestibular lesion. J Neurophysiol, 2014,112(4):870-889

15 Della Santina CC, Potyagaylo V, Migliaccio AA, *et al*. Orientation of human semicircular canals measured by three-dimensional multiplanar CT reconstruction. J Assoc Res Otolaryngol, 2005,6(3):191-206

16 Goldberg JM. Afferent diversity and the organisation of central vestibular pathways. Exp Brain Res, 2000,130(3):277-297
17 Cullen KE, Roy JE. Signal processing in the vestibular system during active versus passive head movements. J Neurophysiol, 2004,91(5):1919-1933
18 Bagnall MW, McElvain LE, Faulstich M, *et al*. Frequency independent synaptic transmission supports a linear vestibular behavior. Neuron, 2008,60(2):343-352
19 Massot C, Chacron MJ, Cullen KE. Information transmission and detection thresholds in the vestibular nuclei: single neurons versus population encoding. J Neurophysiol, 2011,105(4):1798-1814
20 Massot C, Schneider AD, Chacron MJ, *et al*. The vestibular system implements a linear-nonlinear transformation in order to encode self-motion. PloS Biol, 2012,10(7):1-20
21 Sutter Instrument. Pipette Cookbook 2015 P-97 & P-1000 Micropipette Pullers. 2015. Retrieved from http://www.sutter.com/contact/faqs/pipette_cookbook.pdf

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