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VIBRISSA SENSORY NEURONS: LINKING DISTINCT MORPHOLOGY TO SPECIFIC PHYSIOLOGY AND FUNCTION

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Abstract—Rodents use an array of long tactile facial hairs, the vibrissae, to locate and discriminate objects. Each vibrissa is densely innervated by multiple different types of trigeminal (TG) sensory neurons. Based on the sensory ending morphology, there are at least six types of vibrissa innervating neurons; whereas based on electrophysiological recordings, vibrissa neurons are generally classified as rapidly adapting (RA) and slowly adapting (SA), and show different responses to whisking movement and/or touch. There is a clear missing link between the morphologically defined neuronal types and their exact physiological properties and functions. We briefly summarize recent advances and consider single-cell transcriptome profiling, together with optogenetics-assisted *in vivo* electrophysiology, as a way to fill this major gap in our knowledge of the vibrissa sensory system.

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Key words: vibrissa sensory endings, morphological classification, electrophysiological responses, genetic labeling, single-cell RNA-seq, optogenetics.

Contents

MORPHOLOGICAL CLASSIFICATIONS

Each vibrissa is densely innervated by peripheral sensory endings from 100 to 200 sensory neurons (Rice et al., 1986), whose cell bodies reside in the trigeminal ganglion. The myelinated tactile sensory endings innervating the vibrissa follicle sinus complex are morphologically classified into six to seven subtypes including: 1) Merkel endings at the superficial rete ridge collar, 2) transverse lanceolate endings at the level of ring sinus (RS), 3) longitudinal lanceolate endings at the level of RS, 4) Merkel endings in the outer root sheath at the level of RS, 5) club-like endings at the ringwulst, 6) reticular endings at the level of cavernous sinus (CS), and 7) spiny (or Ruffini) endings also at the level of CS (Ebara et al., 2002; Fundin et al., 1997a) (Fig. 1). Distinct features of mechanical stimuli are thought to be differentially converted into neural activities across these subtypes and this combination of activities acts as a unique touch code. Thus, how touch information is deconstructed and encoded by the distinct types of mechanosensory neurons and how the deconstructed information is reintegrated in the brain are vital questions to understand touch perception.

ELECTROPHYSIOLOGICAL STUDIES AND CLASSIFICATIONS

Extracellular recording of TG sensory neurons (blindly) in anesthetized rats has been the model of choice to study response properties of first-order vibrissa afferents (Gibson and Welker, 1983a). Vibrissa neurons encode mechanical forces (e.g., torque) during touch (Bush et al., 2016; Severson et al., 2017), with extremely high spike-timing precision (Bale et al., 2015) and directional selectivity (Lichtenstein et al., 1990). They have been classified as either slowly adapting (SA), rapidly adapting (RA), or mixed SA-RA types according to their response profile following passive whisker deflection with ramp-and-hold stimuli of varying velocity (Gibson and Welker, 1983b; Lichtenstein et al., 1990; Shoykhet et al., 2000; Jones et al., 2004). Angular selectivity differs between RA and SA types, with the former generally showing much sharper tuning (Lichtenstein et al., 1990). It is generally accepted that sensory afferents with Merkel endings are SA, and afferents with lanceolate endings are RA type (Lichtenstein et al., 1990). Although some results implied that sensory neurons with reticular and club-like endings may respectively conform to SA and RA types (Tonomura et al., 2015), their adaption properties, as well as those of spiny/Ruffini cell types remain unresolved.

Since the ramp-and-hold stimuli applied to passive whiskers do not mimic naturally occurring stimuli experienced by rodents performing active whisking while exploring environmental objects, Szwed et al. (2003) recorded TG neuron responses in an ‘‘artificial whisking”

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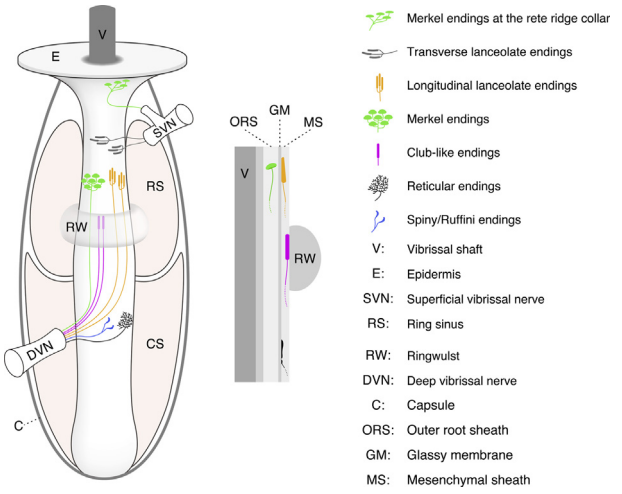

Fig. 1.

paradigm to whisking-in-air or contacting-an-object stimuli in anesthetized rats. Their results classified TG neurons into (1) whisking cells that respond only to whisking, (2) touch cells that respond only to touch, (3) whisking/touch cells that respond to both touch and whisking, and (4) high threshold cells that respond only to strong mechanical stimuli. In all four categories, RA and SA neurons were found. The touch cells could further be divided into four subclasses: contact, pressure, detach, and contact/detach cells. The pressure cells that fire long trains of action potentials as long as the vibrissa presses against the objects were all SA neurons; while the contact/detach cells that fire briefly when the vibrissa touches or detaches from the objects were all RA neurons. This elegant study sheds light on how the firing rates and firing timing of TG neurons can encode object locations (Szwed et al., 2003). How results from "artificial whisking" paradigms translate to natural behavioral is subject to debate, as artificial whisking does not fully recapitulate active whisking conditions. In particular, whisking kinematics are constantly modulated by behavioral and contextual factors such as exploratory head movements and anticipated object location (Towal and Hartmann, 2006; Mitchinson et al., 2007; Voigts et al., 2015). Variable whisking strategies likely impact peripheral encoding. Another crucial issue, namely how any of those electrophysiological (sub-)classes correspond to the 6–7 morphological classes (based on the shapes of sensory endings) remains at that point completely unresolved.

Since recordings in anesthetized rats may not recapitulate neuronal responses in awake animals, Leiser and Moxon (2007) went a step further and performed *in vivo* single-unit recording of TG neurons while rats performed natural whisking. In contrast to previous studies, their results suggest that all TG neurons are whisking/touch cells, or in other words, there are no whisking-in-air-only responsive, or touch-only responsive sensory neurons. All neurons fire more with increasing whisking frequency, and further robustly increase firing when the vibrissae contact objects. SA and RA neurons also exhibited differential patterns of increased firing in response to whisking and touch. However, a few caveats hamper this study. First, for single-unit clustering, a crude dead-time parameter appears to artificially clear-out the refractory period in autocorrelograms. Thus, it cannot be ruled out that neurons with similar waveforms were lumped together in a multi-unit cluster, thereby blurring each unit's functional properties. Second, false-negative events (when whisker touch goes undetected) may have contaminated free-whisking results ("whisking-in-air" condition). Indeed, when using a single top-view camera angle, touch events from vertical whisker movements may be missed (e.g., when touching the floor during exploration). In addition, light whisker touches, with limited bending, where not classified but could nonetheless be a confound in free-whisking results. Beyond those caveats, neurons cannot be identified through blind extracellular recordings, so this study could not bridge the divide between the structure/morphology and functions/encoding properties of vibrissa neurons in awake behaving rodents.

A recent attempt to link morphology with function through blind recording was a heroic *in vivo* intracellular recording followed by neurobiotin labeling of recorded neurons performed by Tonomura et al. (2015). 36 recorded and successfully traced cells from 49 anesthetized rats were vibrissa neurons that included at least 1 neuron for each morphological ending type. Only results from an air puff stimulation (10-second long) were reported, although more results with other types of stimuli may come out from this study in the future. All types of neurons responded to air puff but with different firing rates. The club-like endings exhibited the highest maximal firing frequency compared to lanceolate, Merkel and reticular ending neurons. While extremely valuable, the intracellular recording/tracing experiments are low throughput (one neuron per rat) and, due to TG's location, are too invasive and challenging to be performed in awake preparations. The field needs to and has begun to conduct *in vivo* recording from molecularly identified neurons in awake whisking mice (see below).

IDENTIFYING MOLECULAR MARKERS FOR DIFFERENT TG TOUCH NEURONS

Given current technology, the most logical approach to identify the link between morphology and function is to discover molecular markers that can be used to label a specific morphological class of vibrissa neurons, and perform *in vivo* recording from the identified neurons in awake behaving animals.

Candidate approaches

Recently, significant progress was made in identifying molecular/genetic markers for the body sensory neurons residing in the dorsal root ganglion (DRG). Many of the markers have been used to generate Cre/CreER driver lines that revealed labeling of subsets of low-threshold mechanosensory DRG cells (i.e. touch neurons). A summary of these lines and the sensory ending types they detect is listed in Table 1. So far, in the vibrissal