Electrophysiological properties of maxillary trigeminal Aβ-afferent neurons of rats

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Abstract
Aβ-afferents in maxillary or V2 trigeminal ganglion (TG) neurons are somatosensory neurons that may be involved in both non-nociceptive and nociceptive functions in orofacial regions. However, electrophysiological properties of these V2 trigeminal Aβ-afferent neurons have not been well characterized so far. Here, we used rat ex vivo trigeminal nerve preparations and applied patch-clamp recordings to large-sized V2 TG neurons to characterize their electrophysiological properties. All the cells recorded had afferent conduction velocities in the range of Aβ-afferent conduction speeds. However, these V2 trigeminal Aβ-afferent neurons displayed different action potential (AP) properties. APs showed fast kinetics in some cells but slow kinetics with shoulders in repolarization phases in other cells. Based on the derivatives of voltages in AP repolarization with time (dV/dt), we classified V2 trigeminal Aβ-afferent neurons into four types: type I, type II, type IIIa and type IIIb. Type I V2 trigeminal Aβ-afferent neurons had the largest dV/dt of repolarization, the fastest AP conduction velocities, the shortest AP and afterhyperpolarization (AHP) durations, and the highest AP success rates. In contrast, type IIIb V2 trigeminal Aβ-afferent neurons had the smallest dV/dt of AP repolarization, the slowest AP conduction velocities, the longest AP and AHP durations, and the lowest AP success rates. The type IIIb cells also had significantly lower voltage-activated K⁺ currents. For type II and type IIIa V2 trigeminal Aβ-afferent neurons, AP parameters were in the range between those of type I and type IIIb V2 trigeminal Aβ-afferent neurons. Our electrophysiological classification of V2 trigeminal Aβ-afferent neurons may be useful in future to study their non-nociceptive and nociceptive functions in orofacial regions.

Keywords
Trigeminal, Aβ-afferent, pain, action potentials, orofacial, patch-clamp

Introduction
Primary afferent nerves are commonly classified into C-, Aδ- and Aα/β-afferents (cutaneous afferents) or into group IV, III, II and I fibers (muscle afferents) on the basis of their conduction velocities. The somas of primary afferents, located in the dorsal root ganglions (DRGs) and trigeminal ganglions (TGs), largely vary in size, and are correlated with afferent types. Generally, small- to medium-sized afferent neurons give rise to non-myelinated C-fibers or thinly myelinated Aδ-fibers (or IV- and III-fibers of muscle afferents), whereas large-sized DRG and TG neurons give rise to well myelinated Aβ/Aα-fibers (or II and I-fibers of muscle afferents). Electrophysiological properties and functions of small-sized afferent neurons have been extensively studied. The afferent fibers of most
the DRGs can encode high threshold mechanical and thermal stimuli to function in nociception.\textsuperscript{4,5} The DRG neurons of these afferents are termed nociceptive Aβ-afferent neurons.\textsuperscript{4} For example, in a previous study in cats, it has been shown that although most Aβ-afferents could be activated by gentle mechanical stimulation, a small subset with conduction velocities in the low end of the Aβ afferent range encode nociceptive stimuli.\textsuperscript{6}

Nociceptive Aβ-afferents from DRGs have been suggested to be first responders to noxious stimuli and play an essential role in pain under physiological conditions.\textsuperscript{4,5,7} Many nociceptive Aβ-afferents from DRGs are high threshold mechanoreceptors (HTMRs).\textsuperscript{4,7} It has been hypothesized that under pathological conditions the mechanical threshold of these Aβ-afferent HTMRs may be reduced, leading to mechanical hyperalgesia/alldynia.\textsuperscript{8} On the other hand, non-nociceptive Aβ-afferents from DRGs are mainly LTMRs involved in tactile sensations.\textsuperscript{3} Non-nociceptive Aβ-afferents may also be involved in mechanical allodynia under pathological conditions when central sensitization occurs in the spinal cord dorsal horn.\textsuperscript{9} Thus far, the contribution of Aβ-afferents in sensory disorders involving pathological pain have been poorly studied, partially because limited attempts have been made to differentiate between non-nociceptive and nociceptive Aβ-afferents.

Using intracellular recordings made from DRG neurons in anesthetized animals, previous studies have characterized electrophysiological properties of both non-nociceptive Aβ-afferents and nociceptive Aβ-afferents.\textsuperscript{4,10} Neurons in somatosensory ganglia have been known to be heterogeneous not only in cell size, but also in electrophysiological properties and sensory functions. For example, broad AP spikes with shoulders were commonly found in DRG and TG neurons with unmyelinated C-afferent nociceptors.\textsuperscript{5,11} Similarly, in DRGs, it has been demonstrated that nociceptive afferents in either Aδ- or Aβ-afferent conduction velocity range also exhibit broad AP spikes with shoulders.\textsuperscript{4} In DRGs, nociceptive Aβ-afferents differed from non-nociceptive Aβ-afferents in that the former had greater AP duration, longer AHP duration, and prominent shoulder on their repolarization.\textsuperscript{6,11} These electrophysiological properties are helpful for classifying non-nociceptive-like and nociceptive-like Aβ-afferents of DRG neurons.

Orofacial regions are innervated by maxillary branches (V2 branches) of trigeminal nerves, which can undergo hyper-excitability leading to thermal and mechanical allodynia under pathological conditions.\textsuperscript{12,13} V2 trigeminal nerves only contain sensory nerve fibers but proprioceptive Aα-afferent fibers are not present in V2 trigeminal nerves. Previous electrophysiological studies have mainly focused on small-sized nociceptive-like TG neurons including V2 trigeminal afferent neurons that innervate orofacial regions.\textsuperscript{12,14,15} Yet electrophysiological properties of Aβ-afferent neurons in TGs have not been well characterized so far. In the present study we characterized electrophysiological properties of V2 trigeminal Aβ-afferent neurons by using ex vivo rat trigeminal nerve preparations and patch-clamp recording technique, and found these neurons can be classified into at least 4 subtypes.

### Materials and methods

#### Animals

Sprague-Dawley rats of both males and females aged at 11–15 weeks were used. Animal care and use conformed to NIH guidelines for care and use of experimental animals. Experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Alabama at Birmingham.

#### Patch-clamp recordings from large V2 trigeminal afferent neurons in ex vivo whole-mount trigeminal ganglion preparations

Trigeminal ganglions (TGs) with attached infraorbital nerve bundles (~15 mm) were rapidly dissected out and placed in an ice cold Krebs solution (see below). Connective tissues on the surface of the TGs were carefully removed with a fine forceps. TGs with their infrarostral nerve bundles were then affixed in a recording chamber by a tissue anchor and submerged in a Krebs solution that contained (in mM): 117 NaCl, 3.5 KCl, 2.5 CaCl\textsubscript{2}, 1.2 MgCl\textsubscript{2}, 1.2 NaH\textsubscript{2}PO\textsubscript{4}, 25 NaHCO\textsubscript{3}, and 11 glucose. The Krebs solution was saturated with 95% O\textsubscript{2} and 5% CO\textsubscript{2}, had pH of 7.35, and osmolarity of 324 mOsm, and maintained at room temperature of 24°C. TGs were exposed to 0.05% dispase II and 0.05% collagenase in the Krebs solution for 5 min, then continuously perfused at 2 ml/min with the Krebs solution. Under an infrared-differential interference contrast (IR-DIC microscope), whole-cell patch-clamp recordings were performed on V2 TG neurons ranging 40–55 μm in diameter. Recording electrode internal solution contained (in mM): 105 K-glucosate, 35 KCl, 0.5 CaCl\textsubscript{2}, 2.4 MgCl\textsubscript{2}, 5 EGTA, 10 HEPES, 5 Na\textsubscript{2}ATP and 0.33 GTP-TRIS salt; the pH of the solution was adjusted to 7.35 with KOH. The electrode resistance ranged from 4 to 6 MΩ. The junction potential of the recording condition was 12 mV as calculated based on ionic concentrations of internal and the Krebs bath solutions by using the pCLAMP 10 software (Molecular Devices). After establishing whole-cell access, recordings were first performed under the conventional current-clamp configuration. Action potentials (APs), recorded at the soma of large-sized V2 TG neurons, were evoked at the peripheral end of trigeminal V2 branches (the infraorbital nerves) using a
suction stimulation electrode. The suction stimulation electrode was fire-polished with tip size of ~1 mm in diameter. The peripheral end of the infraorbital nerve was aspirated into the suction stimulation electrode by a negative pressure. APs were evoked by monophasic square wave pulses generated by an electronic stimulator (Master-8) and delivered via a stimulation isolator and the suction stimulation electrode to the peripheral ends of the infraorbital nerves. The duration of the stimulation pulse was 200 μs each pulse. The stimulation intensity was twofold the threshold intensity (30 μA or higher) for eliciting APs from these afferent nerves. APs evoked by the peripheral nerve stimulations were used for determining AP parameters including derivatives of repolarization with time (dV/dt), amplitude, half-width, and afterhyperpolarization duration (AHP). In one set of experiments, APs were elicited by the electrical stimuli at the frequencies of 1, 10, 20, 50, 100, 200, 500, and 1000 Hz. This set of experiments allowed measurement of AP success rate in response to stimuli at different stimulation frequencies.

APs were also recorded by directly injecting depolarizing current into V2 trigeminal Aβ-afferent neurons. This was done under the current-clamp configuration with step currents injected from recording electrodes into the somas of V2 trigeminal Aβ-afferent neurons. The current steps were from −200 pA to 4000 pA with increments of 200 pA per step and the duration of each step was 1 s. APs evoked by current steps were used to determine membrane input resistance, and AP parameters including dV/dt of repolarizations, rheobase, amplitude, half width, and AP thresholds.

Recordings were also performed under the voltage-clamp configuration with neurons held at −72 mV (voltage command of −60 mV). Voltage steps were applied from −102 mV to +58 mV (voltage command of −90 to +70 mV) with increments of 10 mV each step and a step duration of 500 ms. Unless otherwise indicated, membrane voltages mentioned in the texts for this set of experiments have been corrected for the calculated junction potentials of 12 mV.

Signals of current-clamp and voltage-clamp experiments were amplified using a MultiClamp 700B amplifier (Molecular Devices). Signals of current-clamp recordings were low-pass filtered at 2 kHz and sampled at 50 kHz. The sampling at high frequency of 50 kHz is necessary for a high resolution measurement of AP parameters. Signals of voltage-clamp recordings were low-pass filtered at 2 kHz and sampled at 10 kHz. The signals were sampled using the pCLAMP 11 software (Molecular Devices).

**Data analysis**

In the present study TGs were obtained from 6 male and 21 female rats, and the data from both sexes were pooled together as we did not observe any sex differences in the AP properties of V2 trigeminal Aβ-afferent neurons. Conduction velocity was calculated based on the latency of APs and the length of axons. The latency of an AP was measured from the time of stimulation that was marked by a stimulation artifact to the time when the AP was initiated at the recorded TG neuron. The length of the axon was the distance between the stimulation site and the recording site. Input resistance was measured with a -10 mV voltage step from the membrane holding voltage of -72 mV. AP amplitude was measured from AP peak to AP afterhyperpolarization. AP half width was measured as the duration from 50% of AP upstroke to 50% of AP repolarization. AHP duration was measured as the duration between AHP peak and the potential of 80% AHP recovery (i.e. AHP80%). Membrane and AP parameters as well as voltage-activated currents were analyzed using Clampfit 11 software. Unless otherwise indicated, all data are reported as mean ± SEM of n independent observations. Statistical comparisons were made using GraphPad Prism 8 software with *p < 0.05, **p < 0.01, and ***p < 0.001, Student’s t-test or one-way ANOVA with Tukey’s post-hoc tests for multiple groups.

**Results**

The maxillary nerves or V2 branches of the trigeminal nerves are somatosensory nerves that innervate orofacial regions (Figure 1(a)). The somas of these trigeminal afferent nerves are located in the V2 part in the trigeminal ganglion12 (Figure 1(b)). Under an IR-DIC microscope, individual TG neurons in our *ex vivo* TG preparations could be visualized in the V2 TG regions, and these V2 TG neurons showed different cell sizes (Figure 1(b) and (c)). Since in the present study we focused on Aβ-afferent neurons that innervate orofacial regions, we applied patch-clamp recordings to large V2 TG neurons in our *ex vivo* TG preparations (Figure 1(c) and (d)). The V2 TG neurons selected for our patch-clamp recordings had mean diameters ranging from 40 to 55 μm (n = 51). Of these cells, 42 of them (82%) had mean diameters ≥45 μm and 9 of them had mean diameters ≥40 but smaller than 45 μm. Overall, the averaged mean diameters of the recorded V2 TG neurons were 46.0 ± 0.37 μm (n = 51).

To determine if these cells were Aβ-afferent neurons, action potentials (AP) were recorded under the current-clamp configuration from the somas of large-sized V2 TG neurons while electrical stimuli were applied to the peripheral sites of infraorbital nerve bundles (ION, Figure 1(b) and (e)). This allowed us to determine the conduction velocities of these afferents. In each of the V2 TG neurons recorded in the present study, APs appeared after short latencies following the peripheral electrical
stimulation (Figure 1(e)). The conduction velocities of APs were $22.2 \pm 4.2$ m/s for the V2 TG neurons with mean diameters $\geq 40$ and $< 45 \mu m$ ($n = 9$), $21.7 \pm 1.3$ m/s for the V2 TG neurons with mean diameters $45$ and $< 47.5 \mu m$ ($n = 23$), $23.5 \pm 1.7$ m/s for the V2 TG neurons with mean diameters $47.5$ and $< 50 \mu m$ ($n = 13$), and $20.6 \pm 1.7$ m/s for the V2 TG neurons with mean diameters $\geq 50 \mu m$ ($n = 6$). There were no significant differences in the conduction velocities among the cell-size groups of these V2 TG neurons (Figure 1(f)).

The conduction velocities measured from these V2 TG neurons all fell into the range of the conduction speed of Aβ-afferent fibers,$^4$ and we therefore regard them as V2 trigeminal Aβ-afferent neurons.

APs recorded from the V2 trigeminal Aβ-afferent neurons following electrical stimulation of the ION nerves displayed three main types based on the slopes of their repolarization phases (Figure 2(a) to (c)). This was more distinguishable with the derivatives of voltages with time (dV/dt) in the repolarization phases (Figure 2

Figure 1. Large-sized V2 TG neurons and their conduction velocities determined by patch-clamp recordings with ex vivo trigeminal nerve preparations. (a) Illustration of orofacial regions where the V2 branches of trigeminal nerves innervate. (b) Diagram illustrates electrophysiology recording setup. Ex vivo trigeminal nerve preparations with V2 afferent nerve bundle (infraorbital nerves) were used and illustrated in the diagram. Patch-clamp recordings were performed from large-sized neurons in the V2 region of each TG ganglion. A suction stimulation electrode was used to elicit action potentials (APs) by stimulating the infraorbital nerves (ION), the V2 branch of trigeminal nerves. (c) Image shows V2 TG neurons in an ex vivo TG preparation as viewed under a 40X objective. Note that a large-sized TG neuron is located at the center of the field, and recordings were applied to this type of large TG neurons in the present study. (d) Histogram of cell size distribution for the V2 TG neurons recorded in the present studies. (e) Sample trace shows an action potential recorded from a large-sized V2 TG neuron following electrical stimulation applied to ION. Arrow indicates stimulation artifact, which allowed to determine the conduction velocity of the afferent being recorded. (f) Comparison of the conduction velocities of afferent fibers with different soma size groups of $\geq 40$ and $< 45 \mu m$ ($n = 9$), $\geq 45$ and $< 47.5 \mu m$ ($n = 23$), $\geq 47.5$ and $< 50 \mu m$ ($n = 13$), and $\geq 50$ and $< 55 \mu m$ ($n = 6$). In f, each symbol represents an individual experimental observation, mean ± SEM values are also shown; ns, no significant difference.
(a) to (c)). The first type (type I) showed rapid AP repolarization, and the dV/dt in the repolarization phase showed linear acceleration with a single large-sized negative peak (Figure 2(a) and (d)). The second type (type II) showed relatively slower AP repolarization and the dV/dt in the repolarization phase showed nonlinear acceleration with a medium-sized negative peak and a narrow negative shoulder (Figure 2(b) and (e)). The third type (type III) showed very slow AP repolarization with a clear shoulder in the repolarization phase (Figure 2(c)), and the dV/dt in the repolarization phase showed slow acceleration with a broad negative shoulder followed by a small negative peak (Figure 2(b) and (f)). Examination of the negative peaks of the dV/dt in the type III APs revealed two clusters of dV/dt values (Figure 2(g)). We arbitrarily divided them into type IIIa for the APs with relatively larger dV/dt and type IIIb for APs with relatively smaller dV/dt values (Figure 2(g)).
Based on the aforementioned AP types, we attributed these neurons into type I, type II, type IIIa, and type IIIb V2 trigeminal Aβ-afferent neurons accordingly. We compared dV/dt values of APs of the four types of V2 trigeminal Aβ-afferent neurons (Figure 2(h)). As shown in Figure 2(h), the dV/dt values of the negative peaks of type I (280.0 ± 27.1 V/s, n = 6) were significantly higher than those of type II (177.3 ± 4.7 V/s, n = 7), type IIIa (157.1 ± 4.1 V/s, n = 23), and type IIIb (93.2 ± 4.2 V/s, n = 15) V2 trigeminal Aβ-afferent neurons. The dV/dt values of the negative peaks of type IIIb (n = 15) were the smallest and were significantly lower than those of type I, type II and type IIIa V2 trigeminal Aβ-afferent neurons.

We next characterized other properties of these four types of V2 trigeminal Aβ-afferent neurons. There were no significant differences in the cell sizes (Figure 3(a)) and resting membrane potentials (Figure 3(b)) among the four types of V2 trigeminal Aβ-afferent neurons. On the other hand, the conduction velocities of the type IIIb V2 trigeminal Aβ-afferents (16.0 ± 0.9 m/s, n = 15) were significantly slower than the other three types of V2 Aβ-afferents (Figure 3(c)). There were no significant differences in conduction velocities among the type I (29.7 ± 4.6 m/s, n = 6), type II (23.2 ± 1.8 m/s, n = 7) and type IIIa (23.8 ± 1.2 m/s, n = 23) V2 trigeminal Aβ-afferents. AP half widths were the shortest in type I (0.41 ± 0.05 ms, n = 6) and the longest in type IIIb V2 (1.76 ± 0.12 ms, n = 15) trigeminal Aβ-afferent neurons (Figure 3(d)). AP half widths were 0.62 ± 0.02 ms (n = 7) for type II and 0.93 ± 0.03 ms (n = 7) for type IIIa, and were significantly shorter than those of type IIIb V2 trigeminal Aβ-afferent neurons (Figure 3(d)). AP amplitudes were significantly smaller in type I in comparison with type II V2 trigeminal Aβ-afferent neurons, but there were no significant differences among other types of V2 trigeminal Aβ-afferent neurons (Figure 3(e)). AHP80% durations were shown to be the shortest in type I and the longest in type IIIb V2 Aβ-afferent neurons. The AHP80% durations of type IIIb V2 trigeminal Aβ-afferent neurons (179.0 ± 21.0 ms, n = 15) were significantly longer than those of type I (14.1 ± 7.1 ms, n = 6), type II (32.6 ± 6.7 ms, n = 7) and type IIIa (94.9 ± 10.2 ms, n = 22) V2 trigeminal Aβ-afferent neurons (Figure 3(f)).

We determined success rates of AP conduction along different types of V2 trigeminal Aβ-afferents in responses to electrical stimuli at different stimulation frequency. In this set of experiments, APs were elicited by twofold threshold stimuli at the peripheral sites of infraorbital nerves and recordings were made from the somas of the V2 trigeminal Aβ-afferent neurons. At low frequency stimuli of 1 and 10 Hz, APs could be reliably elicited and recorded with a 100% success rate for all four types of V2 trigeminal Aβ-afferents (Figure 4(a) and (b)). At the stimulation frequency of 20 Hz, AP success rates were reduced to 32.2 ± 11.1% (n = 8) in type IIIb V2 trigeminal Aβ-afferents but no significant reduction in AP success rates occurred in other three types of V2 trigeminal Aβ-afferents. At the stimulation frequency of 50 Hz, AP success rates were greatly reduced to 8.1 ± 3.8% (n = 8) in type IIIb V2 trigeminal Aβ-afferents and also significantly reduced to 34.2 ± 5.5% (n = 14) in type IIIa V2 trigeminal Aβ-afferents but AP success rates were not significantly reduced in both type I and type II V2 trigeminal Aβ-afferents. At a high frequency of 100 Hz stimulation, AP success rates were reduced to 25.7 ± 5.8% (n = 7) in type II, to 7.9 ± 2.2% (n = 24) in type IIIa, and to 4.2 ± 1.5% (n = 15) in type IIIb V2 trigeminal Aβ-afferents, but AP success rates of type I V2 trigeminal Aβ-afferents remained to be high (86.2 ± 8.8%, n = 6). However, at the frequency of ≥200 Hz, AP success rates were also significantly reduced in type I V2 trigeminal Aβ-afferents, and almost all the 4 types of V2 trigeminal Aβ-afferents failed to follow the stimuli at the frequency of 1000 Hz (Figure 4(b)). Plotting the AP success rates over stimulation frequencies show sigmoid relationships (Figure 4(c)), and from these curves we determined frequency at which AP success rate or failure rate was at 50% (FS50, Figure 4(c) and (d)). The FS50 values were 167.8 ± 16.8 Hz (n = 6) for type I, 85.1 ± 4.9 Hz (n = 7) for type II, 48.7 ± 3.6 Hz (n = 24) for type IIIa, and 30 ± 6.0 Hz (n = 15) for type IIIb V2 trigeminal Aβ-afferents, respectively.

In the above experiments APs were elicited by applying electrical stimulation to the peripheral sites. We next determined AP properties when APs were elicited by directly injecting depolarizing step currents into the somas of the four types of V2 trigeminal Aβ-afferent neurons. The injection of depolarizing step currents resulted in membrane depolarization and AP firing once the depolarization reached AP thresholds (Figure 5(a) to (e)). The repolarization phase of APs elicited by the step current injections showed features similar to APs elicited by electrical stimulation to the peripheral sites of V2 trigeminal Aβ-afferents. The type I V2 trigeminal Aβ-afferent neurons showed rapid AP repolarization, and the dV/dt in AP repolarization phase showed linear acceleration with a large and sharp negative peak (Figure 5(a)). The type IIIb V2 trigeminal Aβ-afferent neurons showed slow AP repolarization, and the dV/dt in AP repolarization phase showed slow acceleration with a broad negative shoulder followed by a small negative peak (Figure 5(c)). The type II (Figure 5(b)) and type IIIa (not illustrated) V2 trigeminal Aβ-afferent neurons showed features between those of type I and type IIIb. Overall, the dV/dt values of the negative peaks of AP repolarization (Figure 5(d)) were 208.3 ± 22.8 V/s (n = 6) in type I, 120.7 ± 7.9 V/s (n = 7) in type II, 117.9 ± 5.9 V/s (n = 25) in type IIIa, and 79.8 ± 5.5 V/s
(n = 15) in type IIIb V2 trigeminal Aβ-afferent neurons. AP half widths were the shortest in type I (0.44 ± 0.04 ms, n = 6) and longest in type IIIb (2.24 ± 0.30 ms, n = 11) V2 trigeminal Aβ-afferent neurons. AP half widths were 0.72 ± 0.03 ms (n = 7) in type II and 1.35 ± 0.09 ms (n = 18) in type IIIa V2 trigeminal Aβ-afferent neurons, and were significantly shorter than those of type IIIb V2 trigeminal Aβ-afferent neurons (Figure 5(e)). AP thresholds were −39.3 ± 1.45 mV (n = 6), −44.6 ± 1.48 mV (n = 7), −40.2 ± 2.4 mV (n = 18) and −43.4 ± 0.8 mV (n = 11) in type I, type II, type IIIa, and type IIIb V2 trigeminal Aβ-afferent neurons, respectively, and were not significantly different among these V2 trigeminal Aβ-afferent neurons.
Under the voltage-clamp configurations, we examined membrane currents evoked by depolarizing voltage steps in V2 trigeminal Aβ-afferent neurons. For all the four types of V2 trigeminal Aβ-afferent neurons, depolarizing voltage steps evoked transient inward currents at initial stage which were followed by large outward currents (Figure 6(a)). The transient inward currents were voltage-activated Na⁺ currents and the outward currents

(Figure 5(f)). AP amplitudes were shown no significant differences among these V2 trigeminal Aβ-afferent neurons (Figure 5(g)). AP rheobases were relatively lower in type I and type IIIb V2 trigeminal Aβ-afferent neurons in comparison with type IIIa V2 trigeminal Aβ-afferent neurons (Figure 5(h)). There were no significant differences in input resistances of the four different types of V2 trigeminal Aβ-afferent neurons (Figure 5(i)).
were voltage-activated $K^+$ currents. While there were no obvious differences in the kinetics of the outward currents among the four types of V2 trigeminal A$\beta$-afferent neurons (Figure 6(a)), the amplitudes of the outward currents were found to be relatively smaller in type IIIb V2 trigeminal A$\beta$-afferent neurons in comparison with type I and type IIIa V2 trigeminal A$\beta$-afferent neurons (Figure 6(b)).

**Discussion**

In the present study, we have characterized V2 trigeminal A$\beta$-afferent neurons and classified them into four subtypes based on their AP properties. We show that a number of AP parameters are significantly different among V2 trigeminal A$\beta$-afferent neurons. These parameters include the $dV/dt$ of AP repolarization, AP conduction velocities, AP widths, AHP durations, and AP conduction success rates in response to high frequency stimulation. These AP parameters may be useful electrophysiological indicators of functional subtypes of V2 trigeminal A$\beta$-afferents such as non-nociceptive-like and nociceptive-like V2 trigeminal A$\beta$-afferents. The classification of V2 trigeminal A$\beta$-afferent neurons may help future studies on the roles of V2 trigeminal A$\beta$-afferents in different sensory functions and their potential

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**Figure 5.** Properties of action potentials elicited by direct current injections into the somas of V2 A$\beta$-afferent neurons. Three sets of sample traces (solid dark) show APs (top) and their derivatives with time ($dV/dt$, bottom) in a type I (a), a type II (b) and a type IIIb (c) V2 trigeminal A$\beta$-afferent neurons following current injections into the somas of the recorded neurons. The gray dashed traces are APs (top) elicited by peripheral electrical stimulation (top panels) and their $dV/dt$ (bottom). (d) The $dV/dt$ values of negative peaks of repolarization for type I ($n = 6$), type II ($n = 7$), type IIIa ($n = 18$), and type IIIb ($n = 11$) V2 trigeminal A$\beta$-afferent neurons. Summary data of AP half widths (e), AP threshold (f), AP amplitude (g), AP rheobase (h), and input resistance (i) of the four types of V2 trigeminal A$\beta$-afferent neurons. Each symbol represents an individual experimental observation, mean ± SEM is also shown; ns, no significant difference; *$P < 0.05$, **$P < 0.001$, ***$P < 0.001$. 

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involvement in pain such as mechanical allodynia under pathological conditions.

Previous *in vitro* studies on electrophysiological properties of somatosensory neurons were mostly performed with DRG neurons or TG neurons dissociated from their ganglia.\(^{15,16}\) Most of previous electrophysiological studies were performed on small-sized somatosensory neurons.\(^{15,16}\) Since large-sized somatosensory neurons usually do not survive in cell dissociation procedures, electrophysiological properties of large-sized neurons (i.e. A\(\beta\)-afferent neurons) were not well characterized previously. In the present study, *ex vivo* TG preparations were used and large-sized V2 trigeminal afferent neurons were healthy in our TG preparations. This allowed us to apply patch-clamp recordings to characterize electrophysiological properties of V2 trigeminal A\(\beta\)-afferent neurons.

We have considered V2 trigeminal afferents with conduction velocities more than 10 m/s as A\(\beta\)-afferents in the present study. Most V2 trigeminal afferents in the present study had conduction velocities higher than 10 m/s. Previous studies have considered conduction velocity of 10 m/s as the border between A\(\delta\)- and A\(\beta\)-afferent fibers in rats.\(^4\) However, a previous study in rat trigeminal afferent nerves defined A\(\beta\)-afferent fibers as those with conduction velocities more than 14 m/s.\(^{17}\) It should be noted that our experiments were performed at the room temperature of 24°C while previous studies were mostly performed at temperatures between 30°C to 37°C.\(^4,17\) It has been shown in rat sciatic nerves that the conduction velocity varied about 1.2 m/s per degree centigrade over the range of 20–40°C.\(^{18}\) Therefore, all our A\(\beta\) fibers would have conduction

![Figure 6. Voltage-activated outward currents in different types of V2 A\(\beta\)-afferents.](image-url)
velocities well above 14 m/s if our experiments were conducted at conduction velocities higher than 30 °C. In addition to conduction velocity, other membrane and action potential parameters reported in our study may also differ to some extent from those determined at higher temperatures in previous studies. We performed our in vitro patch-clamp recording experiments at the room temperature of 24 °C rather than at higher temperatures between 30 °C to 37 °C because TG neurons could be kept healthy for longer time at the room temperature of 24 °C during our in vitro experiments. In DRG neurons, large-sized neurons were defined as those with soma mean diameters of ≥45 μm, and the large-sized DRG neurons were usually Aβ-afferents. In our V2 trigeminal afferent neurons, in addition to those cells with mean diameters ≥45 μm, cells with mean diameters in the range of 40 to 45 μm were also found to be Aβ-afferents based on the conduction velocity measured in the present study. Since V2 TGs do not contain proprioceptive Aα-aferents, it is a strength of using V2 TGs for determining the properties of Aβ-afferents without unintended inclusion of Aα-aferents.

We show in the present study that among the four types of V2 trigeminal Aβ-afferent neurons the type I neurons have the largest dV/dt of AP repolarization, the shortest AP widths and AHP80 durations, and the highest AP success rates. In contrast, type IIIb V2 trigeminal Aβ-afferent neurons have the smallest dV/dt of repolarization, the longest AP widths and AHP80 durations, and the lowest AP success rates. These discrete AP properties were observed regardless of APs elicited by electrical stimulation at the peripheral endings or at the soma of V2 trigeminal Aβ-afferent neurons. While we can classify V2 trigeminal Aβ-afferent neurons into 4 subtypes based on AP parameters, we have not directly determined whether these subtypes of V2 trigeminal Aβ-afferent neurons are functionally distinct groups of somatosensory neurons. Previous in vivo recordings from the somas of DRG neurons have shown that different functional groups of Aβ-afferent neurons display distinguished AP properties. For example, previous in vivo recordings have shown that LTMR Aβ-afferent neurons in DRGs have APs with rapid kinetics, large dV/dt without inflection in repolarization phase, short AP widths and AHP durations. In our study, the type I V2 trigeminal Aβ-afferent neurons have the AP properties similar to those of LTMR Aβ-afferent DRG neurons shown in the previous studies. However, the numbers of type I neurons were relatively small in the present study, which could be due to a biased sampling of neurons in our recordings. Previous studies also have shown that nociceptive Aβ-afferent neurons in DRGs have APs with slow kinetics, small dV/dt with inflection in repolarization, and longer action potential widths and AHP durations. Nociceptive Aβ-afferent neurons have been found to account for approximately 20% of Aβ-afferent neurons in DRGs of rats. In the present study, we have found that the majority of V2 trigeminal Aβ-afferent neurons, except type I neurons, have APs with slower kinetics, smaller dV/dt with inflection, and longer action potential widths, and longer AHP durations. It is less likely that all V2 trigeminal Aβ-afferent neurons in type II, type IIIa, and type IIIb groups were nociceptive Aβ-afferent neurons. These three types of neurons may include both non-nociceptive and nociceptive V2 trigeminal Aβ-afferents. Previous studies in DRG neurons have observed two subtypes of Aβ-afferent nociceptive neurons, the HTMR (high threshold mechanoreceptors) and the unresponsive Aβ-afferent nociceptive neurons. The HTMR Aβ-afferent nociceptive neurons were shown to have relatively faster AP kinetic while the unresponsive Aβ-afferent nociceptors have very slow AP kinetics. In our V2 trigeminal Aβ-afferent neurons, we have shown that type II V2 trigeminal Aβ-afferent neurons have relatively faster APs similar to those of HTMR DRG neurons identified in the previous studies. On the other hand, type IIIb V2 trigeminal Aβ-afferent neurons had the slowest AP kinetics similar to those of unresponsive nociceptive Aβ-afferent DRG neurons shown in previous studies. In the present study, we show that AP success rates in response to high frequency stimulation are significantly different among the 4 different types of V2 trigeminal Aβ-afferents. To our knowledge, this electrophysiological property of Aβ-afferents has not be characterized in previous studies. We have found that type I V2 trigeminal Aβ-afferents had the highest and type IIIb V2 trigeminal Aβ-afferents had the lowest AP conduction success rates. The responses to high frequency stimulation may be another useful electrophysiological indicator for different functional types of Aβ-afferents. In the present study, we also examined voltage-activated currents in the four types of V2 trigeminal Aβ-afferents. We have found that type IIIb V2 trigeminal Aβ-afferent neurons have relatively smaller voltage-activated K⁺ outward currents in comparison with the other types of V2 trigeminal Aβ-afferent neurons. This may be a factor causing the slower AP repolarization in type IIIb V2 trigeminal Aβ-afferent neurons. More detailed studies on voltage-activated currents in V2 trigeminal Aβ-afferent neurons need to be performed to provide ion channel mechanisms underlying the distinguished AP properties of different types of V2 trigeminal Aβ-afferent neurons. In addition to their roles in both low threshold and high threshold mechanical responses, Aβ-afferent fibers have been suggested to play a key role in mechanical alldynia and spontaneous pain under pathological conditions including tissue inflammation and neuropathy. Furthermore, a previous study using a
pharmacological approach to silence Aβ-afferent fibers has shown the alleviation of neuropathic pain in animal models. Our characterizations of V2 trigeminal Aβ-afferent fibers may help us in future to explore sensory functions of different types of V2 trigeminal Aβ-afferent fibers in orofacial regions under both physiological and pathological conditions.

Author Contributions
JGG conceived and designed the experiments and wrote the paper. YO performed electrophysiology experiments and data analysis. AY, ST and RJV provided technical assistance, and participated in data analysis and discussion.

Declaration of Conflicting Interests
The author(s) declared receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by NIH grants DE018661 and DE023090 to J.G.G.

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