Activity-dependent sensory signal processing in mechanically responsive slowly conducting meningeal afferents

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Uebner M, Carr RW, Messlinger K, De Col R. Activity-dependent sensory signal processing in mechanically responsive slowly conducting meningeal afferents. J Neurophysiol 112: 3077–3085, 2014. First published September 24, 2014; doi:10.1152/jn.00243.2014.—Activity-dependent processes in slowly conducting afferents have been shown to modulate conduction and receptive properties, but it is not known how the frequency of action potential firing determines the responses of such fibers to mechanical stimulation. We examined the responses of slowly conducting meningeal afferents to mechanical stimuli and the influence of preceding action potential activity. In hemisected rat heads with adhering cranial dura mater, recordings were made from meningeal nerves. Dural receptive fields of mechanically sensitive afferent fibers were stimulated with a custom-made electromechano-stimulator. Sinusoidal mechanical stimuli of different stimulus durations and amplitudes were applied to produce either high-frequency (phasic) or low-frequency (tonic) discharges. Most fibers showed slowing of their axonal conduction velocity on electrically evoked (phasic) or low-frequency (tonic) discharges. Most fibers showed slowing of their axonal conduction velocity on electrically evoked activity at ≥2 Hz. In this state, the peak firing frequency of phasic responses to a 250-ms mechanical stimulus was significantly reduced compared with control. In contrast, the frequency of tonic responses induced by mechanical stimuli of >500 ms did not change. In a rare subtype of afferents, which showed conduction velocity speeding during activity, an increase in the phasic responses to mechanical stimuli was observed. Depending on the axonal properties of the afferent fibers, encoding of phasic components of mechanical stimuli is altered according to the immediate firing history. Preceding activity in mechanoreceptors slowing their conduction velocity seems to provide a form of low-pass filtering of action potential discharges predominantly reducing the phasic component. This may improve discrimination between harmless and potentially harmful mechanical stimuli in normal tissue.

electrophysiology; mechanotransduction; nociceptor

THE CRANIAL MENINGES, especially the dura mater encephali, can be regarded as a protective tissue preventing the brain from being lesioned by noxious stimuli of various modalities. Hereby, the nociceptive system of the dura mater detects potentially harmful stimuli to alarm the organism by causing the sensation of headaches. The dura mater is densely innervated by unmyelinated C- and thinly myelinated AΔ-fibers of the trigeminal nerve, most of which can be regarded as polymodal nociceptors (Levy and Strassman 2002). Although polymodal nociceptors encode noxious stimuli of various modalities, the discrimination between harmful and harmless mechanical stimuli might be particularly difficult, taking into account that most meningeal nociceptors display comparably low mechanical thresholds (Levy and Strassman 2002). Indentation or distension of the meninges, which may, for example, occur during fast movements of the head, should only be encoded as nociceptive if they endanger the integrity of the brain or the dura mater itself. Thus encoding mechanisms that are able to filter the responses to stimuli according to their potential harm may be useful properties of meningeal nociceptors.

Most sensory encoding processes are subject to adaptation, a decrease of the action potential discharge during long-lasting stimulation. Adaptation occurs also in C-fiber nociceptors in tissues such as skin (Garell et al. 1996) and cranial dura mater (Levy and Strassman 2002). Adaptation allows sensory endings to maintain their sensitivity to subsequent mechanical stimuli over a wide dynamic range. The question is whether it can also contribute to discrimination between harmful and harmless stimuli.

The ability of sensory neurons to transduce mechanical force into an electrical signal is thought to involve the opening of mechanically sensitive ion channels leading to a generator potential. Using procaine (Katz 1950; Loewenstein and Mendelson 1965) and tetrodotoxin (Loewenstein et al. 1963) to block voltage-gated sodium channels (Na,s), generator potentials have been recorded directly from muscle spindles and Pacinian nerve terminals. Generator potentials in Pacinian corpuscles evoked by mechanical stimulation typically showed a rapid initial depolarization with subsequent adaptation (Gray and Sato 1953; Loewenstein and Mendelson 1965). To date, comparable recordings of generator potentials from sensory endings of unmyelinated axons have not been made primarily due to the small size and indeterminate location of the nerve terminals. Instead, the mechanical response properties of individual unmyelinated fibers have been studied using either action potential activity or patch-clamp techniques applied to their somata under the assumption that the soma is a representative model of the sensory terminal. Mechanical stimulation of the isolated somata as well as the outgrowing neurites (Hu and Lewin 2006) of adult (McCarter et al. 1999) and neonatal (Drew et al. 2002) dorsal root ganglion (DRG) neurons evokes currents that are likely mediated by specific transduction proteins since comparable mechanical stimuli delivered to the somata of sympathetic neurons did not evoke detectable currents (McCarter et al. 1999). Mechanically evoked currents in DRG somata increased in amplitude with increasing mechanical stimulus intensities and, reminiscent of generator potentials, are characterized by a rapid onset with subsequent adaptation (Drew et al. 2002; McCarter et al. 1999; Takahashi and Gotoh 2000). The time course of current adaptation varied across individual neurons but was likely to reflect differing...
rapid (1–50 ms) and slow (1 s) components (Hu and Lewin 2006; Rugiero et al. 2010).

Furthermore, the conversion of generator potentials into action potentials may play an important role for adaption. In small-diameter axons, an activity-dependent reduction in the number of available Na\textsuperscript{+}s has been shown to slow axonal conduction velocity (De Col et al. 2008; Snape et al. 2010). Similarly, inactivation of Na\textsuperscript{+}s causes slow, cumulative adaptation of spike firing generated by repetitive application of 1-s depolarizing pulses in cortical neurons (Fleidervish et al. 1996). In addition to changes in Na\textsuperscript{+} availability, lowering of the Nernst potential for Na\textsuperscript{+} through intraaxonal Na\textsuperscript{+} accumulation during repetitive activity could also contribute to changes in axonal conduction in unmyelinated axons (Endres et al. 1986; Scriven 1981; Tigerholm et al. 2014). Both processes would be expected to increase the threshold for action potential initiation and limit the frequency at which impulses can be generated in axons. Consistent with this, increased activity of mechanically sensitive afferent fibers recently has been found to be accompanied by an increase in mechanical activation thresholds (De Col et al. 2012).

In this study, the impact of preceding action potential firing on the responses to suprathreshold mechanical stimulation in single afferents innervating the rat cranial dura mater was examined.

METHODS

Animal housing and all experimental procedures were carried out in accordance with the guidelines for the welfare of experimental animals as stipulated by the Federal Republic of Germany.

Tissue preparation and experimental conditions. Twenty-five Wistar rats of both sexes with body weights between 250 and 450 g were killed in carbon dioxide atmosphere in a closed container in accordance with German regulations of animal care and treatment. The head was removed from the body, freed of overlying fur, muscle, and other tissue, and the skull was divided with a scalpel along the sagittal suture. The brain was carefully removed without injuring the dura, and the resulting skull halves were placed and kept in synthetic interstitial fluid [SIF; in mM: 145 Na\textsuperscript{+}, 3.5 K\textsuperscript{+}, 1.53 Ca\textsuperscript{2+}, 0.69 Mg\textsuperscript{2+}, 1.67 PO\textsubscript{4}\textsuperscript{3-}, 114 Cl\textsuperscript{-}, 9.64 C\textsubscript{6}H\textsubscript{12}O\textsubscript{6}, 5.55 D(-)-glucose, and 7.6 D(+)-sucrose] for each skull half was fixed in agar within a plastic chamber so that the cranium itself formed the tissue bath. The trigeminal ganglion was carefully removed, and the tentorial nerve (meingeanal branch of V1) or the spinous nerve (meingeanal branch of V2/V3) was fixed of overlying dural connective tissue, cut at the proximal end, and mobilized over a length of ~3 mm.

During preparation and recording, the chamber was perfused at a rate of 6 ml SIF/min. The solution pH was buffered to 7.4 by bubbling with carbon dioxide (95% O\textsubscript{2}-5% CO\textsubscript{2}). The temperature was kept at 35 ± 0.5°C by a flow-through Peltier element integrated in the inlet and regulated by feedback from a thermocouple placed inside the tissue bath. Each skull half was stored in agar within a plastic chamber so that the cranium itself formed the tissue bath. The trigeminal ganglion was carefully removed, and the tentorial nerve (meingeanal branch of V1) or the spinous nerve (meingeanal branch of V2/V3) was fixed of overlying dural connective tissue, cut at the proximal end, and mobilized over a length of ~3 mm.

Recording of action potentials and experimental protocol. The proximal end of the spinous nerve was drawn into a SIF-filled glass pipette (opening ~40 μm) containing an AgCl wire that served as the recording electrode, and a silver wire in the bath was the anode. Neuronal signals were amplified (Axopatch 200A; Axon Instruments), filtered (5-KHz low pass), digitized (Micro1401; Cambridge Electronic Design), and saved on hard disk. Signals were presented on a computer monitor and made audible by a loudspeaker.

After identifying a receptive field by gently touching the dura with von Frey filaments (10 mN, 0.2-mm diameter), a custom-built stimulus was placed onto the dural receptive field. The stimulating probe consisted of a glass capillary (external diameter 150 μm; internal diameter 60 μm) forming the mechanical stimulus probe with a flexible wire electrode inside, which allowed simultaneous mechanical and electrical stimulation (De Col et al. 2012). The force for mechanical stimulation was generated using a solenoid taken from a hard disk drive. Force profiles had a sinusoidal time course. For the identification of single fibers a mechanical stimulus of 10 ms was applied, and for suprathreshold test stimuli stimulation periods of 250–800 ms were used.

Identification of single fibers. At sites where mechanical and electrical stimuli both evoked action potentials of similar form and latency, a collision-like technique was used to ensure that both stimuli were activating one and the same fiber. The procedure has been described previously (De Col et al. 2012). Briefly, electrical and mechanical stimuli were delivered at ever-decreasing delays until the action potential evoked by stimulation with one modality occluded the action potential response evoked by the other modality. When an adequate position was found, the threshold (i.e., the stimulus strength evoking an action potential in 50% of cases) was determined with electrical (1 ms) and mechanical (10 ms) stimuli. In addition, using pulses of 250 ms, the lowest force that was able to evoke more than one action potential was also determined, referred to as the bursting threshold. The conduction distance between recording and stimulating site was estimated by means of an integrated scale in the microscope optics.

Experimental protocol. To study the effect of preceding action potential activity on the responses to mechanical stimuli, different frequencies of electrical stimulation were used to vary axonal conduction velocity within three consecutive stimulation periods of 15 min (see Fig. 4B). In the first period, electrical stimulation was delivered at 0.1 Hz (period 1, precontrol) and five suprathreshold mechanical stimuli were applied separated by a recovery time of at least 3 min. In the second period, a slowing of axonal conduction velocity was induced by increasing the electrical stimulation rate to 2 or 4 Hz (period 2, test frequency) and once again five suprathreshold mechanical stimuli were applied. In the final period, electrical stimulation was reduced to 0.1 Hz (period 3, postcontrol) and the last five suprathreshold mechanical stimuli were applied. The second period was separated from the third one by an interval of 3 min at 0.5-Hz electrical stimulation and one mechanical pulse to observe latency recovery. Following a period of 10 min without any manipulation, the protocol was repeated using an electrical test frequency of 5 Hz. Finally, the tissue was exposed to capsaicin (10^{-6} mol/l).

Data processing and statistics. Stored data were analyzed offline with Spike2 version 6.08 (Cambridge Electronic Design), Microsoft Excel 2003, Microsoft Excel 2007, and STATISTICA 7 (StatSoft). Figures were made using Origin 7 SRI [version 7.0300 (B300); OriginLab 2002].

Values are presented as means and SD in the text or SE in the figures. Repeated-measures ANOVA extended by the Tukey honestly significant difference (HSD) test was used for statistical comparisons as indicated in the text.

RESULTS

Altogether, 29 units with axonal conduction velocities ranging from 0.45 to 5.64 m/s at a temperature of 35°C were examined. The mechanical thresholds varied between 0.3 and 8.0 mN at a stimulus duration of 10 ms. Using a stimulus duration of 250 ms, the bursting threshold (lowest force resulting in >1 action potential) measured in 26 units varied between 0.6 and 4.8 mN. The electrical threshold determined with a 1-ms constant-current pulse varied between 10 and 86 μA. Of 21 units finally tested with
capsaicin \((10^{-6} \text{ mol/l})\), 12 were activated. Twenty-six units exhibited slowing of conduction velocity when challenged with electrical stimulation at 2, 4, or 5 Hz, whereas three fibers showed speeding of their conduction velocity during 4-Hz electrical stimulation.

Mechanical stimulus profiles and their impact on action potential responses. The discharge patterns of individual sensory axons to mechanical stimuli applied as rectangular, ramp, or sinusoidal mechanical stimulus profiles were examined. In response to rectangular mechanical stimuli, an initial phasic burst of action potentials occurred, which was followed by a slowly adapting (SA), more tonic response (Fig. 1A) or adapted to zero (Fig. 1B). Mechanical ramp stimuli of slow and constant velocity (6 and 12 mN/s) evoked largely tonic responses in most units, whereas ramp velocities >100 mN/s elicited tonic-phasic responses in most fibers (Fig. 2A). Using mechanical stimuli with a half-sinusoid profile, response patterns were either dominated by a phasic or a static

![Graph A](attachment:image1.png)  
**A**

![Graph B](attachment:image2.png)  
**B**

**Fig. 1.** Response patterns of dural afferent fibers to rectangular mechanical stimuli. The lower traces show the measured force of mechanical stimuli, and the upper traces the instantaneous (Inst.) frequency of the responses (overlay of 3 stimuli each). The middle traces show examples of evoked discharge to 1 stimulus and the shape of the respective action potential. **A**: responses of a unit to rectangular stimuli of different amplitudes. The highly dynamic increase in force is encoded by a fast-adapting burst of action potentials that passes into a slowly adapting response at lower frequency encoding the static period of the stimulus. Increased force amplitude shifts the response to higher frequencies. **B**: fast-adapting unit with an initial phasic burst of action potentials, activated by rectangular stimuli of different length, which cause nearly identical response patterns.

![Graph A](attachment:image3.png)  
**A**

![Graph B](attachment:image4.png)  
**B**

**Fig. 2.** Response patterns to mechanical stimuli of different stimulus profiles. The lower traces show the measured force of mechanical stimuli, and the upper traces the instantaneous frequency of the responses. **A**: unit stimulated with ramplike mechanical stimuli of different velocities to the same maximum. There is an association between the slope of the ramp and the amplitude of the initial high-frequency impulse pattern (phasic response). **B**: half-sinus-shaped force profiles turned out to be the most reliable stimuli for reproducible responses. By alteration of stimulus amplitude and duration, the direction toward dynamic (high frequencies) or static responses (low frequencies) can be shifted. The upper trace shows responses elicited by stimuli with the same high stimulus amplitude but different duration. The responses shown in the middle trace were elicited with the smaller amplitudes.
component depending on the stimulus duration (Fig. 2B). Short stimuli (≤250 ms) with stimulus speeds >150 mN/s evoked predominantly phasic responses, whereas longer stimuli evoked tonic responses (Fig. 2B).

In 21 fibers using sinusoidal mechanical stimuli of 250-ms duration and amplitudes of at least 2-fold the threshold, a reproducible phasic-tonic response was recorded, which rendered this stimulus type the best for the main experiments (Fig. 2B). To quantify the phasic part of each response to the mechanical stimulus, the “peak firing frequency” was determined as the average of the 5 highest instantaneous frequencies. The peak firing frequency did not vary by >6% on average during the control period. To evoke tonic responses, stimulus durations of >500 ms were used in 13 fibers; the peak firing frequencies did not vary by >10% under the same conditions.

Responses to suprathreshold mechanical stimulation and capsaicin. Responses to suprathreshold mechanical stimulation with a sinusoid half-wave profile of 250-ms duration and amplitudes ranging from 1.4 to 12 mN were quantified according to their peak firing frequency and the period, over which action potentials were generated. Mean values of peak firing frequencies during the first control period ranged from 82 to 214 Hz (n = 22; Fig. 3A). There was no correlation between peak firing frequency and the basal axonal conduction velocity. The responses of six fibers exceeded the stimulation time (Fig. 3, B and C). Four of these fibers were exposed to capsaicin (1 μM) and proved to be responsive. Nine of the remaining fifteen fibers, which ceased firing within the stimulation period, were exposed to capsaicin, of which three were activated (Fig. 3C).

Activity-dependent slowing of conduction velocity associated with a reduction in peak firing frequency to mechanical stimulation. Using sinusoidal half-wave mechanical stimuli of 250-ms duration and amplitudes well above threshold, the effects of preceding spike activity on the electrical latency and the peak firing frequency were examined (Fig. 4A). Electrical stimulation was used to elicit activity at different frequencies, and the responses to the mechanical stimuli were determined every 180 s (Fig. 4B). Using low-frequency electrical stimulation (0.1 Hz), dynamic sinusoidal mechanical stimuli (250 ms) caused a prominent and reproducible phasic response (Fig. 4A). Increasing the rate of electrical stimulation to 4 Hz slowed down axonal conduction. This is evident as an increase in the latency of the action potential after the electrical stimulus (Fig. 4B, middle trace). Mechanical stimuli delivered to the receptive field in this state of slowing produced a pattern of action potentials in which the initial phasic response was reduced. Increasing the electrical stimulus rate from 0.1 to 2 Hz blunted the phasic response evoked by a 250-ms mechanical stimulus, whereas the tonic response to a 600-ms stimulus remained unaffected (Fig. 4C).

On 26 individual fibers, 4 conditions were tested, and the responses were quantified. First, responses to the 250-ms stimuli delivered at 0.1 Hz reached peak firing frequencies of
149.32 ± 37.46 Hz, which were clearly reduced to 125.15 ± 48.76 Hz by 2-Hz electrical stimulation (P < 0.05; n = 11; repeated-measures ANOVA and HSD post hoc test; Fig. 5A, left). Increasing the stimulus rate further to 4 Hz reduced peak firing frequencies from 121.13 ± 40.00 to 82.06 ± 39.37 Hz (P < 0.001; n = 18; Fig. 5A, right). In contrast, responses to mechanical stimuli >500 ms reached peak frequencies of 48.40 ± 14.92 Hz, and an increase in electrical stimulation rate to 2 or 4 Hz did not change peak firing frequencies (50.60 ± 26.11 Hz; n = 14; P = 0.65; Fig. 5A, lower traces). The strong link between conduction velocity slowing (increase of latency) and reduction of the peak firing frequency observed with the 250-ms stimulus is more clear on a time-based representation displayed in Fig. 5B. The course of latency just before mechanical stimulation reflects very well the reciprocal course of the peak firing frequency, which was reduced in the same way as the latency was increased in a strong linear correlation (Fig. 5C).

Fibers without activity-dependent slowing of conduction velocity increasing response frequencies of mechanical stimuli. In contrast to the majority of fibers showing slowing of their conduction velocity with increasing rates of electrical stimulation, 3 of the 29 fibers showed speeding, i.e., a slight decrease in their response latency to an increase in electrical stimulation rate from 0.1 to 4 Hz (Fig. 6B). The absolute magnitude of the decrease in latency was less than the width of the electrical pulse used to activate the fibers. Interestingly, these three fibers also showed an increase in their peak firing frequencies to mechanical stimulation in response to increased rates of electrical stimulation (Fig. 6A). On reducing the rate of electrical stimulation, the peak firing frequency of the response was similarly reduced (Fig. 6A). All three fibers had A6-axonal conduction velocities and did not respond to capsaicin (1 μM).

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Fig. 4. Response behavior to mechanical stimuli dependent on electrically evoked activity. A: response patterns of a unit during increasing electrical stimulation starting at 0.1 Hz (control) up to 5 Hz and back to 0.1 Hz (postcontrol). The upper trace shows that under the control and postcontrol conditions at 0.1 Hz the mechanical stimulus generated high frequencies up to >200 Hz, whereas electrical stimulation at 2, 4, and 5 Hz led to a graded suppression of the higher frequencies (dynamic response). Note: the instantaneous frequency plot of each electrical stimulation level contains the response of at least 5 mechanical stimuli, which applies to all subsequent illustrations of this type. B: stimulation protocol for the experiment shown in A. The lower trace shows the electrical stimulation rate, and the upper trace the mechanical stimulation (half-sinuslike stimuli of 250 ms). The resulting latency (delay between electrical stimulus and the evoked action potential) is depicted in the middle trace. During the 4-Hz electrical stimulation, a general increase in latency (slowing of conduction velocity) can be observed. In addition, a temporary increase in latency induced by each mechanical stimulus was seen only during the 4-Hz electrical stimulation. An increase in latency is not visible during the 0.1-Hz stimulation because the mechanical stimulus immediately following the electrical stimulus causes a burst of action potentials within 1 s, which has no impact on the latency of the next electrical stimulus following 10 s after the previous stimulus. The 10-s interval was long enough for a full recovery from the mechanically induced latency shift. C: mechanical stimuli of 250-ms duration compared with stimuli of 600 ms (lower trace) with the electrical 2-Hz protocol. The 250-ms stimuli elicited a high-frequency dynamic response, which was preferentially suppressed during the 2-Hz electrical stimulation (upper trace). In contrast, in the same unit, the 600-ms stimulus, during which the dynamic response was lacking, caused no suppression of activity during the 2-Hz stimulation (middle trace).
mechanisms may be responsible for both axonal conduction increased action potential traffic. This suggests that common with the slowing of axonal conduction velocity resulting from magnitude of the reduction in peak firing frequency was correlated with the slowing of axonal conduction velocity resulting from increased action potential traffic. This suggests that common mechanisms may be responsible for both axonal conduction velocity slowing and decrease in peak firing frequency in response to mechanical stimulation.

Possible mechanisms involved in the modulation of action potential firing. A decline in the action potential firing rate in response to mechanical stimulation could arise from alterations in stimulus transduction, from changes in encoding the action potential discharge in the axon, or from stimulus impacts altering the cellular environment of the sensory nerve ending (Barker et al. 1982) and/or the property of the plasma membrane. Changes in the impact efficacy of the mechanical stimulus itself are considered to be small, as our experiments indicate a reproducible force profile over periods of stimulation up to several hours. In addition, the response to the same duration and force of mechanical stimuli remains constant over several hours with no significant effects being observed unless the electrical stimulus frequency is altered (Fig. 5B). Effective mechanical transduction measured as the amplitude of repeated mechanically evoked whole cell currents in cultured DRG neurons was similarly stable for up to 3 h (McCartner et al. 1999). Thus it is likely that it is action potential activity itself that affects mechanically evoked firing.

High rates of action potential activity are likely to increase the intracellular sodium concentration in small-diameter axons. This decreases the Nernst potential for Na⁺, which has been suggested to contribute to axonal conduction velocity slowing (Endres et al. 1986; Scriven 1981; Tigerholm et al. 2014). Changes in intracellular Na⁺ are also likely to affect mechanical transducer currents based on the assumption that transduction involves Na⁺. Back as far as the 1950s, receptor potential generation in Pacinian corpuscle has been shown to be mainly dependent on the presence of sodium (Diamond et al. 1958). In recent studies, whole cell recordings of DRG neurons confirmed that mechanically sensitive currents are largely carried by Na⁺ through mechanically gated, large-pore, nonspecific cation channels with reversal potentials close to zero for the SA currents (McCarter and Levine 2006) and close to that for sodium for a rapidly adapting (RA) current component (Hu and Lewin 2006).

An activity-dependent reduction in the number of available Na⁺ has also been associated with an increased current required to initiate action potentials as has been shown in patch-clamp experiments on DRG neurons (Snape et al. 2010).

![Figure 5](http://jn.physiology.org/)

*Fig. 5. Quantitative evaluation of responses to mechanical stimulation dependent on preceding activity. A: mechanically induced peak firing frequencies of 26 fibers stimulated with a stimulus width of either 250 or ≥500 ms during electrical stimulation at 2 Hz (left) or 4 Hz (right). Peak firing frequencies evoked by the 250-ms mechanical stimulus decreased during the 4-Hz stimulation, whereas responses to the longer stimulus lacking the dynamic component show no significant changes at 4 Hz (*P ≤ 0.05, repeated-measures ANOVA and honestly significant difference post hoc test). B: peak firing frequency (frequ.; upper trace) evoked by a 250-ms mechanical stimulus and latency (lower trace) in 18 units. During the electrical 4-Hz stimulation, a general increase in latency (slowing of conduction) is associated with a decrease in peak firing frequency. The latency was measured shortly before and after a mechanical stimulus was applied; mechanical stimuli were repeated every 3 min. C: changes (Δ) in latency plotted against changes in peak firing frequency of the sample of units shown in B. Reduction in peak firing frequency is strongly correlated with increase in conduction latency when a 250-ms mechanical stimulus is applied. Δ latency and Δ peak frequency is the difference between the average latency and the average peak firing frequency before the last and the following mechanically evoked bursts of action potentials. The graph shows the linear fit of the correlation and the 95% confidence intervals; error bars of A–C are SE.*
Activity-dependent increase of mechanically induced responses in a subpopulation of Aδ-fibers. In contrast to the majority of fibers, which showed slowing of their axonal conduction velocity with increasing rates of electrical stimulation, 3 of the 29 fibers exhibited a slight decrease in their conduction velocity with increasing rates of electrical stimulation. The other possibility is that modulation of mechanically evoked responses occurred at the level of action potential initiation. Electrical excitability changes determined in single C-fibers in humans suggest that ongoing activity in these axons increases their excitability in the period 10–200 ms after each action potential (Bostock and Grafe 2003), a feature thought to increase the likelihood of high-frequency firing. This superexcitability is a relative increase in excitability, i.e., a small decrease in threshold superimposed on the global increase in threshold resulting from activity. The superexcitability increases with increasing activity (Bostock and Grafe 1985; George et al. 2007; Zhu et al. 2013). The emergence of a period of superexcitability is likely to counteract or even prevent the decrease of discharge frequency in response to suprathreshold stimuli. The superexcitable period occurs within a short time (10–200 ms) after each discharge in a cluster of action potentials, which corresponds to instantaneous frequencies below 100 Hz and above 5 Hz. Weidner et al. (2002) termed this contrast enhancement. For suprathreshold responses, this implies that the expected global decrease in frequency is compensated for frequencies between 5 and 100 Hz. This fits with our results. Mechanical responses with frequencies beyond 100 Hz could be gradually diminished by increasing the preexisting electrical stimulation, whereas responses with frequencies below 100 Hz would not be suppressed at all (Figs. 4 and 5).

Activity-dependent increase of mechanically induced responses in a subpopulation of Aδ-fibers. In contrast to the majority of fibers, which showed slowing of their axonal conduction velocity with increasing rates of electrical stimulation, 3 of the 29 fibers exhibited a slight decrease in their response latency following an increase in electrical stimulation from 0.1 to 4 Hz (Fig. 6). Although a decrease in response

1. **Fig. 6.** Response behavior in fibers showing speeding during electrical stimulation. A: fiber with low-frequency responses to the mechanical stimulus at control conditions but very strong responses during the 4-Hz stimulation. B: same stimulation protocol as shown in Fig. 4B and same fiber as shown in A. The middle trace shows a latency decrease (speeding of conduction) during the 4-Hz electrical stimulation, whereas each mechanical stimulus caused a small temporary increase in latency.

Preceding action potential activity can thus affect action potential generation without affecting the transduction process.

Action potential activity is likely to change the membrane potential, i.e., axons hyperpolarize under impulse load (Gordon et al. 1990; Rang and Ritchie 1968). Modulation of the transduction process by changes in membrane potential would require a voltage-dependent mechanism. Indeed, recently identified members of mechanically sensitive ion channels observed in the Neuro2A cell line (Piezo1) and DRG neurons (Piezo2) exhibited mechanically induced currents that showed voltage-dependent inactivation (Coste et al. 2010) with repeated bouts of hyperpolarization deactivating channels (Gottlieb and Sachs 2012). Generally, activity tends to hyperpolarize membrane potential in axons and possibly also in sensory endings by activating the Na⁺-K⁺-ATPase (Kobayashi et al. 1997; Morita et al. 1993).

Mechanisms underlying the reduction of peak firing frequency to mechanical stimulation. The mechanisms described so far may be expected to reduce both low- and high-frequency firing. However, we observed a selective damping of high-frequency responses (phasic component) to mechanical stimuli without affecting low-frequency responses (tonic component; Figs. 4 and 5A). This may indicate either separate transduction processes for phasic and tonic responses or an additional process opposing selectively the damping of low frequencies (tonic component). Indeed, for both options, evidence can be found. Rugiero et al. (2010) confirmed the existence of at least two types of mechanically activated currents in DRG neurons, RA and SA currents. The latter group responded mainly to slow mechanical ramps, whereas firing in RA cells was limited by the speed of the stimulation. Interestingly, in this study, RA current inactivation was voltage-dependent, which indicates that changes in membrane potential by preceding action potential activity may influence transduction. One of the molecular counterparts could be the recently discovered Piezo2 ion channel (Coste et al. 2010). Piezo2 is expressed in a subset of DRG neurons and is mechanically sensitive delivering RA, mechanically activated currents. However, it may not be an important mechanotransduction channel in C-fibers because only about one-fourth of DRG neurons immunopositive for Piezo2 were also positive for the nociceptive marker TRPV1, suggesting a mechanonociceptive function (Coste et al. 2010). The other possibility is that modulation of mechanically evoked responses occurred at the level of action potential initiation. Electrical excitability changes determined in single C-fibers in humans suggest that ongoing activity in these axons increases their excitability in the period 10–200 ms after each action potential (Bostock and Grafe 2003), a feature thought to increase the likelihood of high-frequency firing. This superexcitability is a relative increase in excitability, i.e., a small decrease in threshold superimposed on the global increase in threshold resulting from activity. The superexcitability increases with increasing activity (Bostock and Grafe 1985; George et al. 2007; Zhu et al. 2013). The emergence of a period of superexcitability is likely to counteract or even prevent the decrease of discharge frequency in response to suprathreshold stimuli. The superexcitable period occurs within a short time (10–200 ms) after each discharge in a cluster of action potentials, which corresponds to instantaneous frequencies below 100 Hz and above 5 Hz. Weidner et al. (2002) termed this contrast enhancement. For suprathreshold responses, this implies that the expected global decrease in frequency is compensated for frequencies between 5 and 100 Hz. This fits with our results. Mechanical responses with frequencies beyond 100 Hz could be gradually diminished by increasing the preexisting electrical stimulation, whereas responses with frequencies below 100 Hz would not be suppressed at all (Figs. 4 and 5).
latency suggests an increase in axonal conduction velocity, the absolute magnitude of the decrease in latency was less than the width of the electrical pulse used to activate the fibers and thus may represent either a proximal shift in the site of impulse initiation or a more rapid initiation of the action potential. However, these three fibers also showed an increase in their peak firing frequency to mechanical stimulation in response to increased rates of electrical stimulation (Fig. 6). Furthermore, on reducing the rate of electrical stimulation, the peak firing frequency of the phasic response was reduced in the same way (Fig. 6). All three fibers had Aδ-axonal conduction velocities and did not respond to capsaicin (1 μM). Mechanistically, it could also be considered an activity-dependent enhancement of sensitivity through the transduction process. Recently, sensitization of the mammalian mechanically activated ion channel Piezo2 by inflammatory mediators such as bradykinin (Dubin et al. 2012) or through activation of the cyclic AMP sensor EPAC1 (Eijkelkamp et al. 2013) has been reported, but no reports are available indicating sensitization by impulse traffic. However, for Na1.6, which is responsible for action potential conduction in myelinated fibers, repetitive activation has been shown to potentiate activation (Zhou and Goldin 2004). This could offer an explanation for the increase in response frequency in this subset of fibers with conduction velocities in the range of Aδ provided that they express Na1.6.

### Physiological and pathophysiological implications.

Placing the findings in a physiological context, we have previously shown that the impulse load elevates the mechanical threshold of single meningeal afferents. The findings here extend this to show that impulse load also reduces the maximum discharge frequency of afferent fibers in response to mechanical stimuli. These two accommodative features correlate with activity-dependent slowing and represent a form of intrinsic inhibition that occurs in healthy sensory afferents, both meningeal (De Col et al. 2008) and cutaneous afferents (Raymond et al. 1990; Serra et al. 1999; Weidner et al. 1999). These features may enable sensory neurons to improve discrimination between harmful (long-lasting) and harmless (rapid) mechanical stimuli through differential encoding, e.g., prolonged pressure vs. transient pulsatile fluctuations. High action potential frequencies due to repetitive fast transient stimuli may be selectively suppressed before they can be transmitted to the central neurons.

However, during inflammation, nociceptive sensory afferents are disinhibited. For example, experimental sensitization of nociceptors using cutaneous injections of NGF results in a reduction in the amount of activity-dependent slowing in slowly conducting nociceptors (Obreja et al. 2011b) that correlates with a robust and prominent increase in perceived pain on electrical (Obreja et al. 2011a), heat, and mechanical stimulation (Rukwied et al. 2010) following NGF injection in people. Similarly, in a mouse model of diabetic neuropathy, individual sensory C-fibers showed less slowing of axonal conduction velocity in response to 2-Hz stimulation (Sun et al. 2012). In addition, a disinhibition was also observed in sensory afferents from diabetic mice with less dropouts during trains of 2-, 5-, and 10-Hz electrical stimulation illustrating their ability to fire more action potentials at higher frequencies (Sun et al. 2012). It is thus evident that in sensitized and inflamed tissue, the normal accommodative processes that elevate threshold (De Col et al. 2012) and restrict peak firing frequency (Figs. 4 and 5) can be disinhibited. This disinhibition promotes impulse barrages reaching the spinal cord in a process likely to be associated with hyperalgesia and allodynia in the skin and headache for meningeal afferents.

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### DISCLOSURES

The authors declare no conflicts of interest.

### AUTHOR CONTRIBUTIONS

M.U. performed the present work in order to fulfill the requirements for obtaining the degree “Dr. med.” He conducted the experiments, evaluated the data, and drafted the manuscript. R.W.C. and K.M. instructed the experimental work, interpreted the data, and refined the manuscript. R.D.C. designed and supervised the experiments, evaluated and interpreted the data, refined the manuscript, and assembled the figures. All authors approved the final version of the manuscript.

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