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Whisker plucking alters responses of rat trigeminal ganglion neurons

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Abstract

Whisker plucking in developing and adult rats provides a convenient method of temporarily altering tactile input for the purposes of studying experience-dependent plasticity in the somatosensory cortex. Yet, a comprehensive examination of the effect of whisker plucking on the response properties of whisker follicle-innervating trigeminal ganglion (NVg) neurons is lacking. We used extracellular single unit recordings to examine responses of NVg neurons to controlled whisker stimuli in three groups of animals: (1) rats whose whiskers were plucked from birth for 21 days; (2) rats whose whiskers were plucked once at 21 days of age; and (3) control animals. After at least 3 weeks of whisker re-growth, NVg neurons in plucked rats displayed normal, single whisker receptive fields and could be characterized as slowly (SA) or rapidly adapting (RA). The proportion of SA and RA neurons was unaffected by whisker plucking. Both SA and RA NVg neurons in plucked rats displayed normal response latencies and angular tuning but abnormally large responses to whisker movement onsets and offsets. SA neurons were affected to a greater extent than RA neurons. The effect of whisker plucking was more pronounced in animals whose whiskers were plucked repeatedly during development than in rats whose whiskers were plucked once. Individual neurons in plucked animals displayed abnormal periods of prolonged rhythmic firing following deflection onsets and aberrant bursts of activity during the plateau phase of the stimulus. These results indicate that whisker plucking exerts a long-term effect on responses of trigeminal ganglion neurons to peripheral stimulation.

Key words: *Whisker, plucking, sensory deprivation, plasticity, barrel, somatosensory*

Introduction

Whisker plucking has often been used for producing tactile sensory deprivation in the somatosensory system of neonatal and adult rodents (Fox, 1992; Glazewski and Fox, 1996). Whisker removal by plucking is readily accomplished, plucked whiskers re-grow, and whisker plucking, unlike frank follicular injury following cauterization, does not affect the number or axonal myelination of whisker-innervating trigeminal ganglion (NVg) neurons (Li *et al.*, 1995). Two assumptions inherent to this paradigm are that (1) plucking does not alter sensory input generated by the primary afferent neurons and (2) plucking in neonatal and adult animals constitutes an identical experimental manipulation. However, whisker plucking in mice alters follicle morphology (Ibrahim and Wright, 1978a), and repeated traumatic hair removal in humans leads to specific histologic abnormalities within the hair follicle (Bergfeld *et al.*, 2002).

We examined whether whisker plucking alters responses of NVg neurons to movements of the re-grown hair. Whiskers were either repeatedly plucked from birth or plucked once in juvenile animals. Using extracellular single unit recordings and controlled whisker deflections, we found that both experimental manipulations significantly affected the functional response properties of primary afferent neurons. NVg neurons in plucked animals on average responded more robustly to whisker deflections, and a significant percentage of neurons exhibited grossly aberrant response properties. Furthermore, repeated plucking during development produced a different pattern of alterations than a single plucking in juvenile rats. We conclude that whisker plucking alters transmission of sensory information probably by inducing traumatic injury within the follicle. The degree of injury likely differs with age and/or plucking frequency.

Materials and methods

Experiments were conducted on eight (3 male, 5 female) Sprague–Dawley rats. Pups were from litters born on a specified date (Harlan Sprague–Dawley, Indianapolis, IN) and were housed with their mother until P30. All procedures were approved by the Institutional Animal Care and Use Committee. For whisker plucking, rats were anesthetized using isoflurane (IsoFlo, Abbott Laboratories, North Chicago, IL). Each large mystacial vibrissa was individually identified using a dissecting microscope and was plucked by applying steady tension to the base of the whisker hair (Fox, 1992). All large vibrissae (rows A–E, arcs 0–5) were removed. Three animals had their whiskers removed every 2–3 days for 21 days beginning on P1 (P0 = day of birth). Three other animals were anesthetized with similar frequency but only had their whiskers ruffled with forceps during this time period. Upon reaching P21, all six animals had their whiskers plucked. Animals plucked from P1 are denoted as the P1–21 group; rats having their whiskers plucked once are denoted as P21. The whiskers were allowed to grow back for 3 weeks prior to electrophysiological recording, by which time the rats were >40 days old. At the time

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of recordings, the identity of the individual animals as P1 or P1–21 was unknown to the experimenter. Two adult rats >P65 were used as controls.

Surgery and physiologic maintenance

Anesthesia was induced with isoflurane and maintained with halothane (Halocarbon, River Edge, NJ) for the duration of the surgery. A jugular vein catheter and a tracheal cannula were inserted as described previously (Lichtenstein *et al.*, 1990). The animal's skull was exposed, a ground screw was inserted into the bone over the left frontal cortex, and a steel post was attached to the right side of the skull with dental acrylic. Throughout the experiment, the post was used to support the animal's head with unrestricted access to the whiskers on the left side of the face. The area of parietal bone located directly above the left trigeminal ganglion (2.0 mm posterior to bregma, 2.5 mm lateral to midline) was thinned by drilling, and a small section of remaining bone was removed with a scalpel; the dura mater was left intact. The ganglion is located $\approx$10 mm below the dura. Ophthalmic ointment was applied to the eyes to prevent drying of the corneas.

The rat was then transferred to a vibration isolation table, halothane anesthesia was discontinued, and for the remainder of the experiment, the animal was anesthetized with sodium pentobarbital (Nembutal, Abbott Laboratories) administered through the jugular vein catheter. The depth of anesthesia was continuously adjusted to maintain areflexia to foot pinch. The animal's core temperature was kept at 37°C by a servo controlled heating blanket (Harvard Apparatus, Cambridge, MA). Experiments were terminated with a lethal dose of sodium pentobarbital.

Recordings, stimulus control, and data acquisition

Extracellular recordings from NVg neurons were obtained using stainless steel microelectrodes (4–6 MΩ at 1 kHz, FHC, Brunswick, ME). Single units were identified on the basis of spike amplitude and waveform criteria and digitized using a time and window discriminator. The whisker activating the unit, the principal whisker (PW), was identified using a hand-held probe. The PW was then deflected with a piezoelectric stimulator (Simons, 1983) at 2 s intervals in eight directions in 45° increments (0° = caudal, 90° = dorsal). Each deflection was repeated ten times, and all 80 deflections were presented in a random sequence. The stimulator was attached to the whisker 10 mm from the skin surface and was calibrated to deliver a 1 mm deflection at the tip. Stimulus onset and offset velocities were $\approx$125 mm/s. Spike times were collected with a 100 μs resolution during a 500 ms time period symmetrically bracketing a 200 ms ramp-and-hold stimulus.

Data analyses

Spike time stamps were converted to peri-stimulus time histograms (PSTHs) having 1 ms bins. PSTHs for individual neurons were analyzed separately and also combined into population PSTHs. The latter were used to determine 25 ms long time windows appropriate for measuring responses to deflection onset (ON) and offset (OFF). Twenty-five millisecond long time windows capture the entire transient responses of NVg neurons to whisker movement onsets and offsets. ON and OFF response magnitudes were defined as the number of spikes discharged by a neuron during these time epochs. We computed mean ON and OFF responses for the maximally and minimally effective deflection angles and the average response over all eight angles. Initial firing rates were computed as the number of spikes discharged during the first 5 ms of the ON response. Responses to steady-state deflection of the whisker (plateau) were determined using a 100 ms epoch during the static portion of the stimulus. A neuron was classified as slowly adapting (SA) if its plateau response at the deflection angle evoking the highest plateau activity significantly exceeded spontaneous firing (*t*-test, one tail $p<0.025$; see Lichtenstein *et al.*, 1990). All other neurons were classified as rapidly adapting (RA).

Distributions of observed values were compared using χ^2 analyses, and distribution means were compared using Student's *t*-tests. Two-tailed probability values <0.05 were considered significant unless noted otherwise.

Results

Whiskers regrew at variable rates in animals subjected to repeated whisker plucking from birth. Individual whiskers required at least 3 days of re-growth before achieving sufficient length to need re-plucking. Re-grown whiskers were often abnormally shaped, and some (usually $\approx$2 per animal) were so misshapen after at least 3 weeks of re-growth as to preclude attachment of the piezoelectric whisker stimulator. Most re-grown whiskers, however, appeared relatively normal in both the P1–21 and P21 groups. Here, we make no attempt to relate electrophysiological findings to the physical characteristics of the individual whisker hairs.

Responses of NVg neurons to whisker deflections were examined in control, P21, and P1–21 rats ($n = 80$, 105, and 105 neurons, respectively). Whisker plucking resulted in a mixture of normal and altered response properties in NVg neurons. Normal response properties include single whisker receptive fields, low spontaneous firing rates (see Minnery and Simons, 2003), and large transient responses to stimulus onsets and offsets (Fig. 1). The proportions of slowly and rapidly adapting neurons were equivalent among control and plucked groups ($\approx$75% SA, $\approx$25% RA) and identical to previously reported results from this laboratory (Lichtenstein *et al.*, 1990; Shoykhet *et al.*, 2000). Angular tuning of NVg neurons, measured either as the number of angles evoking a mean response statistically smaller than the one at the maximally effective angle (see Lichtenstein *et al.*, 1990) or as a ratio of the maximal angle response to the response averaged over all eight angles, remained unchanged. In addition, several temporal response properties were unaffected by plucking. Average response latency and the time to the 50th percentile spike in the ON response window were equivalent among all groups.

Quantitative comparisons based on analyses of individual neurons revealed that plucking and re-growth was associated with greater evoked firing rates in both RA and SA neuronal populations (Fig. 2). Differences were apparent even within the first 5 ms of the ON response as initial firing rates of both SA and RA neurons were higher in rats whose whiskers were plucked from birth than in control animals (data not shown). The magnitude of the entire ON response in RA neurons was also increased in P1–21 animals but not in P21 rats (Fig. 2 A–C). In contrast, responses of SA cells were affected even by a single episode of plucking, suggesting that plucking has a greater impact on SA than on RA neurons. ON response magnitudes of SA neurons, averaged over all deflection angles (Fig. 2A), were larger in P21 and P1–21 rats than in control animals (*t*-tests, $p < 0.05$). A similar increase in the size of the evoked ON response in SA neurons was observed in both groups of plucked animals at the maximally effective angle (Fig. 2B). At the minimally effective angle, however,

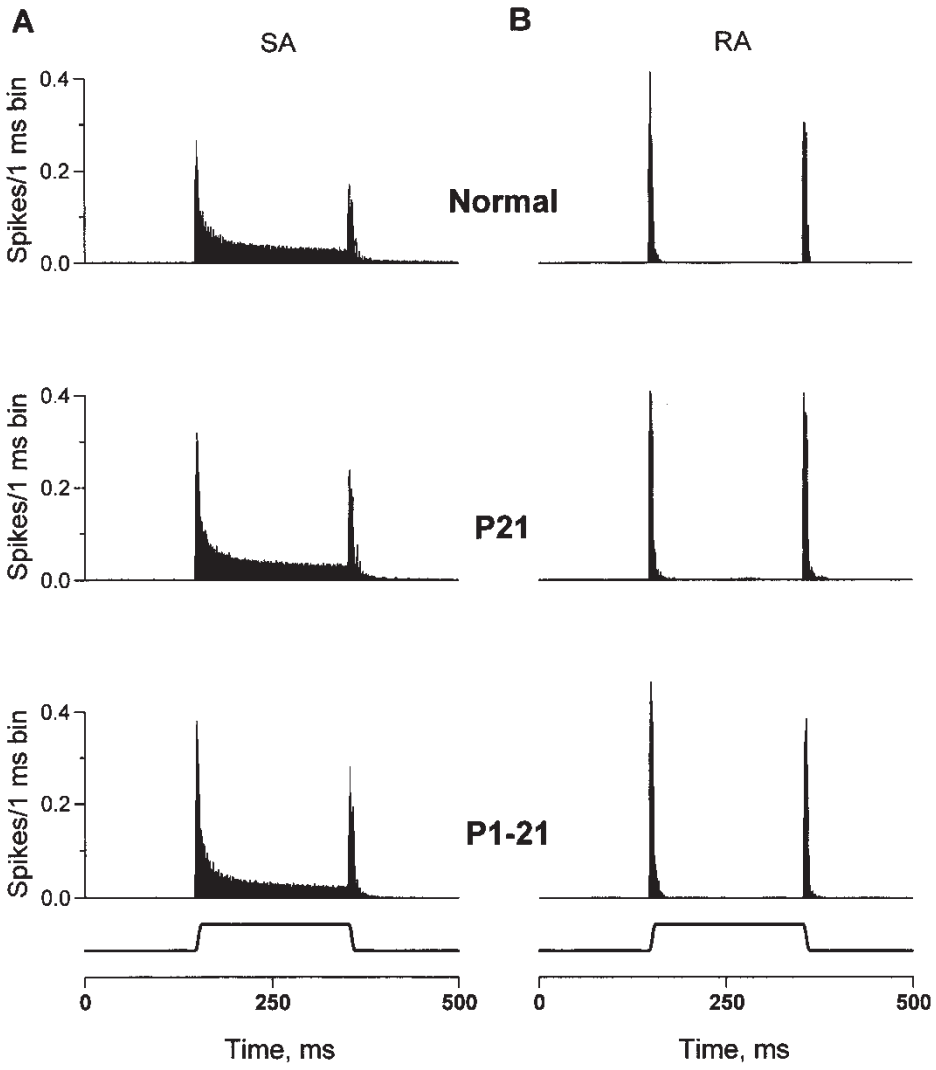

FIGURE 1. Population peri-stimulus time histograms (PSTHs) of responses of SA (A) and RA (B) neurons to whisker deflection in normal, P21, and P1–21 rats. Responses are averaged over all deflection angles. The scale on the ordinate axes in (A) applies to (B). The ramp-and-hold stimuli are shown schematically below the PSTHs. The sample sizes are (SA/RA): Normal = 54/25, P21 = 81/24, P1–21 = 75/30.

the increase in ON response magnitudes of SA neurons was observed only in animals plucked from birth but not in animals whose vibrissae were plucked once on P21 (Fig. 2C, $p < 0.001$). Plucking also resulted in larger than normal OFF responses in SA cells primarily due to the greater magnitude of responses at minimally effective angles (Fig. 2 D–F). Plateau responses in SA neurons were similar among the three groups ($p > 0.05$).

Plucking-induced increases in ON response magnitudes of NVg neurons were observed in five out of the eight deflection angles (t-tests, $p < 0.05$). The three directions unaffected by plucking were spatially contiguous, centered on the caudal direction of initial whisker movement ($0° \pm 45°$). Perhaps coincidentally, the deep vibrissal nerve carrying the afferent fibers enters the whisker follicle from its posteriordorsal aspect (Rice *et al.*, 1986).

The firing patterns of neurons in plucked animals were often abnormal. Some RA neurons showed aberrant burst activity during the plateau phase of the stimulus (Fig. 3C), and this was observed more often (~12%) in the P21 group. More commonly, neurons in plucked animals displayed long periods of abnormal firing following deflection onsets (Fig. 3B). We quantified this property by examining response magnitudes and inter-spike intervals (ISIs) during the first 50 ms of the plateau response. While the average response magnitudes were equivalent among the three groups (t-tests, $p > 0.05$), there was a significantly greater proportion of short ISIs (<5 ms) in both groups of plucked animals than in control rats (χ^2, $p \ll 0.001$). Together, the two results suggest that as a result of plucking, spikes are redistributed towards a more rhythmic firing pattern during the first 50 ms of the plateau response.

Discussion

We examined the effects of whisker plucking on responses of primary afferent neurons in young rats. We found that the degree of plucking-induced trauma to the follicle, evidenced by bleeding and removal of variable amounts of connective tissue along with the whisker hair, differed considerably,

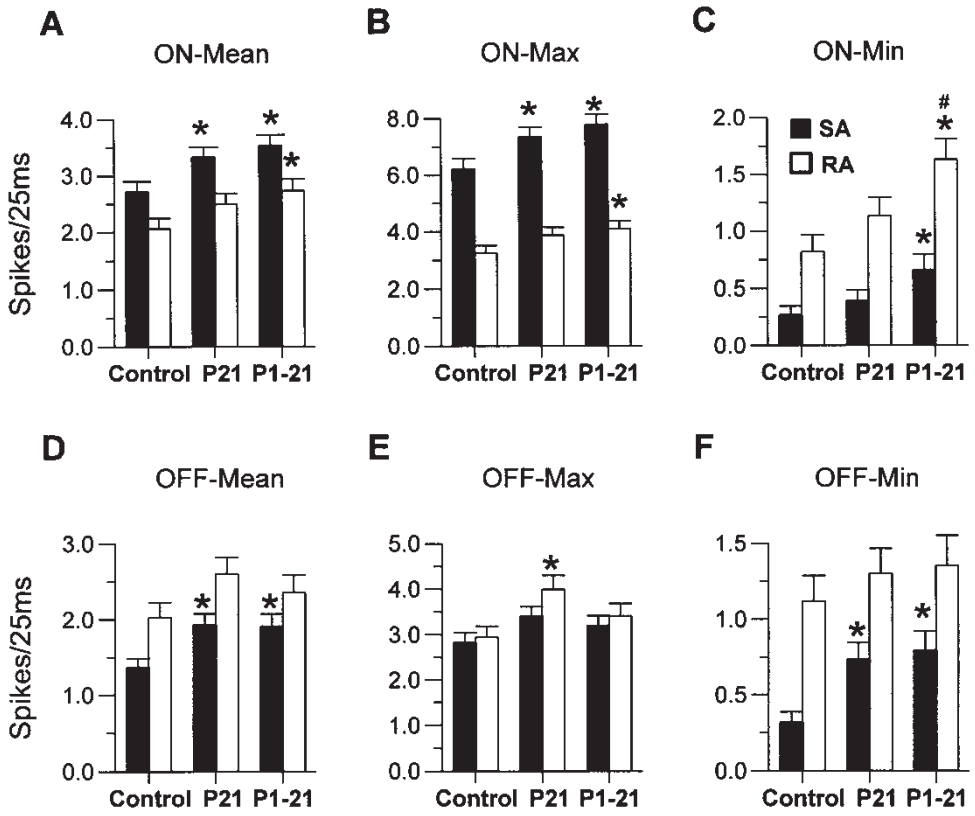

FIGURE 2. Response magnitudes of SA neurons (solid bars) and RA neurons (open bars) to deflection onsets and offsets. (A) ON response magnitudes averaged over all deflection angles. (B) ON response magnitudes for the maximally effective angles. (C) ON response magnitudes for the minimally effective angles. (D) OFF response magnitudes averaged over all deflection angles. (E) OFF response magnitudes for the maximally effective angles. (F) OFF response magnitudes for the minimally effective angles. Asterisks denote statistically significant differences from normal values (see Materials and methods). ⋆ denotes a statistically significant difference between values from P21 and P1–21 rats.

even when performed by the same investigator. Moreover, the time of whisker emergence from the plucked follicle, the apparent rate of growth, and the physical nature of the re-growing hairs varied among different animals and among different whiskers in the same animal. One factor that could determine whisker re-growth is the tension applied to the hair during whisker removal. The minimum necessary tension may in turn depend on the age of the animal, the location of the whisker in the mystacial pad, and the previous plucking history. In addition, an earlier study determined that the rate of whisker re-growth depends on the stage of the hair cycle at the time of plucking (Ibrahim and Wright, 1978b).

Trigeminal ganglion neurons responded to whisker deflections more robustly in plucked animals than in control rats. Initial firing rates of NVg neurons, known to represent stimulus parameters that influence responses of neurons in the cerebral cortex (Pinto *et al.*, 2000; Shoykhet *et al.*, 2000), were higher in plucked than in control animals. Plucking was also associated with abnormal rhythmic firing during the time period immediately following the transient response to movement onset. Effects of repeated plucking from birth for 21 days differed from those produced by a single plucking episode at P21. Thus, whisker plucking produces detectable and persistent abnormalities in the responses of NVg neurons and cannot be assumed to represent equivalent experimental modifications in rats of different ages.

Plucking-induced increases in response magnitude observed in this study contrasts with the finding of Glazewski *et al.* (1998) that 2–3 episodes of whisker plucking over the course of 6–8 days in rats aged 28–35 days do not affect response levels of NVg neurons. This discrepancy may reflect differences in the whisker stimuli used to evaluate response properties. Deflections employed by Glazewski *et al.* (1998) were significantly smaller in amplitude than those employed here ($\approx 1^\circ$ vs 5.7°) and were delivered only in one direction (see Armstrong-James and Fox, 1987). Both deflection amplitude and direction are known to affect responses of NVg neurons (Gibson and Welker, 1983; Lichenstein *et al.*, 1990; Shoykhet *et al.*, 2000), and the use of larger amplitude, multi-angle deflections may have optimized detection of abnormalities.

What are the potential mechanisms by which whisker plucking alters responses of NVg neurons? Several mechanisms, although theoretically possible, can be eliminated on the basis of response properties that remain unaffected by whisker plucking. The gross structure of the deep vibrissal nerve innervating the whisker follicle is likely preserved, inasmuch as receptive fields of NVg neurons in plucked animals, like those in control rats, are confined to a single whisker. It remains unknown whether the

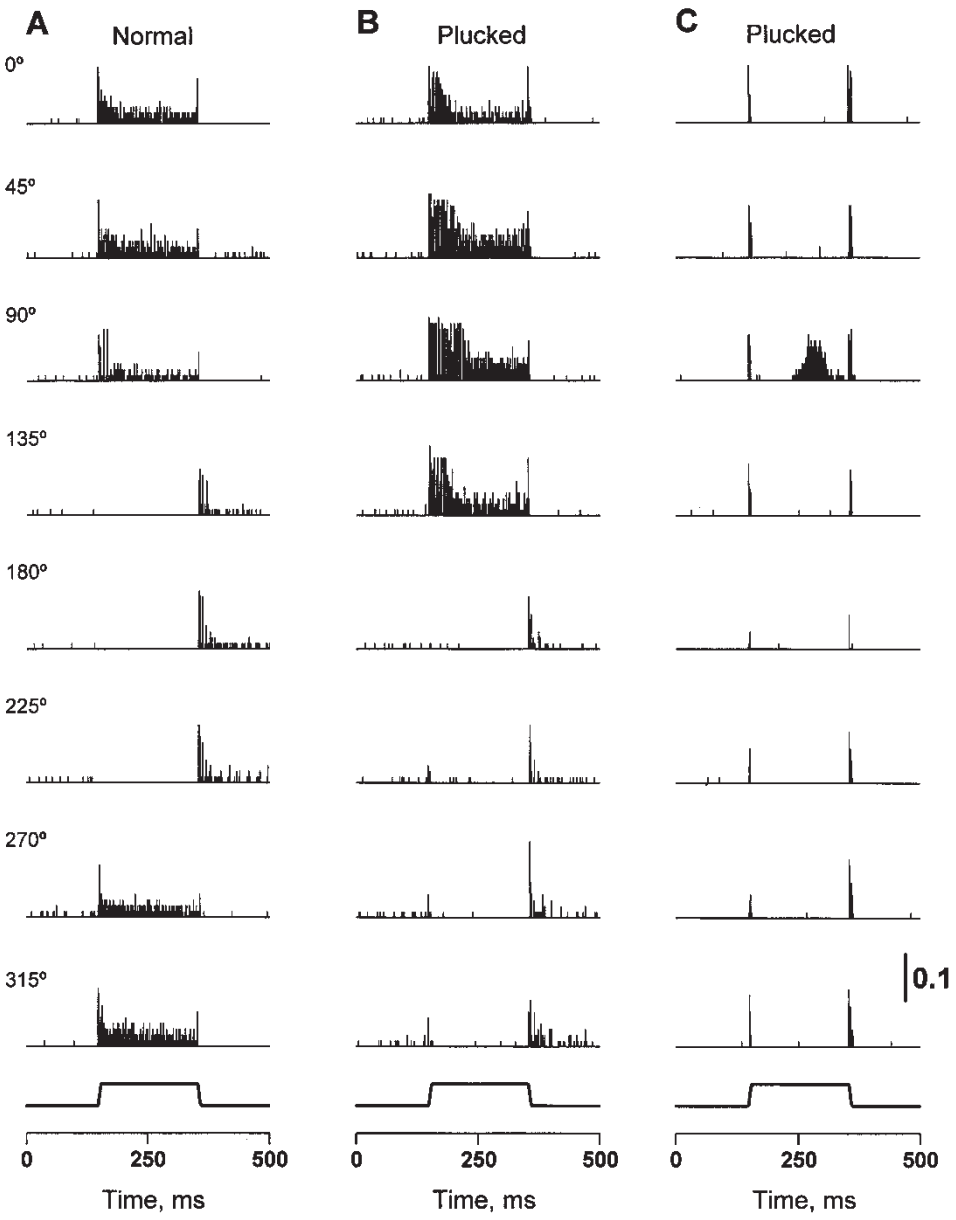

FIGURE 3. Peri-stimulus time histograms (PSTHs) showing responses of three individual neurons to whisker deflections in eight directions. The direction of whisker movement is indicated on the left (0° = caudal, 90° = dorsal). The cell in (A) was recorded from a normal rat and the cells in (B) and (C) from two different P21 rats. The scale bar represents 0.1 spikes/1 ms bin and applies to all PSTHs. The ramp-and-hold stimuli are shown schematically below the PSTHs. Note that the neuron in (B) is slowly adapting and displays an abnormally prolonged response to whisker movement onset at the most effective angles. The neuron in (C) is rapidly adapting but displays a pronounced period of crescendo–decrescendo firing during sustained whisker deflection dorsally.

anatomical structure of nerve endings within the follicle is disturbed by whisker plucking, requiring subsequent re-growth. Regardless of whether or not the re-growth of nerve terminal is required for restoration of function, the circumferential spread of individual nerve endings within the follicle probably remains unchanged because whisker plucking does not affect the angular tuning of NVg neurons (see Rice *et al.*, 1986; Lichtenstein *et al.*, 1990; Ebara *et al.*, 2002). Whisker plucking also likely preserves the physiologic identity of nerve endings and their parent cell bodies as the proportions of SA and RA neurons in the NVg population are equivalent between the plucked and the control groups. The SA vs the RA nature of neuronal response to sustained stimuli is determined early in life by genetic and/or molecular factors intrinsic to the cell (Woodbury *et al.*, 2001; Woodbury and Koerber, 2003). Lastly, plucking-induced alterations in responses of NVg neurons are probably not due to long-lasting changes in the myelination status of primary afferent axons as response latencies also remain unaffected by plucking (see also Li *et al.*, 1995).

Plucking-induced alterations in responses of NVg neurons likely occur as a consequence of modest injury to the whisker follicle. In rodents, repeated plucking produces damage to the follicular epithelium and to follicular melanocytes (Ibrahim and Wright, 1978a). In humans, hair plucking damages the local vasculature and the mesenchymal sheath of the dermal papilla and leads to inflammation

(Hordinsky *et al.*, 1999). Inflammation and injury may induce changes in the ion channel composition of nerve receptor endings, rendering primary afferent neurons more excitable (Yoshimura and de Groat, 1999; Bove *et al.*, 2003). Plucking may also alter the mechanical properties of the whisker hair, the hair follicle, and the connective tissue within the follicle. Formation of non-elastic scar tissue, for example, might result in more efficient transmission of deflection energy to nerve terminals within the follicle and lead to greater evoked firing rates and abnormal firing patterns. Regardless of the specific mechanism, whisker plucking results in a persistent change in the functional properties of NVg neurons limiting its usefulness as a means of temporary sensory deprivation.

References

Armstrong-James, M., and K. Fox (1987) Spatiotemporal convergence and divergence in the rat S1 ''barrel'' cortex. *J Comp Neurol* **263:** 265–281.

Bergfeld, W., F. Mulinari-Brenner, K. McCarron, and C. Embi (2002) The combined utilization of clinical and histological findings in the diagnosis of trichotillomania. *J Cutan Pathol* **4:** 207–214.

Bove, G.M., B.J. Ransil, H.C. Lin, and J.G. Leem (2003) Inflammation induces ectopic mechanical sensitivity in axons of nociceptors innervating deep tissues. *J Neurophysiol* **90:** 1949–1955.

Ebara, S., K. Kumamoto, T. Matsuura, J.E. Mazurkiewicz, and F.L. Rice (2002) Similarities and differences in the innervation of mystacial vibrissal follicle–sinus complexes in the rat and cat: a confocal microscopic study. *J Comp Neurol* **449:** 103–119.

Fox, K. (1992) A critical period for experience-dependent synaptic plasticity in rat barrel cortex. *J Neurosci* **12:** 1826–1838.

Gibson, J.M., and W.I. Welker (1983) Quantitative studies of stimulus coding in first-order vibrissa afferents of rats. 2. Adaptation and coding of stimulus parameters. *Somatosens Res* **1:** 95–117.

Glazewski, S., and K. Fox (1996) Time course of experience-dependent synaptic potentiation and depression in barrel cortex of adolescent rats. *J Neurophysiol* **75:** 1714–1729.

Glazewski, S., M. McKenna, M. Jacquin, and K. Fox (1998) Experience-dependent depression of vibrissae responses in adolescent rat barrel cortex. *Eur J Neurosci* **10:** 2107–2116.

Hordinsky, M., M. Ericson, D. Snow, C. Boeck, and W.S. Lee (1999) Peribulbar innervation and substance P expression following nonpermanent injury to the human scalp hair follicle. *J Investig Dermatol Symp Proc* **4:** 316–319.

Ibrahim, L., and E.A. Wright (1978a) The long term effect of repeated pluckings on the function of the mouse vibrissal follicles. *Br J Dermatol* **99:** 371–376.

Ibrahim, L., and E.A. Wright (1978b) The effect of a single plucking at different times in the hair cycle on the growth of individual mouse vibrissae. *Br J Dermatol* **99:** 365–370.

Li, X., S. Glazewski, X. Lin, R. Elde, and K. Fox (1995) Effect of vibrissae deprivation on follicle innervation, neuropeptide synthesis in the trigeminal ganglion, and S1 barrel cortex plasticity. *J Comp Neurol* **357:** 465–481.

Lichtenstein, S.H., G.E. Carvell, and D.J. Simons (1990) Responses of rat trigeminal ganglion neurons to movements of vibrissae in different directions. *Somatosens Mot Res* **7:** 47–65.

Minnery, B.S., and D.J. Simons (2003) Response transformation and receptive field synthesis in the lemniscal trigeminothalamic circuit. *J Neurophysiol* **89:** 40–56.

Pinto, D.J., J.C. Brumberg, and D.J. Simons (2000) Circuit dynamics and coding strategies in rodent somatosensory cortex. *J Neurophysiol* **83:** 1158–1166.

Rice, F.L., A. Mance, and B.L. Munger (1986) A comparative light microscopic analysis of the sensory innervation of the mystacial pad. I. Innervation of the vibrissal follicle–sinus complexes. *J Comp Neurol* **252:** 154–174.

Shoykhet, M., D. Doherty, and D.J. Simons (2000) Coding of deflection velocity and amplitude by whisker primary afferent neurons: implications for higher level processing. *Somatosens Mot Res* **17:** 171–180.

Simons, D.J. (1983) Multi-whisker stimulation and its effects on vibrissa units in rat SmI barrel cortex. *Brain Res* **276:** 178–182.

Woodbury, C.J., and H.R. Koerber (2003) Widespread projections from myelinated nociceptors throughout the substantia gelatinosa provide novel insights into neonatal hypersensitivity. *J Neurosci* **23:** 601–610.

Woodbury, C.J., A.M. Ritter, and H.R. Koerber (2001) Central anatomy of individual rapidly adapting low-threshold mechanoreceptors innervating the hairy skin of newborn mice: early maturation of hair follicle afferents. *J Comp Neurol* **436:** 304–323.

Yoshimura, N., and W.C. de Groat (1999) Increased excitability of afferent neurons innervating rat urinary bladder after chronic bladder inflammation. *J Neurosci* **19:** 4644–4653.