Peripheral nerve damage does not alter release properties of developing central trigeminal afferents

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Lo F-S, Erzurumlu RS. Peripheral nerve damage does not alter release properties of developing central trigeminal afferents. J Neurophysiol 105: 1681–1688, 2011. First published February 9, 2011; doi:10.1152/jn.00833.2010.—The infraorbital branch of the trigeminal nerve (ION) is essential in whisker-specific neural patterning (“barrelettes”) in the principal nucleus of the trigeminal nerve (PrV). The barrelettes are formed by the ION terminal arbors, somata, and dendrites of the PrV cells; they are abolished after neonatal damage to the ION. Physiological studies show that disruption of the barrelettes is accompanied by conversion of functional synapses into silent synapses in the PrV. In this study, we used whole cell recordings with a paired-pulse stimulation protocol and MK-801 blocking rate to estimate the presynaptic release probability (Pr) of ION central trigeminal afferent terminals in the PrV. We investigated Pr during postnatal development, following neonatal ION damage, and determined whether conversion of functional synapses into silent synapses after peripheral denervation results from changes in Pr. The paired-pulse ratio (PPR) was quite variable ranging from 40% (paired-pulse depression) to 175% (paired-pulse facilitation). The results from paired-pulse test were confirmed by MK-801 blocking rate experiments. The nonuniform PPRs did not show target cell specificity and developmental regulation. The distribution of PPRs fit nicely to Gaussian function with a peak at ~100%. In addition, neonatal ION transections did not alter the distribution pattern of PPR in their central terminals, suggesting that the conversion from functional synapses into silent synapses in the peripherally denervated PrV is not caused by changes in the Pr.

paired-pulse facilitation; paired pulse depression; MK-801; sensory deafferentation

A MAJOR CONSEQUENCE OF NEONATAL peripheral sensory nerve injury is synaptic plasticity in the central nervous system. Neonatal denervation of the whisker pad (up to postnatal day 3) results in loss of trigeminal afferent patterning and nonspecific symmetric distribution of barrelette cell dendrites (see Woolsey 1990 for review; Arends and Jacquin 1993; Lo and Erzurumlu 2002). These structural alterations are accompanied by conversion of functional synapses into silent synapses (Lo and Erzurumlu 2007). The principal nucleus of the trigeminal nerve (PrV) contains three major cell types: barrelette, interbarrelette neurons, and GABAergic neurons with distinct morphological and physiological characteristics. Cell class-specific membrane properties are present at postnatal day 1 (Lo et al. 1999; Lo and Erzurumlu 2001), and they do not change after infraorbital nerve (ION) transection, even though structural alterations take place in the PrV in the form of absence of barrelettes (Lo and Erzurumlu 2001). Most of the synapses are already functional in the PrV at birth, with postsynaptic N-methyl-D-aspartate (NMDA) and D,L-α-amino-3-hydroxy-5-methylisoxazole-propionic acid (AMPA) receptors (Lo and Erzurumlu 2007). After neonatal ION transection, the central branches of the ION still engage in synaptic transmission with the exception that synchronized synaptic inputs induce a sustained depolarization (plateau potential) that is mediated by L-type Ca2+ channels (Lo and Erzurumlu 2002). In addition, minimal stimulation tests show that most functional synapses switch to silent synapses without functional AMPA receptors (Lo and Erzurumlu 2007). However, suprathreshold to maximal stimulation of the trigeminal tract (TrV) induces AMPA receptor-mediated postsynaptic responses in both developing and denervated PrV neurons (Lo and Zhao 2011). Morphological and physiological responses to neonatal ION transection in the PrV raise the question whether or not physiological properties of trigeminal afferent terminals change after damage to the peripheral trigeminal axons.

Transmitter release probability (Pr) is an important index of the presynaptic terminal function. It is critical for defining synaptic strength and short-term plasticity. The average size of the postsynaptic response is proportional to the Pr. The Pr value has been used to identify the first order relays and higher order relays in the thalamus (Reichova and Sherman 2004; Sherman 2007). In the trigeminal pathway, the higher relay stations [the ventral posterior medial nucleus of the thalamus and layer IV excitatory neurons in the barrel cortex] exclusively receive high Pr terminals that result in paired-pulse depression (PPD) (Castro-Alamancos 2002; Laurent et al. 2002; Deschenes et al. 2003; Yanagisawa et al. 2004; Fontanez and Porter 2006; Lu et al. 2006; Iwasato et al. 2008; Lee and Sherman 2008; Wang and Zhang 2008; Zhu 2009). However, the properties of afferent terminals in the PrV have not been investigated. We used paired-pulse test and MK-801 blocking rate to estimate the Pr of the trigeminal afferent terminals. For paired-pulse test, if the Pr is high, the postsynaptic response by the second stimulus is always depressed by the first stimulus (PPD) because of vesicle depletion. If the Pr is low, the second response is facilitated by the first stimulus [paired-pulse facilitation (PPF)] due to residual presynaptic Ca2+ (Thomson 2000; Zuker and Regehr 2002; Maggi et al. 2004; Yanagisawa et al. 2004). MK-801 is an irreversible, use-dependent, open-channel NMDA blocker. If the Pr is high, MK-801 blocks NMDA receptor-mediated excitatory postsynaptic currents (EPSCs) rapidly. If the Pr is low, MK-801 blocks synaptic transmission slowly (Hessler et al. 1993; Rosenmund et al. 1993). Here, we studied: 1) the characteristics of presynaptic ION terminals in the developing PrV; 2) the effects of peripheral nerve damage on the Pr of central trigeminal terminals;
and 3) whether the conversion of functional synapses into silent synapses after peripheral denervation results from changes in the Pr.

METHODS

Intraorbital nerve transection. Postnatal day 0 Sprague-Dawley rat pups were anesthetized by hypothermia, and a unilateral skin incision was made caudal to the right whisker pad. The ION was visualized and cut with sterile microscissors between the eye and the caudal edge of the whisker pad. After recovery from anesthesia, the pups were returned to their home cage. All animal handling was in accordance with a protocol approved by the University of Maryland, Baltimore, Animal Use and Care Committee.

Brain slice preparation. Rat pups at postnatal day 0 to postnatal day 14 were deeply anesthetized with fluothane and then euthanized by decapitation. The brain was removed and immersed in cold (4°C), sucrose-based artificial cerebrospinal fluid (aCSF; in mM: 234 sucrose, 2.5 KCl, 1.25 NaH2PO4, 10 MgSO4, 24 NaHCO3, 11 glucose, and 0.5CaCl2) bubbled with 95% O2-5% CO2 (pH 7.4). The brain stem was embedded in 2% agar and cut into 400-μm thick transverse sections with a vibrotome (Electron Microscopy Sciences). Slices containing the PrV were placed in a submerged-type recording chamber (Fine Science Tools) and continuously perfused (2 ml/min) with normal aCSF (in mM: 124 NaCl, 2.5 KCl, 1.25 NaH2PO4, 2 MgSO4, 26 NaHCO3, 10 glucose, and 2 CaCl2; pH 7.4) at room temperature. During recording, bicuculline (10 μM) or picrotoxin (50 μM; Sigma, St. Louis, MO) was added to the aCSF to block GABAergic responses.

Electrophysiological methods. Recordings began at least 1 h after incubation in normal aCSF. Whole-cell patch micropipettes were pulled horizontally in four stages from borosilicate glass (WPI, K150F-4) with a P-87 puller (Sutter Instrument). The patch electrodes were backfilled with a cesium-based solution containing (in mM): 115 CsMeSO3, 10 NaCl, 1 KCl, 4 MgCl2, 1 CaCl2, 10 EGTA, 20 HEPES, 3 Na2-ATP, 0.5 Na2-GTP, and 0.1 spermine (pH 7.25) (CsMeSO3 and spermine were purchased from Sigma) with a tip resistance of 5–9 MΩ. Neurons in the ventral part of the PrV (barrelette region) were blindly patched with the techniques as described previously (Lo et al. 1999). In brief, patch-electrode resistance was monitored in Bridge mode of Axoclamp 2B amplifier by measuring the voltage drop induced by a current pulse (∼100 pA, 200 ms). An increase in resistance of 20–50 MΩ was taken as a sign that the tip of the electrode contacted the surface of a neuron. A steady negative pressure was applied with a 5-ml syringe to form a gigahm seal. Then, a brief suction was used to break into the neuronal soma. The formation of whole cell configuration was indicated by a sudden drop in seal resistance and a DC drop of >55 mV. After “break-in,” the serial resistance was completely compensated with bridge balance. We only collected data from cells with resting membrane potential negative to −55 mV and input resistance >200 MΩ with an Instrutek ITC-16 interface unit and stored on a Pentium 4 computer with Pulses (HEKA) software program. Both current clamp and continuous voltage clamp were performed on recorded cells. Different DC pulse protocols were used to induce active conductances of PrV cells. Barrelette cells were identified by their A-type K⁺ conductance that was gradually blocked by Cs⁺ dialyzed from the patch electrode ~3 min later. Interbarrelette cells were characterized by T-type low threshold Ca²⁺ conductance (Lo et al. 1999).

A pair of fine-tip stimulating electrodes (0.5 MΩ, WPI, IRM33A05KT; tip diameter: 2–3 μm, separated by 300 μm) was inserted into the TrV lateral to the ventral PrV (barrelette region). We stimulated the TrV by passing current pulses (0.2- to 0.3-ms duration, <500 μA) through the electrodes at 0.33 Hz. PrV neurons were voltage-clamped at +60 mV to show the EPSCs in the presence of GABAergic antagonist. A paired-pulse protocol was used to test Pr.

The interstimulus interval (ISI) ranged from 50 to 750 ms to determine the time course of PPF or PPD.

MK-801 experiments. NMDA receptor-mediated EPSCs were isolated at a holding potential of +60 mV in the presence of 6,7-dinitroquinoxaline-2,3-dione (10 μM) and picrotoxin (50 μM). Micropipettes were filled with an additional 5 mM BAPTA to prevent induction of long-term potentiation (Bender et al. 2006). The paired-pulse ratio (PPR) was determined first, and then MK-801 (5 μM) was applied without stimulation for 10 min. Stimulation of TrV resumed at 0.1 Hz to show the blocking rate of MK-801. The amplitude of EPSCs was normalized and fitted with double exponential correlation with SigmaPlot software.

Data analysis and statistics. We first averaged 10 traces of the paired EPSCs at the same ISI and then measured the peak amplitude of each EPSC (the difference between the point just before stimulus artifact and the peak of EPSC). This peak amplitude was actually the amplitude of AMPA receptor-mediated EPSC. We also measured the amplitude of NMDA receptor-mediated EPSC at 30 ms after stimulus (Lo and Zhao 2011). The EPSC induced by the first stimulus was identified as the mean EPSC1, while that by the second stimulus, as the mean EPSC2. The PPR = mean EPSC2/mean EPSC1 × 100 (Kim and Alger 2001). PPD was defined when the PPR was <100%, while PPF was >100%. Linear correlation between mean EPSC1 and mean EPSC2 and double exponential correlation of MK-801 blocking rate were determined with SigmaPlot software. Aged data were expressed as means ± SE. A Student’s t-test was used to determine statistical significance. A χ²-test was used to determine the difference between two groups, and an ANOVA test was used to compare multiple mean values. Matlab Tool Box was used to fit Gaussian distribution and bimodal distribution of PPRs. Goodness-of-fit of the Gaussian distribution was verified with the Jarque-Bera (JB) test.

RESULTS

We used a paired-pulse protocol to estimate Prs of trigeminal central afferents. The PPR was not uniform ranging broadly from 40 to 175%. At first, we used the commonly used criterion to define PPD (<100%) and PPF (>100%) and analyzed their properties separately. Then, we pooled all PPRs to determine their distribution pattern.

Time course of PPD. We determined the time course of PPD in 10 neurons by changing the ISI from 50 to 750 ms. Example records are given in Fig. 1A. Each record is average of 10 traces. When the ISI was 50 ms, EPSC1 and EPSC2 largely overlapped so that the peak value of EPSC2 was much higher than that of EPSC1. This raised the possibility that the depression of EPSC2 might result from postsynaptic current saturation. Along with the increase in ISI to 200 and 500 ms, the overlap of EPSCs gradually diminished. The averaged time course of PPD (Fig. 1C) indicated that the PPR did not change significantly at 50–200 ms ISI (P > 0.5). Thus the depression of EPSC2 cannot be attributed to the saturation of postsynaptic current.

PPD is independent of stimulus intensity. We investigated the changes in PPD magnitude at ISI of 200 ms induced by progressively increasing stimulus intensity. We plotted the amplitude of EPSC2 induced by increasing intensity against the amplitude of corresponding EPSC1 in eight neurons. One example is presented in Fig. 1E. The amplitudes of EPSC2 and EPSC1 induced by different stimulus intensities were linearly correlated (R = 0.85; P < 0.0001). All tested neurons (n = 8) showed similar high correlation (R = 0.85 ± 0.02; P < 0.0001), indicating that the magnitude of PPD is independent of stimulus intensity.
Time course of PPF and its independence of stimulus intensity. We determined the time course of PPF in seven neurons. One example is presented in Fig. 1B showing PPF at different ISIs. The averaged time course (Fig. 1D) revealed similar magnitude of PPF at ISI 50–200 ms (P > 0.45; n = 7). Then, PPF declined gradually following the increased ISIs. Thus we selected 200 ms ISI in the subsequent PPF experiments. An example plot of amplitude of EPSC2 against EPSC1 induced by different stimulus intensities (Fig. 1F) showed linear correlation (R = 0.97; P < 0.0001). All tested neurons (n = 7) showed similar high correlation (R = 0.89 ± 0.02; P < 0.0001), suggesting that the magnitude of PPF is also independent of stimulus intensity.

AMPA receptor- and NMDA receptor-mediated EPSCs show similar PPR. At a holding potential of +60 mV, the amplitude of AMPA-EPSC was always larger than that of NMDA-EPSC, i.e., AMPA-to-NMDA ratio >1, in both developing and denervated PrV (Lo and Zhao 2011). The peak value of the EPSC corresponded to the amplitude of the AMPA-EPSC. To rule out the possibility that AMPA and NMDA synapses are not tightly linked and may be regulated differentially, we measured the PPRs from both AMPA-EPSCs and NMDA-EPSCs of 20 neurons (5 for each PPD and PPF in the normal and denervated PrV). For PPD, the averaged PPR measured from AMPA-EPSC was 73.4 ± 4.8% (Fig. 2A, Control black bar), while that from NMDA-EPSC was 65.0 ± 7.2% (Fig. 2A, Control white bar) in the normal PrV. In the denervated PrV, averaged PPRs were 70.5 ± 7.7% for AMPA-EPSCs (Fig. 2A, IO Cut black bar) and 66.2 ± 8.9% (Fig. 2A, IO Cut white bar) for NMDA-EPSCs. An ANOVA test showed no significant difference between them (P > 0.83). For PPF, the averaged PPR was 130.2 ± 6.4% for AMPA-EPSC (Fig. 2B, Control black bar) and 127.1 ± 7.4% for NMDA-EPSC (Fig. 2B, Control white bar) in the normal PrV. In the denervated PrV, corresponding PPRs were 125.5 ± 6.3% (Fig. 2B, IO Cut black bar) and 135.7 ± 12.0% (Fig. 2B, IO Cut white bar). An ANOVA test showed no significant difference between groups (P > 0.82). Thus AMPA-EPSC and NMDA-EPSC of the same neuron exhibited similar PPR.

PPD and PPF are confirmed by MK-801 blocking rate. Because changes in PPR do not necessarily reflect changes in the probability of neurotransmitter release (Hanse and Gustafsson 2001; Kim and Alger 2001), we applied MK-801 to neurons showing PPD (n = 7) and PPF (n = 7) in the normal PrV. In the presence of MK-801 (5 μM), NMDA-EPSCs declined with repetitive stimulation. The EPSC decay could be fitted with double exponential correlation (Rosenmund et al. 1993). Figure 2C presents two examples to show the difference in MK-801 blocking rate between PPF and PPD in the normal PrV. The neuron showing PPF exhibits a slow decay after MK-801 application, while the neuron showing PPD exhibits a fast decay. Double exponential fitting gave two time constants: τ1 and τ2. For PPD neurons, τ1 = 7.3 ± 1.2 stimuli and τ2 = 350.2 ± 111.7 stimuli. For PPF neurons, τ1 = 52.9 ± 6.8 stimuli, which was significantly longer than that of PPD neurons (P < 0.0001), while τ2 = 508.3 ± 151.6 stimuli, which was not different (P > 0.55) from PPD neurons. Thus both paired-pulse test and MK-801 blocking rate lead to similar results. Additionally, we performed MK-801 blocking test on four neurons (2 for each PPD and PPF) in the denervated PrV. Two examples are given in Fig. 2D. A χ2-test showed no significant difference (P > 0.80) in τ1 and τ2 of MK-801 blocking rate between the normal and the denervated PrV.

Lack of target cell specificity in release properties of central trigeminal afferents in the PrV. The pharmacological and release properties of presynaptic terminals are determined, at
least in part, by their target cells in the hippocampus and neocortex, (Reyes et al. 1998; Scanziani et al. 1998; Watanabe et al. 2005; Sun et al. 2005; Suzuki and Bekkers 2006). The presynaptic terminals from the same parent axon can mediate PPF in one type of target neuron while mediating PPD in a different type of target neuron (Reyes et al. 1998; Scanziani et al. 1998). We tested whether there is a similar difference between synapses made onto barrelette and interbarrelette neurons, which are excitatory neurons in the PrV. Both barrelette and interbarrelette neurons exhibited either PPD or PPF. Because release properties of presynaptic trigeminal afferents are not developmentally regulated (see below), we pooled data from all tested postnatal ages together. In the normal PrV, the averaged PPD magnitude in barrelette neurons was $83.1 \pm 2.9\%$ (Fig. 3A, PPD black bar; $n = 21$), while that in the interbarrelette neurons was $76.2 \pm 2.7\%$ (Fig. 3A, PPD white bar; $n = 25$). The magnitude of PPD in both types of neurons was not significantly different ($P > 0.09$). Similarly, the PPF magnitude in barrelette neurons was $126.6 \pm 3.3\%$ (Fig. 3A, PPF black bar; $n = 24$), while that in interbarrelette neurons was $129.8 \pm 4.5\%$ (Fig. 3A, PPF white bar; $n = 19$), which was about the same as barrelette neurons ($P > 0.57$). Then, we pooled PPRs in each cell classes. The PPR for barrelette neurons was $106.3 \pm 3.9\%$ (Fig. 3A, pool black bar; $n = 45$), while PPR for interbarrelette neurons was $99.3 \pm 3.3\%$ (Fig. 3A, pool white bar; $n = 44$), which was not significantly different.

![Fig. 3. Lack of target cell specificity in the trigeminal afferents of the developing and denervated PrV.](image)

**A:** In the developing PrV, the averaged PPD magnitude in barrelette neurons (PPD black bar) is not significantly different ($P > 0.09$) from that in the interbarrelette neurons (PPD white bar). PPF magnitude in barrelette neurons (PPF black bar) is similar to ($P > 0.57$) that in interbarrelette neurons (PPF white bar). When we pooled PPRs of each cell classes, the PPR for barrelette neurons (Pool black bar) is not significantly different from PPR for interbarrelette neurons (Pool white bar).

![Fig. 2. Mechanisms underlying PPD and PPF.](image)

**A:** averaged paired-pulse ratios (PPRs) of neurons showing PPD in the normal and denervated principal nucleus of the trigeminal nerve (PrV). Black bars are PPRs measured from DL-α-amino-3-hydroxy-5-methylisoxazole-propionic acid (AMPA) receptor-mediated EPSCs, while white bars are from N-methyl-D-aspartate (NMDA) receptor-mediated EPSCs. IO cut, infraorbital nerve cut. There is no difference between them ($P > 0.8$, ANOVA test). **B:** averaged PPRs of neurons showing PPF in the normal and denervated PrV. There is no difference between them ($P > 0.82$, ANOVA test). **C:** 2 examples of MK-801 blocking experiments in the normal PrV. Decays of NMDA receptor-mediated EPSC can be fitted as double exponential correlation with high R values. Note that the blockade of PPF neuron is slower than that of PPD neuron. **D:** 2 examples of MK-801 blocking experiments in the denervated PrV showing similar blocking rate to those in C.
different from barrelette neurons. It should be pointed out that the pooled PPRs from both classes of neurons were \( \sim 100\% \) suggesting that the contribution of PPD and that of PPF were about equal. We conclude that there is no target cell specificity with respect to presynaptic release properties of central trigeminal terminals in the PrV.

**Presynaptic Pr of the central trigeminal afferents is not regulated during postnatal development.** Pr of thalamocortical terminals undergoes a developmental change in the barrel cortex during the critical period for pattern formation (Yanagisawa et al. 2004). We investigated whether this is also the case in the PrV, the first relay station of the trigeminal pathway. We plotted PPRs against postnatal ages (Fig. 4 in the PrV, the first relay station of the trigeminal pathway). We plotted PPRs against postnatal ages (Fig. 4A). No linear or nonlinear correlation \( (R = 0.17; P > 0.57) \) was found. An ANOVA test showed that the variance of the PPR was not significant \( (P = 0.446) \) between different ages. Thus, unlike the barrel cortex, the Pr remains constant during postnatal development in the PrV; this is perhaps due to the relative maturity of synapses in the PrV at birth (Lo et al. 1999; Lo and Erzurumlu 2007). It can be seen from Fig. 4A that PPRs seem to range symmetrically from low to high value.

**Gaussian distribution of PPRs in the PrV.** Our analysis showed that PPRs range symmetrically from a low-to-high value (Fig. 4A). We then plotted the PPR distribution (Fig. 4C). The histogram of PPR distribution was continuous, symmetrical, and bell shaped. It could not fit into a bimodal distribution but fitted nicely to Gaussian function. The JB test is a goodness-of-fit measure of departure from Gaussian distribution. The JB test showed \( P = 0.001 \). The fitted curve ranged from 0 to 200% with a mean 102.9 ± 3.1%. Because the peak of the curve is \( \sim 100\% \), it indicates that quite a few terminals show neither PPD nor PPF. The SD is 29.1%; thus 68% terminals have the PPRs between 73.8 and 132.0% (means ± SD). Only 16% terminals (means ± 2 ± 3 SD) have PPRs <73.8 or >132.0%. This further suggests that Pr of central trigeminal afferents is continuously distributed over a wide range with a preponderance of middle Pr values. On a linear \( x \)-axis, the distribution of PPRs showed a symmetrical histogram (Fig. 4C), but the histogram was skewed towards PPD when we plotted on a logarithmic \( x \)-axis (see Supplemental Materials, Supplemental Fig. S1; Supplemental Material for this article is available online at the J Neurophysiol website).

**Neonatal denervation does not change the Pr of central trigeminal afferents.** We investigated whether peripheral sensory nerve injury alters the release properties of the central branch terminals of the affected ganglion cells. In the denervated PrV, both barrelette and interbarrelette neurons showed similar magnitudes of PPD and PPF. The averaged magnitude of PPD in barrelette neurons was 75.2 ± 4.3% (Fig. 3B, PPD black bar; \( n = 21 \)), while that in interbarrelette neurons was 69.2 ± 4.7% (Fig. 3B, PPD white bar; \( n = 14 \)). There was no significant difference between the two cell types \( (P > 0.37) \). In addition, there was no significant difference between normal PrV and peripherally denervated PrV \( (P > 0.14) \). The averaged magnitude of PPF in barrelette neurons was 129.2 ± 4.5% (Fig. 3B, PPF black bar; \( n = 19 \)), while that in interbarrelette neurons was 133.7 ± 8.2% (Fig. 3B, PPF white bar; \( n = 9 \)), which is similar to that in barrelette neurons \( (P > 0.62) \). In addition, the magnitude of PPF in the denervated PrV was not different from that in the normal PrV \( (P > 0.39) \). Then, we pooled PPRs in each cell classes. The PPR for barrelette neurons was 100.8 ± 5.3% (Fig. 3B, Pool black bar; \( n = 45 \)), while PPR for interbarrelette neurons was 94.4 ± 7.8% (Fig. 3B, Pool white bar; \( n = 23 \)), which was not significantly different from barrelette neurons \( (P > 0.49) \). Thus there is no target cell specificity in the denervated PrV. In addition, the

![Fig. 4. PPRs are not developmentally regulated in the normal and denervated PrV. A: there is no correlation between the PPRs and postnatal ages in the normal PrV. B: there is no correlation between PPRs and postnatal ages in the denervated PrV. C: distribution of PPRs in the normal PrV fits Gaussian function \( (P = 0.001) \). Fitting curve covers from 0 to 200% with a peak at \( \sim 100\% \). D: distribution of PPRs in the denervated PrV fits Gaussian function \( (P = 0.001) \) that is similar to that in C.](image-url)
pooled PPRs in each cell classes in the denervated PrV did not differ from those in the developing PrV \( (P > 0.40) \). Biologically, the fewer number of interbarrelette cells does not have significance because all cell types are affected following ION damage and there is no selective survival of a specific class of cells in the PrV (Miller and Kuhn 1997; Lo and Erzurumlu 2001, 2002). In terms of specific sample sizes, the \( \chi^2 \)-test showed that the proportion of barrelette and interbarrelette cells in the normal and denervated PrV (Fig. 3) is not significantly different \( (P = 0.11) \).

We plotted all PPRs against different postnatal ages (Fig. 4B). There was no correlation (linear or nonlinear; \( R = 0.10; P > 0.76 \)), suggesting that there is no developmental change in the Pr of the trigeminal afferents. The histogram of PPRs in the denervated PrV could be fitted nicely by Gaussian function with \( P = 0.001 \) (Fig. 4D). The fitted curve coverage from 0 to 200% with a mean of 98.5 \( \pm \) 4.4% that was not significantly different from normal PrV \( (P > 0.40) \). The histogram of PPRs plotted on a logarithmic x-axis skewed towards PPD with a peak \( \sim 100\% \) (Supplemental Fig. S2). We conclude that the release properties of the trigeminal central terminals are not altered after damage to their peripheral counterparts. These results also indicate that the conversion from functional to silent synapses after neonatal denervation (Lo and Erzurumlu 2007) does not result from presynaptic changes in transmitter Pr.

DISCUSSION

The present results reveal that the mechanisms underlying postnatal synaptic refinement and plasticity differ between the first relay station of the whisker-barrel pathway, the PrV, and the higher relay stations (VPM and barrel cortex). First, we found that, unlike the inputs to the VPM and barrel cortex, central trigeminal axons exhibit heterogeneous Prs that vary continuously over a broad range. Second, in contrast to the barrel cortex, presynaptic Pr remains constant during postnatal development in the PrV. Third, our findings rule out presynaptic transmitter release-related events in conversion of functional synapses into silent synapses in the PrV following neonatal peripheral denervation. Finally, neonatal damage to the peripheral axons of the trigeminal ganglion (TG) cells does not appear to affect release properties of their central axons in the PrV.

The presynaptic transmitter Pr of afferent pathways is often classified as high Pr or low Pr. Based on the results from MK-801 blocking rate, an indirect measurement of Pr, hippocampal synapses have been classified as low Pr and high Pr (Hessler et al. 1993; Rosenmund et al. 1993). However, the Schaffer collateral synapses on CA1 pyramidal cells show a wide range of Prs (Hanse and Gustafsson 2001). Further studies using minimal stimulation techniques, the styryl dye FM-1–43 uptake, and mathematic analyses indicate that hippocampal synapses have Prs that range continuously over all possible probabilities \((<0.1 \text{ to } 1.0)\). Because the continuous Pr distribution skew heavily toward the low Pr value, neither Gaussian function nor bimodal function fits the Pr distribution (Allen and Stevens 1994; Murthy et al. 1997; Huang and Stevens 1997). The Prs have also been investigated in the thalamocortical reciprocal circuitry. It has been proposed that in the sensory thalamic nuclei the first order relay neurons always receive high Pr afferent terminals (“driver”) that result in PPD, while higher order relay neurons receive low Pr terminals (“modulators”) that lead to PPF (Reichova and Sherman 2004; Sherman 2007). In the trigeminal pathway, VPM and layer IV barrel cortex excitatory neurons exclusively receive high Pr terminals that result in PPD (Castro-Alamancos 2002; Laurent et al. 2002; Deschenes et al. 2003; Yanagisawa et al. 2004; Fontanez and Porter 2006; Lu et al. 2006; Iwasato et al. 2008; Lee and Sherman 2008; Wang and Zhang 2008; Zhu 2009). The present results reveal that in the first trigeminal relay station (PrV), whisker-specific trigeminal afferents have heterogeneous release properties. The continuous symmetrical bell-shaped distribution of PPRs fits nicely to the Gaussian function with a peak at \( \sim 100\% \), suggesting that the afferent terminals in the PrV are different from those in the hippocampus, the somatosensory thalamus, or the barrel cortex. To our knowledge, this Gaussian PPR distribution pattern is described for the first time in the central nervous system.

The mechanisms underlying the difference between the afferent release properties in the PrV vs. downstream neural circuits in the thalamus and cortex are not known. The diversity of presynaptic performance depends on multiple mechanisms, such as the variation in synaptic morphology, \( Ca^{2+} \) channel subtypes of presynaptic terminals, and presynaptic proteins [for review see Thomson (2000b); Craig and Boudin (2001); Atwood and Karunanithi (2002); Reid et al. (2003)]. We do not know the morphological and functional variations of presynaptic trigeminal terminals in the PrV, the composition of presynaptic \( Ca^{2+} \) channels, and whether there are differences in presynaptic proteins. However, the perfect Gaussian distribution of PPRs in the PrV (without any bias such as that seen in the hippocampus) suggests heterogeneity of the trigeminal terminals in the PrV.

Many brain structures receive low Pr and high Pr afferent terminals from different types of cells (Li et al. 2003; Yanagisawa et al. 2004; Reichova and Sherman 2004; Zhu 2009). In the piriform cortex, different postsynaptic response patterns of the two main classes of layer II neurons (superficial pyramidal and semilunar) are mediated by presynaptic terminals of the lateral olfactory tract with different release probabilities (Suzuki and Bekkers 2006). Postsynaptic firing pattern may be modulated by the short-term synaptic plasticity induced by different Prs (Suzuki and Bekkers 2006). Trigeminal afferent terminals originate from different classes of TG cells. TG cell responses to passive whisker deflection can be roughly divided into two types: rapidly adapting (RA) and slowly adapting (SA; Shoykhet et al. 2003). The rapidly adapting cells exhibit only phasic ON response to whisker deflection. The afferent terminals of RA ganglion cells probably have high Pr, so that the preceding spike depresses the synaptic transmission of subsequent spikes due to vesicle depletion (Thomson 2000a; Zuker and Regehr 2002). The SA cells show both phasic ON and tonic plateau responses to whisker deflection (Shoykhet et al. 2003). Their afferent terminals possibly have low Pr, so that the preceding spike facilitates synaptic transmission of subsequent spikes via the residual presynaptic \( Ca^{2+} \) (Thomson 2000a; Zuker and Regehr 2002). Jones et al. (2004) reported that between pure RA and pure SA responses to whisker deflection, mixed RA/SA responses varied and formed a stimulus-dependent continuum. Our present results support this finding and suggest that the continuous distribution of central
trigeminal afferent Prs plays an important role in the transmission of continuous TG response profiles to the postsynaptic PrV neurons.

Most ION-PrV synapses of the rat are functional at birth (Lo et al. 1999; Lo and Erzurumlu 2007). ION transaction on postnatal day 0 leads to several activity-dependent morphological and physiological changes in the PrV. Whisker-specific patterns (barrelettes) are abolished [see Woolsey (1990) for review] with reoriented dendritic tree of barrelette neurons (Lo and Erzurumlu 2002). Cell death in the PrV significantly increases, so that the number of cells in the PrV reduces by one-third (Miller and Kuhn 1997). However, the remaining PrV neurons of all three classes maintain their electrophysiological and morphological characteristics (Lo and Erzurumlu 2001, 2002) and establish many new synapses with the TrV fibers (reactive synaptogenesis) within 5 days after lesion (Lo et al. 2011). A striking physiological change is the conversion of functional synapses into silent synapses (Lo and Erzurumlu 2007). Silent synapses may involve both pre- and postsynaptic mechanisms. Several models of presynaptic silent synapses have been proposed [reviewed by Kullmann (2003); Voronin and Cherubini (2003) 2004; Atasoy and Kavalali (2006)]. One model suggests that both AMPA and NMDA receptors are functional but that silent synapses result from low Pr from the presynaptic site. Increasing Pr with different methods leads to conversion from silent into functional synapses (Gasparini et al. 2000; Maggi et al. 2003). The present study shows no changes in the presynaptic Pr after neonatal denervation, suggesting that silent synapses are not associated with presynaptic release properties.

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