Behavioral/Systems/Cognitive

NMDA Receptor-Dependent Regulation of Axonal and Dendritic Branching

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In the rodent trigeminal principal nucleus (PrV), trigeminal afferent terminals and postsynaptic cells form discrete modules (“barrelettes”) that replicate the patterned array of whiskers and sinus hairs on the snout. Barrelette neurons of the PrV relay whisker-specific patterns to the contralateral thalamus and, subsequently, to the primary somatosensory barrel cortex. Genetic impairment of NMDA receptor (NMDAR) function blocks development of barrelettes in the PrV. Underlying cellular and functional defects are not known. Here, we examined morphological differentiation of whisker afferents, dendritic differentiation of barrelette cells, and their electrophysiological properties in mice with genetic perturbations of the essential subunit NR1 of NMDARs. We show that in NR1 gene knock-down (KD) and knock-out mice, whisker afferents begin their embryonic development normally but, over time, fail to segregate into patches, and instead they develop exuberant terminal arbors spanning most of the PrV. Postnatal NR1KD barrelette cells, with significantly reduced NMDA currents, retain their membrane and synaptic properties but develop longer dendrites with no orientation preference. These results indicate that NMDARs regulate growth of presynaptic terminal arbors and postsynaptic dendritic branching, thereby leading to consolidation of synapses and patterning of presynaptic and postsynaptic elements.

Key words: barrelettes; transgenic mice; trigeminal afferents; trigeminal principal nucleus; membrane properties; pattern formation

Introduction

In the first relay station of the rodent trigeminal pathway principal nucleus (PrV), whisker afferent terminals form discrete patches of terminals that replicate the patterned array of the whiskers on the snout (Erzurumlu and Jhaveri, 1992). Afferent patterning is detected by select sets of postsynaptic neurons, trigeminothalamic projection or “barrel” neurons, which orient their dendrites toward discrete patches of trigeminal afferent terminals. As a result, whisker-specific barrelette units are formed (Ma and Woolsey, 1984; Bates and Killackey, 1985; Ma, 1993). PrV barrelette cells convey these patterns to the thalamic barrels and consequently to the somatosensory barrel cortex (Woolsey and Van der Loos, 1970; Van der Loos, 1976; Killackey and Fleming, 1985; Erzurumlu and Jhaveri, 1990; Senft and Woolsey, 1991). Lesions of the whisker follicles, or the branch of the trigeminal nerve innervating them, irreversibly alter or abolish patterns in the PrV and upstream somatosensory centers during a critical period in development (Belford and Killackey, 1980; Durham and Woolsey, 1984; Woolsey, 1990; O’Leary et al., 1994).

Cellular and molecular mechanisms underlying patterning of presynaptic terminals and barrelette cell dendrites are unknown. Neural activity, in particular NMDA receptor (NMDAR)-mediated activity, is an essential player in development of whisker-specific patterns in the PrV. Targeted deletion of the NMDARI (NR1) (Li et al., 1994) or NR2B (Kutsuwada et al., 1996) subunit genes of NMDARs, or transgenic reduction of NR1 subunit expression (Iwasato et al., 1997), produced mice with no whisker patterning in the PrV, whereas a full array of whiskers were present on the snout. To gain insight into cellular mechanisms of pattern deficits, we studied the development of whisker afferents in the PrV of NR1 knock-out (KO) and NR1 knock-down (KD) mice during the critical period of pattern formation. We next analyzed electrophysiological properties and dendritic patterning of barrelette neurons in postnatal NR1KD mice. Our results show that although membrane properties of barrelette neurons remain unchanged in transgenic mice, their dendritic differentiation and terminal arbor fields of their presynaptic partners arriving from whisker follicles change dramatically.

Materials and Methods

Animals. NR1KD mice were generated as described previously (Iwasato et al., 1997). Crossing transgenic founders with NR1+/− mice (Li et al., 1994) led to incorporation of the NR1 transgene into NR1+/- strain. NR1−/- mice carrying NR1 transgene have 70–80% reduction of NR1 expression (Iwasato et al., 1997). These mice were originally reported as “NR1−/- LTg+/-” mice; here, we refer to them as NR1KD mice. In the present study, normal mice (C57BL/6) and mice carrying the NR1 transgene were used as controls.

Genotype analysis was performed by PCR using the DNA samples extracted from the tail. Oligo sequences designed for NR1 and Neo were used (Iwasato et al., 1997). PCR products were resolved on a 2% agarose gel. Neo oligo pair gives a 280 bp fragment, and NR1 oligo pair gives 240 bp (normal NR1 gene) and 160 bp (NR1 transgene) fragments.

All animal handling was in accordance with a protocol approved by the
Histology. Mice were given an overdose of sodium pentobarbital and perfused transcardially with 4% paraformaldehyde in 0.1M PB, pH 7.4. Brains were removed and placed in the same fixative overnight. After cryoprotection in 30% sucrose in PB for 2 d, 90-μm-thick coronal sections through the brainstem were taken from P5, P14, and P21 NR1KD and control mice. All chemicals were purchased from Sigma (St. Louis, MO). Sections were incubated in cytochrome oxidase (CO) reaction solution (in mg/ml: 0.5 cytochrome c, 40 sucrose, 0.5 diamino-benzidine, in PB) at 37°C in a shaker incubator for 6–8 h. Staining of CO histochemistry enabled us to visualize PrV boundaries and barrelette patterns.

To visualize PrV neurons, the Golgi–Cox impregnation method was used (Datwani et al., 2002). Brainstem samples from 4% paraformaldehyde-perfused animals were immersed into impregnation solution [mixture of solution A (1.0 g of potassium dichromate and 1.0 g of mercuric chloride in 85 ml of distilled water) with solution B (0.8 g of potassium chromate and 0.5 g of sodium tungstate in 20 ml of distilled water)] at room temperature for 6–8 d. After impregnation, the specimens were cryoprotected in 30% sucrose for 3 d. Frozen sections were cut on sliding microtome at 200–300 μm. Sections were collected and passed through 15% ammonium hydroxide (EM Science, Gibbstown, NJ) for 30 min and Kodak fixative solution (Eastman Kodak, Rochester, NY) for 15 min and then rinsed thoroughly in distilled water. Sections were then counterstained with cresyl violet, dehydrated through a series of alcohol, and mounted with De-pex (Electron Microscopy Sciences, Washington, PA).

Morphometric analyses. Impregnated neurons were examined under a Nikon (Tokyo, Japan) light microscope with a 40× lens and reconstructed using a drawing tube. Images of neurons were then scanned at 300 dots per inch and standardized and stored in Adobe Photoshop 7.0 (Adobe Systems, San Jose, CA). In this study, we adopted the terminology used by Ohara and Havton (1994). The dendrite derived directly from the soma is the first-order branch or primary dendrite. The daughter branches arising from that are second-order branches, and so on. The point at which a dendrite gives rise to two daughter branches is called a bifurcation node. The distance from the soma or a bifurcation node to the next node is called the segment length. Termination of a dendrite is called an ending.

For morphometric analysis of the dendrites, soma size and area covered by the dendritic tree were measured from the two-dimensional display by MetaVue program. The number of primary dendrites, bifurcation nodes, and endings were counted manually. To examine the dendritic tree orientation, endings were marked, and the greatest angle between the two furthest endings was measured. A neuron with ≥75% of its endings falling into one quadrant was designated as a neuron with selective orientation. Otherwise, the neuron was classified as a symmetrical neuron. The concentric sphere method of Sholl (1953) was used to analyze dendritic branching patterns. Briefly, concentric spheres of a constant interval, 10 μm, were brought over each cell, and the cell was oriented with the center of the soma as the origin; the dendrogram was prepared accordingly. Intersections of spheres and dendrites of different orders were counted. The position (between two rings) and order of bifurcation nodes and endings were also noted. The number of branches

Figure 1. Absence of whisker-specific patterns in NR1KD mice. Cytochrome oxidase histochemistry reveals barrelette patterns in control PrV at P5, P14, and P21, whereas the patterns are absent in NR1KD mice at all ages. Barrelette rows corresponding to whisker rows a–e are indicated in control PrV. All micrographs are from coronal sections and are oriented the same way, with lateral to the right and dorsal to the top. TR, Trigeminal tract. Scale bar, 500 μm.

Louisiana State University Health Sciences Center Animal Use and Care Committee.

1,1'-Dioctodecyl-3,3,3',3'-tetramethylindo-carbocyanine perchlorate labeling. Control, NR1KO, and NR1KD mice [embryonic day 15 (E15) to postnatal day 5 (P5)] were perfused transcardially with phosphate buffer (PB), followed by 4% paraformaldehyde. A small crystal of 1,1'-dioctodecyl-3,3,3',3'-tetramethylindo-carbocyanine perchlorate (DiI) (Molecular Probes, Eugene, OR) was placed into one whisker follicle on the snout. Samples were kept in an incubator at 37°C for 2–3 weeks. The brainstems were sectioned horizontally by vibratome (VT1000S; Leica, Nussloch, Germany) at a thickness of 300 μm. Pictures at different focal planes were taken from fluorescent microscope by CoolSnap camera (Photometrics, Tucson, AZ) with MetaVue program (Universal Imaging, Downingtown, PA). DiI-labeled single axons were then reconstructed from composite pictures. All of the single axons emanating from the trigeminal tract with their terminal arbors were included in the study. Cases with neighboring axons leaving the tract and entering the brainstem trigeminal complex and those in which there were overlapping terminal fields were not included in our analyses.
and the length of segments were measured and analyzed into orders. Segment lengths were measured by tracing the dendritic segments from one bifurcation point to another (or to the tip of the last segment) with the use of MetaVue program. Our dendritic segment measurements were done from two-dimensional reconstructions of dendritic fields of neurons embedded in 2- to 300-μm-thick sections; therefore, they do not reflect absolute values for dendritic segment length, but they are nevertheless informative for comparisons between control and knock-down cases.

Electrophysiology. For electrophysiological recordings, NR1KD and control mice (P7–P13) were anesthetized with Fluothane and killed by decapitation. Brains were removed and immersed in cold (4°C) artificial CSF (ACSF) bubbled with 95% O₂ and 5% CO₂. Brains were then embedded in 2% agar and cut into 400-μm-thick transverse sections with a vibratome. After 2–4 h of incubation in ACSF at room temperature, slices containing the PrV were transferred into a submerged-type recording chamber and perfused continuously (>2 ml/min) with oxygenized ACSF at room temperature. Whole-cell recordings were then made as described previously (Lo and Er-zurumlu, 2001, 2002). Voltage-clamp was performed with patch electrode filled with Cs-based solution. AMPA receptor-mediated and NMDA receptor-mediated EPSCs were induced by maximal stimulation of the trigeminal tract. The AMPA receptor-mediated EPSC is identified by voltage clamping at −70 mV in the presence of 10 μM bicuculline. The NMDA receptor-mediated EPSC is isolated by voltage clamping at +40 mV in the presence of 10 μM bicuculline and 25 μM DNQX, an AMPA receptor antagonist.

Results

Lack of barrelette patterns in the PrV of NR1-deficient mice

In normal mouse PrV, barrelette patterns appear at approximately P2–P3 and are consolidated by P5 (Ma, 1993). NR1KO mice die shortly after birth because of respiratory problems. When parturition is blocked by terbutaline injections to the pregnant dam and the pups are delivered 2 d past normal gestation period (equivalent to P2), barrelettes are absent in NR1KO mice (Li et al., 1994). Transgenic expression of low levels of NR1 in the NR1KO background “rescues” postnatal lethality, and in these (NR1KD) mice (Iwasato et al., 1997), barrelettes never form (Fig. 1). Consequently, barreloids in the thalamus and barrels in the somatosensory cortex are absent, although these animals have a normal set of whiskers and display whisking behavior. The underlying cellular defects could involve differentiation of whisker-specific afferent terminals in the PrV, dendritic development of barrelette cells, or synaptic communication and electrophysiological properties of PrV neurons. First, we examined the developmental differentiation of whisker-specific trigeminal afferent terminals in the PrV of NR1KO and NR1KD mice and then electrophysiological properties of barrelette neurons and their dendritic differentiation in NR1KD mice.

Exuberant axonal arborization in NR1 mutant PrV

Trigeminal ganglion axons invade the developing whisker fields at approximately E10 in the mouse, and their central processes lay down the trigeminal tract in the brainstem by E13 (Stainier and Gilbert, 1990). After the establishment of the central tract, trigeminal axons emit radial collaterals into the brainstem trigeminal nuclei and begin forming whisker-specific patchy terminals by E15. We placed tiny DiI crystals into individual whisker follicles of E15, E17, P0, and P5 mice. We used four lines of mice as follows: NR1KO, NR1KD, wild-type (C57BL/6), and C57BL/6NR1transgene mice. The latter two groups were pooled as controls.

Figure 2. Development of whisker afferent terminal arbors in the PrV. A, Whisker-specific trigeminal afferents begin arborizing in the PrV at E15, and the arborization patterns are similar in control and NMDAR-deficient mice. At E17, there is a notable expansion of terminal arbors in the mutant PrV. By P0, terminal arbors in PrV are expansive in NR1KO mice and much larger than those in NR1KD and control mice. At P5, the terminal fields in the NR1KD PrV are significantly larger than those in controls and never develop segregated patches. Arrowheads indicate the parent axon emerging from the trigeminal tract. All axons are oriented the same way, with the position of the central trigeminal tract (lateral) to the right and rostral to the top. Scale bar, 100 μm. B, Areal extent of central trigeminal afferent arbors. Arbor area is measured by outlining the tips of eachafferent from two-dimensional reconstruction. There is no significant difference among control, NR1KD, and NR1KO afferents at E15; however, from E17 and on, NR1KD and NR1KO terminals have significantly enlarged compared with the controls. NR1KD terminal areas are larger than NR1KO at E17 and P0; the differences are significant (p < 0.05 and p < 0.001, respectively). C, Branch tips of central trigeminal afferent arbors. The number of branch tips shows no differences among the control, NR1KD, and NR1KO cases at E15. By E17, NR1KO cases have significantly increased terminal tips compared with both control and NR1KD. From P0 and on, NR1KD terminals have more branches than controls (p < 0.001). A horizontal bar is used to represent the mean of each group. Significant differences are indicated by asterisks (**p < 0.05; ***p < 0.001; Student’s t test).
nal arbor field is emergent in NR1KD and specifically in NR1KO cases (Fig. 2A, B). At the time of birth, the whisker afferent arbors are the largest and most complex in NR1KO mice and conspicuously larger in NR1KD animals in comparison with controls. By P5, which is after the end of the critical period for whisker lesion-induced morphological plasticity (Woolsey, 1990), trigeminal terminal arbors in the NR1KD PrV occupy a fivefold larger area than those in control cases (Fig. 2A, B). Quantitative analyses of the afferent arbors and the number of branch tips between E15 and P5 revealed that there is no significant difference between the controls and NR1-deficient PrV at E15 (Fig. 2B, C). However, significant differences in all of the measured arbor parameters are evident in NR1KO mice from E17 onward and are also seen in the postnatal NR1KD mice (Fig. 2B, C). Clearly, widespread terminal arbors, increased branch tips, and overlapping distribution of whisker afferents within the PrV of NR1KO and NR1KD mice are major defects that contribute to the absence of barrelette patterns (Fig. 1).

Membrane properties and synaptic response of barrelette neurons in PrV

Barrelette neurons of the PrV are the major trigeminothalamic projection neurons, and they alone convey the whisker-specific neural pattern template to the ventrobasal thalamus and subsequently to the primary somatosensory barrel cortex (Killackey and Fleming, 1985; Erzurumlu and Jhaveri, 1990). To determine whether intrinsic membrane properties and synaptic responses of barrelette neurons were altered in NR1-deficient mice, we performed electrophysiological recordings in the PrV of postnatal NR1KD and control mice. We could not use NR1KO mice, because they die after birth as a result of respiratory failure (Li et al., 1994). Barrelette neurons from the earliest postnatal ages to maturity typically have an A-type K⁺ conductance (Iₐ) when the membrane potential is depolarized after hyperpolarization, leading to delayed sodium spikes (Lo et al., 1999). This is not altered in NR1KD barrelette neurons (Fig. 3A). Thus, membrane properties of barrelette neurons are preserved in NR1KD PrV.

Synaptic responses were recorded from barrelette neurons after trigeminal tract stimulation (Fig. 3B, C). An EPSP-IPSP sequence is induced in control and NR1KD barrelette neurons, indicating that excitatory and inhibitory synaptic connections are present (Fig. 3B). Voltage-clamp analyses reveal that the excitatory response is mediated by AMPA and NMDA receptors (Fig. 3C). However, in NR1KD neurons, the amplitude of NMDAR-mediated responses is significantly smaller than control neurons (24.1 ± 5.6 vs 130.4 ± 16.5 pA, p < 0.005), thus confirming 80% reduction of NMDAR function.

In NR1KD mice, intrinsic membrane properties and synaptic responses of barrelette neurons are not altered, other than an 80% reduction in the NMDA component of the EPSCs. Their presynaptic partners form diffuse and widespread terminal arbors that span multiple barrelette domains. How does this affect dendritic arbor and patterning of barrelette neurons?

Reduced NMDAR function disrupts dendritic differentiation and patterning of barrelette neurons in the PrV

Barrelette patterns are readily visualized by use of routine cytochrome oxidase histochemistry or Nissl stains (Fig. 4A). On average, barrelettes have a ~10 μm wall and a ~30–40 μm center (Fig. 4A, asterisks). We used the Golgi heavy metal impregnation technique (Datwani et al., 2002) to reveal the morphological details of the PrV neurons (Fig. 4B). Small barrelette neurons form the barrelette wall and display selective dendritic orientation (Fig. 4B, arrow) toward trigeminal afferent terminal patches. Large interbarrelette neurons have extensive dendritic fields covering multiple barrelettes (Fig. 4B, arrowheads). Because P14 is well beyond the critical period for barrelette development, and dendrites of PrV cells have acquired their mature characteristics, we chose this time point to examine the dendritic differentiation of barrelette neurons. A total of 87 barrelette neurons (control, n = 40; NR1KD, n = 47) from the whisker representation area of the PrV were examined. We measured various morphometric parameters (Table 1) and determined dendritic projections and the orientation preference of dendritic trees (Fig. 4). Sholl analysis (Sholl, 1953) was applied to reveal the complexity and distribution of the dendrites (Fig. 5A–C). Finally, we plotted numbers of dendritic branches and segment lengths for each dendritic order (Fig. 5D, E).

Soma sizes of barrelette cells in control and NR1KD mice are similar (Table 1). Normally, barrelette neurons have three primary dendrites emanating from the soma (Table 1), and their dendritic fields are restricted and oriented toward the barrelette centers (Fig. 4C, E, F). NR1KD barrelette neurons usually have...
four primary dendrites (Table 1) that radiate in all directions from the soma (Fig. 4D, E). Most of the barrelette neurons (85%) in NR1KD PrV do not have orientation preference (Fig. 4F).

Sholl analysis (Sholl, 1953) revealed that the dendrites of NR1KD barrelette neurons have more proximal (10 µm) intersections (Fig. 5A), because NR1KD neurons have more primary dendrites. However, the dendrites of NR1KD barrelette neurons formed fewer branches (Fig. 5B, D), especially at higher orders, giving significantly reduced branch number beyond the fifth order and resulting in less dendritic orders and smaller total branch point numbers (Table 1). Although the total number of terminal endings in NR1KD barrelette neurons is comparable with control cells (Table 1), their distribution is markedly different. In NR1KD barrelette neurons, some of the terminal endings are located beyond 50 µm from the soma (Fig. 5C). This is because their dendrites have longer segments (Fig. 5E), causing a 35% increase in the total dendritic length (Fig. 5E′) and more intersections at distal regions (Fig. 5A).

Consequently, NR1KD barrelette neurons have much larger dendritic fields (Table 1) (3.4-fold larger dendritic fields than those of control cells). Overall, NR1KD barrelette neuron dendrites show little or no orientation preference, have longer segments, and have fewer high-order branches, indicating that NMDAR-mediated mechanisms play a major role in dendritic sculpting, complexity, and orientation.

Discussion

Our results indicate that neural activity-mediated by the NMDARs plays a significant role in presynaptic terminal and postsynaptic dendritic sculpting in the first relay nucleus of the mouse trigeminal somatosensory pathway. Recently, we reported that in NR1KD mice, there is a significant reduction in the volume of the PrV (24.4%) and similar volumetric reductions in its thalamic target, ventroposteromedial nucleus (25.5%) (Lee and Erzurumlu, 2005). Ongoing studies are aimed at determining whether such volumetric reductions in its thalamic target, ventroposteromedial nucleus (25.5%) (Lee and Erzurumlu, 2005). Ongoing studies are aimed at determining whether such volumetric reductions in these nuclei are a consequence of pronounced cell death. Increased arboreal span of trigeminal terminals and expansion of dendritic fields of barrelette neurons found in the present study are cellular defects/anomalies that accompany areal shrinkage of the nucleus itself. Despite severe defects in morphological differentiation of NMDAR-deficient PrV, barrelette cells re-
tained their intrinsic membrane properties and normal synaptic transmission, albeit reduced NMDA currents. By virtue of the expansion of whisker afferent terminal arbors and aberrant organization of barrelette cell dendrites, individual barrelette neurons, which normally respond to inputs from a single whisker, must now respond to overlapping inputs from multiple whiskers and subsequently relay this information to the ventrobasal thalamus and primary somatosensory cortex, where whisker-related patterns also fail to develop. Although these structural changes implicate functional alterations in synaptic transmission between the PrV and thalamus and subsequently in the barrel cortex, this prediction remains to be tested by electrophysiological recordings from upstream targets.

Patterning of neural connections along the rodent whisker-barrel pathway depends on inputs from the sensory periphery during a critical period in development. When the whisker follicles or the infraorbital nerve innervating them are damaged at early postnatal ages (to P3), the barrelette patterns (and upstream neural patterns) are predictably altered or abolished (Woolsey, 1990; O’Leary et al., 1994). Excitatory and inhibitory circuits in basal lamina and barrelette cell dendrites fail to orient their dendrites. Instead, they reorient their terminal arbor growth is exaggerated, their terminal arbor growth is exaggerated, and patterning into discrete clusters is impaired. Postsynaptic dendritic defects could be a passive response to expanded presynaptic terminal arbors. In cortex-specific NR1 KO mice, whisker-specific thalamocortical axon arbors are also expansive but display localized concentrations of terminal boutons (Lee et al., 2005), yet even in these regions of terminal concentrations, layer IV spiny stellate (barrel) cells fail to orient their dendrites. Instead, they develop longer, nonoriented dendritic trees (Datwani et al., 2002). During the process of concurrent addition and pruning of presynaptic terminal and postsynaptic dendritic branches, NMDAR-mediated activity could act as a stop/stabilization signal, thereby contributing to their localization and patterning. In the mouse barrel cortex, expansion of terminal fields of thalamocortical axons has also been noted for other mutants such as the barrelless (brl; adenyl cyclase type 1 KO) and monoamine oxidase A (MAOA) KO mice, which show cortical pattern defects but not in subcortical trigeminal centers (Welker et al., 1996; Rebsam et al., 2002). Although details of dendritic differentiation of barrel cells in these mutants have not been charted, it is likely that other molecules, independently or cooperatively with NMDARs, can also affect restriction and patterning of whisker-related afferent terminals. A recent study showed that mice lacking Drg11, a homeodomain transcription factor, exhibit trigeminal pattern defects strikingly similar to those observed in NR1 KD mice (Ding et al., 2003). These mice fail to develop whisker-related patterns in the PrV, ventroposteromedial thalamic nucleus, and the somatosensory cortex. Single-whisker afferent terminal and barrelette cell dendritic differentiation analyses in these mice are not yet available, but clearly this transcription

| Table 1. Morphometric analyses of barrelette neurons |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Soma size (μm²) | Covered area (μm²) | Primary dendrites | Bifurcation nodes | Terminal endings | Dendritic orders |
| Control         | 81.2 ± 3.2      | 618 ± 39.7      | 2.7 ± 0.1       | 17 ± 0.8       | 20 ± 0.8         | 7.6 ± 0.3       |
| NR1 KD          | 84.9 ± 3.2      | 2093 ± 121.0**  | 3.7 ± 0.2**     | 14 ± 0.8*      | 18 ± 0.9         | 6.1 ± 0.2**     |

Results are mean ± SEM. Significant differences are indicated by asterisks (*p < 0.05; **p < 0.001; Student’s t test).

Figure 5. Dendritic complexity and branching pattern of barrelette neurons. A, NR1 KD barrelette neurons have a larger number of intersections at the most proximal and distal parts; B, Both control and NR1 KD barrelette neurons bifurcate their dendrites proximally. In control neurons, the distribution curve drops sharply from the peak. There are fewer dendritic bifurcations in NR1 KD neurons; thus, the distribution curve of NR1 KD neurons is more flat. C, Most of the dendritic terminals of control and NR1 KD barrelette neurons are located between 10 and 40 μm from the soma center, and the peak is between 20 and 30 μm. However, in NR1 KD barrelette neurons, some of the terminal endings are located beyond 50 μm from the soma. D, Dendrites of NR1 KD barrelette neurons have more branches at lower orders, whereas the branch number is reduced significantly at higher orders in comparison with the controls. E, Comparisons of the segment length of each dendritic order show that in NR1 KD neurons, segments of the first to sixth orders are significantly longer, resulting in a 35% increase in total dendritic length (E’). Results are mean ± SEM. Significant differences are indicated by asterisks (*p < 0.05; **p < 0.01; ***p < 0.001; Student’s t test).
factor is essential for proper development of and barrelette patter-ning in the PrV and its upstream targets. Presently, there is no known relationship between Drg11 and NMDA receptors.

Comparison of presynaptic arbor differentiation between control, NR1KO, and NR1KD animals suggests the presence of a threshold level of NMDA receptor function below which morphological differentiation is affected, whereas synaptic transmission is not. Presently, this threshold and the signaling pathways downstream from NMDA receptors used in clustering of whisker afferent terminals and dendrites of their postsynaptic partners are not known. Morphological defects at both the presynaptic and postsynaptic sites indicate active communication via antero-\(\text{grade}\) and \(\text{retrograde}\) signaling mechanisms. Nitric oxide (NO), brain-derived neurotrophic factor (BDNF), and arachidonic acid (AA) have all been implicated as potential retrograde signals that might affect structural differentiation of presynaptic terminals in other systems (for review, see Schmidt, 2004). To our knowledge, none of these molecules are known to be specifically expressed by PrV barrelette neurons. NO synthase distribution in the rat brainstem trigeminal complex does not correspond to the thala-mic projection (barrelette) neurons (Dohrn et al., 1994), and nicotinamide adenine dinucleotide phosphate-diaphorase-positive elements seen in the PrV are of dorsal raphe origin (Simpson et al., 2003). BDNF expression in the developing mouse PrV has not been detailed, although a subset of trigeminal ganglion cells are endowed with tyrosine kinase receptor B and depend on survival for BDNF (Ernfors et al., 1994). Nothing is known about the potential role of AA as a retrograde messenger in this system.

We do not know whether defects in NR1KD mice arise from reduced levels of NMDAR function presynaptically, postsynaptically, or both. One line of preliminary evidence suggests that levels of postsynaptic NMDAR function rather than presynap-tic plays the central role. In NR1KD mice, there are differential levels of expression of NR1 between the PrV and spinal trigeminal nucleus interpolaris, the latter having higher expression (Iwasato et al., 1997). Whisker afferents bifurcate as they enter the central trigeminal tract, and one branch heads for the PrV and the other for the spinal trigeminal nucleus where they both form whisker-specific patterns (Jacquin et al., 1993). In NR1KD mice, these terminals in the spinal trigeminal nucleus interpolaris form rudimentary, patterned arbors, whereas their sister branches fail to form any patterns in the PrV (Lee J-I. Lee and R. S. Erzurumlu, unpublished observations). Thus, postsynaptic NMDAR signaling is more likely to play a significant role in neuronal patterning. Observations from cortex-specific NR1 KO mice also confirm this idea. In these mutants, thalamocortical afferents with intact NMDARs develop exuberant terminal arbors in the barrel cortex, and layer IV stellate cells fail to orient their dendrites and grow longer dendritic segments (Lee et al., 2005).

During the past several years, a number of molecules downstream from NMDAR-initiated Ca\(^{2+}\) entry into neurons and those that act cooperatively with NMDARs at the postsynaptic density have been noted in modulating dendritic cytoskeletal dyna-mics, spine morphology, and presynaptic terminal sculpting (Carroll and Zuki, 2002; Scheiffele, 2003; Wenthold et al., 2003). Calcium/calmodulin-dependent protein kinase II (Wu and Cline, 1998; Zou and Cline, 1999), neurophins and neurexins (Nguyen and Sudhof, 1997; Scheiffele et al., 1999), and Eph pro-teins (Daiva et al., 2000) are among these. It remains to be det-ermined how these molecules, or others yet to be identified, partic-ipate in choreography of neuronal patterning and underlying communication between the whisker-specific afferent terminals and their partners in the PrV.

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