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Neonatal Sensory Nerve Injury-Induced Synaptic Plasticity In The Trigeminal Principal Sensory Nucleus

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Abstract

Sensory deprivation studies in neonatal mammals, such as monocular eye closure, whisker trimming, chemical blockade of the olfactory epithelium have revealed the importance of sensory inputs in brain wiring during distinct critical periods. But very few studies have paid attention to the effects of neonatal peripheral sensory nerve damage on synaptic wiring of the central nervous system (CNS) circuits. Peripheral somatosensory nerves differ from other special sensory afferents in that they are more prone to crush or severance because of their locations in the body. Unlike the visual and auditory afferents, these nerves show regenerative capabilities after damage. Uniquely, damage to a somatosensory peripheral nerve does not only block activity incoming from the sensory receptors but also mediates injury-induced neuro- and glial chemical signals to the brain through the uninjured central axons of the primary sensory neurons. These chemical signals can have both far more and longer lasting effects than sensory blockade alone. Here we review studies which focus on the consequences of neonatal peripheral sensory nerve damage in the principal sensory nucleus of the brainstem trigeminal complex.

Keywords

Infraorbital nerve; silent synapses; astrocytes; reactive synaptogenesis; whisker-barrel system

Neonatal Sensory Nerve Damage

Obstetric injuries to the brachial plexus during birth and orofacial injuries and fractures in young children are common occurrences which can lead to permanent brachial plexus palsy or trigeminal nerve pathologies (see reviews by Alcalá-Galiano et al., 2008; Eggensperger Wymann et al., 2008; Sandmire et al., 2008; Vyas et al., 2008). While these neurological cases are extensively studied at the peripheral nerve level, their CNS consequences remain largely unknown. In pain research, drastic changes in the physiology and adult responsiveness of spinal dorsal horn pain circuits following neonatal peripheral nerve

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damage or foot incisions have been noted (reviewed in Fitzgerald and Walker, 2009; Baccei, 2011). Increasing numbers of studies are now indicating how peripheral nerve damage or injury in neonates can alter peripheral and spinal nociceptive circuits and neuropathic pain states; these studies are recently reviewed in detail (Fitzgerald and McKelvey, 2015, this issue; Baccei et al., 2015, this issue).

Defining the molecular and cellular consequences of peripheral nerve damage in the developing CNS is critical for a better understanding of the neural response to injury while it is under construction. The rodent trigeminal system (whisker-to-barrel cortex pathway) is an excellent model for studying cellular and molecular mechanisms which underlie both the patterning of neural connections and their plasticity that follow damage to a purely sensory peripheral nerve.

The first relay station of the system *en route* to the thalamus and the neocortex is the trigeminal principal sensory nucleus (PrV) of the brainstem trigeminal complex (BSTC). The BSTC consists of the PrV, the spinal trigeminal subnuclei (oralis, interpolaris, and caudalis), the motor trigeminal nucleus, and the mesencephalic trigeminal nucleus. The ventral portion of the PrV receives central trigeminal axons of the infraorbital nerve (ION), which innervates the 5 rows of whiskers and perioral sinus hairs in common laboratory rodents.

Sensory nerve (ION) damage during perinatal periods dramatically alters patterning in the PrV and leads to long-lasting synaptic changes. This is an invaluable, model system to investigate CNS consequences of peripheral nerve injuries in neonates, at a time when the brain wiring is in progress. To better understand the effects of neonatal sensory nerve damage on the PrV, analyzing the developmental organization and function in this somatosensory nucleus representing the orofacial peripheral fields is of critical importance.

Development and Organization of the Rodent Trigeminal System and the PrV

The infraorbital (IO) branch of the maxillary division of the trigeminal nerve is an exclusively sensory nerve. The IO innervates all of the whiskers on the snout. Central axons of the ION convey whisker-specific information to the trigeminal brainstem. Through interaction of target-derived molecular cues and receptors, the trigeminal ganglion (TG) neurons that contribute to the ION establish a topographic and patterned map of the whisker follicles in the trigeminal brainstem (reviewed in Erzurumlu et al., 2006, 2010). Central branches of the ION afferents develop synaptic terminal clusters, which replicate the patterned organization of the whiskers (Erzurumlu and Jhaveri, 1992; Waite et al., 2000). While whisker-specific patterning in the brainstem emerges by E19-20 in the rat (Chiaia et al., 1992; Waite et al., 2000), it emerges at the time of birth in mice (Ma, 1993). Terminal arbors, at this stage have synaptically active boutons, and the postsynaptic responses in the PrV show a predominant N-Methyl-D-Aspartate (NMDA) component (Waite et al., 2000).

Postsynaptic target neurons in several nuclei of the BSTC organize with respect to the discrete, whisker-specific patterning of afferent terminals, that, in turn, form the barrelettes

(Bates and Killackey, 1985; Belford and Killackey, 1979; Erzurumlu et al., 1980; Ma, 1993; Ma and Woolsey, 1984). The mouse and rat PrV appears as a peanut shaped structure, located laterally in coronal sections through the brainstem. The ventral part of the nucleus, largely derived from rhombomere 3 (Oury et al., 2006), contains the barrelettes with the facial axis represented in an inverted fashion (Figure 1).

The PrV cells receive other inputs in addition to the ION central axons, such as excitatory and inhibitory inputs from caudal brainstem trigeminal nuclei (Furuta et al., 2008; Martin et al., 2014), serotonergic raphe (Lee et al., 2008; Kirifides et al., 2001) and noradrenalinergic locus coeruleus afferents (Simpson et al., 1997, 1999) and corticotrigeminal inputs from the somatosensory cortex (Malmierca et al., 2014; Sanchez-Jimenez et al., 2009, 2013).

The barrelette neurons of the PrV play a key role in transmitting the whisker-related patterning to the contralateral thalamus and the ventroposteromedial nucleus (VPM). Decades ago, Killackey and Fleming (1985) reported that lesion of the neonatal PrV, but not the spinal trigeminal nucleus, abolishes pattern formation in the VPM and barrel cortex. Genetic manipulation studies have also confirmed this finding in the mouse: thalamic and cortical patterns fail to develop when barrelette patterns are absent in the PrV but not in the spinal trigeminal nucleus (Iwasato et al., 1997).

In the VPM, thalamocortical projection neurons recognize the patterning of incoming PrV trigeminothalamic afferents and form barreloids and, in turn, form the thalamocortical axons (TCAs) arising from the barreloids neurons that transmit the patterns to the layer 4 of the primary somatosensory (SI) cortex (Erzurumlu and Jhaveri, 1990). Recognizing the discrete, patterned distribution of TCA terminals, layer 4 spiny stellate cells orient their dendrites towards them, forming cylindrical aggregates--the barrels. This process became eminently clear in a recent in vivo time-lapse imaging study (Mizuno et al., 2014). Much of this pattern formation involves glutamatergic transmission, NMDA and metabotropic glutamate receptors, and downstream or upstream molecular signals, such as adenylyl cyclase type I or phospholipase C (reviewed in Erzurumlu and Gaspar, 2012; Erzurumlu and Kind, 2001; Wu et al., 2011).

Several genetic manipulations in mice targeting functional NMDARs have shown that following their loss of function, these patterns do not develop and are subsequently absent in downstream paths, the thalamus and the cortex (Iwasato et al., 1997; Kutsuwada et al., 1996; Li et al., 1994; Rudhard et al., 2003). In the absence of functional NMDARs, TG afferents target properly and form a gross topographic alignment in the brainstem (Li et al., 1994). This aspect of facial map formation involves axon guidance and intrinsic molecular cues (reviewed in Erzurumlu et al., 2006, 2010). However, when NMDAR function is genetically perturbed, barrelette patterns do not develop and dendritic orientation of barrelettes cells fail to form (Lee et al., 2005).

Physiological Properties of the PrV Neurons

The ventral PrV contains three classes of neurons: (1) Barrelette cells, (2) Interbarrelette cells, and (3) inhibitory interneurons. Polarized, asymmetrical dendritic orientation and whisker-specific patterning are morphological characteristics of barrelette neurons. These

cells can be distinguished further by their electrophysiological properties (Figure 2). They display a transient K^+ (I_A) current and receive monosynaptic excitatory and disynaptic inhibitory inputs when the trigeminal tract is stimulated (TrV) (Lo et al., 1999).

Interbarrelette neurons have dendritic trees which span multiple barrelettes. They are distinguished by the low-threshold T-type Ca^{2+} current (I_T) and receive excitatory inputs from multiple sources (Lo et al., 1999). The small GABAergic interneurons provide disynaptic inhibition to the barrelette neurons (Lo et al., 1999) (Figure 2).

Barrelette and interbarrelette cells can be identified by their morphological and electrophysiological characteristics as early as postnatal day 1 (P1). There are no changes in the resting potential, composition of active conductances, and Na^+ spikes of both barrelette and interbarrelette cells during postnatal development (P1–P13) (Lo and Erzurumlu, 2001).

In brainstem slice preparations, stimulation of different sites of the central trigeminal tract (TrV) induces two types of responses in the same barrelette cell (Lo et al., 1999) (Figure 2). The first type is an EPSP-IPSP sequence. Pharmacological analyses show that the EPSP is mediated by both AMPA and NMDA receptors. The IPSP is mediated by GABA_A receptors. The latency of the EPSP is nearly constant in response to different stimulus intensities, suggesting that barrelette cells receive monosynaptic excitation from the TrV. The IPSP is mediated in all likelihood by a disynaptic, feed-forward inhibitory circuit. The second type of stimulation is an IPSP alone. The latency of the IPSP is also in the disynaptic range; therefore it must be mediated from a lateral inhibitory circuit. Interestingly, the same group of GABAergic interneurons mediates both feed-forward and lateral inhibition. It is reasonable to infer that barrelette cells receive excitation and feed-forward inhibition from the principal whisker and lateral inhibition from adjacent whiskers (Lo et al., 1999).

In the context of injury to peripheral ION axons and their regenerative propensity, we must take into account the conduction and transmitter release properties of the central TG axons. The transmitter release probability of TG central terminals is determined by a paired-pulse protocol. The paired-pulse ratio (PPR) is quite variable, ranging from paired-pulse depression (PPD, $PPR < 1$) to paired-pulse facilitation (PPF, $PPR > 1$). The distribution of PPRs fits perfectly to Gaussian function with a peak at 100% (Lo and Erzurumlu, 2011). Jones et al. (2004) reported that whisker-related responses of TG cells form a stimulus-dependent continuum. The continuous distribution of PPRs may play an important role in the transmission of continuous TG response profiles to the postsynaptic PrV neurons. The Gaussian distribution of PPRs is a unique property in the first relay station in the trigeminal central pathway, because layer 4 barrel cortex excitatory neurons and thalamic VPM neurons exclusively receive high probability terminals that result in PPD (Castro-Alamancos, 2002; Deschênes et al., 2003; Fontanez and Porter, 2006; Iwasato et al., 2008; Laurent et al., 2002; Lee and Sherman, 2008; Lu et al., 2006; Wang and Zhang, 2008; Yanagisawa et al., 2004; Zhu, 2009).

Synaptic connections between the TG and the PrV undergo postnatal refinement, which can be assessed physiologically. Multiple input index analysis (MII), an electrophysiological approach, is commonly used to assess the convergence of afferent fibers onto single target

neurons. This analysis protocol; has been used in numerous systems (Arsenault and Zhang, 2006; Crepel and Mariani, 1976; Lo et al., 2002; Lu and Constantine-Paton, 2004; Mariani and Changeux, 1981). The criterion for MII is the "stepwise graded character" or "abrupt jump in the amplitude" of postsynaptic responses upon progressively increasing stimulus intensity. MII analyses in the PrV have shown that about 30% of afferent connections are eliminated within 2 postnatal weeks (Lo et al., 2011).

Effects of Neonatal ION Transection on Physiological Properties of the PrV

The unique whisker-specific neural patterning, which starts in the PrV and ends in the SI cortex, takes place during a short period in the late embryonic and the early postnatal periods. Damage to the ION or whisker follicle cautery during postnatal (P) life up to day 3 irreversibly alters the patterning of the system (reviewed in Erzurumlu and Gaspar, 2012; Erzurumlu and Kind, 2001) (Figure 3). Since the pioneering study by Van der Loos and Woolsey (1973), several groups have repeatedly demonstrated that when a row of whisker follicles are cauterized at birth, barrels which represent that row shrink and fuse, while the barrels corresponding to the neighboring whisker follicle rows expand, a phenomenon known as structural plasticity of the whisker-barrel system. More dramatically, if the ION is transected or damaged at birth barrels do not develop at all and the TCA terminals form aberrant, nonspecific islands of terminal arbor fields. (Jacquin and Rhoades, 1983). In short, the critical period for structural plasticity ends after postnatal day 3 in commonly studied laboratory rodents (mouse, rat, hamster).

Neonatal ION injury also results in dendritic tree orientation defects in barrelette cells (Arends and Jacquin, 1993; Lo and Erzurumlu, 2001) and cell death in the TG (Aldskogius and Arvidsson, 1978; Aldskogius et al., 1985; Sugimoto et al., 1998, 2004) and the PrV (Miller, 1999; Miller and Kuhn, 1997). Physiological studies show reactive synaptogenesis (Lo et al., 2011) and AMPA receptor endocytosis (Lo and Erzurumlu, 2007) after transection of afferent nerve, i.e., ION, from the whisker pad. The cellular mechanisms that underlay these changes have been partially disclosed in the last decade.

TG cells have two types of activities: low frequency spontaneous and high frequency sensory activity (Minnery and Simons, 2003; Shoykhet et al., 2003). ION damage eliminates high frequency sensory afferents to the PrV and leaves spontaneous activity intact. Hence all changes after ION transection are whiskersensory activity-dependent. The effect of TG spontaneous activity on PrV properties remains unknown.

While neonatal ION transection leads to the death of 9–10% of the TG cells (Sugimoto et al., 1998, 2004), many survive and their central axons connecting to the brainstem are functional. Paired-pulse protocol shows that PPRs have a wide range and fit nicely to Gaussian function with a peak at 100% (Lo and Erzurumlu, 2011) that is the same as normal animals. Thus, the transmitter release function of the central terminals of the surviving TG cells is unaffected by ION transection.

Neonatal ION transection results in trans-synaptic degeneration in the PrV, such that the number of cells in the PrV reduces by one-third and barrelettes fail to form (Lo and Erzurumlu, 2002; Miller, 1999; Miller and Kuhn, 1997). Barrelette cells normally display

skewed dendritic orientation directed towards whisker-specific trigeminal afferent terminal patches; after ION damage this bias is lost or does not develop (Lo and Erzurumlu, 2002). But basic membrane properties of the “deafferented” barrelette and interbarrelette cells are similar to those seen in the PrV with intact ION (Lo and Erzurumlu, 2001). Clearly, development of the intrinsic membrane properties in the PrV does not depend on normal synaptic inputs during the critical period of pattern formation.

Peripheral Nerve Injury-Induced Reactive Synaptogenesis in the CNS

In the mature CNS, denervation results in rapid synaptic loss followed by a prolonged period of new synapse formation. This form of neural plasticity has been referred to as “reactive synaptogenesis,” implying that it is a reaction to denervation (Collazos-Castro and Nieto-Sampedro, 2001; Cotman et al., 1981; Hamori, 1990; Matthews et al., 1976). In the mature state, the time course of reactive synaptogenesis varies among different central pathways. For example, in the entorhinal cortex-dentate gyrus pathway, reactive synaptogenesis starts on post-lesion day 6 and persists for more than a month (Collazos-Castro and Nieto-Sampedro, 2001; Cotman et al., 1981; Marrone et al., 2004). In the dorsal column lemniscal pathway, reactive synaptogenesis begins in the thalamus about a month after lesion and continues for several weeks (Wells and Tripp, 1987a, 1987b). Not much is known about reactive synaptogenesis during the development of the somatosensory pathways, particularly following peripheral nerve injuries.

In normal PrV barrelette cells, at resting potential (~–60 mV), stimulation of the TrV with maximal intensity induces an EPSP-IPSP sequence. Following ION transection, however, deafferented barrelette cells respond to maximal stimulation of the TrV with a long lasting depolarization known as plateau potential. L-type high threshold Ca^{2+} channels mediate this plateau potential, because it is blocked by L-type Ca^{2+} channel blocker nitrendipine. Pharmacological manipulations show that the plateau potential is triggered by NMDAR-mediated EPSP, which depolarizes membrane potential above –40 mV (Lo and Erzurumlu, 2002). This finding suggests that the postsynaptic excitatory response in barrelette cells increases after ION transection. Further study with MII analysis has demonstrated that the increased postsynaptic excitation results from an increase in excitatory connections from TG cells to single barrelette cells (Figure 3). The estimated number of convergent TG cells (MII) gradually increases from 2 to 5 days after ION transection and remains unchanged in the second postnatal week. This analysis indicates rapid convergent synaptogenesis between surviving TG cells and barrelettes cells after ION transection, much akin to reactive synaptogenesis in the deafferented brain regions (Collazos-Castro and Nieto-Sampedro, 2001; Cotman and Anderson, 1988; Deller and Frotscher, 1997; Dieringer, 1995; Hamori, 1990; Marrone and Petit, 2002; Matthews et al., 1976).

The reactive synaptogenesis in the deafferented PrV is not only restricted by the critical period of structural plasticity, because ION transection after the critical period still leads to increased MII with a similar time course (Lo et al., 2011).

“Silent” Synapses in the Deafferented PrV

Failure to form or dissolution of whisker-specific barrelette patterns in the denervated PrV is accompanied by an increase in afferent convergence upon individual barrelettes cells. Paradoxically, this TG input convergence does not entail functional synapses. In fact, most functional synapses convert to silent synapses (Lo and Erzurumlu, 2007). Silent synapses have been identified as synapses, which show NMDA, but an absence of AMPA receptor response in hippocampal and cortical slices (Isaac et al., 1995, 1997; Liao et al., 1995). Activity-dependent synaptic modification and the conversion of silent synapses into functional synapses involve “exocytosis” or movement of AMPARs within the membrane from extrasynaptic sites to the postsynaptic density. AMPAR “endocytosis.” Modification diminishes the AMPAR pools from the postsynaptic surface and contributes to LTD, while AMPAR exocytosis or trafficking back to the postsynaptic site contributes to LTP (Ashby et al., 2004; Brecht and Nicoll, 2003; Malenka and Malenka, 2002; Sheng and Lee, 2003). High frequency tetanus switches silent synapses into functional synapses.

About 80% of the synapses in the PrV are functional within the first postnatal week. Minimal stimulation of the TrV elicits EPSCs at holding potentials of both -70 mV and $+60$ mV. This finding indicates that both AMPAR and NMDAR are functional. About 20% are silent synapses, in which almost no AMPAR-mediated EPSCs at -70 mV, while NMDAR-mediated EPSCs are normal at $+60$ mV. Three days after ION transection, most functional synapses become silent synapses (Figure 3). Without sensory inputs from the whiskers, silent synapses persist through the second postnatal week, indicating that the maintenance of AMPAR function depends on whisker sensory inputs. High-frequency (50 Hz) electrical stimulation of the afferent pathway during membrane depolarization to relief Mg^{2+} blockade of NMDARs, mimics sensory input and restores synaptic function. But low-frequency (1 Hz) stimulation has no such effect. Therefore, AMPAR trafficking (exocytosis) in the developing PrV depends on NMDAR-mediated high frequency incoming activities (Lo and Erzurumlu, 2007). Silent synapses result from a lack of surface expression of AMPARs (endocytosis). An application of glycine promotes rapid delivery of AMPARs to the postsynaptic density (Lu et al., 2001) and converts silent synapses into functional synapses (Lo and Erzurumlu, 2007). While maturation of synapses requires high frequency inputs, synaptogenesis and NMDAR trafficking may depend upon low frequency spontaneous activity (Shoykhet et al., 2003) in TG neurons.

Aftermath of crush injuries and nerve regeneration

A question of clinical significance begs whether or not these structural and functional changes are reversible after regeneration of ION fibers. Unfortunately, the regeneration of sectioned ION is a slow process and never complete (Jeno and László, 2002; Kis et al., 1999; Renehan and Munger, 1986; Waite, 1984; Waite and Cragg, 1982). Nevertheless, crush injury leads to a partial blockade of nerve function and rapid functional recovery by detecting postsynaptic responses induced by peripheral stimuli (Kis et al., 1999; Renehan and Munger, 1986; Waite and Cragg, 1982). Therefore, neonatal ION crush serves as an ideal model to study the reversibility of synaptic remodeling.

Previously, functional recovery of crushed ION was estimated by responses in the brain to whisker deflection. It is impossible to determine the time course of recovery of ION function quantitatively. A novel *in vitro* cranial cup preparation meets the needs for accurate quantifying conductive function of crushed ION (Lo et al., 2014). In this preparation, TG cells are not mechanically damaged and can be kept alive *in vitro* for more than 8 h. Intracellular recordings from TG cells indicate that the rat TG cells are divided into F-type and S-type classes, according to the shape and duration of the action potential (AP) and after hyperpolarization (AHP) (Cabanès et al., 2002). The APs of TG cells are mediated by both Na⁺ and Ca²⁺ channels. TG cells that innervate the whisker pad are identified by testing the response to stimulation of ION. Stimulation of ION induces an AP with fixed latency in whisker-innervating TG cells. Both F- and S-type TG cells innervate the whisker pad with similar latencies. Therefore, the conduction velocity of peripheral branch of both F-type and S-type rat TG cells is about the same (Lo et al., 2014).

The ratio of recorded S-type/F-type TG cells from normal TG (control) is similar to that from ION crushed TG, indicating that mechanical crush affects both types TG cells equally. Intracellular recordings reveal that 68% of recorded TG cells innervate the whisker pad in control TG. The proportion of recorded whisker pad-innervating cells of both cell types serves as an index of ION conductive function. Immediately after the crush, about 2/3 ION fibers are blocked in contrast with 100% blockade after ION transection. There is no significant ION functional recovery at post-injury day 3. Partial recovery is found at post-injury 5 days, and complete recovery at post-injury day 7. Significantly, crushed ION fibers recover conductive function after post-injury day 3, the critical period of PrV pattern plasticity (Lo et al., 2014).

Similar to ION transection, nerve crush injury leads to an increase in the incidence of silent synapses. In the normal PrV of first postnatal week, only 20% of the cells reveal silent synapses lacking AMPAR-mediated EPSCs at -70 mV (Lo and Erzurumlu, 2007). The incidence of silent synapses increased to 69% at post-injury days 2–3 when ION function is not recovered. The incidence after ION crush is ~3.5 times that of the control, while the incidence after ION transection is 92.9%, that is, ~4.6 time of the control (Lo and Erzurumlu, 2007). This is in line with the result of partial blockade of ION function after crush. At post-injury day 5–7, when ION function has basically resumed, the incidence of silent synapses drops to 27%, significantly different from post-injury day 2–3, but about the same as the control. Therefore, the increase in the incidence of silent synapses (AMPA endocytosis) after ION crush is reversible (Lo et al., 2014).

Mechanisms of Peripheral Nerve Injury-Induced Synaptic Plasticity in the CNS

What are the mechanisms underlying synaptic plasticity in the CNS following peripheral nerve injury? A fortuitous observation in the Prv following neonatal ION injury is a dramatic increase in astrocytosis, evidenced by glial fibrillary acidic protein (GFAP) immunostaining (Lo et al., 2011, 2014) (Figure 4). Astrocytes play an active role in the development and maintenance of synapses and in reactive synaptogenesis following brain injury (Ullian et al., 2004). *In vitro* studies show that retinal ganglion cells, spinal motor

neurons, and inhibitory interneurons show more synaptogenesis when cocultured with astrocytes or in astrocyte-conditioned media (Elmariah et al., 2005; Pfrieger and Barres, 1997; Reddy et al., 2003; Ullian et al., 2001, 2004). Processes of astrocytes often engulf synapses and are well positioned to sense changes in the synaptic environment; they play an active role in synapse formation and in the regulation of pre- and postsynaptic function by communicating with their neuronal partners through soluble factors that they secrete (reviewed in Allen and Barres, 2005).

Virtually nothing is known about the glial response in the PrV during the period of reactive synaptic plasticity, but strong evidence suggests that they participate in the development of silent synapses and their conversion to functional ones in other systems. Astrocytes secrete thrombospondins (TSPs) that promote synaptogenesis in the CNS. TSP family members TSP1 and TSP2 are trimers and interact with cell-surface receptors, cytokines, growth factors, and proteases (reviewed in Adams, 2001). In the developing brain, TSP-1 and -2 are expressed to a high degree during the peak period of synaptogenesis, but are downregulated in the adult (Christopherson et al., 2005). Genetic deletion of these proteins in mice results in 30% fewer synapses in their brains (reviewed in Allen and Barres, 2005). TSP-induced synapses are apparently “silent synapses” that contain NMDA receptors but lack AMPA receptors (Allen and Barres, 2005) much like the case in the neonatally denervated PrV (Lo and Erzurumlu, 2007).

Pharmacological and morphological approaches suggest that synaptogenesis is mediated by astrocytes via a series of biochemical cascades. Transsynaptic cell death in the PrV following ION transection leads to an increase in extracellular ATP concentration. ATP activates purinergic receptors on astrocytes and switches quiescent astrocytes into reactive ones with upregulated GFAP (astrocyte marker) expression (Figure 4). Reactive astrocytes release some molecules such as thrombospondins (TSPs) that promote synaptogenesis (see Allen, 2013; Risher and Eroglu, 2012; Ullian et al., 2004 for reviews). Several pieces of evidence indicate: 1) Reactive synaptogenesis requires activation of purinergic receptors. Reactive Blue 2 (RB-2), an antagonist of P2Y₄ receptor, just partially blocks reactive synaptogenesis and upregulation of GFAP expression in the deafferented PrV. Suramin, an antagonist for P2X and P2Y receptors, completely blocks synaptogenesis and GFAP expression. 2) The synaptogenesis requires functional astrocytes because sodium fluoroacetate, an inhibitor of astrocyte function, completely blocks synaptogenesis but stimulates GFAP expression of the whole brain. And 3) TSPs are mediators for synaptogenesis because Gabapentin, a blocker of TSPs, completely blocks synaptogenesis but it has no effect on GFAP expression, because it acts downstream the reaction of astrocytes (Lo et al., 2011). Similar to ION transection, ION crush also results in reaction of astrocytes. At post-injury day 3, there is a dramatic increase in astrocyte numbers, length of arborized astrocyte processes and astrocyte process complexity. However, this difference is no longer evident at P10 when functional recovery has taken place. In addition, with ionized calcium binding adaptor molecule 1 (Iba-1) immunostaining of microglia, there is a pronounced increase in the numbers of microglia with larger cell bodies in the ION crushed PrV at P3 but no longer evident by P10. ION crush-induced reaction of astrocytes and microglia is reversible after ION functional recovery (Lo et al., 2014). The effect of reactive astrocytes and microglia on short-term synaptic plasticity remains unknown. That point notwithstanding, it is clear that ION

crush induced temporary changes in silent synapses and glia reaction are not subjected to critical period of PrV pattern plasticity.

In sharp contrast with the aforementioned results, the disruption of barrelettes induced by ION crush is not reversible. Barrelettes labeled by immunocytochemistry for VGlut1 (label for sensory afferent terminals) and cytochrome oxidase histochemistry (a generic barrelette marker) are disrupted at P3 and not recovered at P10. This point suggests that the ION function recovers after P3 the critical period of barrelette formation (Lo et al., 2014).

Transsynaptic responses to neonatal sensory nerve damage

When sensory nerve damage alters synaptic circuitry at the first relay station in the CNS it is not difficult to envisage that other downstream target regions might also be affected. Surprisingly, very little is known about transregional effects of neonatal ION lesions, other than transsynaptic cell death and loss of whisker-specific neuronal patterns. Ongoing studies in our laboratory show that neonatal ION damage leads to silent synapses in the VPM and convergence of multiple PrV afferent terminals onto individual VPM barreloid neurons. Astrocytic responses are also found in the ION receipt zone of the VPM and in extensive areas of the neocortex (Figure 4). We need further studies to elucidate how far and to which brain regions neonatal ION damage effects reach and whether they are all reversible following functional recovery of the nerve and, if so to what extent are they reversible.

Conclusions

The neonatal brain is highly malleable and responds to peripheral sensory nerve injury by altered connectivity and synaptic arrangements. Some of these altered circuitry functions can be reversed while others are changed irreversibly. The laboratory rodent whisker-barrel system is an excellent model to investigate peripheral nerve injury-induced synaptic plasticity in the neonatal brain.

Both transection and crush injuries of the infraorbital nerve, a purely sensory nerve, lead to dissolution of whisker-specific neural patterns or a failure to form in the trigeminal brainstem. There is a critical window for this morphological effect. Physiologically, while the intrinsic membrane and synaptic properties of the trigeminal principal sensory nucleus neurons do not change, their functional synapses are converted to silent synapses and individual neurons receive multiple trigeminal afferents. These reactive synaptic circuitry changes are not confined by the critical period and travel in waves across synapses to downstream trigeminal stations in the thalamus and cortex.

Specific cellular and molecular mechanisms underlying reactive synaptogenesis and conversion of functional synapses to silent synapses in response to neonatal nerve injury are largely unknown. Astroglial responses parallel these synaptic changes, which indicate their potential involvement in signaling mechanisms.

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Abbreviations

AMPA	α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AHP	After hyperpolarization
AP	Action potential
BSTC	Brainstem trigeminal complex
CO	Cytochrome oxidase
EPSP	Excitatory postsynaptic potential
GABA	Gamma aminobutyric acid
Iba-1	Ionized calcium binding adaptor molecule
ION	Infraorbital branch of the maxillary division of the trigeminal nerve
IPSP	Inhibitory postsynaptic potential
LTD	Long-term depression
LTP	Long-term potentiation
NMDA	N-Methyl-D-Aspartate
P	Postnatal day
PPD	Paired-pulse depression
MII	Multiple input index analysis
PPF	Paired-pulse facilitation
PPR	Paired-pulse ratio
PrV	Principal sensory nucleus of the trigeminal nerve
SI	Primary somatosensory cortex
TCA	thalamocortical axon
TG	Trigeminal ganglion
TrV	Central trigeminal tract
VGlut1	Vesicular glutamate transporter 1
VPM	ventroposteromedial nucleus of the thalamus

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Highlights

- Damage to a purely sensory nerve (ION) in neonatal mammals leads to disruption of patterning of sensory maps in the CNS and in substantial synaptic reorganization.
- In the whisker-barrel system of rats and mice, neonatal infraorbital nerve lesions lead to conversion of functional synapses to silent synapses by losing functional AMPA receptors and convergence of multiple trigeminal afferents on single barrelettes neurons.
- Injury-induced synaptic plasticity does not follow the timing of the critical period for structural plasticity.
- Effects of neonatal sensory nerve transection and crush are quite different in the CNS.
- Artificial stimulation of the central trigeminal tract in ways that mimic natural stimulation of the ION can revert silent synapses into functional synapses.
- Neonatal nerve injury-induced reactive synaptogenesis and emergence of silent synapses extends across synaptic relays.
- Neuron-astrocyte signaling probably play a major role in signaling of peripheral nerve injury across multiple levels of the neonatal brain.

Figure 1. Barrelettes and the physiological properties of PrV neurons

A. PrV appears as a peanut-shaped structure in coronal sections through the brainstem. The five rows of whiskers on the face are represented in an inverted fashion in the PrV with the dorsal most whiskers represented ventrally and the tip of the nose medially. The nucleus is bordered dorsomedially by the motor trigeminal nucleus and laterally by the central trigeminal tract (TrV); d: dorsal, l: lateral. Cytochrome oxidase histochemistry reveals the barrelettes patterns in the ventral half of the nucleus. **B.** Schematic representation of the barrelettes as cellular modules (top) and cytochrome oxidase dense patches (bottom). **C.** Three types of PrV cells in the barrelettes region. The drawings illustrate barrelettes (dark blue) interbarrelette (light blue) and GABAergic interneurons (red). Whole-cell recording in barrelette neuron shows A-type potassium conductance upon membrane depolarization (dark blue record). The same membrane depolarization induces a low threshold spike mediated by T-type calcium channels (light blue record) in interbarrelette neuron. GABAergic neuron gives fast spiking firing to the membrane depolarization (red record).

Figure 2. Neuronal circuitry in the PrV

Activation of trigeminal ION central axon *S1* induces a monosynaptic EPSP and a disynaptic feed-forward IPSP in the recorded barrelette neuron (dark blue) via GABAergic interneuron (red). Activation of *S2* gives rise to a disynaptic lateral IPSP alone in the same barrelette neuron. Stimulation of both *S1* and *S2* with increasing intensity results in stepwise amplitude increase in both EPSP and IPSP. However, the step number of IPSC is larger than that of EPSP, suggesting the GABAergic interneuron receives trigeminal inputs from more fibers than the recorded barrelette neuron, i.e., both feed-forward and lateral inhibition are

mediated by the same group GABAergic interneurons. Similarly, the step number of EPSP in interbarrelette neuron (light blue) is larger than that of barrelette neuron, suggesting that interbarrelette neuron receives more trigeminal inputs than barrelette neuron.

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Figure 3. Effects of neonatal ION damage in the PrV

A. Illustration of the organization of whiskers and corresponding barrelettes in the PrV. **B.** A similar illustration depicting the effects of IO nerve cut or nerve crush on barrelettes, there are no longer whisker-related patterns. **C, D,** Cytochrome oxidase histochemistry reveals barrelettes in postnatal PrV (**C**) and their absence following ION damage (**D**). Scale bar = 300 μ m. **E, F,** similar micrographs showing vesicular glutamate 1 (VGLut1) immunolabeling showing how trigeminal afferents in the ION projection zone of the PrV no longer show immunoreactivity after neonatal nerve damage. VGLut1 is used as a marker for trigeminal afferent axons and their terminals. The immunoreactive patches seen at the very ventral part of the PrV correspond to supraorbital whiskers innervated by the ophthalmic component of the trigeminal nerve. The two major effects of neonatal ION damage are increased convergence of trigeminal afferents onto single barrelettes cells as demonstrated by MII (**G, H**), and conversion of functional synapses to silent synapses (**I, J**). **G.** On the intact side, MII of trigeminal inputs to barrelette neurons is low, as an example here MII=4. **H.** After neonatal ION transection, MII increases rapidly. The example here shows MII=9. Note that the current calibrations are different between **G** and **H**. **I.** On the intact side, TG-PrV synapses are functional in 80% of barrelette neurons, just like the example showing minimal stimulation induces EPSCs at both +60 mV and -70 mV. **J.** Three days after ION transection, functional synapses convert into silent synapses in >80% barrelette neurons. Minimal stimulation evokes EPSCs only at +60 mV, almost no EPSC at -70 mV, i.e., lacking functional AMPA receptors.

Figure 4. Immediate astrocytic response to neonatal ION damage all along the trigeminal pathway

A, B, Comparison of GFAP labeling in the denervated and unaffected PrVs from the same brain following unilateral neonatal ION damage. Asterisks indicate the borders of the ION-recipient zone of the PrV. **C, D,** Higher magnification views from the control and denervated PrV. Scale bar = 250 μ m for **A, B,** and 20 μ m for **C, D.** **E.** Section through the thalamus showing conspicuous increase in GFAP immunostaining in the ION input recipient zone (red circle) of the VPM contralateral to the ION damaged side. **F.** A distinct GFAP

immunopositivity is detectable in the contralateral VPM even at low magnification views of the brain sections. **G, H**, two representative sections showing large areas of the parietal cortex, including the barrel cortex, with high levels of GFAP immunoreactivity. These astrocytic responses occur rather fast after neonatal ION damage. The mechanisms underlying this transregional signaling, astrocytic response, and the consequences are largely unknown.