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Synaptic plasticity in the trigeminal principal nucleus during the period of barrelette formation and consolidation

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Abstract

We examined whether the postsynaptic responses of cells in the principal sensory nucleus of the trigeminal nerve (PrV) are subject to long-term changes in synaptic strength, and if such changes were correlated the whisker-specific patterning during and just after the critical period for pattern formation. We used an in vitro brainstem preparation in which the trigeminal ganglion (TG) and PrV remained attached. By electrically activating TG afferents, we evoked large-amplitude extracellular field potentials. These responses were postsynaptic in origin and blocked by the glutamate antagonist, DNQX. At P1, a time when barrelettes are consolidating, high frequency stimulation of their afferents led to an immediate (<1 min) and long-lasting (90 min) reduction (35%) in the amplitude of the evoked response. At P3–7, when the pattern of barrelettes have stabilized, the same form of tetanus led to an immediate and long-lasting increase (40%) in the amplitude of the response. Both forms of synaptic plasticity were mediated by the activation of L-type Ca^{2+} channels. Application of the L-type channel blocker, nitrendipine, led to a complete blockade of any the tetanus induced changes. These associative processes may regulate the patterning and maintenance of whisker-specific patterns in the brainstem trigeminal nuclei.

Keywords

Long-term depression; Long-term potentiation; L-type calcium channel; Synaptic response; Brainstem trigeminal nucleus

Patterned and topographic organization of neural connections is an essential structural substrate for processing sensory information in the vertebrate nervous system. We have been investigating cellular mechanisms underlying the establishment and refinement of such a neural network in the rodent trigeminal system [9,15,24,26]. In the first relay station of the trigeminal pathway (brainstem trigeminal complex -BSTC-), primary afferent arbors and a subset of BSTC neurons form the 'barrelettes' which replicate the patterned arrangement of the whisker follicles on the snout [31,35]. These patterns are evident with routine histological stains in the principal sensory nucleus (PrV) and components of the spinal nucleus of the trigeminal nerve. It is the trigeminal afferent terminals that first bring the whisker-related patterns to the brainstem and instruct barrelette cells to orient their dendrites towards them [33]. Whisker-specific patterning in the BSTC is present as early as E19 in the rat [3], and shortly after birth in mice [29]. Furthermore, these patterns are subject to structural modification during a critical period in development [11,12,35]. Barrelette neurons of the PrV project to the contralateral ventroposteromedial nucleus (VPM) of the

thalamus, where they relay the patterns to VPM cells and consequently to the primary somatosensory, 'barrel' cortex [2,8,10,19].

The cellular mechanisms by which patterning of presynaptic afferents develop and how these patterns are translated to their postsynaptic counterparts are not well understood. A major hypothesis in explaining how patterned neural connections develop is that coordinated activity in presynaptic inputs and its detection by the postsynaptic target cells lead to selective, long-term strengthening of specific sets of synapses, i.e., the 'Hebbian synapse' [6,14,18,32]. Within this conceptual framework, long term changes in synaptic efficacy either in the form of potentiation (LTP) or depression (LTD) are believed to reflect the strengthening and consolidation of coincident inputs and the weakening and elimination of less active or asynchronous inputs, respectively. In the present study we examined whether the postsynaptic responses of barrelette cells in the PrV are subject to LTP and/or LTD, and if such changes are correlated with whisker-specific neural patterning and consolidation during and after the critical period for pattern formation.

Postnatal rat pups (P0–7) were euthanized by inhalation of halothane. The brains were taken out and placed in cold artificial cerebrospinal fluid (ACSF, 126 mM NaCl, 2.5 mM KCl, 2 mM CaCl₂, 2 mM MgSO₄, 26 mM NaHCO₃, 1.25 mM NaHPO₄ and 10 mM dextrose, pH: 7.4). The brainstems were dissected out with the trigeminal ganglia (TG) attached on both sides. Coronal slices (approximately 500 μ m) through the PrV with the ganglion attached were then prepared under a stereomicroscope (Fig. 1). These preparations were transferred to a temperature controlled recording chamber at an interface of warm (34°C) humidified air (95% O₂, 5% CO₂) and ACSF. The slices were viewed through this stereomicroscope and under fiber optic illumination, the boundaries of the PrV and the regions containing the barrelettes are readily identified. Bipolar stimulating electrodes (Pt Irteflon coated) were placed in the trigeminal ganglion (TG) or in the trigeminal tract adjacent to the PrV. Recording electrodes (borosilicate glass with a capillary fiber in the lumen 0.4 mm inner diameter and 1.0 mm outer diameter) were filled with 2 M KAC, and had a final impedance of 2–5 megohms.

By electrically activating TG afferents, we could evoke large-amplitude extracellular field potentials in Prv (Fig. 2). These field potentials were synaptically evoked and postsynaptic in origin [16,33]. Fig. 2A shows that it was sometimes possible to evoke action potentials that rode the negative peak of the field potential. Moreover, these field potentials were sensitive to glutamatergic receptor antagonists. Fig. 2B shows that bath application (10 μ m) of 6,7-dinitroquinoxaline-2,3-dione (DNQX) virtually abolished the response ($n=3$). Fig. 2C also reveals field potentials had an NMDA component. Bath application (50 μ m) of D-2-amino-5-phosphovaleric acid (APV) led to a 20–40% reduction in amplitude of the response ($n=3$). In contrast to their sensitivity to glutamate blockers, the application of the L-type Ca²⁺ channel blocker, nitrendipine (50 μ m) had no effect on the amplitude of the field potential ($n=3$).

To determine whether high frequency stimulation of TG afferents led to long term changes in synaptic efficacy we measured the amplitude of field responses in the barrelette region of PrV before and after tetanus. Baseline measurements were evoked by delivering a single shock (0.1–1.0 mA, 0.3 ms) to trigeminal axons once every 30 s for 10 min. For these measurements, we used a stimulus intensity that produced a field potential that was one half to two thirds of the maximal response. Examples of pre-tetanus responses obtained are shown as red traces in Fig. 2D,E. The amplitude plots illustrated in Fig. 3, indicate that baseline responses were stable and showed minimal variability. Following a 10 min baseline period we then issued six stimulus trains once every thirty s. Each train was 1 s in duration and consisted of 50 stimulus pulses (0.1–1.0 mA, 0.3 ms) delivered at a rate of 50 Hz.

Examples of post-tetanus response are shown as blue traces in Fig. 2D,E. The amplitude plots in Fig. 3 show that at P1 (Fig. 3A), this form of high frequency stimulation led to an immediate (<1 min) and long-lasting (> 90 min) *reduction* in the amplitude of the evoked response ($n=3$, Wilcoxon matched pair signed rank test, $P<0.05$). However, between P3–7, the identical form of stimulation led to an immediate and long-lasting *increase* in the amplitude of the response ($n=7$, Wilcoxon matched pair signed rank test, $P<0.05$). Both forms of plasticity were mediated by the activation of L-type Ca^{2+} channels. Fig. 4C indicates that bath application of the L-type Ca^{2+} antagonist nitrendipine blocked all tetanus induced changes in synaptic efficacy ($n=3$). Interestingly, application of the NMDA receptor blocker APV did not alter the amplitude or polarity of any tetanus induced changes ($n=3$, not shown).

The results of the present experiment are summarized in Fig. 4. At 60 min post-tetanus, the amplitude of the evoked response for untreated P1 and P3–7 animals was 65% and 140% of baseline values, respectively. Those treated with nitrendipine showed no form of synaptic plasticity (100% of baseline values) and were significantly different from untreated controls (Mann–Whitney U-test, $P<0.001$).

These results indicate that at P1 when barrelettes are in their formative stages, high frequency stimulation of TG afferents produces a long-term depression in synaptic responses. Between P3–7 when barrelettes are consolidating, heightened activity of TG axons leads to long-term potentiation in responses. It is widely believed long-term changes in synaptic efficacy herald the refinement of developing sensory connections [5,7,20,21].

During perinatal development, central processes of IO nerve axons form a patterned array of synaptic terminals in the trigeminal brainstem. Somehow, these patterns are recognized by a subset of brainstem trigeminal neurons but not others [26]. Barrelette cells recognize their patterned input and consequently orient their dendritic arbors towards the focalized, whisker-specific, afferent arbors. One mechanism by which this could occur is to have post-synaptic cells serve as co-incident detectors [4,32]. Within this context, LTD can serve as a mechanism to selectively eliminate multiple whisker inputs and LTP to consolidate inputs that are co-active. Presently we do not have direct evidence to support this contention. However, the prevalence of LTD during early phases of pattern formation, and switch to LTP during pattern consolidation indicates that these associative processes may regulate the patterning and maintenance of whisker-specific patterns in the brainstem trigeminal nuclei.

While NMDA receptors have been implicated as a potential substrate for mediating activity dependent consolidation of barrelettes in PrV [17,22,23] our results suggest that other neural elements such as high threshold L-type Ca^{2+} channels play an important role. The L-type Ca^{2+} channel blocker nitrendipine led to complete blockade of the long term changes in synaptic modification. These results suggest that influx of Ca^{2+} through L-type channels is necessary and sufficient to induce activity dependent changes in synaptic efficacy. The role of these channels in mediating synaptic plasticity has precedence. L-type Ca^{2+} channel activity can induce depression and potentiation in central structures [1,27,30,36]. In some of these structures, strong postsynaptic activity evoked by NMDA activity or by the spatial or temporal summation of EPSPs leads to the activation of large plateau-like depolarizations (30–60 mV and 200–700 ms). These potentials are mediated by the activation of L-type Ca^{2+} channels [27,28]. Indeed, there is now evidence showing a similar form of activity in barrelette cells of PrV [25]. It is the activity-dependent sequestration of intracellular Ca^{2+} through these channels that could perhaps activate a number of signal transduction pathways that lead to both functional and structural manifestations of synapse stabilization [1,13,34].

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Fig. 1. Brainstem trigeminal ganglion preparation. (A) Low power view showing the preparation. (B) High power view illustrating the location of the barrellete region in PrV and our experimental approach. By stimulating the trigeminal ganglion we can record synaptic responses in the barrellete region of the PrV. PrV=principal nucleus of V, TG=trigeminal ganglion, ION=infraorbital nerve. Scale bars are 1 mm.

Fig. 2.

Examples of extracellular field potentials recorded in PrV evoked by TG stimulation. (A) Extracellular field potentials (red traces) as well as action potentials are evoked by electrical stimulation of the trigeminal tract at low (red trace) and high (blue traces) levels of stimulus intensity. At relatively low levels of stimulation a large (negative in polarity) amplitude response is evoked. At higher stimulus intensities, action potentials ride the crest of the field potential. (B) Examples of responses recorded before (red trace) and after (blue trace) bath application of the DNQX. This AMPA antagonist greatly attenuates the response indicating that field potentials are postsynaptic in origin and mediated by glutamatergic transmission. (C) Examples of responses recorded before (red trace) and after (blue trace) APV application. The NMDA antagonist reduces the amplitude of the response indicating that responses have both NMDA and nonNMDA components. (D) Examples of responses recorded before (red trace) and after nitrendipine (blue trace) application. The L-type Ca^{2+} antagonist has no effect on the synaptically evoked response. (D,E) Field potentials evoked before (red traces) and after (blue traces) high frequency stimulation (6 1-s trains at 50 Hz deliver once every 30 s) of the trigeminal tract. At P1 HFS leads to a decrease in the amplitude of the response. However at older ages (P3–P7), the identical form of stimulation leads to an increase in the amplitude of the response.

Fig. 3.

Plots showing the amplitude of the field potential measured 10 min before and up to 90 min after high frequency stimulation of the trigeminal tract. Each point represents amplitude as a percentage of the pre-tetanus baseline response. The vertical dashed lines depict when tetanus was delivered. Left panels illustrate single, representative examples and right panels show summary plots. Top panels: At P1, HFS leads to a long-term depression in the amplitude of the field potential. Middle panels: At P3–7 the identical form of tetanus results in a potentiation of the response. Bottom panels: Bath application of the L-type Ca^{2+} antagonist nitrendipine blocks tetanus-induced changes.

Fig. 4.

Summary plot showing tetanus induced changes in the amplitude of the the field response at P1, P3–7 and in the presence of nitrendipine. Bars depict mean values obtained 60 min post-tetanus.