Keeping in Check Painful Synapses in Central Amygdala

Keith Tully,[1] Yan Li,[1] and Vadim Y. Bolshakov[1,*]
[1]Department of Psychiatry, McLean Hospital, Harvard Medical School, 115 Mill Street, Belmont, MA 02478, USA
*Correspondence: vadimb@mclean.harvard.edu
DOI 10.1016/j.neuron.2007.11.011

Glutamatergic projections from the parabrachial nucleus to the central amygdala are implicated in pain transmission. In this issue of *Neuron*, Delaney et al. identify a new form of adrenergic modulation at these synapses, demonstrating that noradrenaline-induced suppression of glutamate release is mediated by a decrease in the number of sites of synaptic transmission without changes in probability of release.

The role of the amygdala in mediating emotional aspects of behavioral responses and pain processing is well established (LeDoux, 2000; Neugebauer et al., 2003; Maren and Quirk, 2004). It has been repeatedly demonstrated that different amygdala-based behavioral processes are under the modulatory control of noradrenergic inputs originating in brain stem areas, such as the locus coeruleus (McGaugh, 2000). The noradrenaline release in the amygdala during stressful or emotionally significant events appears to be needed for the stabilization of emotional memory traces, facilitating transition of short-term memory to the long-term form, a process called memory consolidation. Recent studies provide evidence that noradrenaline can promote synaptic plasticity needed for fear learning either at the neuronal network level, by changing the balance between excitatory and inhibitory inputs (Tully et al., 2007), or by facilitating postsynaptic trafficking of AMPA glutamate receptors (Hu et al., 2007).

Nociceptive information can be delivered to the amygdala, comprised of several interconnected nuclei, by way of projections from the somatosensory cortex and thalamus, and also through direct inputs from the parabrachial nucleus of the brainstem. Neurons in the pontine parabrachial nucleus (PB), responsive to nociceptive stimulation, project to the central nucleus of the amygdala (CeA) (Sarhan et al., 2005), specifically to its capsular and lateral divisions (CeAC and CeAL, respectively). PB conveys nociceptive information to the CeA through the spino-parabrachioamygdaloid pathway, linking the spinal cord, PB, and CeA, which is an output amygdala nucleus. Thus, these projections are directly implicated in pain transmission. Both the CeAC and CeAL receive massive noradrenergic innervation. There is evidence that such ascending noradrenergic projections can decrease pain sensation under stress (Yoshimura and Furue, 2006), but how noradrenaline may exert this analgesic action is not well understood. The impressive new study by Delaney et al. (2007) in this issue of *Neuron* provides important mechanistic clues helping to explain this phenomenon.

Fibers originating in the parabrachial nucleus terminate on neurons in the CeAL where they form large basket-like perisomatic synapses (Sarhan et al., 2005). Using a "minimal stimulation" approach, the authors recorded monosynaptic glutamatergic synaptic responses in the CeAL evoked by single-axon stimulation of fibers originating in PB in the ventral amygdaloid fiber tract in brain slices. Single-fiber stimulation in this pathway resulted in unusually large unitary synaptic responses consisting of 8–10 quanta of neurotransmitter. It is not clear, however, whether multiquantal responses were due to activation of multiple synaptic contacts formed by the branching axon only or they were also, at least in part, due to multiquantal release at individual sites of synaptic transmission. Regardless, single-fiber stimulation has produced large excitatory postsynaptic currents (EPSCs) that were rapidly suppressed by exogenously applied noradrenaline. Under more physiological conditions of current-clamp recording, when recorded neurons are allowed to depolarize, single-fiber presynaptic stimuli triggered spike firing in CeAL neurons that was also suppressed by noradrenaline. Noradrenergic inhibition at the PB-CeAL synapses was mediated by α_2-adrenoreceptors, as it was blocked and mimicked by the specific antagonist and agonist of this receptor subtype, respectively. What is the synaptic site of such modulatory actions of noradrenaline? Recording asynchronous single quanta synaptic responses induced by stimulation of parabrachial fibers under conditions when extracellular strontium was substituted for calcium, the authors demonstrated that the quantal amplitude was not decreased by noradrenaline, while the size of multiquantal evoked responses was diminished, consistent with the presynaptic mechanism of noradrenaline-induced suppression of glutamatergic inputs to CeAL neurons. Nevertheless, the paired-pulse ratio, inversely depending on basal probability of release (Pr), remained unchanged in the presence of the neuromodulator. This might indicate that the effect of noradrenaline on glutamatergic neurotransmission at the PB-CeAL synapses is associated with a decrease in the number of sites of synaptic transmission without changes in Pr.

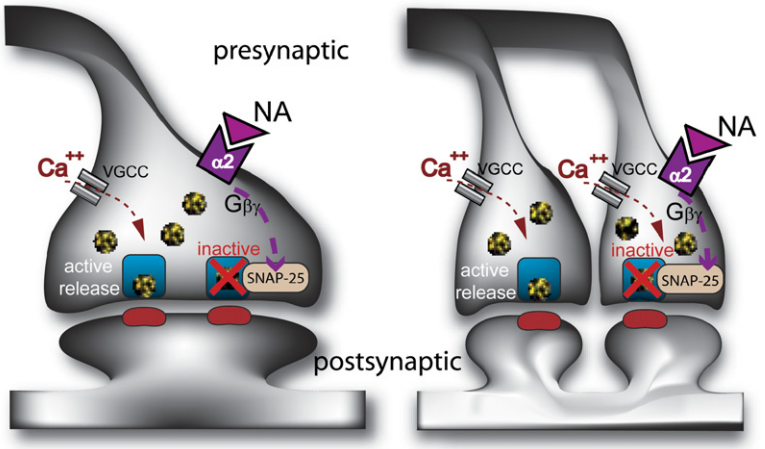

Figure 1. Potential Mechanisms for Noradrenaline-Induced Suppression of Neurotransmission at the Glutamatergic PB-CeAL Synapses

Activation of presynaptic α_2 receptors by noradrenaline causes inactivation of some of the active zones at synapses where multiquantal release may occur (left panel). Alternatively, it could also block release at the fraction of synapses containing single release sites (right panel). Under both scenarios, the effects on release are mediated by direct interactions of G$\beta\gamma$ with the release machinery independently of presynaptic calcium influx.

The previous quantitative studies of anatomical connections between physiologically identified pre- and postsynaptic neurons indicate that a single axon may form multiple synaptic contacts on a target neuron through axonal branching (e.g., see Markram et al., 1997). However, with minimal stimulation techniques and criteria used in the present work to identify single-fiber responses, one could not exclude a possibility that more than one fiber was stimulated if different fibers had identical or very similar excitability. Since noradrenaline can change membrane excitability by activating potassium conductance in nerve terminals, it is feasible that some of the effect on release could be mediated by decreases in the number of fibers activated by electric stimulation. Additionally, multiquantal release observed at the PB-CeAL synapses under baseline conditions could be due to release of multiple vesicles of glutamate from a single active zone. NA could then decrease the number of quanta released by a presynaptic action potential at individual sites of synaptic transmission. If basket terminals form multiple synaptic contacts, with each contact releasing in most cases a single quantum of neurotransmitter, then the effect of noradrenaline could result from inactivation of some of the axonal branches. If the probability of release at individual release sites is very low and multiple sites are activated in response to presynaptic activity, then paired-pulse ratio might be unchanged by the neuromodulator (noradrenaline). The sensitivity of this measure (paired-pulse ratio) to changes in Pr might be insufficient if Pr is low at individual release sites and/or noradrenaline has a specific effect on sites with the lower Pr. Electron-microscopic studies performed in combination with electrophysiological recordings might be needed to address these issues directly.

Similar to many other neurotransmitters in the brain, noradrenaline activates G protein-coupled receptors (GPCRs) in nerve terminals. The ability of presynaptic GPCRs to decrease neurotransmitter release at central synapses is well documented. Most commonly, such effects on neurotransmission are associated with decreased probability of release, either due to the direct effect on the voltage-gated calcium channels or the effect on potassium conductance in the nerve terminal's membrane, also leading to the decreased calcium influx. As evoked release at central synapses steeply depends on intraterminal calcium concentration, any decreases in calcium influx lead to decreased Pr and diminished synaptic strength. However, the PB-CeAL synapses utilize a very different mechanism of modulation because the effects of noradrenaline on neurotransmission did not involve decreases in presynaptic calcium influx. It was directly demonstrated in the experiments with a low-affinity calcium indicator, loaded into the PB terminals. Thus, noradrenaline had no affect on Pr, but apparently decreased the number of sites of synaptic transmission. It is well known that α_2 adrenoreceptors, shown to mediate the effects of noradrenaline in this study, are coupled to Gi/o type G proteins. Consistent with this, the authors found that pretreatment of slices with pertussis toxin prevented the inhibitory action of noradrenaline on release. To explore further how the effects on release could be mediated, they loaded presynaptic terminals with the membrane-permeable G$\beta\gamma$-binding peptide mSIRK and found that this treatment blocked presynaptic inhibition, while mSIRK did not affect neurotransmission when loaded into postsynaptic neurons. These observations indicate that the effects of G protein in presynaptic terminals were mediated by G$\beta\gamma$ subunits acting on neurotransmitter release downstream of calcium influx. This is similar to the results of previously published studies in which G$\beta\gamma$ have been shown to decrease release independently of calcium influx (Blackmer et al., 2001). Consistent with earlier findings (Gerachshenko et al., 2005), cleaving the G$\beta\gamma$ binding sites on the SNAP-25 with botulinum toxin A diminished the effects of noradrenaline at the studied synapses, indicating that the effects on release were due to direct interactions of G$\beta\gamma$ with the machinery of neurotransmitter release. It remains to be determined, however, why some of the sites of synaptic transmission get inactivated in the presence of noradrenaline while others remain active (Figure 1).

Delaney et al. (2007) provide convincing evidence that the observations made with exogenously applied

noradrenaline are functionally relevant. They showed directly that endogenous noradrenaline, released by short trains of high-frequency stimulation delivered to noradrenergic fibers in slices, produced suppression of neurotransmission at the PB-CeAL synapses mediated by α_2-adrenoreceptor activation. What is the potential role of the newly described form of presynaptic modulation by noradrenaline? A key feature of many central synapses is that neurotransmission at the level of single release sites is somewhat unreliable (Cowan et al., 2001), with only a fraction of presynaptic action potentials resulting in release of neurotransmitter quanta. However, neuronal spiking in the brain often occurs in bursts, suggesting that trains of action potentials invade nerve terminals. During short trains of presynaptic impulses, probability of release increases in response to subsequent pulses due to accumulation of residual calcium that was not removed from the terminal after the previous action potential. Thus, during neuromodulator-evoked suppression of release, an inhibitory action might be, at least in part, relieved in the course of repetitive presynaptic firing. The newly identified mechanism of presynaptic modulation is insensitive to the increased levels of presynaptic activity, as it does not depend on presynaptic calcium influx and does not implicate decreases in probability of release. Thus, the biological role of such a mechanism might be to maintain a necessary level of analgesia under a stressful situation, despite high levels of presynaptic firing.

Synaptic mechanisms and organization may vary widely between different cell types and brain structures. What's more, they can change, both by activity and neuromodulators, in a brain region- and neuromodulator-specific fashion. It has been previously shown that the PB-CeAL synapses can undergo LTP, which is associated with increased Pr (Lopez de Armentia and Sah, 2007). It might be important to explore whether the noradrenaline-induced suppression of glutamate release in these synapses interacts with LTP mechanisms. It would also be interesting to determine whether the modulatory mechanism at the PB-CeAL synapses, identified in the exciting work of Delaney et al. (2007), is specific to the actions of noradrenaline or whether similar effects could be observed with other neurotransmitters. If it is noradrenaline specific, it would be a very interesting example linking an identified mechanism of presynaptic plasticity to biologically meaningful behavioral responses. Although additional work is clearly needed, this study furthers our knowledge of how neurotransmission at central synapses can be modulated.

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