Cholinergic modulation of synaptic properties of cortical layer VI input to posteromedial thalamic nucleus of the rat investigated *in vitro*

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The second order somatosensory thalamic nucleus (posteromedial nucleus, PoM) receives excitatory projection from layer VI of somatosensory cortex. While it is known that layer VI cortical input to first order, ventrobasal nucleus (VB) is modulated by cholinergic projections from the brainstem, no such data exists concerning the PoM nucleus. In order to study if layer VI corticothalamic transmission to PoM is also modulated we used patch-clamp recording in thalamocortical slices from the rat's brain. Excitatory postsynaptic potentials (EPSPs) were evoked in PoM cells by trains of 5 electrical pulses at 20 Hz frequency applied to corticothalamic fibers. After carbachol was applied to mimic activation of the cholinergic neuromodulatory system corticothalamic EPSP amplitudes were reduced, while facilitation of EPSP amplitudes was enhanced for each next pulse in the series. Such cholinergic control of layer VI corticothalamic synapses in PoM may be used as gain modulator for the transfer of the peripheral sensory information to the cortex.

Key words: posteromedial thalamic nucleus, corticothalamic input, cholinergic modulation, synaptic facilitation

The somatosensory thalamus receives ascending, excitatory projections from the periphery that bring sensory information to be relayed to the neocortex. These fibers are however outnumbered by the massive recurrent feedback projection from the somatosensory cortex (Rouiller and Welker 2000). Depending on the exact types of their afferent projections the thalamic cells were classified as first and higher order relay neurons. First order relays receive their driving input from the periphery and are believed to transmit information to the cortex, which in turn can modulate their activity through layer VI efferents. In contrast, higher order thalamic relays receive driver input from cortical layer V and are thought to transmit information from one cortical area to another, thus forming a cortico-thalamo-cortical pathway (Guillery and Sherman 2002).

In the rat these two types of somatosensory relay cells are located in separate nuclei. The neocortical projection to the ventrobasal nucleus (VB) originates from layer VI of the primary somatosensory cortex (S1) while the posteromedial nucleus (PoM) receives excitatory inputs from both layer VI and V of S1 (Hoogland et al. 1991, Veinante et al. 2000) and additional projections from the secondary somatosensory (S2), motor and insular cortices (Lévesque et al. 1996a,b, Liao et al. 2010, Veinante et al. 2000). Most previous studies on synaptic integration in the thalamus have focused on first order nuclei such as VB. Much less is known about the thalamic relay in higher order thalamic nuclei such as PoM but different experimental findings suggest that synaptic integration is more complicated in higher, as compared to first order nuclei.

It is well known that the function of the thalamic nuclei is modulated by cholinergic input from brainstem (McCormick 1992). It has an important role for the transition between sleep and wakefulness, as well as in setting different arousal levels (Steriade 2004). Cholinergic modulatory influences on firing properties of thalamic neurons during behavioral activation have been extensively described

(McCormick 1992, McCormick and Bal 1997). However the detailed effects of this modulatory system on synaptic transmission within the thalamus, in particular in higher order nuclei, are much less understood. The aim of the present experiments was to investigate if cholinergic modulation affects synaptic transmission from layer VI neurons in S1 to PoM cells.
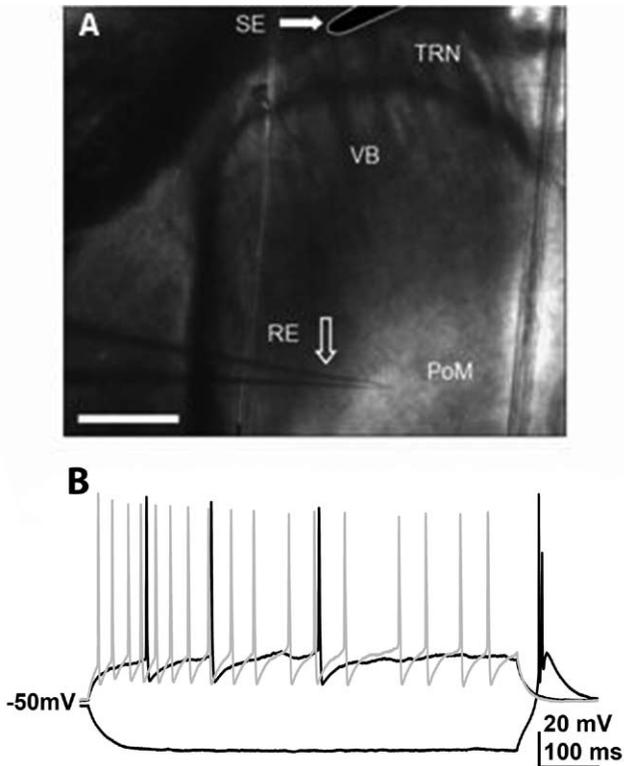

Fig. 1. (A) Low magnification (1.4 ×) image of the somatosensory thalamic area with recording electrode in PoM and stimulation electrode in the internal capsule. (VB) ventrobasal nucleus with characteristic stripes of dense fibers; (PoM) posteromedial nucleus recognized by uniform, brighter area; (IC) internal capsule; (TRN) thalamic reticular nucleus; (RE) recording electrode (indicated by an empty white arrow); (SE) stimulating electrode (indicated by a solid white arrow; to enhance visibility of SE it was outlined in white). Scale bar is 0.5 mm. The two vertical threads of the mesh holding the slice can be seen throughout the photograph. (B) Examples of responses of a typical PoM neuron to intracellular depolarizing and hyperpolarizing pulses (−200, +200 and +300 pA). Among two responses for depolarizing pulses the one in gray corresponds to +300 pA. Note that the tonic firing rate (34 Hz) and the spike frequency during the burst (227 Hz) are similar to the values calculated by Landisman and Connors (2007).

All experiments were approved by the Local Ethic Commission and were performed in accordance with the European Communities Council Directive of 24 November 1986.

Three- to four-week old Wistar rats were used in the experiments. Under deep Isoflurane anesthesia rats were decapitated and brains were removed and immersed in cold (between −1.5°C and −1°C) NaCl-free artificial cerebrospinal fluid (ACSF) of the following composition (in mM): KCl 3, NaH_2PO_4 1.25, $NaHCO_3$ 24, $MgSO_4$ 4, $CaCl_2$ 0.5, d-glucose 10, sucrose 219 (300–308 mOsm). In this solution sodium chloride was substituted by sucrose to improve the viability of thalamic neurons in slices. Thalamocortical slices (350 µm thick) (Agmon and Connors 1991, Land and Kandler 2002) were prepared using a Leica VT1000S vibrating blade microtome. Such slices are especially suitable for selective studies of synapses made on PoM cells by axons from cortical layer VI, because descending fibers from layer V are mostly cut-off (Landisman and Connors 2007). Slices were incubated for 30 min (at 31°C) and then at room temperature for at least 1 hour. The ASCF in the incubation chamber contained (in mM): NaCl 126, KCl 3, NaH_2PO_4 1.25, $NaHCO_3$ 24, $MgSO_4$ 3, $CaCl_2$ 1, d-glucose 10. After recovery, individual slices were transferred to the recording chamber, with circulating (2–2.5 ml/min), warm (31–32°C) ASCF of similar composition to the incubation solution except for $MgSO_4$ and $CaCl_2$ that both were 2 mM. All solutions were saturated with 95% O_2–5% CO_2. Bicuculline methiodide (10 µM) was used to block $GABA_A$ receptors and eliminate the recurrent inhibitory influence from thalamic reticular nucleus (TRN).

Region of PoM nucleus was readily distinguished from VB, TRN and the internal capsule when using a low-magnification objective (1.4×) with an Olympus BX61WI microscope (Fig. 1). A high-magnification, water immersion objective was used for visualizing individual neurons. Visually-guided whole-cell voltage recordings were performed from PoM neurons using electrodes (3–6 MΩ) pulled from standard-wall (1.2 mm outer diameter) borosilicate glass capillaries. The electrodes were filled with (in mM): 120 K-gluc, 10 HEPES, 0.1 EGTA, 4 KCl, 2 NaCl, 4 ATP-Mg, 0.5 GTP-Na2, 10 phosphocreatine (Tris salt). pH was adjusted to 7.25 with KOH and osmolarity to 285–290 mOsm by adding sucrose. The membrane voltage values (including resting ones) were not corrected for the

junction potential (15 mV for the used pipette solution; as calculated in pCLAMP) and the average firing threshold for the recorded cells was −34 mV. To evoke excitatory postsynaptic potentials (EPSPs), 5 electrical pulses (200 µs duration) at 20 Hz frequency were applied through a concentric stimulating electrode placed at the corticothalamic fiber tract in the internal capsule. Stimulation current ranged from 150 to 500 µA. Stimulation intensities were chosen in a way to have measurable first EPSP after its reduction following carbachol application. For this reason we set the first EPSP amplitude in control condition between 1–2 mV. The amplitudes of consecutive EPSPs were estimated according to their individual baselines determined as an average voltage measured within the period of 1–2 ms preceding the rising phase of the given postsynaptic potential. The electrical train stimuli were repeated 8–22 times (with 15 s inter-trial intervals) for obtaining a baseline before carbachol (both, muscarinic and nicotinic receptors agonist, 6–8 µM) was added to the circulating ACSF and the series of stimulation repeated again 16–50 times. In most of the slices the cortex was cut-off to prevent the activation of the whole thalamo-cortico-thalamic loop. All chemicals were obtained from Sigma (St Luis, USA). Throughout the text, the averaged data were presented as means ± SEM. Means were compared with Wilcoxon Singed Rank Test for paired comparisons.

The recordings from PoM cells (n=9) revealed an average resting membrane potential of −61.3 ± 1.0 mV and membrane resistance of 83.3 ± 6.0 MΩ. The average resting potential measured in our experiment was similar to the value obtained by Landisman and Connors (2007). The action potential responses to 500 ms rectangular depolarizing or hyperpolarizing current pulses were typical for thalamic neurons (Jahnsen and Llinas 1984): at hyperpolarized membrane potential (below −60 mV) low threshold calcium spikes generated a burst of sodium spikes (bursting mode) and at depolarized membrane potential (above −60 mV) standard sodium spike response to the current injection (tonic mode). The recorded PoM cells exhibited mean tonic firing rate of 32.6 ± 5.8 Hz for +300 pA injected current pulse (500 ms duration), and mean firing frequency during a burst of 214 ± 8.1 Hz (n=9, see also examples in Fig. 1). These values are at the range of the data obtained by Landisman and Connors (2007), giving additional support for the notion that the cells recorded in our experiments were located in PoM.

In order to avoid calcium spikes generation during electrical stimulation of cortico-thalamic fibers, the baseline membrane voltage was adjusted to a relatively depolarized value (−56 mV). The 20 Hz stimulation of corticothalamic fibers evoked excitatory postsynaptic potentials that were facilitating in all recorded cells (see example in Fig. 2, black trace) (Lindström and Wróbel 1990, Granseth et al. 2002, Granseth 2004). That is, while the first stimulation impulse in the train evoked an EPSP of low amplitude, the amplitude of the second EPSP was elevated more than three times. Amplitudes of subsequent EPSPs were further enhanced with each stimulation, although less than for the first two stimulations (see group average in Fig. 3, black trace).

When the cholinergic agonist carbachol was added to the bath we observed a depolarizing shift in the membrane potential (9.1 ± 1.3 mV) which was compensated by adding negative DC current that brought the membrane potential to the same baseline value as in the control condition.

As compared to a control conditions, carbachol substantially decreased the amplitude of all postsynaptic responses evoked by the train stimulation. The amplitude reduction (almost 3-fold) was most pronounced for the first EPSP in the train. This is clearly visible
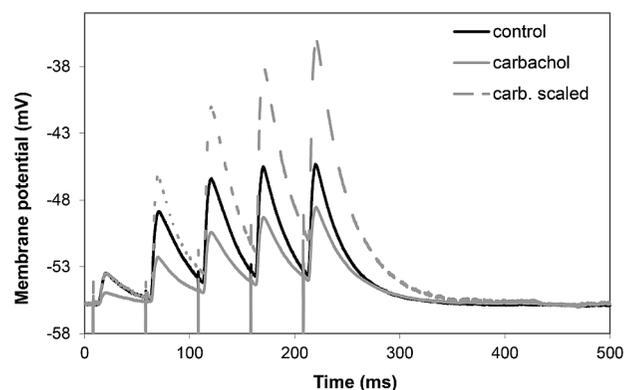

Fig. 2. Representative examples of facilitating synaptic responses of PoM cell to stimulation of corticothalamic axons before and after application of carbachol. Average results for one cell: "control" – 11 repetitions of the stimulation train without carbachol (with bicuculline); "carbachol" – 33 repetitions with carbachol in the bath. In both cases all repetitions recorded in this cell were used for averaging. Additionally, response obtained during carbachol application was rescaled, so that the first EPSP's amplitude was equal to the "control" one ("carb. scaled").

for the averaged response (gray trace) from a representative cell in Fig. 2. The following postsynaptic potentials were less affected - the final amplitude of the last EPSP was only 1.5 times smaller than in control situation. Thus, in parallel to the reduction of the overall EPSP amplitude, carbachol application resulted in a significant increase of the facilitation of the following responses relative to the amplitude of the first EPSP in a train. This is evident when responses obtained with carbachol are rescaled to match the amplitude of the first EPSP (Fig. 2, dashed trace).

To quantify the cholinergic modulatory effect on the corticothalamic EPSPs, we averaged the EPSP amplitudes for the PoM neurons (n=9). The plot of average EPSP amplitudes in Figure 3 is similar to the individual trace in Figure 2. On an average, the amplitude of the first EPSP after carbachol application was 3.7 times smaller than in control condition (0.29 ± 0.08 mV *versus* 1.06 ± 0.18 mV; $P<0.01$, Wilcoxon test). Much weaker responses were also evoked by the remaining pulses in the train. For successive EPSPs, the normalized mean amplitudes with carbachol were 1.49 ± 0.32, 2.33 ± 0.43, 2.83 ± 0.46, and 3.33 ± 0.45 mV, respectively, whereas corresponding control values were 4.19 ± 0.43, 5.55 ± 0.43, 6.00 ± 0.46, and 6.43 ± 0.41 mV ($P<0.01$, Wilcoxon test – for each "drug-control" amplitude pair).
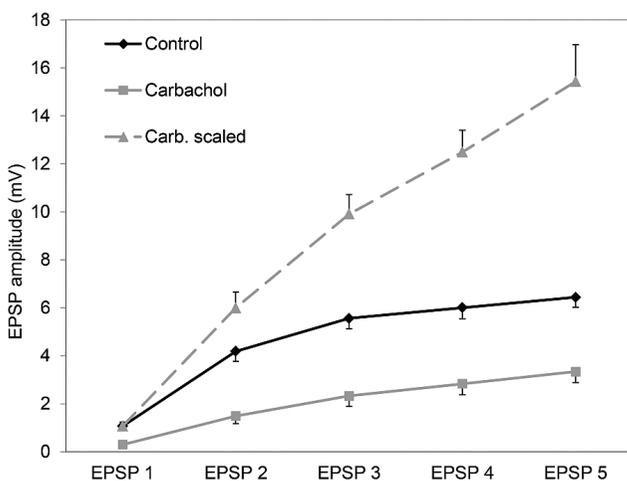

Fig. 3. Average amplitudes of the consecutive EPSPs in the train, measured for the group of 9 cells studied with carbachol. The plot obtained from individually scaled postsynaptic responses (as in Fig. 2) is included as well. Error bars indicate SEMs. "Control–carbachol" and "control–carbachol scaled" differences were significant for each EPSP ($P<0.01$).

Despite their smaller amplitude, responses evoked by the train of stimuli under the influence of cholinergic agonist showed significantly stronger facilitatory effect. This is evidenced by the relative change in amplitude between the preceding and next pulse in the stimulus train. The biggest increase of amplitudes was for the first two ($EPSP_2/EPSP_1$), both during carbachol application (6.1 ± 0.56) as well as in control condition (4.22 ± 0.3). This increment was 44.4% larger with cholinergic agonist than without it ($P<0.01$, Wilcoxon test). For the subsequent pulses in the train the values obtained during carbachol application were also significantly ($P<0.01$, Wilcoxon test) bigger than for control condition (increments were: 26.2%, 17.9%, and 13.3%, respectively).

When the EPSP amplitudes obtained with cholinergic agonist were rescaled to match the amplitudes of the first EPSPs in both conditions, increase of synaptic facilitatory effect for the whole train became clearly visible (Fig. 3 – dashed gray line). Before averaging, the amplitudes were rescaled individually for each cell. The enhanced facilitation with carbachol made the rescaled EPSP amplitudes appear significantly larger than controls ($P<0.01$, Wilcoxon test). Moreover, the scaled plot obtained during carbachol is consistently steeper than in control condition. In particular, when we compared the relative increase in EPSP amplitude at the end of the train (the averaged $EPSP_5/EPSP_1$ ratio), we obtained a much higher value of 17.5 ± 3.1 for carbachol compared 6.9 ± 0.8 in control condition ($P<0.01$, Wilcoxon test). In other words, EPSPs were 2.5 times more enhanced during the stimulation train with cholinergic agonist than without.

To summarize, in this study we examined the cholinergic influence on the synaptic properties of corticothalamic input from layer VI to PoM. Our results demonstrate that carbachol significantly depresses corticothalamic EPSPs evoked in PoM cells by a train consisting of 5 pulses at 20 Hz frequency, with the biggest decrease measured for the first EPSP. At the same time successive EPSPs facilitate substantially more with the most pronounced effect for the first pair of postsynaptic responses.

Our results in PoM are similar to data obtained previously for VB (Castro-Alamancos and Calcagnotto 2001). Thus, the cholinergic modulation of corticothalamic synaptic transmission from layer VI is similar in first- and higher order somatosensory nuclei. This suggests that this pattern of modulation could be a general

mode of action of acetylcholine and can be valid for other thalamic nuclei as well. The functional role of cholinergic modulation of corticothalamic synapses might be to enhance the dynamic gain control mechanism previously proposed for the corticothalamic feedback (Lindström and Wróbel 1990, Granseth et al. 2002, Granseth 2004). According to this hypothesis, the enhanced facilitation of corticothalamic input induced by acetylcholine (occurring most probably during behavioral arousal and attentive state) could enhance the gain for the stream of peripheral information on its way to the cortex. Our results support also the notion that neuromodulatory systems may play a significant role in spike timing based information coding (Ponulak and Kasiński 2011).

The question arises whether pre- or postsynaptic mechanism is responsible for the observed effects of cholinergic influence. It has been shown that facilitation at corticothalamic synapses can be (at least to large extent) accounted for by a presynaptic increase in transmitter release probability (Granseth et al. 2002, Granseth and Lindström 2003). It has been shown for many types of synapses that if an action potential is followed by another action potential within a few hundred milliseconds, the probability that a synaptic vesicle is released is elevated for the second action potential (for review see Zucker and Regehr 2002). Such facilitation of transmitter release becomes more prominent if the initial release probability is lower (Manabe et al. 1993). In the light of these data we hypothesize that the mechanism of cholinergic modulation of corticothalamic transmission in PoM cells is presynaptic. According to such hypothesis, activation of presynaptic cholinergic receptors at the synapses from layer VI cortical neurons to PoM cells will reduce the probability that an action potential will trigger the release of glutamate. This in turn will enhance facilitation of the following stimuli. This idea is supported by observations that various cholinergic agents acting presynaptically can decrease excitatory transmission in the hippocampus (Scanziani et al. 1995) in the ventral striatum (Pennartz and Lopes da Silva 1994) and, interestingly, also inhibitory GABAergic transmission in the thalamus (Masri et al. 2006). Modulation of synaptic transmission may also depend on kinetics of synaptic currents (Mozrzymas 2008) and on properties of vesicular transporter systems (Liguz-Lecznar and Skangiel-Kramska 2007).

It is important to note that the well known depolarizing postsynaptic effects of acetylcholine on thalamic neurons (reviewed in McCormick 1992) should not influence our results, because depolarization caused by carbachol in our experiments was compensated to assure the same driving force for ions responsible for the EPSPs. However, two postsynaptic mechanisms: receptors desensitization and saturation have been recently added to the palette of factors that could potentially affect the temporal dynamics of synaptic transmission (Blitz et al. 2004). One of them – receptor saturation – has in fact been shown to weaken facilitation of corticothalamic synapse in VB (Sun and Beierlein 2011). If similar mechanism is present at the corticothalamic synapse in PoM, lower amount of neurotransmitter released during depressed responses under carbachol influence would likely prevent the saturation of postsynaptic receptors and in effect enhance the apparent facilitation. Future experiments need to address if receptor saturation is also present in PoM cortico-thalamic synapse.

The other issue requiring more experiments is to elucidate which type of cholinergic receptors are involved in modulation of corticothalamic synapses in PoM. It is worth to note that in VB acetylcholine also depresses corticothalamic synapses, and increases paired-pulse facilitation, as shown by Castro-Alamancos and Calcagnotto (2001) and Nagumo with colleagues (2011). However, the results of these two research groups differ regarding the type of receptors (muscarinic in the first study and nicotinic in the second one) underlying the mechanism of the described effects. Nagumo with colleagues (2011) have suggested that the reason might come from the different ages of the experimental animals used (2–3 weeks *versus* over 7 weeks old mice). Nicotinic receptor expression usually decreases and muscarinic receptor expression increases during postnatal development (Fiedler et al. 1987). Moreover, abundant muscarinic M2 receptors were found in higher order thalamic nuclei of adult rats (Barthó et al. 2002).

To conclude, activation of acetylcholine receptors depresses transmission in corticothalamic synapses at PoM, simultaneously increasing frequency facilitation. Whether mechanism of this modulation is pre- or postsynaptic and which group of cholinergic receptors is involved in this process requires further studies.

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