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Research Report

# Cholecystokinin action on layer 6b neurons in somatosensory cortex

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ABSTRACT

Layer 6b in neocortex is a distinct sublamina at the ventral portion of layer 6. Corticothalamic projections arise from 6b neurons, but few studies have examined the functional properties of these cells. In the present study we examined the actions of cholecystokinin (CCK) on layer 6b neocortical neurons using whole-cell patch clamp recording techniques. We found that the general CCK receptor agonist CCK8S (sulfated CCK octapeptide) strongly depolarized the neurons, and this action persisted in the presence of tetrodotoxin, suggesting a postsynaptic site of action. The excitatory actions of CCK8S were mimicked by the selective $CCK_B$ receptor agonist CCK4, and attenuated by the selective $CCK_B$ receptor antagonist L365260, indicating a role for $CCK_B$ receptors. Voltage-clamp recordings revealed that CCK8S produced a slow inward current associated with a decreased conductance with a reversal potential near the $K^+$ equilibrium potential. In addition, intracellular cesium also blocked the inward current, suggesting the involvement of a $K^+$ conductance, likely $K_{leak}$. Our data indicate that CCK, acting via $CCK_B$ receptors, produces a long-lasting excitation of layer 6b neocortical neurons, and this action may play a critical role in modulation of corticothalamic circuit activity.



## 1. Introduction

Corticothalamic neurons modulate information transfer through the thalamus by influencing firing mode and synchronization of relay neurons as well as modulate thalamocortical rhythms (Sherman and Guillery, 1996; Godwin et al., 1996; Sillito and Jones, 2002; Sillito et al., 1994; Steriade, 2001; Blumenfeld and McCormick, 2000; Bal et al., 2000). Corticothalamic innervation arises from deep layer 5 or 6 glutamatergic neocortical neurons (Bourassa et al., 1995; Killackey and Sherman, 2003). These excitatory neurons can activate both ionotropic and metabotropic glutamate receptors on thalamic relay neurons leading to both short- and long-term synaptic responses (Sherman and Guillery, 1996; Reichova and Sherman, 2004; Kao and Coulter, 1997; McCormick and von Krosigk, 1992; Turner and Salt, 1998; Alexander and Godwin, 2005).

Layer 6 can be subdivided into two distinct laminae: layers 6a and 6b. Layer 6b has also been referred to as layer VII, subplate, or subgriseal layer and these neurons and these neurons project to thalamus in rodents (Clancy and Cauller,

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*Abbreviation:* CCK, cholecystokinin

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1999; Killackey and Sherman, 2003). The morphology of layer 6b neurons is more diverse than that of layer 6a neurons in that 6b neurons have apical dendrites that extend in horizontal or oblique directions (Killackey and Sherman, 2003; Torres-Reveron and Friedlander, 2007), and some 6b neurons lack apical dendrites altogether (Clancy and Cauller, 1999; Andjelic et al., 2009). In addition, certain proteins are predominantly expressed within layer 6b such as orphan nuclear receptor Nurr1 and neurexophilin 3 (Arimatsu et al., 2003; Beglopoulos et al., 2005).

There is limited understanding of the functional significance of layer 6b as a distinct layer (Torres-Reveron and Friedlander, 2007). It is speculated that layer 6b neurons may modulate arousal or wakefulness because the neuropeptide orexin produces strong excitation of layer 6b neurons and little effect on cortical neurons in other layers (Bayer et al., 2004; Sakurai, 2007). A source of orexin in the brain are hypothalamic neurons, and these cells are strongly excited by an anorexinergic neuropeptide, cholecystokinin (CCK, Tsujino et al., 2005).

CCK was initially found in gut and later widespread within the brain (Crawley and Corwin, 1994). In the thalamocortical circuit, CCK is present in some corticothalamic and thalamocortical projection neurons. CCK receptors are localized in both deep layers of neocortex as well as thalamus (Burgunder and Young, 1990; Mercer et al., 2000; Mercer and Beart, 2004; Schiffmann and Vanderhaeghen, 1991; Zarbin et al., 1983). In thalamus, CCK selectively depolarizes GABA-containing thalamic reticular nucleus

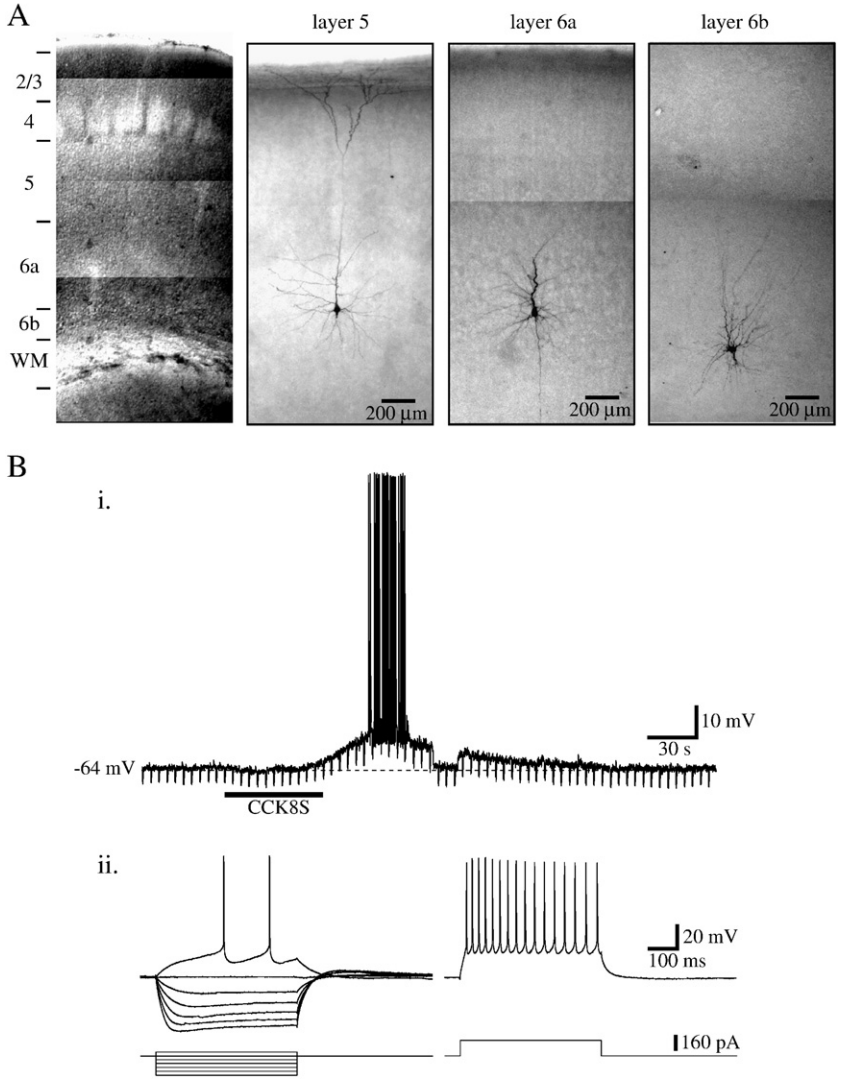


**Fig. 1 – CCK depolarizes layer 6b neurons. A: *Left*: Low power image of somatosensory cortex in coronal slice. Layer 6b can be identified from the image above white matter. *Right*: Biocytin-filled morphologies representative of layer 5, 6a and 6b neurons. B: i. In a current-clamp recording from a layer 6b neuron in control conditions, CCK8S (1.25 µM, 60 s) depolarizes the membrane leading to action potential discharge. The short downward deflections are voltage responses to short hyperpolarizing current pulses (–15 pA, 500 ms). The prolonged hyperpolarization to baseline levels is produced by adding hyperpolarizing current and serves to determine voltage-independent alterations in the transient hyperpolarizing steps. ii. Voltage responses and firing pattern to the hyperpolarizing and depolarizing DC current steps to the neuron.**