Schizophrenia, Hypocretin (Orexin), and the Thalamocortical Activating System

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Diminished connectivity between midline-intralaminar thalamic nuclei and prefrontal cortex has been suggested to contribute to cognitive deficits that are detectable even in early stages of schizophrenia. The midline-intralaminar relay cells comprise the final link in the ascending arousal pathway and are selectively excited by the wake-promoting peptides hypocretin 1 and 2 (orexin A and B). This excitation occurs both at the level of the relay cell bodies and their axon terminals within prefrontal cortex. In rat brain slices, the release of glutamate from midline-intralaminar thalamocortical terminals induces excitatory postsynaptic currents (EPSCs) in layer V pyramidal cells in prefrontal cortex. When hypocretin is infused into medial prefrontal cortex of behaving animals, it improves performance in a complex cognitive task requiring divided attention. Chronic restraint stress causes atrophy of the apical dendritic arbors in layer V prefrontal pyramidal cells and leads to a reduction in hypocretin-induced EPSCs, indicating impairment in excitatory thalamocortical transmission. Thus, taken together with evidence for an underlying loss of excitatory thalamocortical connectivity in schizophrenia, stress in this illness could further exacerbate a breakdown in cortical processing of incoming information from the ascending arousal system.

Key words: attention/dendrite/EPSC/prefrontal/stress

Introduction

Abnormalities in prefrontal cortex have been implicated in schizophrenia and may be responsible for deficits in executive functions such as attention and working memory. Recent studies have suggested that a loss in connectivity between prefrontal cortex and midline-anterior thalamus may contribute to the cognitive deficits in early stages of schizophrenia. This hypothesis is supported by evidence from magnetic resonance imaging scans in patients with first-episode schizophrenia indicating that fiber pathways in the anterior limb of the internal capsule, which connect midline and anterior thalamic nuclei to prefrontal cortex, are reduced in volume.1 These findings are consistent with earlier proposals that connectivity between thalamic nuclei and prefrontal cortical areas may be impaired in schizophrenic patients.2–5

A major component of the thalamocortical input to prefrontal cortex is comprised of projections from midline and intralaminar thalamic relay neurons.6 This projection is unique in that it coordinates activity levels broadly across cerebral cortex and supports attention and awareness,6,7 rather than simply relaying one type of sensory information to one particular cortical region. As described below, the peptides hypocretin 1 and 2 (orexins A and B) selectively excite thalamic midline-intralaminar neurons8 and also their projections in prefrontal cortex.9,10 Distinct hypocretin projections to the thalamus and prefrontal cortex11 suggest that the ability of hypocretin to excite both the cell body and nerve terminal may not be redundant but instead may be involved in coordinating networks of bursting neurons.12 Midline-intralaminar neurons are unusual in that they burst during wakefulness,13 when hypocretin levels are elevated,14,15 instead of bursting during slow-wave sleep–like specific sensory thalamic neurons. The ability of hypocretin to excite the final synapse of the ascending arousal system suggests a role for prefrontal release of hypocretin in executive tasks such as attention and working memory, which are impaired in schizophrenia.

Thalamocortical Activating System

The midline-intralaminar nuclei of the thalamus are part of the ascending arousal system. Functional imaging studies have shown that activation of these nuclei leads to higher levels of arousal and attention.3,16 They have long been considered “nonspecific” because they project broadly within frontal cortex and can recruit other areas of cortex to become active.17 However, the different groups of nuclei appear to have fairly specific domains within which they promote attention and arousal: visceral-limbic functions (dorsal group), cognitive functions...
many brainstem neurons that, in turn, project to the thalamus as well as the cortex (figure 1). Thus, hypocretin neurons can have both a direct and indirect influence upon thalamocortical transmission.

Hypocretin and Antipsychotic Drugs Excite Midline-Intralaminar Thalamic Neurons

Hypocretin has been shown selectively to depolarize midline-intralaminar thalamic neurons and not sensory thalamic neurons. Such specificity is congruent with previous observations that hypocretin fibers avoid specific sensory thalamic nuclei, terminating predominantly in midline-intralaminar nuclei such as rhomboid and central medial.21 Midline-intralaminar nuclei show prominent expression of the hypocretin receptor 2,24,25 which is very sensitive to both hypocretin peptides. Bayer et al.8 examined the electrophysiological effects of hypocretin-1 and -2 peptides on thalamic neurons in brain slice with whole-cell recording.8 Both peptides potently depolarized neurons in midline-intralaminar nuclei with EC50 less than 20 nanomolar but did not depolarize neurons in sensory thalamic nuclei.

Interestingly, acute administration of either typical or atypical antipsychotic drugs activates midline thalamic neurons,26,27 as measured by increased Fos-like protein expression. The direct and indirect mechanisms contributing to these changes are not well understood. However, it has been suggested that atypical antipsychotics may increase thalamic activation indirectly through activation of hypocretin (orexin) projection neurons.17 Interactions between the hypocretin system and antipsychotic medicines also have been recently demonstrated in the ventral tegmental area, where blocking the effects of endogenous hypocretin with an antagonist of orexin (hypocretin)-1 receptors is able to prevent the usual increase in the number of spontaneously active dopaminergic neurons after administration of antipsychotic drugs.28

Hypocretin Excites Thalamocortical Terminals in Prefrontal Cortex

Recent work has illustrated the importance of thalamocortical transmission in facilitating recurrent network activity and cortical up states.29 The ability of hypocretin directly to excite thalamocortical terminals in prefrontal cortex3,10 appears to work to enhance some aspects of attention. One potential mechanism may involve the susceptibility of thalamocortical terminals to the generation of terminal spikes, which would promote bursting in the projecting thalamic neurons through antidromic transmission.12,19 It has been suggested that neurotransmitters released in the terminal field could thus influence the bursting of groups of neurons in a distant brain region. Our recent work suggests that hypocretin released in prefrontal cortex could act in this manner. Recent work

**Fig. 1.** A schematic depicting hypocretin projections to brain areas involved in maintaining arousal (adapted from Sutcliffe and deLecea23). In the thalamus, hypocretin axons predominantly project to midline-intralaminar thalamic nuclei. Prefrontal cortex appears to receive a unique and strong hypocretin projection compared with other cortical areas. Hypocretin neurons also project to many lower brain areas involved in wakefulness, including tuberomammillary nucleus (TMN), dorsal raphe nucleus (DRN), locus coerules (LC), pontine reticular formation (PRF), and prepontine tegmental area (PPT).
shows that hypocretin can also directly excite a population of neurons in cortical layer VIb. The function of these very deep cortical neurons is not well understood, but they are believed to send superficial corticocortical projections.

In examining the effects of hypocretin in prefrontal cortex, we found that there was very little direct effect of hypocretin on layer V prefrontal neurons. In prefrontal slice, we recorded from layer V pyramidal neurons by whole-cell patch clamp and applied hypocretin in the bath. Hypocretin had little effect on the resting membrane potential or the holding current, but potentiated synaptic release of glutamate onto these cells. This effect was observed in voltage clamp as a large increase in spontaneous excitatory postsynaptic currents (sEPSCs) illustrated in figure 2. Blocking action potentials with tetrodotoxin (TTX) eliminated the hypocretin-induced increase in sEPSCs. This indirect effect of hypocretin occurred preferentially in medial prefrontal cortex, a region that has been shown to receive a greater density of hypocretin projections than other cortical regions.

We started to suspect that hypocretin was able to induce terminal spikes in thalamocortical terminals because prior thalamic lesions greatly suppressed this effect. Furthermore, the hypocretin-elicited increase in sEPSCs could be suppressed by agonists of mu-opioid receptors, such as DAMGO. Mu-opioid agonists are known to be able to suppress thalamic cortical but not excitatory corticocortical transmission. Our model of this process is shown in figure 2B. Hypocretin receptor 2 is abundant in midline/intralaminal thalamic neurons, which project to prefrontal cortex. Hypocretin stimulation appears to depolarize thalamocortical terminals to threshold for spiking. This effect can be physiologically opposed by stimulation of the G<sub>i</sub>/G<sub>o</sub>-coupled mu-opioid receptors.

**Two-Photon Calcium Imaging Studies**

Although the above studies are suggestive of a direct action of hypocretin upon midline/intralaminal terminals, this hypothesis required more rigorous testing. We used two-photon imaging to identify directly the synapses at which hypocretin induced glutamate release. First, we filled layer V pyramidal neurons with 2 dyes—a green calcium indicator, Oregon green BAPTA-1, and a red control dye, Alexa 594. This combination of dyes allows us to image postsynaptic calcium transients at single dendritic spines. Under baseline conditions, the dendrite and spines appear orange, but they can turn yellow (indicative of an increase in the green to red ratio) when an influx of calcium increases the brightness of the green calcium indicator. We found that hypocretin induced glutamate release onto a small percentage of spines on branches of the apical dendrites of prefrontal layer V pyramidal neurons.

**Spines tended to be on higher order branches located in midlayer V or in layer I of medial prefrontal cortex. Although the apical dendritic location of the responding spines would be congruent with excitation of direct thalamic input, these findings do not identify which glutamatergic projections are excited by hypocretin.**

To determine whether hypocretin specifically activated synapses receiving thalamic inputs, we labeled thalamocortical projections to prefrontal cortex by electroporating the thalamus in vivo with the retrograde tracer Phaseolus vulgaris conjugated to a green fluorescent
marker, Alexa 388. Several days later, to allow for anterograde transport to terminals, slices were examined for thalamocortical labeling. After filling layer V pyramidal neurons with the combination of dyes described before, we found scattered appositions in the $x$-$y$ plane between green-labeled thalamic boutons and orange-labeled dendritic spines. Just as only a fraction of spines responded to hypocretin in our previous experiments, apparent appositions between labeled boutons and spines were infrequent and occurred primarily on higher order branches of the apical dendrites in layers V and I. We imaged these spines to observe the effects of hypocretin, as shown in figure 3.

Hypocretin induced calcium transients at 7 out of 8 spines appearing to be in apposition to labeled thalamic boutons but only at 2 out of 26 control spines that were in focus but not in apparent apposition to a labeled bouton. The latter spines may potentially be the recipients of intact synaptic input from deep layer VIb neurons that are also excited by hypocretin. These experiments provide strong convergent evidence that hypocretin excites thalamocortical synapses in prefrontal cortex. Furthermore, the finding that only specific subsectors of the apical dendritic field were affected by hypocretin proved to be critical for subsequent studies on the effects of stress on thalamocortical transmission in prefrontal cortex (see below).

**Effects of Hypocretin and Nicotine on Arousal and Attention**

Hypocretin levels have been shown to correlate with states of attention and arousal, with high levels observed during alertness and periods of physical activity. We have found that hypocretin infused into prefrontal cortex improves performance on a task of sustained and divided attention, specifically at the most demanding, shortest, target duration. The underlying mechanism for this effect appears to be that hypocretin excites single, identified thalamocortical synapses in prefrontal cortex in the absence of direct postsynaptic effects on layer II/III or layer V pyramidal neurons. By inducing ectopic spikes in thalamocortical terminals, hypocretin released in prefrontal cortex has the potential to affect alertness and attention in vivo.

Recently, it has been shown that people with narcolepsy—who lack hypocretin—have selective deficits in the executive attention network of the prefrontal cortex. While older literature suggests that cognitive deficits in narcolepsy may simply be a result of tiredness, Rieger et al in 2003 show that the selective deficit in divided attention persists affect controlling for alertness. Furthermore, another recent study has showed abnormal frontal cortex physiology in narcoleptics during preattentive and attentive tasks.

We also found that nicotine excites high-affinity nicotinic acetylcholine receptors on the same thalamocortical terminals as are activated by hypocretin and results in a large increase in glutamate release onto prefrontal layer V pyramidal neurons. In conjunction with the hypocretin experiments, we replicated a recent study that showed that direct infusion of nicotine into prefrontal cortex improves performance of rats on a task of divided attention. Interestingly, nicotine has repeatedly been shown to enhance attention. It has been proposed that the high nicotine use in schizophrenia represents a
form of self-medication to improve attention and working memory (see Kumari and Postma\textsuperscript{37}). Similarly, the activation of hypocretin inputs to the thalamus or prefrontal cortex could contribute to the therapeutic effects of antipsychotic drugs, at least of the atypical category\textsuperscript{11}.

Stress and Hypocretin Transmission in Prefrontal Cortex

Stress has been implicated in the pathogenesis of various psychiatric illnesses including schizophrenia (see Walker and DiFrancesco\textsuperscript{38}). Stress and activation of glucocorticoid pathways also exacerbate symptoms of schizophrenia\textsuperscript{39}. Recently, several laboratories have shown that repeated restraint stress in rats leads to dendritic atrophy of layer II/III pyramidal cells in medial prefrontal cortex (mPFC\textsuperscript{40–42}), an effect mimicked by high levels of corticosterone\textsuperscript{43}. Notably, these atrophic changes are restricted to the apical dendritic field. These apical tufts of layer V neurons are the target of the midline-intralaminar glutamatergic terminals that are directly excited by hypocretin\textsuperscript{10} and potentially the target of axons from the deep layer VIb neurons that are also excited by hypocretin\textsuperscript{30}. These findings suggest that excitatory transmission modulated by hypocretin might be impaired by stress-induced atrophy in the apical dendritic region.

Using whole-cell recording and 2-photon laser scanning of neurobiotin-labeled cells in rat brain slices, we correlated electrophysiological and morphological changes in the same layer V cells following repeated immobilization stress\textsuperscript{44}. This data is summarized in figure 4. In brain slices from rats exposed to repeated mild restraint stress (20 minutes/d for 7 days), there was a reduction in sEPSC

![Fig. 4](image-url). Frequency of hypocretin (hcrt)-induced spontaneous excitatory postsynaptic currents (sEPSCs) and total length of apical tuft branches are reduced in layer V pyramidal cells after repeated immobilization stress (20 minutes/d, 7 days). Z-stack projections showing the apical tuft for a layer V pyramidal cell in control (A) and stressed (B) groups. In each case, white boxes outline a distal branch, expanded in top right of projection image. sEPSC responses to hcrt are shown at bottom right of projection images. Bar graphs (C) give mean showing reduced total apical tuft branch length associated with decreased frequency of hcrt-induced sEPSCs. These changes are accompanied by a decrease in dendritic spine density and an increase in segmentation/beading at apical tuft dendritic tips; * $P < .05$, **$P < .01$. 

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responses to hypocretin, suggesting a deficit in thalamic inputs that normally innervate the apical dendrite. Post-hoc morphological analysis in the labeled cells revealed a similar magnitude of reduction in total apical tuft dendritic branch length. Specific morphological changes included a decrease in dendritic spine density and an increase in bead-like segmentation in distal branches of the apical tuft because they extend through layer I to the pial membrane. Chronic administration of the stress-elevated hormone corticosterone mimicked both the electrophysiological and morphological changes observed with stress.

Recent work from Winsky-Sommerer et al. shows that activation of the excitatory CRF inputs to hypocretin neurons can increase stress-related arousal. The reduction of hypocretin responses that we see in prefrontal layer V neurons after chronic stress could result from a neurobiological adaptation to ameliorate stress-induced hyperarousal.

**Conclusions**

Hypocretin can excite the thalamocortical arousal pathway at 2 levels. It selectively depolarizes neurons in the midline-intralaminar nuclei of the thalamus, and it also directly excites the axon terminals of these cells within prefrontal cortex. Because the hypocretin responses are markers of characterized thalamocortical synaptic inputs to mPFC, this suggests that apical dendritic atrophy and the associated loss of excitatory synapses may contribute to stress-related attentional and other cognitive deficits that occur in schizophrenia. Taken together with evidence for diminished connectivity between the midline thalamus and prefrontal cortex in schizophrenia, stress-induced dendritic atrophy could further exacerbate a breakdown in cortical processing of incoming information from the ascending arousal system. In turn, atrophic changes in reciprocal corticothalamic connections could disrupt normal feedback regulation of sensory filtering at a thalamic level.

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**References**


