Orexin Projections of the Hypothalamus

I. Indirect Projections from the Suprachiasmatic Nucleus to Major Arousal-Promoting Cell Groups In Rat: Implications for the Circadian Control of Behavioural State. (2005)
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Abstract—The circadian clock housed in the suprachiasmatic nucleus (SCN) controls various circadian rhythms including daily sleep–wake cycles. Using dual tract-tracing, we recently showed that the medial preoptic area (MPA), subparaventricular zone (SPVZ) and dorsomedial hypothalamic nucleus (DMH) are well positioned to relay SCN output to two key sleep-promoting nuclei, namely, the ventrolateral and median preoptic nuclei. The present study examined the possibility that these three nuclei may link the SCN with wake regulatory neuronal groups. Biotinylated dextran-amine with or without cholera toxin B subunit was injected into selected main targets of SCN efferents; the retrograde labeling in the SCN was previously analyzed. Here, anterograde labeling was analyzed in immunohistochemically identified cholinergic, orexin/hypocretin-containing and aminergic cell groups. Tracer injections into the MPA, SPVZ and DMH resulted in moderate to dense anterograde labeling of varicose fibers in the orexin field and the tuberomammillary nucleus. The locus coeruleus, particularly the dendritic field, contained moderate anterograde labeling from the MPA and DMH. The ventral tegmental area, dorsal raphe nucleus, and laterodorsal tegmental nucleus all showed moderate anterograde labeling from the DMH. The substantia innominata showed moderate anterograde labeling from the MPA. These results suggest that the MPA, SPVZ and DMH are possible relay nuclei for indirect SCN projections not only to sleep-promoting preoptic nuclei as previously shown, but also to wake-regulatory cell groups throughout the brain. In the absence of major direct SCN projections to most of these sleep/wake-regulatory regions, indirect neuronal pathways probably play an important role in the circadian control of sleep–wake cycles and other physiological functions.

Fig. 2. Examples of retrograde labeling in the SCN and anterograde labeling in selected centers of the arousal system following BDA+CTB injection into the MPA (A–D; case 482) and the SPVZ (E–H; case 517). All images are ipsilateral to the injection site (i.e. right side of the brain). Photomicrographs of the injection site revealed for CTB and for BDA in case 482 have been shown in Deurveilher et al. (2002). (A) In the SCN, CTB-labeled neurons (arrows) are distributed mostly in the shell rather than the core region. The shell and core regions are depicted in dotted lines based on vasopressin and NPY immunoreactivity, respectively, from a separate series of sections. Several retrogradely labeled neurons are also seen in adjacent regions, particularly dorsomedial to the SCN. (B) In the basal forebrain, BDA-labeled fibers and terminals, rated as “moderate-dense” in density, are present in the substantia innominata. Most terminals appear to be of the en passant type. (C) In the perifornical area, BDA-labeled varicose axons (arrows) are mixed with preprohypocretin-ir or orexin perikarya (brown cells). (D) In the dorsal tuberomammillary nucleus, many BDA-labeled bouton-like swellings (arrows) are closely associated with HDC-ir or histaminergic neurons (brown cells). The inset shows one such HDC-ir neuron (arrowhead) along with a closely apposing BDA-labeled varicose axon at a higher magnification. (E) BDA injection site in the SPVZ. The hole at the center of the injection site is an artifact due to tissue eck. (F) In the SCN, CTB-labeled neurons are present in both the shell and core regions (examples of labeled neurons indicated by black and white arrows, respectively). Note that the SCN core contains NPY-ir fibers (brown). (G) In the perifornical area, most BDA-labeled terminals (black) do not show obvious association with orexinergic or preprohypocretin-ir perikarya (brown cells). A retrogradely labeled neuron is also present (black cell). (H) In the locus coeruleus, a few BDA-labeled fibers (arrows) are seen in the dendritic (LCD) and the core regions of the locus coeruleus (LCC; brown somata and dendrites) visualized with TH immunoreactivity. For other abbreviations, see Table 1. Scale bars 100 m (A–D, F); 500 m (E); 50 m (G, H); 20 m (inset in D).
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Hypocretinergic (orexinergic) neurons in the lateral hypothalamus project to motor columns in the lumbar spinal cord. Consequently, we sought to determine whether the hypocretinergic system modulates the electrical activity of motoneurons. Using in vivo intracellular recording techniques, we examined the response of spinal motoneurons in the cat to electrical stimulation of the lateral hypothalamus. In addition, we examined the membrane potential response to orthodromic stimulation and intracellular current injection before and after both hypothalamic stimulation and the juxtacellular application of hypocretin-1. It was found that (1) hypothalamic stimulation produced a complex sequence of depolarizing–hyperpolarizing potentials in spinal motoneurons; (2) the depolarizing potentials decreased in amplitude after the application of SB-334867, a hypocretin type 1 receptor antagonist; (3) the EPSP induced by dorsal root stimulation was not affected by the application of SB-334867; (4) subthreshold stimulation of dorsal roots and intracellular depolarizing current steps produced spike potentials when applied in concert to stimulation of the hypothalamus or after the local application of hypocretin-1; (5) the juxtacellular application of hypocretin-1 induced motoneuron depolarization and, frequently, high-frequency discharge; (6) hypocretin-1 produced a significant decrease in rheobase (36%), membrane time constant (16.4%), and the equalizing time constant (23.3%); (7) in a small number of motoneurons, hypocretin-1 produced an increase in the synaptic noise; and (8) the input resistance was not affected after hypocretin-1. The juxtacellular application of vehicle (saline) and denatured hypocretin-1 did not produce changes in the preceding electrophysiological properties.

We conclude that hypothalamic hypocretinergic neurons are capable of modulating the activity of lumbar motoneurons through presynaptic and postsynaptic mechanisms. The lack of hypocretin-induced facilitation of motoneurons may be a critical component of the pathophysiology of cataplexy.

Figure 3. The depolarizing responses of lumbar motoneurons to hypothalamic stimulation decrease in amplitude after the juxtacellular application of SB-334867, an antagonist of Hcrt type 1 receptors. The stimulation of the perifornical hypothalamus induced a depolarizing response that consisted of early and late EPSPs (A, straight and curved arrows, respectively). After the application of SB-334867 onto the recorded motoneuron, the early and late hypothalamically induced EPSPs decreased by 46.8 and 37.1%, respectively. No evident change occurred in the late IPSP after the application of SB-334867. Whereas SB-334867 decreased the synaptic response to hypothalamic stimulation (A and top traces in B and C), it did not affect the reflex EPSP (bottom traces in B and C). The records in A were obtained from a hamstrings motoneuron using a K-citrate-filled micropipette; those in B and C were obtained from a triceps surae cell using a KCl-filled micropipette. Each trace is an average of 10–20 sweeps.
Orexin-expressing neurons in the hypothalamus project throughout the neuraxis and are involved in regulation of the sleep/wake cycle, food intake, and autonomic functions. Here we specifically analyze the anatomical organization of orexin projections to the dorsal vagal complex (DVC) and raphé pallidus and effects on ingestive behavior and autonomic functions of local orexin-A administration in nonanesthetized rats. **Retrograde tracing experiments revealed that as many as 20% of hypothalamic orexin neurons project to the DVC, where they form straight varicose axon profiles, some of which are in close anatomical apposition with tyrosine hydroxylase (TH), glucagon-like peptide-1, γ-aminobutyric acid-, and nitric oxide synthase-immunoreactive neurons in a nonselective manner. Similar contacts were frequently observed with neurons of the nucleus of the solitary tract whose activation by gastrointestinal food stimuli was demonstrated by the expression of nuclear c-Fos immunoreactivity.** Orexin-A administration to the fourth ventricle induced significant Fos-expression throughout the DVC compared with saline control injections, with about 20–25% of TH-ir neurons among the stimulated ones. Fourth ventricular orexin injections also significantly stimulated chow and water intake in nonfood-deprived rats. Direct bilateral injections of orexin into the DVC increased intake of palatable high-fat pellets. Orexin-ir fibers also innervated raphe’ pallidus. Fourth ventricular orexin-A (1 nmol) activated Fos expression in the raphé pallidus and C1/A1 catecholaminergic neurons in the ventral medulla and increased body temperature, heart rate, and locomotor activity. The results confirm that hypothalomedullary orexin projections are involved in a variety of physiological functions, including ingestive behavior and sympathetic outflow.
Hypocretin/orexin neurons give rise to an extensive projection system, portions of which innervate multiple regions associated with the regulation of behavioral state. These regions include the locus coeruleus, medial septal area, medial preoptic area, and substantia innominata. Evidence indicates that hypocretin modulates behavioral state via actions within each of these terminal fields. To understand better the circuitry underlying hypocretin-dependent modulation of behavioral state, the present study characterized the degree to which there exists: 1) lateralization of hypocretin efferents to basal forebrain and brainstem arousal-related regions, 2) topographic organization of basal forebrain- and brainstem-projecting hypocretin neurons, and 3) collateralization of individual hypocretin neurons to these arousal-related terminal fields. These studies utilized combined immunohistochemical identification of hypocretin neurons with single or double retrograde tracing from the locus coeruleus, medial preoptic area, medial septal area, and substantia innominata. Results indicate that approximately 80% of hypocretin efferents to basal forebrain regions project ipsilaterally, whereas projections to the locus coeruleus are more bilateral (65%). There was a slight preference for basal forebrain-projecting hypocretin neurons to be distributed within the medial half of the hypocretin cell group. In contrast, hypocretin neurons projecting to the locus coeruleus were located primarily within the dorsal half of the hypocretin cell group. Finally, a large proportion of hypocretin neurons appear to project simultaneously to at least two of the examined terminal fields. These latter observations suggest coordinated actions of hypocretin across multiple arousal-related regions.

Fig. 7. Photomicrographs depicting retrogradely labeled and prepro-HCRT-ir neurons from one rat infused with FG in MPOA and with WGA in LC. Shown are FG labeled (FG; blue), prepro-HCRT-ir (HCRT; brown), and WGA labeled (WGA; black granules) within the perifornical region of the lateral hypothalamus. Boxes with corresponding letters in A indicate neurons displayed at higher magnification in B and C. A: Photomicrograph depicting individual HCRT neurons that project to MPOA (FG-HCRT), LC (WGA-HCRT), or both MPOA and LC (TRIPLE) as well as non-HCRT neurons that project to MPOA (FG). B,C: Photomicrographs of the same section as in A. Scale bars 50 m in A; 20 m in B,C.
V. Exclusive Postsynaptic Action of Hypocretin-Orexin on Sublayer 6b Cortical Neurons.
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The hypocretin–orexin (hcrt–orx) neurons are thought to maintain wakefulness because their loss results in narcolepsy. This role may be fulfilled by the excitatory action that the hcrt–orx peptide exerts on multiple brainstem and forebrain systems that, in turn, promote cortical activation. Here, we examined whether hcrt–orx may also exert a postsynaptic excitatory action at the level of the cortex, where hcrt–orx fibers project. However, we found that neurons in layers 2–5 in the primary somatosensory cortex (SSp) were unresponsive to hcrt–orx. We then found that although all neurons tested in sublayer 6a were also unresponsive to hcrt–orx, all those tested in sublayer 6b were highly sensitive to the peptide. The sublayer selectivity of hcrt–orx was not restricted to the somatosensory cortex, because it was also found to be present in the primary visual cortex, the motor cortex, and the cingulate cortex. In the SSp, in which the hcrt–orx effect was investigated further, it was demonstrated to be postsynaptic, to result from an interaction with Hcrtr2–OX2 receptors and to depend on the closure of a potassium conductance. Similar to the selectivity of action in the thalamus, where hcrt–orx excites the nonspecific thalamocortical projection neurons and not the specific sensory relay neurons, here in the cortex, it excites a specific subset of cortical neurons which, through corticocortical projections, may also be involved in promoting widespread cortical activation.

Figure 1. Exclusive action of hcrt–orx on cortical neurons of sublayer 6b in the SSp. A, Toluidin blue counterstained cortical slice slab containing two recorded neurons (arrowheads) labeled with neurobiotin in sublayers 6a and 6b (separated by a horizontal band of fibers; *) and the responses of which to hcrt–orx are shown in D and E. B1, Absence of response to hcrt–orx of a neuron in layer 2/3. B2, Summarized results showing that all neurons of layer 2/3 were unresponsive to hcrt–orx. n represents the total number of cells tested. C1, Absence of response to hcrt–orx of a neuron in layer 4/5. C2, Summarized results showing that most neurons of layer 2/3 were unresponsive to hcrt–orx (see Results). D1, Absence of response to hcrt–orx of a neuron in layer 6a. D2, Summarized results showing that most neurons of layer 6a were unresponsive to hcrt–orx (see Results). E1, Depolarizing response to hcrt–orx of a neuron in layer 6b. E2, Summarized results showing that all neurons of layer 6b were depolarized by hcrt–orx. F1, F2, Enlargement of neurons 1 and 2 from A. Scale bar, 15 m. G, Increase in PSPs in a layer 5 neuron (middle panel) is impeded in the presence of a low calcium–high magnesium ACSF. Calibration: 5 mV, 2 sec. CC, Corpus callosum.

Figure 4. Exclusive action of hcrt–orx in sublayer 6b in primary visual cortex, primary motor cortex, and cingulate cortex. A, B, Excitatory action of hcrt–orx on sublayer 6b neurons of the primary visual cortex (A), primary motor cortex (B1), and cingulate cortex (B2). Scale bar, 15 m (in all insets showing infrared images of recorded cells). AQ, Aqueduct; CP, caudate–putamen; DG, dentate gyrus; LS, lateral septum; LV, lateral ventricle; PAG, periaqueductal gray.