

## Normal Role of Hypocretin/Orexin

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### I. Introduction

There is now no doubt that hypocretin/orexin (Hcrt) deficiency is the cause of most narcolepsy in humans (1,2). It is also clear that mutations of the Hcrt system cause genetic narcolepsy in several animal species (3,4). Narcolepsy in these Hcrt deficiency situations is characterized by sleepiness during the normally active period and losses of muscle tone during waking periods called cataplexy. We know from observations in humans and in canine narcoleptics that such cataplectic episodes are accompanied by unimpaired consciousness, and it is reasonable to expect that this is also the case in rodent Hcrt mutant models.

These findings lead to the conclusion that one of the functions of Hcrt is to prevent the symptoms of narcolepsy. In this chapter I will address the question of how this function of Hcrt might be achieved and consider evidence bearing on a more general role of Hcrt in behavioral control. It is unlikely that simply defining the differences in behavior between narcoleptic and normal individuals will illuminate all of the functions of Hcrt. It is virtually certain that a variety of brain systems compensate for the postnatal loss of Hcrt in humans by occupying vacated synaptic sites and by up and down regulation of receptors whose activity is altered by the loss of Hcrt. It is also likely that Hcrt mutants have developmental alterations that modify, and perhaps ameliorate, the effects of Hcrt mutations, thus masking some of the normal functions of Hcrt.

An effective alternate strategy for determining the role of Hcrt is to observe fluctuations in its release in normal animals, in which the role will not be obfuscated by compensatory reorganization. Such observations, including studies of release using microdialysis and cerebrospinal fluid (CSF) assays, will indicate when this peptide is released. Ultimately, it may be possible to bridge the gulf between information gained from such observations and the pathology of narcolepsy, by better understanding the neurological adjustments that result from Hcrt dysfunction.

One early theory of Hcrt function was that it was an "orexigenic" or appetite-stimulating compound. This was based on initial observations that high doses of Hcrt injected into the lateral hypothalamus, a region whose stimulation is known to induce feeding, increased food intake. However, subsequent infusion studies did not always support these initial observations (5). We tested the hypothesis that

Hcrt was involved in the regulation of food intake by studying the release of Hcrt in dogs under baseline conditions, after 48 hours of food deprivation and at various times after feeding (6). To our surprise, we found that these manipulations had very small effects that were statistically insignificant. However, when these same normal animals were sleep deprived for as little as 24 h, Hcrt levels increased by an average of >70%. Further analysis showed that it was not the sleep loss per se that drove the Hcrt level up. Rather, the activity that was induced to prevent sleep, as measured by actigraphs worn by the dogs, was most tightly correlated with the rise in Hcrt level. We followed up on this finding by increasing motor activity with vigorous play with the animals for up to 2 hours. This again produced a 60% to 70% increase in Hcrt level compared to levels in the same animals during alert waking, with the increase in individual trials correlated with activity level. We conclude from these studies that, in contrast to food deprivation and consumption, motor activity is tightly correlated with Hcrt release (6,7).

This finding is compatible with what we know about the connections of Hcrt cells. The most massive extrahypothalamic projection of the Hcrt system is to the locus coeruleus.<sup>5</sup> We found that microinjection of Hcrt into the locus coeruleus produced a striking increase in muscle tone (7). In other work, we showed that locus coeruleus activity is tightly linked to muscle tone, and that in narcoleptic dogs locus coeruleus cells cease discharge immediately prior to and during cataplexy (8). The locus coeruleus facilitates muscle tone and a group of cells in the medial medulla inhibit muscle tone. These two systems are tightly and reciprocally linked (9). One can therefore see that loss of Hcrt will interrupt a pathway that increases muscle tone by direct facilitation and simultaneous disinhibition.

One can explain cataplexy, even if one does not invoke potential neural reorganizations in Hcrt deficient animals, by hypothesizing that Hcrt neurons are normally activated phasically during certain emotional stimuli to maintain muscle tone. In the absence of this compensatory excitation, the underlying reduction in tone accompanying strong emotional stimuli is revealed. In humans, the most common triggers for cataplexy: laughter, anger and surprise are consistently accompanied by motor changes even in normal individuals, as exemplified by "doubling over with laughter," becoming speechless or stammering with anger, dropping objects when surprised or shocked, and so on. In narcoleptics with cataplexy, these same conditions elicit complete, rather than partial, suppressions of muscle tone.

Our findings and other studies that show a strong relationship between motor activity and Hcrt release are certainly not the last word in the analysis of the behavioral role of Hcrt. Although it appears clear that Hcrt release is elevated during motor activity, many questions remain. Is Hcrt related to all movements or only to particular types of movements? For example, do Hcrt levels increase to the same extent in rhythmic movements such as grooming as they do in exploration? Is Hcrt release associated with, for example, head vs. limb movements? Is Hcrt release more tightly linked to ipsilateral vs. contralateral movements? Does Hcrt level increase in relation to movement intensity? Is Hcrt release related to the emotional aspects or to cognitive changes not present in quiet waking but characterizing motorically active states? Such questions can best be answered by recording from identified Hcrt neurons in freely moving animals, a challenge that has not yet been met.

Whatever the fine-grained analysis of Hcrt activity and motor activity reveals, we do know that Hcrt can act directly on motoneurons. Hcrt neurons project to motoneurons, and when Hcrt is microinjected into motoneuron pools it produces a profound motor excitation. However, this excitation is completely blocked by glutamate antagonists (10). This indicates that Hcrt acts through glutamate at the motoneuronal level. It is possible that Hcrt modulation of glutamate release may be a major mode of Hcrt action. We have shown that intravenous injection of Hcrt produces release of glutamate in Hcrt innervated regions as exemplified by the amygdala, but not in regions not innervated by Hcrt, as exemplified by the cerebellum (11).

Apart from its facilitation of motor systems and its correlated activation of fore-brain waking systems, Hcrt is likely to function in the coordination of monoaminergic and cholinergic systems. Individual Hcrt neurons have projections to multiple aminergic systems, strongly suggesting a coordination role (5).

A key effect of the lack of coordination of monoaminergic cells by Hcrt deficiency can be seen in cataplexy. In narcoleptic dogs, whose cataplexy attacks are behaviorally and pharmacologically indistinguishable from those in human narcolepsy, a striking dyscoordination of monoaminergic activity is seen. In the normal animal, histamine and norepinephrine cells are both tonically active throughout waking. Arousing stimuli activate these cell groups and there is some reduction in activity during quiet waking. In sleep, both cell groups cease activity. A striking finding is that, although these two cell groups increase and decrease discharge more or less simultaneously in all waking-sleep states in normal animals, they do not do this in narcoleptic animals. We have recently shown that in cataplexy, histamine cells increase activity, as might be expected in normal animals during excitement, but norepinephrine cells completely cease activity (12). Thus, either through the direct action of Hcrt on norepinephrine and histamine neurons, or through neurological reorganization subsequent to Hcrt dysfunction, this coordinated activity is lost, resulting in the dissociated firing of norepinephrine and histamine cells that is linked to cataplexy. We hypothesize that the loss of norepinephrine release causes the loss of muscle tone of cataplexy, whereas the maintained activity of histamine cells produces continued consciousness. In contrast the cessation of activity in both of these cell groups results in sleep, with a correlated reduction of muscle tone.

## **II. Conclusion**

Much remains to be done in order to elucidate the role of Hcrt in normal behavior. The Hcrt system interacts closely with monoaminergic and amino acid systems. A link to motor activity is clear, and a link to certain types of motivated behaviors is likely, but the precise relationship underlying these links is not yet understood.

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