# AN APPROACH TO DETERMINING THE FUNCTIONS OF HYPOCRETIN (OREXIN)

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## 1. INTRODUCTION

Many papers on hypocretins/orexins begin with a list of the functions they regulate. Included in these lists are some of the following: sleep, food intake, water intake, gastric acid secretion, blood pressure, heart rate, movement, muscle tone, arousal, release of lutenizing hormone, corticosterone, insulin, growth hormone and prolactin. It is also reported that hypocretin/orexin coordinates monoamine, acetylcholine and amino acid release. Finally it is well established that the loss of hypocretin neurons is linked to narcolepsy (reviewed in ref. 1). One can anticipate that with further research the "laundry list" of hypocretin functions will continue to grow.

Although the finding of links between hypocretin/orexin and a wide range of behaviors and physiological changes, is useful, it is obvious that a simple catalog of hypocretin/orexin relations does not provide a fundamental insight into its function(s). It is analogous to saying that the biceps muscle is involved in eating, drinking, motor activity, sexual behavior; sleep etc., because its activity is strongly modulated during all of these behaviors. In terms of its putative arousal functions, it is not sufficient to say that hypocretin/orexin is arousal related. A number of brain systems are active during arousal.[2,3] Does hypocretin/orexin play a unique role? How does hypocretin's role in each of these behaviors or control mechanisms differ from that of other neurotransmitter systems? How does the loss of hypocretin/orexin explain the symptoms of narcolepsy? Is abnormal hypocretin/orexin function involved in all cases of human narcolepsy, including those with normal hypocretin/orexin levels in the CSF and no mutation of the hypocretin/orexin system?[4,5,6] The theme of this chapter is that some answers are beginning to emerge from the hypocretin/orexin literature, although much remains to be done.

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## 2. ARE HYPOCRETIN CELLS HOMOGENEOUS?

One key question that must be addressed is whether all hypocretin/orexin neurons have the same function or whether there may be subcategories of hypocretin/orexin neurons dedicated to different physiological or behavioral functions. An analogy may be drawn to the dorsal raphe serotonergic, posterior hypothalamic histaminergic and locus coeruleus noradrenergic neurons. Each one of these cell groups has a fairly homogeneous population of cells in terms of size and neurochemical phenotype. Existing evidence suggests that all these cell groups show a similar "sleep-off pattern of discharge, i.e. they discharge tonically during waking, greatly reduce activity in nonREM sleep and cease activity in REM sleep.[7] Although many cells in these groups send multiple axonal projections to more rostral and more caudal regions, there is some specificity. For example more caudally placed locus coeruleus cells are more likely to have caudal projections than more rostrally located cells.[8,9] It should also be noted that although cells in these cell groups may have similar projections, local presynaptic mechanisms may strongly modulate release,[10,11] so that for example serotonergic cells may release 5HT on one side of the brain but not on the other.[12]

We need to consider the possibility that the hypocretin/orexin cell population may also have subgroups with different projection and perhaps even different activity patters and behavioral/physiological relations. In our early human work we measured cell size and found that the different hypocretin/orexin subpopulations had differing mean sizes. For example the lateral hypocretin/orexin cells were approximately 80 % larger in cross-sectional area than the dorsomedial cell group (Nienhuis and Siegel, unpublished data), hypocretin/orexin release may be modulated at target zones independently of discharge rate, as is the case with dopamine, serotonin containing and other cell groups. Currently the only studies of hypocretin/orexin release have looked at CSF levels or microdialysates in specific brain regions.[13] These observations necessarily reflect the overall changes in hypocretin/orexin release. However, individual hypocretin/orexin neurons could have discharge or release patterns which differ from this overall pattern. Until we can identify individual hypocretin/orexin neurons *in vivo* we will not be able to directly address these questions. Nevertheless studies of hypocretin/orexin release provide important clues to the nature of hypocretin/orexin release.

## 3. REGULATION OF HYPOCRETIN/OREXIN RELEASE

In studies of normal and of narcoleptic dogs, we have found that hypocretin/orexin level is not simply a property of a given animal or a function of time of day. Rather, it is closely tied to behavior.

Because of the link between hypocretin/orexin and narcolepsy, we first investigated the effects of sleep deprivation and consequent sleepiness on hypocretin/orexin level. We found that sleep deprivation for 24 hours, executed by walking the dogs whenever they began to go to sleep produced a 70% increase in hypocretin/orexin levels relative to dogs whose exercise was "yoked" to that of the experimental animals.[14] We measured activity actigraphically and found, not surprisingly, that the experimental animals had fewer periods of extended inactivity. To control for the increased activity of the sleep deprived animals, we compared animals that were kept awake for a 2 hour period, an interval that does not require forced locomotion, to animals that were active in a yard for the same 2

hour period. We found that this manipulation produced the same elevation of hypocretin/orexin level produced by sleep deprivation (Figure 1). We found the same increase in hypocretin/orexin level with activity in the normal cat.[13] Thus, the most parsimonious conclusion is that the activity rather than the sleep loss was the cause of the elevation of hypocretin/orexin level after sleep deprivation.

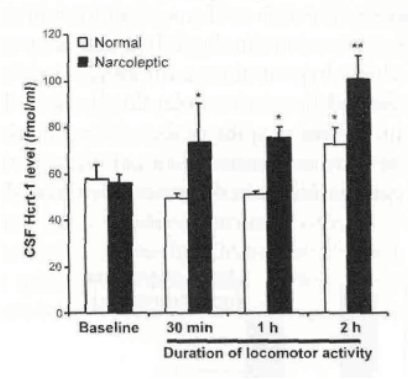


**Figure 1** Exercise elevates hypocretin/orexin levels measured in the cerebrospinal fluid in both normal and narcoleptic dogs.[14]

We next subjected normal dogs to 48 h of food deprivation (we used dogs in these studies because of the ease of drawing adequate volumes of CSF from dogs in comparison to smaller animals). "Orexigenic" compounds (i.e. compounds stimulating eating), such as neuropeptide Y increase in concentration with food deprivation (reviewed in ref 1). We found no such increase in hypocretin/orexin level with food deprivation (Figure 2).

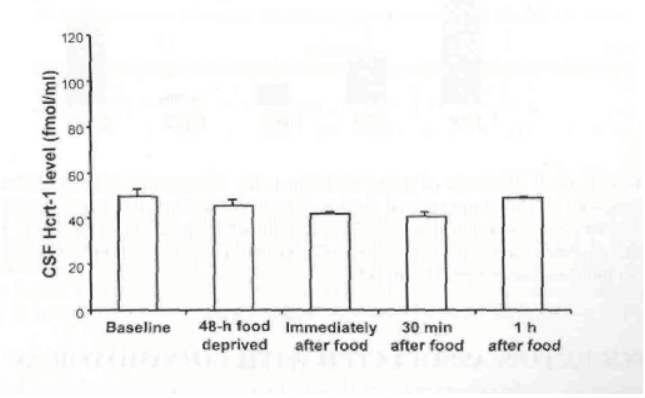


**Figure** 2. Food deprivation does not alter hypocretin level, and eating after food deprivation does not significantly alter hypocretin/orexin level in contrast to other "orexigenic" compounds whose levels are significantly elevated with food deprivation.[14] A similar elevation of hypocretin/orexin level with motor activity was observed in normal cats. [13]

We also saw no significant change in hypocretin/orexin level after feeding at the end of the deprivation period.[14] These results are inconsistent with the hypothesis that hypocretin/orexin release is tightly linked to food intake. However, in the context of our activity findings, if a food-deprived animal became more active under conditions of food deprivation, it would be predicted that hypocretin/orexin level would rise. Other data that is inconsistent with an orexigenic role of hypocretin/orexin is the lack of anorexia or reduced weight in the hypocretin/orexin ligand knockout mouse,[15] the obesity of the ataxin mutant mouse in which hypocretin/orexin cells degenerate postnatally[16] and the obesity tendency in unmedicated human narcoleptics.[17] Both the narcoleptic human and ataxin mutant animals gain weight despite *reduced* food intake.[1] This obesity can be explained by the reduced activity and consequent caloric expenditure, but is inconsistent with the hypothesis that hypocretin/orexin deficient mice would be anorexic.

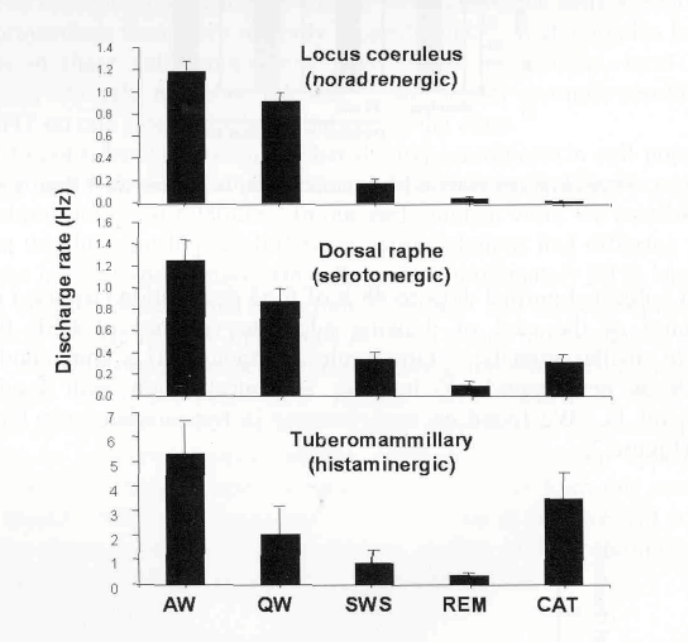


**Figure** 3. Sleep waking cycle discharge of monoaminergic cells. Monoaminergic cells behave similarly across normal sleep cycles, with all showing maximal discharge in active waking, decreased discharge in quiet waking, greatly reduced discharge in sow wave sleep (SWS) and minimal discharge in REM sleep. In cataplexy, noradrenergic locus coeruleus cells are "off," whereas histaminergic cells are "on" and serotonergic dorsal raphe cells have an intermediate pattern (From ref 7).

## 4. ARE HYPOCRETINS ASSOCIATED WITH LOCOMOTOR ACTIVITY?

A final point of reference for developing hypotheses as to the underlying function(s) of hypocretin/orexin cells is careful observation of animals and humans without hypocretin/orexin neurons or with mutations affecting hypocretin/orexin release or postsynaptic response to the peptide. These are narcoleptic animals. Genetically narcoleptic dogs do not weigh less than age and breed matched controls (unpublished

observations (John, Wu and Siegel). It is unclear if they move less than controls, but clearly they do not exhibit the prolonged periods of activity that characterize normal animals. A typical symptom of human narcolepsy is periods of daytime immobility (i.e. naps) coupled with interrupted nighttime sleep.[18] A second symptom of narcolepsy is sudden losses of muscle tone, without loss of consciousness (cataplexy). These losses are linked to cessation of activity in locus coeruleus cells),[19] reduced activity in serotonergic cells[20] and maintained or increased activity in histaminergic cells (Figure 3). They are also linked to activation of medial medullary motor inhibitory cells (Figure 4).[21]

All of these motor links support an underlying connection of hypocretin/orexin release to motor activity. This link can explain many of the phenomena attributed to hypocretin/orexin cells. It is important to measure motor activity and muscle tone in any in vivo examination of hypocretin/orexin release correlates. Only such measurements can separate motor activity from other putative correlates of hypocretin/orexin activity.

Although the data discussed above link hypocretin/orexin release to motor activity, they still leave important questions unanswered. What aspect of motor activity is most closely linked to hypocretin/orexin release? Is it the types of movement, rhythmic vs.

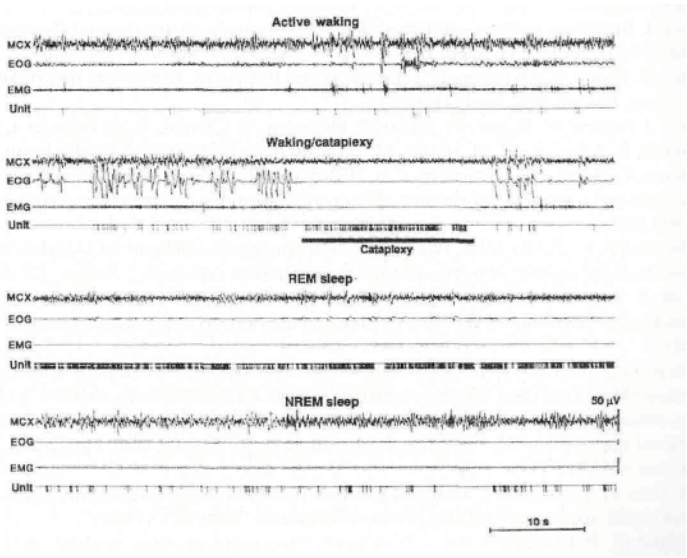


**Figure** 4. Medullary cataplexy-on cells. Most brainstem cells are active in waking and REM sleep. However the "cataplexy-on" cell type illustrated is inactive during waking with movements but is maximally active in REM sleep and immediately prior to and during cataplexy attacks in narcolepsy. These cells are likely to trigger cataplexy by active inhibition of motoneruons, acting in concert with disfacilitation produced by the cessation of activity in noradrenergic cells during cataplexy and REM sleep, as is shown in Figure 3 (From ref 21).

exploratory, rapid vs. slow? Is muscle tone itself the key variable? Is it simply the level of muscle tone? Is it the velocity of movement? Is it the continuity of movement? Is it the emotions that accompany activity? Is it the alertness that accompanies movement? The last two questions might be addressed by comparing the hypocretin/orexin release in animals moving at differing speeds on a treadmill. If movement per se is the key element there should be a lawful relationship. If it is the accompanying excitement, one might

expect small or even absent increases in hypocretin/orexin release with prolonged rhythmic movement.

More data are needed to separate these and other potential correlates of hypocretin/orexin cell activity. Current in vivo studies of such phenomena have been performed by CSF extraction of by microdialysis. Such techniques do not permit a fine-grained analysis of hypocretin/orexin cell activity. Thus an important advance would be the in vivo recording of identification of hypocretin/orexin cells in relation to behavior. Techniques to identify such cells in vivo using the results of pioneering in vitro work are becoming available. We can look forward to a clarification of the underlying function(s) of hypocretin/orexin cells once these techniques are successful.

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