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The Use of Saporin Conjugates to Dissect Neurons Responsible for Sleep and Wakefulness

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INTRODUCTION

Virtually all organisms manifest regular periods of behavioral quiescence and activity. In mammals and birds, these periods have evolved into regular episodes of wakefulness and sleep. The sleep period itself has further differentiated into two distinct states, slow wave sleep (SWS) and rapid eye movement (REM) sleep. In humans, SWS has further differentiated into four distinct stages, each identified by a specific pattern on the electroencephalogram (EEG). REM sleep is very similar to wakefulness in many ways; the main difference is that there is behavioral quiescence during REM sleep. For this reason, REM sleep is often referred to as *paradoxical sleep*.

The normal human sleep pattern is characterized by a progression through the four SWS stages, followed by REM sleep. This pattern lasts for about 90 min and is repeated about four to five times during the night. Sleep occupies nearly a third of our life. During this period, we are totally unproductive, useless, and defenseless. Yet, we cannot survive without sleep *(1)*.

What brain region is responsible for generating these states of consciousness? To answer this question, researchers have relied on the classical "slash-and-burn" method. Historically, the transection and electrolytic lesion method quickly identified the regions of the brain subserving wakefulness, SWS, and REM sleep. The weakness of this method is that it destroys fibers

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of passage (transection), and specific neurons cannot be identified. Excitatory amino acids, such as ibotenic and kainic acid, are improvements in that they lesion cells without damaging fibers of passage. However, the disadvantage is that the lesion cannot be limited to a particular cell type, only to a region. Indeed, we have begun to realize that it is necessary to target a specific neuronal phenotype because neurons serving states of consciousness are intermingled with neurons responsible for other functions.

The saporin conjugate is a marked improvement over other lesion methods in that a specific cell type can be targeted *(2,3)*. Elsewhere in this book, other investigators have discussed advantages of saporin, and we respectfully refer the reader to those chapters. In our efforts, we are using saporin-based neurotoxins to lesion specific neuronal phenotypes and thereby create a circuit model of sleep generation. For instance, 192 immunoglobulin G-saporin (192 IgG-sap) lesions basal forebrain (BF) cholinergic neurons, and antidopamine β-hydroxylase-saporin lesions only noradrenergic neurons such as those of the locus coeruleus (LC). Both types of neurons have been proposed as key components to sustain alertness.

HYPOCRETIN–OREXIN–SAPORIN CONJUGATE

We have now created a new saporin conjugate, hypocretin 2-saporin (HCRT-2-sap), to lesion HCRT/OX (orexin) receptor-containing neurons *(4)*. The impetus for creating this neurotoxin was the discovery that the neuropeptide hypocretin, which is also known as orexin (OX), was linked to the sleep disorder narcolepsy *(5,6)*. HCRT/OX was discovered by two independent groups using different approaches *(7–9)*. In the central nervous system, HCRT/OX-containing neurons are located only in the lateral hypothalamus, from where they project to the entire brain and spinal cord, providing especially heavy innervation to the arousal populations.

Two OX receptors have been identified, and the distribution of the receptor messenger ribonucleic acid (mRNA) *(10,11)* and protein *(12)* have been determined. Orexin 1 (HCRT-1) receptor (OX-1R/HCRT-1R) mRNA is more abundant in ventromedial hypothalamic nucleus, hippocampal formation, dorsal raphe, and LC. In the rat, OX-2R mRNA is mainly expressed in cerebral cortex, nucleus accumbens, subthalamic and paraventricular thalamic nuclei, and posterior pretectal nuclei *(10,13)*. In the pontine brainstem, OX-1Rs are located in the LC *(13)*. The LC receives the heaviest projection of OX-containing fibers, and intraventricular administration of OX A or HCRT-2/OX B excites LC neurons *(14,15)*. OX-containing terminals are also found in areas implicated in wakefulness, such as the LC, tuberomammillary nucleus (TMN), the dorsal raphe, and the BF *(7)*. Because