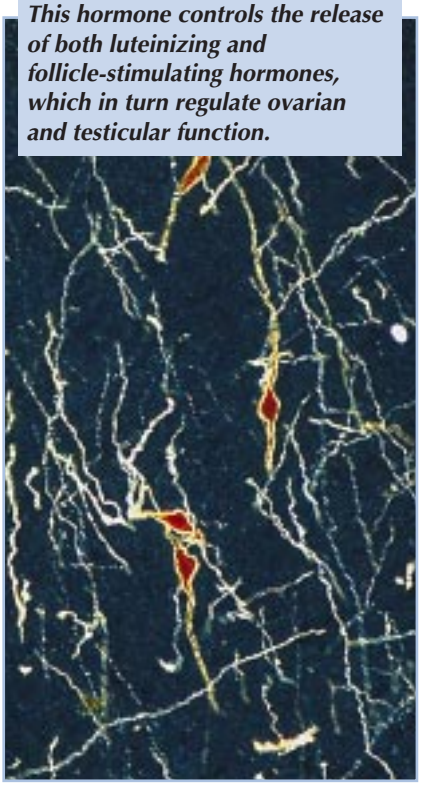
BIOPHOTONICS POST SCRIPTS

by Benjamin D. Butkus, Associate News Editor

Imaging on a gland scale



A dark-field image reveals axons (yellow) and cell bodies (red outlined in yellow) of reproductive neuroendocrine neurons, responsible for producing luteinizing hormone-releasing hormone, also known as LH-RH. This hormone controls the release of both luteinizing and follicle-stimulating hormones, which in turn regulate ovarian and testicular function.

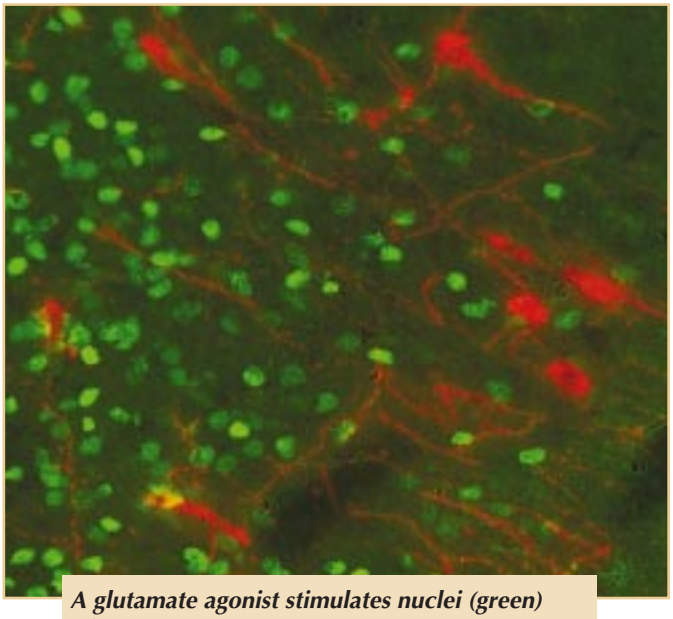
The body's messenger system comprises two main couriers: the neural network, which effects immediate, short-term, localized change, and the endocrine system, which induces more delayed, long-term changes throughout the body. Gloria E. Hoffman and colleagues at the University of Maryland School of Medicine in Baltimore are studying the important interplay between these two agents of change, broadly referred to as the neuroendocrine system.

Specifically, the researchers are attempting to solve two mysteries of the neuroendocrine system. First, by studying its central nervous system command center, the hypothalamus, they are attempting to elucidate sexual dimorphism in neurons and how the brain regulates the reproductive neuroendocrine cycle — or, in simpler terms, why females have hormonal cycles and males don't. Second, they are examining how reproductive hormones protect the brain during injurious states such as temporal lobe epilepsy and multiple sclerosis.

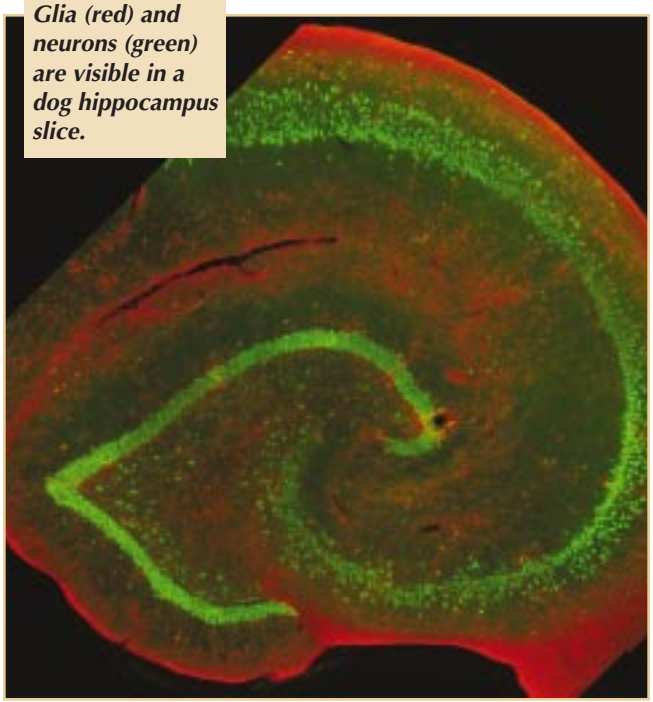
The scientists search for the answers to such questions by capturing images of brain tissue from various organisms. They use a SensiCamQE high-performance CCD camera from The Cooke Corp. of Auburn Hills, Mich., in conjunction with techniques that include spinning-disk confocal fluorescence microscopy with a broad palette of fluorophores, such as the Cy and Alexa dyes, as well as dark-field, Nomarski and bright-field microscopy — either alone or in combination with fluorescence.

Hoffman said that the camera's sensitivity is particularly important for low-light techniques such as spinning-disk confocal and dark-field microscopy, while its resolution helps them identify hard-to-distinguish structures such as clusters of messenger RNA in the cytoplasm and nucleus, which provide evidence of specific gene activity. In addition, the camera's image-capture speed allows them to quickly acquire and integrate color images with a liquid crystal adapter, essentially providing a live multiple-color image.

All of the accompanying images were captured with the camera. □



A glutamate agonist stimulates nuclei (green) of select LH-RH-producing neurons (red).



Glia (red) and neurons (green) are visible in a dog hippocampus slice.

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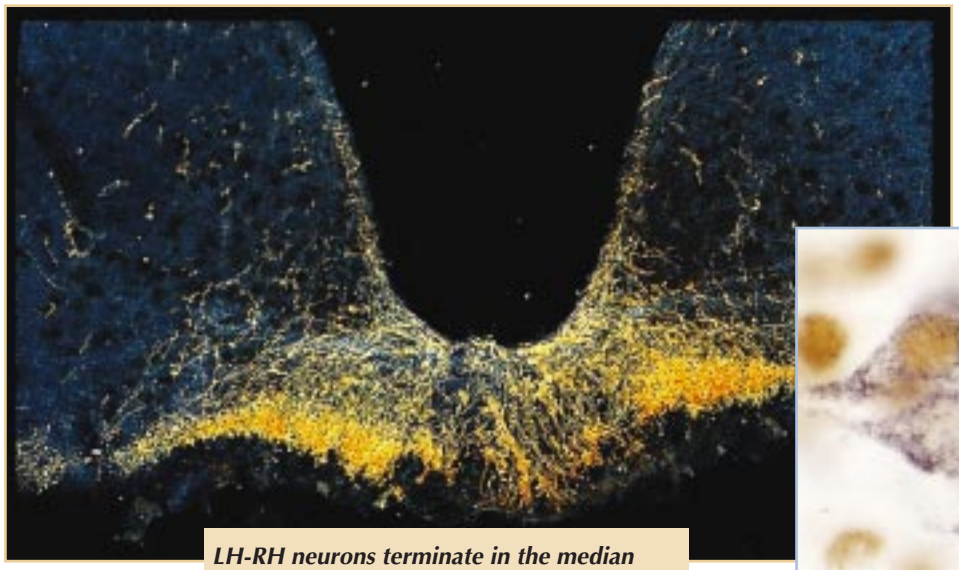
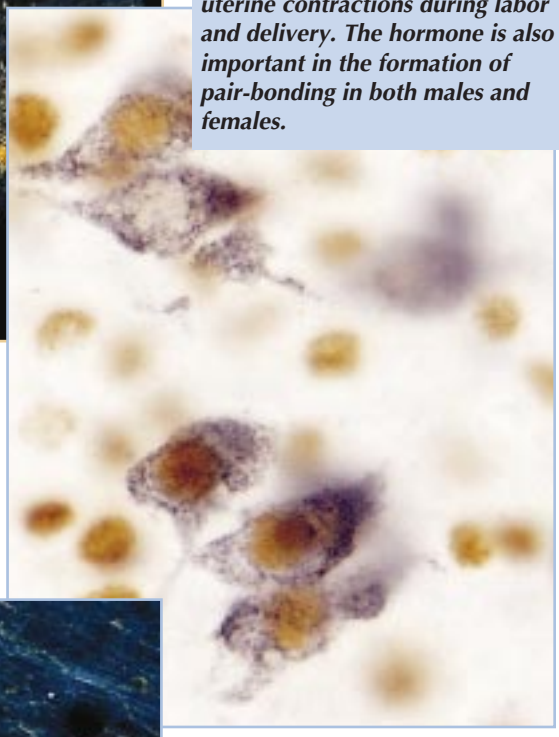
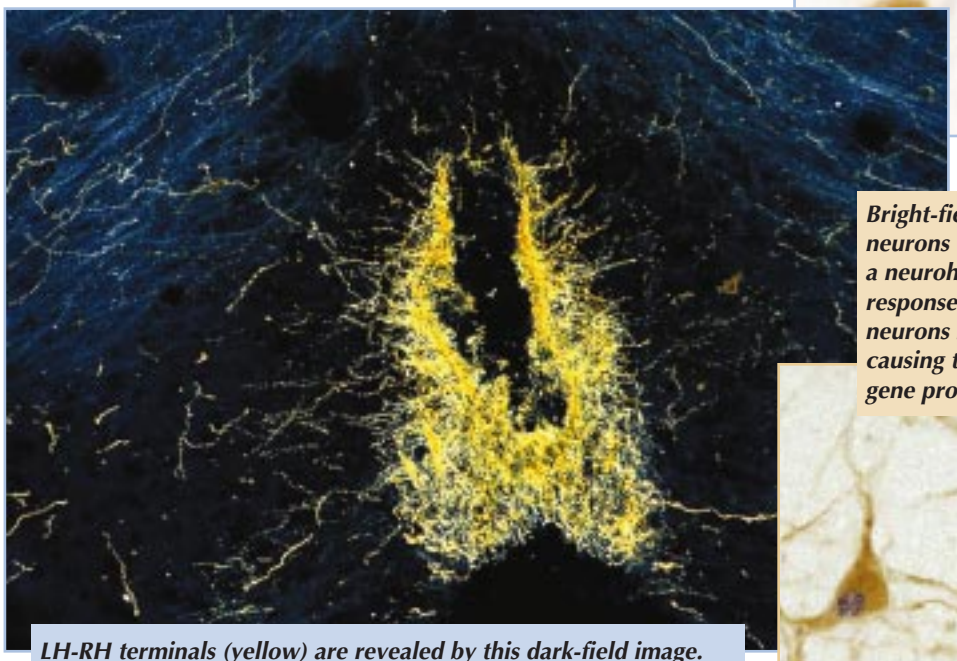


LH-RH neurons terminate in the median eminence of the hypothalamus (dense yellow-orange). In the median eminence, blood vessels drain into the anterior pituitary gland, where LH-RH stimulates the release of luteinizing and follicle-stimulating hormones.

Stimulation of neurons expressing the mRNA for the hypothalamic hormone oxytocin (blue grains are RNA clusters) results in the expression of the gene for the Fos protein (brown nuclei), which is often associated with increased neuronal firing. Oxytocin is required for the milk letdown reflex during lactation and for uterine contractions during labor and delivery. The hormone is also important in the formation of pair-bonding in both males and females.





LH-RH terminals (yellow) are revealed by this dark-field image. The terminals are abundant in the organum vasculosum of lamina terminalis. This area is the remnant of a neural tube region that, like the median eminence, lies outside the blood-brain barrier and functions as one of the brain's "windows" for conveying signals from the periphery.

Bright-field imaging of the brain stem shows neurons containing norepinephrine (brown), a neurohormone usually released in response to hypotension or stress. Some neurons have been stimulated by hypoxia, causing them to express an immediate early gene product (black nuclei).