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Multiphoton imaging of the glutamatergic signaling dynamics in the suprachiasmatic nucleus.

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**Multiphoton imaging of the glutamatergic signaling dynamics in the suprachiasmatic nucleus.**

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**Abstract**

Circadian rhythms in mammals are generated and controlled by the suprachiasmatic nucleus (SCN). Signals of solar cycles are transmitted to the SCN via the monosynaptic retino-hypothalamic tract that releases neurotransmitter glutamate to activate the SCN acceptor cells. In the SCN brain slice preparations glutamate application resets the clock with a phase delay early night and phase advance late night<sup>1</sup>. Early night glutamate induces a phase delay via the release of the gaseous messenger nitric oxide<sup>1</sup> and, consequently, intracellular calcium release from ryanodine channels (CICR)<sup>2</sup>. We utilized the method of the multiphoton microscopy of the adult rat brain slice to visualize the effects of glutamate on the key components of the glutamate-induced phase delay signaling. Glutamate-induced calcium transients were visualized with calcium fluorescence dyes, Calcium Green and Fluo 4. Nitric oxide release was visualized with DAF fluorescence dye. The morphological organization of the SCN cells in the living brain slice was studied with the membrane dye Laurdan, and these data were used to estimate the size of the structures visualized with calcium and NO fluorescence probes in the SCN. These novel approaches to imaging the dynamics of the glutamatergic signaling cascade in living adult brain slices reveal new mechanistic insights. Supported by: NS33240 and NS35859. (1.) Ding, J. M. et al. *Science* 266, 1713-1717 (1994). (2.) Ding, J. M. et al. *Nature* 394, 381-384 (1998).