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Neuroscience Letters 401 (2006) 209–213
Neuroscience Letters
www.elsevier.com/locate/neulet

Light-induced Fos expression is attenuated in the suprachiasmatic nucleus of serotonin 1B receptor knockout mice

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Received 18 January 2006; received in revised form 28 February 2006; accepted 8 March 2006

Abstract

The hypothalamic suprachiasmatic nucleus (SCN) is a circadian oscillator that receives a dense serotonergic innervation from the median raphe nucleus. Serotonin (5-HT) modulates the effects of light on circadian behavior by acting on 5-HT_{1B} receptors on retinohypothalamic (RHT) terminals in the SCN. Activation of 5-HT_{1B} presynaptic receptors on RHT terminals inhibits glutamate release. However, 5-HT_{1B} receptor knockout (5-HT_{1B} KO) mice have attenuated behavioral responses to light [P.J. Sollars, M.D. Ogilvie, A.M. Simpson, G.E. Pickard, Photic entrainment is altered in the 5-HT_{1B} receptor knockout mouse, J. Biol. Rhythms 21 (2006) 21–32]. To assess the cellular response of the 5-HT_{1B} KO SCN to light, light-induced Fos expression was analyzed in 5-HT_{1B} KO and wild-type (WT) mice. In addition, the distribution of melanopsin containing retinal ganglion cells that contribute the majority of axons to the RHT was examined in 5-HT_{1B} KO mice and compared to that of WT mice. Light-induced Fos expression in the SCN was reduced in 5-HT_{1B} KO mice compared to WT mice at circadian time (CT) 16 and CT 23 in a manner similar to the reduction previously described in light-induced behavioral phase shifts. The number of melanopsin retinal ganglion cells was similar in WT and 5-HT_{1B} KO mice. These data taken together with previous data suggest that functional removal of the 5-HT_{1B} receptor results in reduced functional light input to the SCN.

Keywords: Circadian rhythm; Suprachiasmatic nucleus; Retinohypothalamic tract; 5-HT_{1B} receptor; Melanopsin; Fos

The suprachiasmatic nucleus (SCN) is a circadian oscillator that derives functional utility from its ability to be entrained to the 24 h environmental day/night cycle. Entrainment provides stable and appropriate phasing of rhythmic behavior with the environment, thereby, in effect, enabling recognition of local time [20]. Entrainment of the SCN circadian system to the day/night cycle is accomplished by a daily resetting mechanism in which light exposure during the early subjective night produces phase delays and light during the late subjective night generates phase advances [5].

Photic signals that reset the circadian clock are transmitted from the retina to the SCN via the retinohypothalamic tract (RHT) [13]. The retinal ganglion cells that contribute to the RHT use glutamate as a neurotransmitter [6] and the majority of these neurons are intrinsically photosensitive due to their expression of melanopsin [10]. The SCN also receives serotonergic afferent fibers from the median raphe nucleus of the midbrain that are coextensive with RHT fibers in the ventral SCN; destruction of the serotonergic innervation of the SCN amplifies the effects of light on circadian behavior [14,16,28].

The serotonin 1B (5-HT_{1B}) receptor subtype is one of several 5-HT receptor subtypes found in the SCN [1]. The 5-HT_{1B} receptor is located on RHT terminals [18] and activation of these presynaptic 5-HT_{1B} receptors in vivo attenuates light-induced: (1) behavioral phase shifts [17,19]; (2) suppression of pineal melatonin [24]; and (3) SCN gene expression [8,19,27]. 5-HT_{1B} receptor agonists inhibit optic nerve-evoked glutamatergic excitatory postsynaptic currents (EPSCs) in the SCN in vitro [18,29] and these responses are eliminated in 5-HT_{1B} receptor knockout (KO) mice [29].

There is substantial experimental evidence indicating that activation of 5-HT_{1B} presynaptic receptors on RHT terminals reduces photic input to the SCN and thus it might be predicted that the circadian system of mice lacking functional 5-HT_{1B} receptors may be more responsive to light. However, it has recently been shown that 5-HT_{1B} KO mice have an attenuated response to light evidenced by an altered phase angle of entrainment and reduced light-induced phase shifts of circadian

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doi:10.1016/j.neulet.2006.03.012

activity rhythms [31]. It is not yet known why the circadian system of 5-HT_{1B} KO mice is less responsive to light. If 5-HT_{1B} KO mice have a reduced number of melanopsin-expressing retinal ganglion cells that contribute to the innervation of the SCN, [7,15,32] such a reduction could be responsible for the attenuated behavioral response to light observed in these animals. Alternatively, increased GABA transmission in the SCN could also contribute to this phenotype [4,31]. To investigate further the effect of light on the circadian system in the 5-HT_{1B} KO mouse, we examined light-induced Fos expression in the SCN, a cellular correlate of light-induced behavioral phase shifts [9,22]. In addition, the number of melanopsin-containing retinal ganglion cells was also examined in 5-HT_{1B} KO mice and compared to that of wild-type controls.

5-HT_{1B} KO and wild-type (WT) mice (males, 6–10 weeks old at the beginning of the experiment) were used. 5-HT_{1B} KO mice [26] on the C57BL/6 genetic background, were generously provided by Dr. René Hen (Columbia University). A colony was maintained in our laboratory under a 12:12 light:dark cycle (100 lx light:0 lx dark) along with WT mice (C57BL/6J) obtained from Jackson Laboratory (Bar Harbor, ME); animals were housed 3–4 per cage with food and water available ad libitum. All procedures used in these studies adhered to the guidelines approved by the Colorado State University Animal Care and Use Committee and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratories Animals.

Animals were transferred to individual cages equipped with an activity wheel (diameter 11 cm), with food and water constantly available and were maintained in light-tight chambers (six cages to a chamber). Light inside the chamber was provided by a single 48 in., 40-W fluorescent bulb controlled by an external timer and animals were initially maintained on a 12L:12D cycle for at least 2 weeks. The light:dark cycle was discontinued and animals were housed thereafter in constant darkness (DD).

Wheel-running activity was monitored as previously described [30] to determine each animal's circadian phase. Briefly, wheel revolution data were collected in 5-min bins and activity records were generated in the standard manner, with each day's activity presented beneath that of the previous day. Data were analyzed using CIRCADIA software (Dr. Ralph Mistlberger, Simon Fraser University) running on a Power Mac 7600/132 computer.

Fos expression in the SCN was determined as previously described [19]. Briefly, after at least 10 days in DD conditions, animals were removed from their wheel-running cages in the dark with the aid of infrared night-vision goggles (ITT-NE5001 generation 3, GT Distributors, Austin, TX) and transferred to a light stimulation apparatus designed for this purpose. Animals received light pulses (10 min of white light at 20 lx) at either circadian time (CT) 16 or 23. The onset of wheel-running activity is designated as CT 12 and was used as a phase reference point. Control animals (no photic stimulation) were treated identically except that the shutter of the light source remained closed during the 10 min the animals were in the light stimulation apparatus. After light- or sham-light stimulation, animals were returned to their wheel-running cages in DD and 90 min after the beginning of the light pulse, were anesthetized (sodium pentobarbital, 80 mg/kg) in the dark, perfused with 0.9% saline followed by 4% paraformaldehyde in phosphate buffer (0.1 M, pH 7.4) and the brain dissected out. Brains were post-fixed overnight at 4 °C in the same fixative containing 30% sucrose and 30 μm coronal sections through the rostrocaudal extent of the SCN were collected on a freezing microtome and processed for the immunocytochemical demonstration of Fos. Fos was detected using a rabbit antiserum (Oncogene, AB-5) diluted 1:10,000 and a secondary goat anti-rabbit IgG conjugated to Alexa 488 (Molecular Probes). Tissue was examined using a Leica DMRA light microscope equipped with epifluorescence and all Fos immunopositive-nuclei throughout the rostrocaudal extent of both SCN were counted and summed by an observer blind to the genotype of the animals. Some animals used in this study had previously received a single light pulse at either CT 16 or CT 23 and behavioral phase shifts were monitored (see [31]). This treatment occurred at least 14 days prior to the light pulse they received for the immunocytochemical analysis of Fos in the current study.

Retinas of 5-HT_{1B} KO and WT mice were examined for melanopsin immunoreactivity at the light microscopic level as previously described [2] using a well characterized anti-melanopsin antibody (UF006 generously provided by Dr. Ignacio Provencio, University of Virginia); secondary goat anti-rabbit IgG conjugated to Alexa-594 was used to visualize melanopsin-labeled cells. Immunostained retinas were flat mounted on microscope slides and examined using a Leica DMRA light microscope equipped with epifluorescence. All melanopsin immunopositive retinal ganglion cells observed within a grid 500 μm × 500 μm at 10 randomly selected locations on each retina were counted by an investigator blind to the genotype of the animals.

Differences were analyzed for significance using the Student's *t*-test and data are expressed as mean ± S.E.M.

Light pulses at CT 16 and CT 23 induced Fos expression in the SCN in WT and 5-HT_{1B} KO mice. The number of light-induced Fos-immunoreactive cells in the SCN of 5-HT_{1B} KO mice at CT 16 (353 ± 89, $n = 9$) was less than the number of light-induced Fos-immunoreactive cells in the SCN of WT mice at CT 16 (943 ± 333, $n = 6$) although this difference did not reach statistical significance ($p = 0.054$) (Fig. 1B). At CT 23 the number of light-induced Fos-immunoreactive cells in the SCN of 5-HT_{1B} KO mice (566 ± 224, $n = 5$) was also less than the number of light-induced Fos-immunoreactive cells in the SCN of WT mice (2693 ± 137, $n = 4$) and this difference was statistically significant ($p < 0.001$) (Fig. 1B). The reductions in light-induced Fos expression in the SCN of 5-HT_{1B} KO mice at CT 16 and CT 23 paralleled the reductions in light-induced behavioral phase shifts at CT 16 and CT 23 in WT and 5-HT_{1B} KO mice previously reported using similar stimuli (10 min, white light at 20 lx; [31]). The light-induced behavioral phase shift data (Fig. 1A) are presented together with the light-induced Fos data from the current study for comparison (Fig. 1A and B). A very small number of Fos-immunoreactive neurons were observed at CT 16 and CT 23 in the SCN of animals that did not receive photic stimulation (WT $n = 2$, 149 and 170 cells and KO $n = 2$, 22 and 116 cells at