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Circadian rhythmicity in serotonin transporter knockout mice

Raina D. Pang [a,*], Daniel P. Holschneider [a,b,c,d,e], Joseph D. Miller [e]

[a] Graduate Program in Neuroscience, University of Southern California, United States
[b] Department of Psychiatry and Behavioral Science, University of Southern California, United States
[c] Department of Neurology, University of Southern California, United States
[d] Department of Biomedical Engineering, University of Southern California, United States
[e] Department of Cell and Neurobiology, University of Southern California, United States

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ABSTRACT

Aims: Serotonin transporter knockout (5-HTT KO) mice exhibit elevated basal extracellular serotonin, increased depressive-like behaviors and increased rapid eye movement sleep. Because abnormalities of circadian rhythms are associated with mood disorders, we tested the hypothesis that 5-HTT KO mice would have altered circadian rhythmicity.
Main methods: Homecage locomotor activity was recorded in wild-type (WT) and KO mice under a standard 12:12 light–dark cycle. After 4 weeks of recording, mice received a one-hour light pulse at circadian time (CT) 14 and then were kept under constant darkness for 3 weeks.
Key findings: There were no significant differences in amplitude, period, acrophase or total home cage locomotor activity between WT and KO mice during the 12:12 light–dark cycle or during constant darkness. The mean phase delay to a CT 14 light pulse was significantly attenuated in KO compared to WT mice.
Significance: Acute increases in serotonin have been reported to attenuate photic phase shifts. The current study demonstrates that this effect is maintained in the face of a lifelong absence of 5-HTT.
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Introduction

The mammalian circadian clock located in the suprachiasmatic nucleus (SCN) is thought to be modulated by serotonin (5-HT) (Prosser et al., 1993). It is well-known that serotonergic agonists can phase shift the circadian clock in the SCN in vitro (Prosser et al., 1993) and in vivo (Challet et al., 1998; Edgar et al., 1993). Furthermore, acute pharmacological increases in 5-HT have been shown to attenuate the responsivity of animals to photic phase shifts (Challet et al., 2001; Gannon and Millan, 2007) and destruction of serotonergic fibers increases the magnitude of photic phase shifts (Bradbury et al., 1997). Serotonergic effects on photic phase shifting may relate to the believed role of 5-HT in inhibiting the release of glutamate from retinal terminals and decreasing the responsiveness of retinorecipient cells in the SCN (Rea and Pickard, 2000; Ying and Rusak, 1994). What is less clear are the effects of lifelong increases in 5-HT levels on circadian rhythms and phase shifts.

In this study, we examine the effect of lifelong elevated 5-HT levels on circadian rhythms in locomotion in the serotonin transporter knockout (5-HTT KO) mouse model. 5-HTT KO mice have provided insight into the effects 5-HTT has on physiological function and behavior (for review see Murphy and Lesch, 2008). These mice have greatly reduced, but not wholly absent 5-HT clearance (Baganz et al., 2008; Zhou et al., 2002), an observation similar, though not identical, to findings in the human subjects carrying the low expressing forms ('s' or 'L_G') of the serotonin transporter length polymorphic region (5-HTTLPR) (Greenberg et al., 1999). On a behavioral level, 5-HTT KO mice and humans with the low-expressing form of 5-HTTLPR display increased susceptibility to depressive behavior (Alexandre et al., 2006; Caspi et al., 2010), and an increased stress hormone response (Gotlib et al., 2008; Tjurmina et al., 2002).

Abnormalities of circadian rhythms, sleep architecture, and phase advances or delays are commonly associated with mood disorders (Manber and Chambers, 2009; Mistlberger et al., 2000). Changes in sleep quality and insomnia are noted in association with the 5-HTTLPR polymorphism (Brummett et al., 2007; Deuschle et al., 2010). Alterations in sleep architecture, specifically increased frequency and duration of rapid eye movement sleep (REM) (Alexandre et al., 2006; Wisor et al., 2003), have also been reported in 5-HTT KO mice. To our knowledge, this is the first report examining basal circadian rhythms and photic phase shifting in the 5-HTT KO mouse. Since the phase response curve for serotonergic agonists appears to have a larger phase advance region than delay region (Edgar et al., 1993), we hypothesized that 5-HTT KO mice would be relatively phase-advanced in constant conditions relative to WT mice. Furthermore, we hypothesized that the photic phase shift would be attenuated in KO animals.

* Corresponding author at: 1333 San Pablo St., McKibben Annex, MCA-B1, Los Angeles, CA 90033, United States. Tel.: +1 323 442 1586; fax: +1 323 442 1587.
E-mail address: rpang@usc.edu (R.D. Pang).

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Materials and methods

Ethics statement

All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Southern California (Animal Welfare Assurance # A3518-01, USC protocol # 11093).

Animals

5-HTT KO mice were backcrossed onto a C57BL/6 background for greater than 15 generations from an original mixed background [129/P1ReJ (ES cells), C57BL/6J and CD-1] (Bengel et al., 1998; Salichon et al., 2001). KO mice were bred at USC from homozygous pairs obtained from Taconic (Taconic, Hudson, NY, USA). Prior to the start of experiments, mice were housed in groups of three to four animals under standard conditions. At the start of circadian recordings adult male mice were individually housed. Genotyping was confirmed by Transnetyx, Inc. (Cordova, TN, USA) from tail snips obtained post-mortem with primer sequences obtained from Taconic (m5-HTT-C: 5′ TGA ATT CTC AGA AAG TGC TGT C 3′, m5-HTT-D: 5′ CTT TTT GCT GAC TGG AGT ACA G 3′, neo3a: 5′ CAG CGC ATC GCC TTC TAT C 3′).

Circadian rhythms

A modified Aschoff type II design was employed to measure basal circadian rhythms and response to a circadian time (CT) 14 light pulse. Transparent cages (31 cm × 18 cm × 13 cm) containing the individually housed mice (5-HTT KO: n = 11, average age = 14.5 ± 0.5 weeks; WT: n = 14, average age = 15.7 ± 0.6 weeks) were placed within frames containing three infra-red photo beams spaced 8.8 cm apart (San Diego Instruments, San Diego, CA, USA). Locomotion indicated by photo beam breaks was recorded electronically using PAS software (San Diego Instruments, San Diego, CA, USA). After one week of habituation to the room, baseline recording (12-hour light, 12-hour dark; LD 12:12) was started. After four weeks on the LD 12:12 schedule, animals were given a light pulse 14 h after light onset (1 h duration, CT 14, 150 lux). Mice were maintained in constant darkness for three weeks while activity continued to be recorded.

Behavioral analysis

Periods, amplitudes, activity counts, and acrophases were analyzed separately for the light/dark schedule and constant darkness conditions using ClockLab (Actimetrics, Wilmette, IL, USA). Means for the KO and WT mice were compared using a *t*-test for independent groups (significance defined as $p<0.05$). To minimize carry over effects from the light pulse, the first five days post-light pulse were considered transient days and excluded from the free running analysis. Period was determined by measuring the slope of a regression line on the actogram in ClockLab and by the F-periodogram (Actimetrics, Wilmette, IL, USA). An amplitude measure was calculated using the F-periodogram in ClockLab (Actimetrics, Wilmette, IL, USA). Briefly, a signal to noise ratio was generated by dividing the modified F-value at the dominant period by the noise value at that period. Activity counts were automatically generated based on the number of beam breaks. The acrophase was determined from the time at which the maximum in the locomotor rhythm occurred in either the light/dark cycle or constant darkness. Phase shifts were determined by comparing the difference in the fitted regression lines before and after a light pulse was given.

Results

No significant differences in amplitude, period, acrophase or total activity counts were seen between WT and KO mice during the standard 12:12 light–dark cycle (Table 1; $p>0.05$). There was also no significant difference in amplitude, period, acrophase or total activity counts between WT and KO mice during constant darkness (Table 1; $p>0.05$). The mean phase delay to a light pulse was significantly attenuated in KO compared to WT mice (Fig. 1 and Table 1, $p<0.05$).

Discussion

This study investigated the effects of lifelong elevation of 5-HT levels on circadian rhythms and locomotor patterns in 5-HTT KO mice. Effects of the gene on circadian parameters were investigated in homozygous KO mice rather then heterozygous mice to determine the effects of the greatest elevation of 5-HT on circadian indices. Although heterozygous mice show a similar reduction in 5-HTT binding (about a 50% reduction) as in human studies investigating the short allele of the 5-HTT gene (Bengel et al., 1998; Lesch et al., 1996), they display similar 5-HT levels as WT mice, necessitating the use of homozygotes to maximize our chances of seeing an effect.

Since adult 5-HTT KO mice exhibit elevated basal extracellular 5-HT over that of WT controls (Mathews et al., 2004), we expected to see differences in the organization of circadian rhythms in this preparation. In general, we found the 5-HTT KO mice to have surprisingly normal homecage locomotor activity and circadian rhythms (both in LD 12:12 and constant darkness). KO mice compared to WT mice did not show significant differences in acrophase in constant darkness. This is different from what we hypothesized based on knowledge of acute administration of serotonergics (Edgar et al., 1993). This provides further evidence that lifelong alterations in serotonergic function do not have the same effect as acute alterations. This might represent compensatory changes in the 5-HTT KO mice, which result in a normalization of the locomotor and circadian patterns, even in a state of elevated 5-HT. The effects of elevated 5-HT resulting from 5-HTT KO on REM sleep were also shown to be related to developmental effects rather than elevated levels of 5-HT in adulthood (Alexandre et al., 2006).

A previous report found 5-HTT KO mice to be hypolocomotory (Holmes et al., 2002). In this study, however, we found no significant difference in spontaneous homecage locomotor activity, which is consistent with studies of 5-HTT KO rats (Linder et al., 2008). The current study looked at homecage locomotor activity over several weeks, whereas a prior report looked at a 24-hour period following one day of acclimation (Holmes et al., 2002). Shorter sampling of behavioral activity may have been influenced by anxiety-like behavior, which is a characteristic of the 5-HTT KO animals (Holmes et al., 2003; Pang et al., 2011).

We did see a significant attenuation in the photic phase shift in the 5-HTT KO mice. This is consistent with data from acute increases in 5-HT neuronal activity, which have been shown to attenuate photic phase shifts (Challet et al., 2001; Gannon and Millan, 2007; Mistlberger et al., 2002). There is strong evidence for a number of 5-HT receptors in the SCN, namely 5-HT1A, 5-HT1B, 5-HT2C and 5-HT7 (Lovenberg et al., 1993; Roca et al., 1993). Regulation of the phase shift to light seems to depend primarily on pre- and postsynaptic 5-HT1A and inhibitory presynaptic 5-HT1B receptors in the retinohypothalamic tract (RHT) (Pickard

Table 1
Circadian rhythm parameters (mean ± S.E.M.) during a LD 12:12 cycle and in total darkness for male 5-HTT KO (n = 11) and WT mice (n = 14). Significant genotypic differences ($p<.05$*) were observed in phase shift to a light pulse.

		Period (h)	Amplitude	Activity counts	Phase shift (h)
12 h/12 h	KO	23.99 ± 0.01	1.46 ± 0.08	1010 ± 42	
Light/dark	WT	23.99 ± 0.0	1.5 ± 0.08	1141 ± 79	
Constant	KO	23.84 ± 0.03	1.36 ± 0.04	962 ± 77	−1.00 ± 0.25*
Darkness	WT	23.77 ± 0.02	1.29 ± 0.06	867 ± 89	−1.78 ± 0.21*

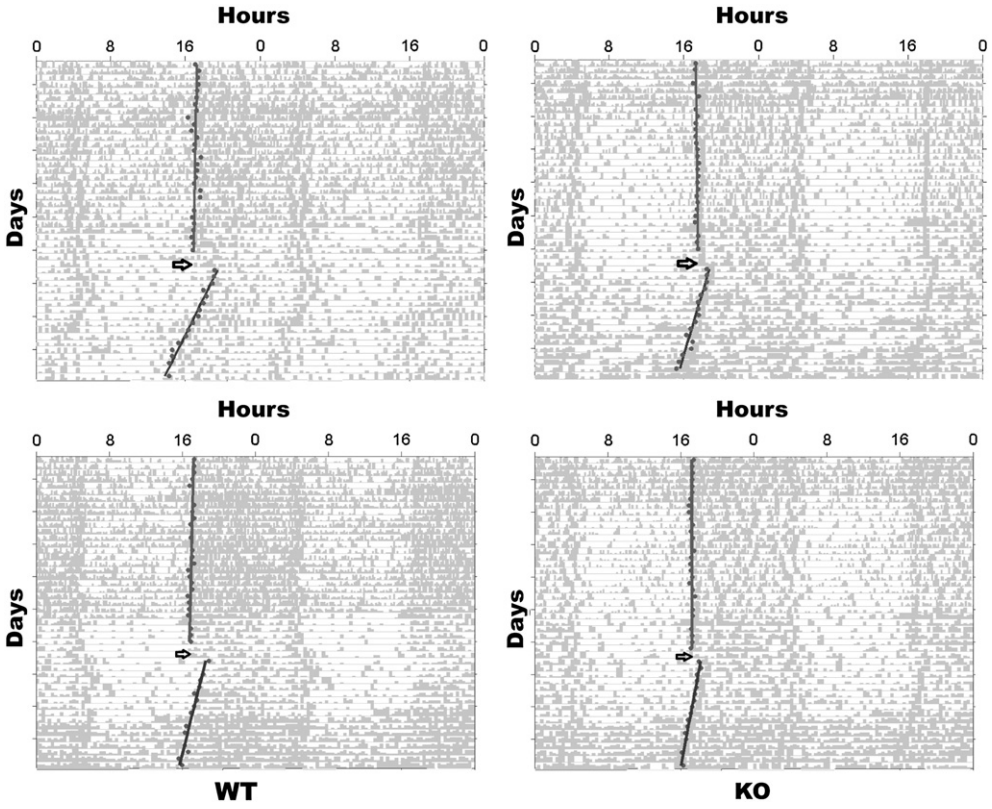

Fig. 1. Sample actograms: Sample of double plotted actograms of homecage locomotor activity in two WT and two KO mice. After four weeks on standard 12:12 light–dark schedule, animals were given a 1 hour light pulse (indicated by the arrow) 2 hours after lights off. Thereafter, mice were maintained in constant darkness for three weeks while activity continued to be recorded.

et al., 1996). Evidence suggests that heightened 5-HT tonus can downregulate 5-HT1A (Bouali et al., 2003) and 5-HT2C (Moya et al., 2011), but no evidence exists for 5-HT induced downregulation of 5-HT7. The involvement of 5-HT7 cannot be excluded, but if 5-HT7 is likewise refractory to elevated 5-HT, more widespread alteration in circadian period and phase angle would be expected (Lovenberg et al., 1993).

The simplest explanation of our work is that the 5-HT1B is refractory to downregulation by elevated 5-HT tonus in the 5-HT transporter knockout resulting in elevated 5-HT1B receptor activation, inhibition of the RHT and a diminished phase shift to light. Diminished, rather than augmented phase shifts to nocturnal light pulses have been reported in mice lacking the 5-HT1B receptor (Sollars et al., 2006). Since the 5-HT1B receptor is desensitized or downregulated in the 5-HTT KO mouse (Shanahan et al., 2009), it is possible that the attenuation of the photic phase shift in this study reflects a similar functional change in that receptor at some point in the afferent input to the SCN. The chronic effects of heightened 5-HT tonus would then be quite similar to the acute effect of such elevation, i.e. a reduced photic phase shift (Rea and Pickard, 2000; Ying and Rusak, 1994).

The mice used in this study were obtained through homozygous breeding pairs. Homozygous breeding raises the possibility that effects seen on circadian rhythms and photic phase shifting could relate to differences in maternal effects rather than alterations in serotonergic levels per se. We acknowledge the limitations to this type of breeding, but maternal effects on circadian behavior should be minimal. While studies show that during fetal development there is maternal coordination of the phase of the fetal circadian clock (Reppert and Schwartz, 1983), circadian rhythms in murine pups develop in the absence of a functional maternal circadian clock (Jud and Albrecht, 2006; Reppert and Schwartz, 1986). These studies suggest that the light–dark cycle probably is the dominant postnatal influence on circadian rhythms.

Conclusions

Basal circadian parameters and homecage locomotor activity remained unchanged in 5-HTT KO mice. This may reflect compensatory changes that normalize these parameters. Challenge with a light pulse, however, unmasked a diminished ability to respond with a phase shift.

Conflict of interest statement

None.

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