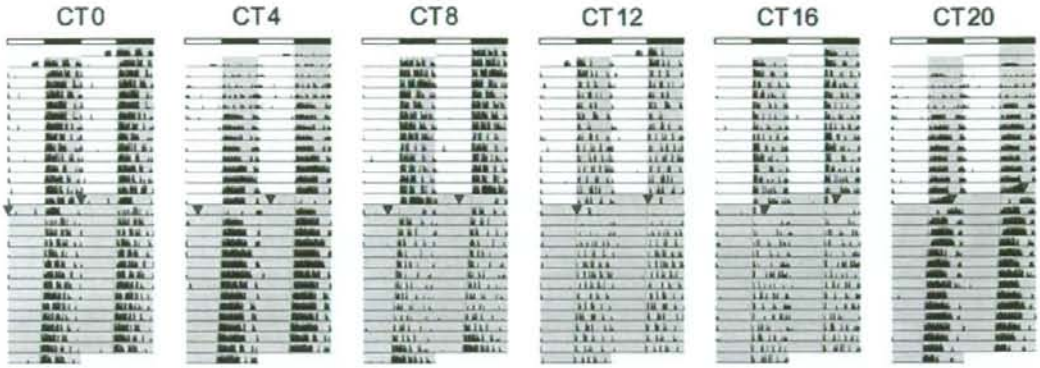

Figure 3. No influence of ECS on the circadian clock. Double-plotted activity records of representative mice receiving one ECS (red triangles) at different times of the day: circadian time (CT) 0, 4, 8, 12, 16, and 20. CT0–12 is the subjective light phase, and CT12–24 is the subjective dark phase. The light and dark periods are indicated by white and gray backgrounds, respectively. Onset of activity shown by green lines was used as the reference phase for the determination of phase shift.
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Materials and Methods

Animals

All the mutant POLG Tg mice used were heterozygotes. Male mutant mice were used for mating to avoid possible transmission of mtDNA mutations from the maternal side, although expression of the transgene is restricted in neuronal tissues. Genomic DNA was isolated from tail biopsies, and the genotyping was performed by multiplex PCR using the two sets of primers: Fw, 5′-TGG TGA AAC AGT TGA ATC TTC C-3′; Rv, 5′-GTC AGG AGA TTG GTG ATC TGC-3′; and Fw, 5′-AGT GAG TTG AAA GCC ATG GTG-3′; Rv, 5′-GTG GTT GAA CTG CAT CAG TAG G-3′. Controls were non-Tg littermates whenever possible. Mice of older than 26 weeks of age were used to measure wheel-running activity, because mtDNA defects were not accumulated in the young mutPOLG Tg mice [7]. The Wako Animal Experiment Committee, RIKEN, approved all animal experiment protocols.

Long-term recording of wheel-running activity and statistic analyses

Recoding and analyses of wheel-running activity were performed as described previously [7]. Wheel-running activity (three counts per rotation) was recorded every 10 min by an online PC computer system (O'Hara & Co., Tokyo, Japan).

The delayed activity index is defined as a percent of the activity during the first 3 h of the light period divided by the total activity during the previous dark period (12 h). Anticipatory activity index is defined as a percent of the activity during the last 3 h of the light period divided by the activity during the following dark period (12 h). Day-to-day variation in wheel-running activity was estimated by the unevenness in daily wheel-running activity, which is the total number of counts of wheel running per day. The equation used to calculate day-to-day variation was described in our previous report [7]. Wilcoxon signed-rank tests were used to test for any difference in these indices before versus after the treatment. Values of $p < 0.05$ were considered statistically significant.

Electroconvulsive stimulation

Animals were handled on the day before the first ECS to become adjusted to immobilization in a small cylinder and the application of ear-clip electrodes. Ear-clip electrodes were dampened with saline and attached to the pinnae, and electroshock (12–50 mA, 60 pulses/sec, 0.5-msec pulse width, 1-sec duration) was delivered using an electroconvulsive device (Ugo Basile, Comerio, Italy) and a bipolar square-wave pulse generator (Ugo Basile). A tonic seizure was characterized by extension of all limbs and forward head extension that lasted for about 10 sec. We titrated the dose (current amplitude) upward in 2-mA steps beginning at 10–15 mA, which depended on body weight. Because repeated ECS increased the seizure threshold, we administered ECS of the last threshold intensity, and we titrated the intensity upward if the animal did not exhibit a tonic seizure. Mice were treated 6 times in 2 weeks in the first experiment (male Tg, $n=8$; male non-Tg, $n=7$, 37–48 (44.6±3.8) weeks old at the start of recording; female Tg, $n=8$; and female non-Tg, $n=7$, 37–53 (44.3±5.6) weeks old) and 3 times in 1 week in the second experiment (male Tg, $n=9$; male non-Tg, $n=6$, 32–50 (42.8±7.1) weeks old; female Tg, $n=8$; and female non-Tg, $n=2$, 26–48 (40.0±6.8) weeks old). In the second experiment, to prevent death due to respiratory failure, we placed mice in a plastic bag filled with oxygen gas for about 1 min before delivering the electroshock. The oxygen gas was supplied to mice after ECS until they began breathing regularly. To investigate the influence of ECS on the circadian clock, in a third experiment we delivered a single ECS at various times of day under dim red light. The mice were placed in constant darkness before delivering ECS. The light conditions were shown in Fig. 3.

Acknowledgments

Author Contributions

Conceived and designed the experiments: TKasahara TKato. Performed the experiments: TKasahara MK TM MI. Analyzed the data: TKasahara TKato. Wrote the paper: TKasahara TKato.

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