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Neuropeptide Y-deficient mice show altered circadian response to simulated natural photoperiod

Hyun Jung Kim and **Mary Harrington**
Neuroscience Program, Smith College, Northampton, Massachusetts

Abstract

Circadian rhythms, endogenously generated by the suprachiasmatic nucleus (SCN), can be synchronized to a variety of photic and non-photic environmental stimuli. Neuropeptide Y (NPY) is produced in the intergeniculate leaflet (IGL) and known to mediate both photic and non-photic influences on the SCN. We recently found that *npy*-/- mice were slower to shift their locomotor activity onset to the new time of light offset when photoperiod was abruptly changed from light/dark (LD) cycle 18:6 to LD 6:18. In the present study, we measured the locomotor response of *npy*-/- mice to gradual changes in photoperiod (4 min a day) for 141 days (LD 16:8 changing to LD 8:16), mimicking external LD cycles in nature. When the photoperiod approached LD 8:16, *npy*-/- mice showed a significantly delayed onset of activity compared to wild-type mice. Activity patterns disintegrated into multiple bouts and intensity of activity decreased as the photoperiod changed and these changes were more pronounced in *npy*-/- mice. Our results lend further support to the idea that NPY is involved in circadian entrainment responses to seasonal photoperiod changes.

Keywords

Neuropeptide Y; Photoperiod; Circadian rhythm; Intergeniculate leaflet; Entrainment

1. INTRODUCTION

Photoperiodic responses depend on circadian entrainment. In nature, animals are exposed to daily changes in the time of dawn and dusk. These small changes in day length are translated into changes in the duration of nocturnal secretion of pineal melatonin, an important signal for transduction of the environmental daylength to adaptive physiological responses (Goldman, 2001). Duration of melatonin secretion is strongly correlated with duration of nocturnal locomotor activity in hamsters, and changes in phase of locomotor activity relative to the light:dark cycle are paralleled by changes in phase of melatonin secretion to the light:dark cycle (Elliott and Tamarkin, 1994). The phase relationship between the changing external day:night cycle and the internal circadian clock allows animals to detect seasonal changes (Elliott, 1976; Goldman, 2001).

Whereas entrainment is largely mediated via the retino-hypothalamic tract input to suprachiasmatic nucleus (SCN) of the hypothalamus, an input pathway from the intergeniculate

To whom correspondence should be addressed: M. E. Harrington, Neuroscience Program, Smith College, Northampton, MA 01063 Tel: (413) 585-3925 E-mail: E-mail: mharring@email.smith.edu.

leaflet (IGL) of the thalamus appears to play an important role in entrainment and photoperiodic responses to short days. In Siberian hamsters, IGL ablation resulted in significantly shorter total duration of activity (alpha) and more negative phase angles of entrainment when exposed to a decreasing simulated natural photoperiod (Freeman et al., 2004). These changes in circadian entrainment were associated with reduced likelihood of molting to a winter pelage (Freeman et al., 2004). IGL-ablated hamsters were reported to demonstrate reduced hibernation after transfer to cold temperatures and a short photoperiod (Menet et al., 2003). A role for the IGL in discriminating dusk and dawn and integrating photoperiodic information by the SCN has been suggested by other studies (Jacob et al., 1999; Menet et al., 2001; Shinohara et al., 1993; Smale and Morin, 1990).

The IGL is thought to mediate these influences on the SCN through neuropeptide Y (NPY) along with other neurotransmitters (Harrington, 1997). The Syrian hamster shows an increase in the number of neurons expressing NPY when hamsters are housed under short photoperiods (Jacob et al., 1998). We recently found that *npy*-deficient (*npy*-/-) mice were slower to shift their locomotor activity onset to the new light/dark cycle than WT mice when photoperiod was altered abruptly from LD 18:6 to LD 6:18 (Harrington et al., 2007). In that study the mice were not housed with running wheels during the time of the switch in photoperiod, but the motion sensor activity records indicated the *npy*-/- mice took approximately 10 days longer to re-entrain to the short day photoperiod, and adopted a more negative phase angle of entrainment under LD 6:18 relative to WT animals (i.e., time of activity onset was delayed relative to WT). In the present study, we examined whether *npy*-/- mice would show different timing of activity onset when photoperiod very gradually changed from LD 16:8 to LD 8:16, more realistically mimicking photoperiodic changes in nature. We also examined the wheel-running records for changes in intensity and fragmentation of daily activity.

2. RESULTS

Baseline measures were taken while WT and *npy*-/- mice were entrained to LD 16:8 for 2 weeks and no differences were observed between the groups. Following this, we changed the day length by 4 min a day (delayed the light offset by 2 min and advanced the light onset by 2 min) until reaching LD 8:16. Locomotor activity of WT and *npy*-/- mice as assessed with running-wheel records in response to these gradual changes revealed differences in the change of the phase angle of entrainment, intensity of locomotor activity, and fragmentation of daily activity into multiple bouts (Fig 1).

The phase angle of entrainment gradually become more negative as the photoperiod shortened, and *npy*-/- mice showed a significantly delayed onset of activity compared to WT mice (the difference of phase angle of entrainment between LD 16:8 and LD 8:16 ; WT -15.4 min vs. *npy*-/- mice -42.9 min; $t(17) = -2.11$, $P < 0.05$) (Fig 2).

The intensity of locomotor activity, measured as the number of running-wheel revolutions per min (rev/min), decreased as photoperiod decreased, with *npy*-/- mice showing a more dramatic reduction in activity (*npy*-/- mice decreased by 9.25 ± 14.56 rev/min; WT mice decreased by 4.54 ± 19.35 rev/min; $t(16) = -2.41$, $P < 0.05$) (Fig 3A). Groups were significantly different on Days 71-77, 103-109, and 135-141. The reduction in intensity of activity was accompanied by fragmentation of daily wheel-running activity. The number of daily activity bouts increased as the dark period increased, and the number of bouts in records from *npy*-/- mice was significantly greater than that seen in records from WT mice on Days 39-45 and Days 135-141 (see Fig 3). The final number of bouts for WT mice was 4.38 ± 0.42 bouts/day vs. *npy*-/- 5.58 ± 0.34 bouts/day; $t(18) = 2.26$, $P < 0.05$). Total duration of activity (alpha) increased as photoperiod decreased for both groups similarly ($P > 0.5$) (Fig 3B). Thus, our results suggest

that *npy*-/-mice show reduced level of activity and increased rate at which activity fragments into multiple bouts within the prolonged dark phase as photoperiod decreases.

3. DISCUSSION

Our results support that NPY is involved in photoperiodic time measurement and responsiveness. We replicated our prior finding that npy-/- mice entrain to LD 8:16 with a delayed phase of entrainment (Harrington et al., 2007). This finding is apparently robust in that the prior study used an abrupt change of photoperiod and no access to running wheels, but produced the same difference in phase of entrainment. We interpret these changes in phase angle of entrainment as arising from altered circadian period and photic and non-photic responsiveness in npy-/- mice (Harrington et al., 2007). IGL ablation produced a similar effect in Siberian hamsters (Freeman et al., 2004). The hamsters in that study also showed shorter total duration of activity following IGL ablation, whereas our npy-/- mice did not differ in this measure, although by the end of our experiment the measures of activity duration showed high variability. The npy-/-mice showed reduced intensity of activity and greater fragmentation of wheel-running activity into multiple bouts as compared to the WT mice. Since our study continued over a 20-week period, we could not avoid the possibility that changes in locomotor activity might be age-related; our animals were more than 180 days old by the end of the experiment. Several studies indicated that considerable fragmentation in the diurnal rhythm of locomotor activity with advanced age in golden hamsters and C57BL/6 mice (Valentinuzzi et al., 1996; Penev et al., 1997). The activity fragmentation might also be related to weight gain during the photoperiod experiment. Although the amount of body weight gain as day length shortened was not statistically different between two animal groups, average body weight of *npy-/-* mice was always larger than that of WT (see Supplementary Figure 1). If age or body weight explain our results then it would be necessary to posit that npy-/- mice are more susceptible than WT mice to the effects of these variables on circadian parameters; at this moment we do not know of any evidence that suggests this to be the case.

Because the npy-/- model is a mouse, not a reproductively photoperiodic animal, one might question the relevance of our findings. We believe that the behavioral changes mice show in response to a change in photoperiod, such as a change in phase angle of entrainment, are fundamentally related to the reproductive responses of animals such as hamsters that show major changes in reproductive response depending on the photoperiod. On the other hand it is an open question if the role of the NPY input pathway is the same in both animals; our results suggest that there are at least some commonalities in the role of this pathway in modulating circadian responses to photoperiod.

The results of IGL ablation revealed a role of the IGL in synchronizing physiological seasonal adjustments (e.g., gonadal regression, and pelage molt) elicited by exposure to decreasing day lengths (Freeman et al., 2004) and in processing nonphotic cues that can alter the photoperiodic response (Freeman et al., 2006). Moreover, the number of NPY mRNA containing IGL neurons significantly increased in a short photoperiod compared to those kept in a long photoperiod (Jacob et al., 1989). Many prior studies of IGL function were conducted in long photoperiod but these results suggest this input pathway to the SCN may play a more important role in regulating circadian responses to short photoperiod.

4. EXPERIMENTAL PROCEDURES

Animals

The 129S6 *npy*-/- mice were back-crossed for 15 generations onto a C57BL/6 background at Taconic Farms (Germantown, NY; B6.129-*npy*^{tm1} N15) and provided by E. Maratos-Flier (Harvard Medical School, Boston, MA) (Segal-Liberman et al., 1990). Mice were weaned at

3 wk and housed under LD 12:12. Male WT mice (n = 8) and littermate *npy-/-* mice (n = 12) were 6 ± 1 weeks of age at the start of the experiment. Food and water were available ad libitum. The experimental protocol was reviewed and approved by the Smith College Institutional Animal Care and Use Committee.

Locomotor Activity Measurement

Mice were housed in cages fitted with running wheels (15 cm in diameter). Wheel revolutions were constantly monitored using Clocklab computer software, with 1-min sampling epochs (Actimetrics, Evanston, IL). Mice were initially placed in LD 16:8 for 14 days until they showed synchronized circadian rhythms (lights on 0500-2100). An automated light timer (Thermo Fisher Scientific Inc., Waltham, MA) was programmed to simulate natural photoperiod changes experienced from July 16 to November 16 at 55°N latitude. The time of light offset was advanced by 2 min a day, and the time of light onset was delayed by 2 min a day until the light/dark cycle reached LD 8:16 (lights on 0900-1700). Total duration of the study was 141 days, including the initial 14 days of baseline. Standard overhead room lighting (50 lux) used for entrainment and maintenance, and dim red light (<.1 lux) was used for routine checks of the animals.

Behavioral Analysis and Statistics

The patterns of wheel-running activity, recorded over a 16-week period of *npy-/-* mice were compared with that of WT animals. Each activity pattern was characterized by several parameters, including time of onset and offset, total duration of the activity phase, intensity, and total number of wheel revolutions. All parameters were determined with Clocklab software. The phase angle of entrainment was defined as the difference in hours between the time of light offset and activity onset. A positive value indicates that activity onset preceded light offset, whereas negative value indicates that activity began subsequent to light offset. We applied the procedure proposed by Penev et al. (1997) to define a minimal bout criterion interval (between-bout time criterion) using the activity data of WT mice (n = 8) over 7-day period before the photoperiod treatment. A block size of 3 min and threshold of 4 counts/min were optimal to determine the bout criterion interval for the experiment. The resulting function confirmed the presence of discrete clusters of wheel-running behavior in mice activity records and was used to select a 27-min bout criterion interval (Fig 3C).

All results throughout the text are reported as means ± standard error (SE). For comparison of the two groups over time, data were analyzed by analysis of variance (ANOVA) followed by post-hoc tests of Dunnet or Bonferroni multiple comparison. All statistical tests were carried out using MatLab. A value of $p < 0.05$ was regarded as statistically significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

NPY
: Neuropeptide Y

LD
: light-dark

SCN
: Suprachiasmatic nucleus

RHT
: retinohypothalamic tract

GHT

geniculohypothalamic tract

IGL

intergeniculate leaflet

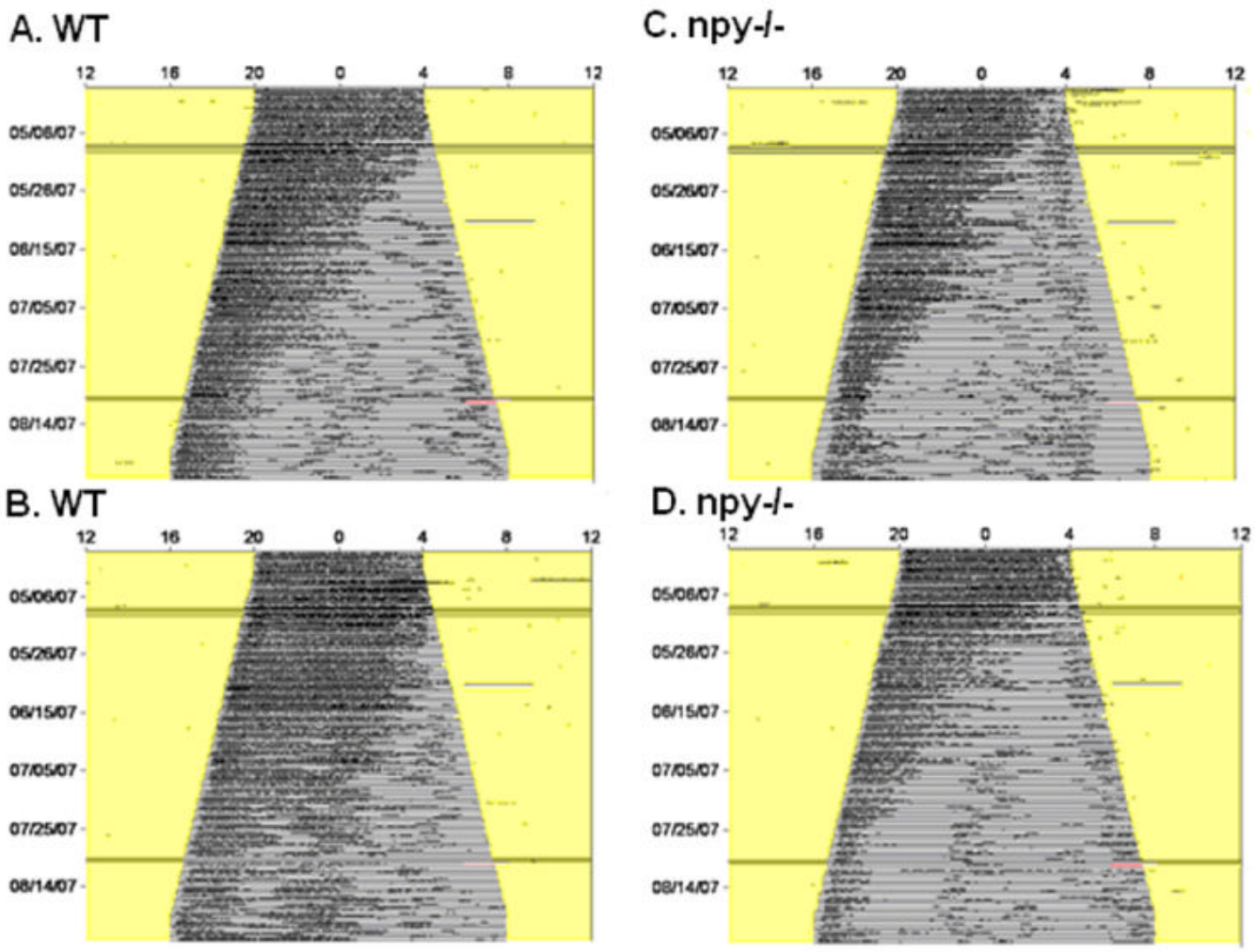

Figure 1.

Representative actograms of WT (A and B) and *npy-/-* (C and D) mice. Actograms show days on the *y*-axis and 24 hours on the *x*-axis. The activity levels are marked by black bars and the number of wheel revolution is plotted in 6-min bins. The three black lines across each actogram indicate compute malfunctioning, but the light cycle was not affected by the computer problems on these days. Notice that activity of both groups started to fragment into smaller bouts as the day length gradually changed from LD 16:8 to LD 8:16.

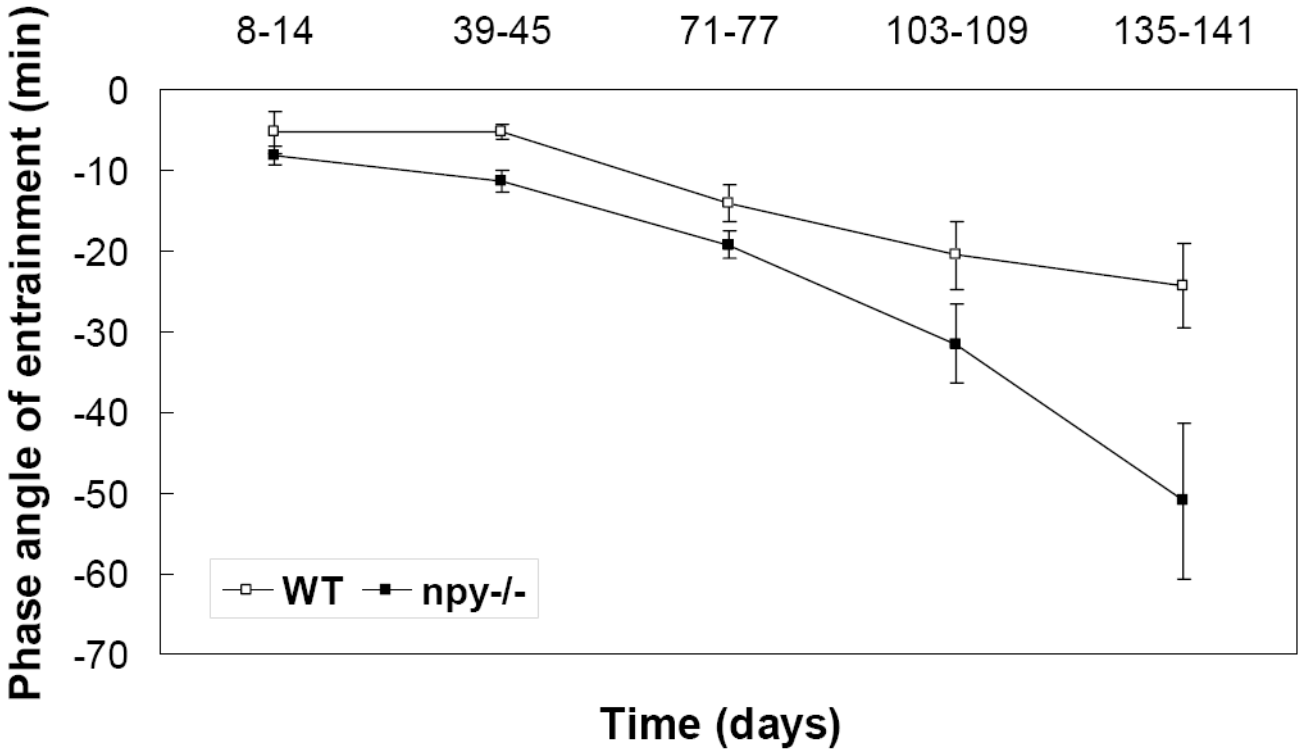

Figure 2.

Delayed phase angle of entrainment. Phase angles of entrainment was recorded over 5 one-week periods selected at 3-5 week intervals throughout the experiment, and the mean (± S.E.M.) was used to compare between two groups. In the final week (134-141 days) of the experiment, the *npy*-/- mice showed a significantly delayed onset of activity compared to WT mice (the difference of phase angle of entrainment between start point and end point of the treatment WT -15.4 min vs. *npy*-/- mice -42.9 min; $t(17) = -2.11$, $P < 0.05$). Groups did not significantly differ before this time point.

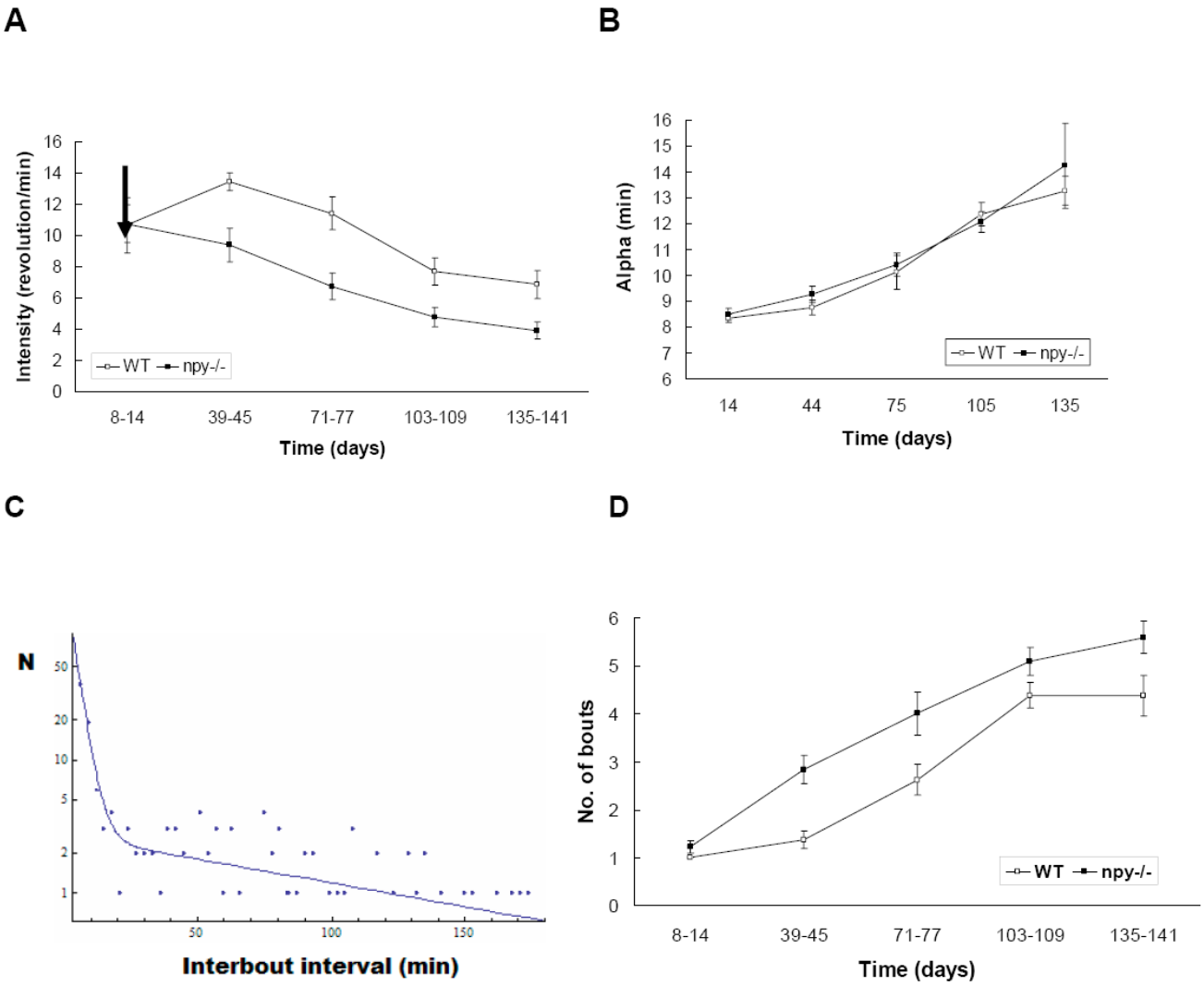

Figure 3.

(A) Intensity of activity was measured as average wheel running counts per min. Mean (± S.E.M.) intensity data recorded for one week, and five data points were selected with 3-5 week intervals throughout the experiment. (B) Mean (± S.E.M.) of total duration of activity (alpha) of WT and *npy*-/- mice at 5 time points was selected from 141 days of experiment. (C) First 3h segment of the log-survivorship curve based on the analysis of intervals between discrete wheel-running events recorded over a period of 14 days from 8 young male WT mice under LD 16:8. On the X axis we plot results for varied interbout intervals and the Y axis shows the number of instances showing that interval, using a block size of 3 min and a threshold of 4 counts/min. The position where the slope changes dramatically defines the minimum time between bouts of activity, giving our 27-min bout criterion interval (see Penev et al., 1997). (D) The numbers of daily activity bouts (mean ± S.E.M.) recorded over 5 weeks selected throughout the photoperiod treatment. The number of bouts showed an increase throughout the experiment, but *npy*-/- mice exhibited more activity fragments compared to WT (the final number of bouts WT 4.38 ± 0.42 vs. *npy*-/- 5.58 ± 0.34; $t(18) = 2.26$, $P < 0.05$).