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Perinatal photoperiod imprints the circadian clock

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Using real-time gene expression imaging and behavioral analysis, we found that the perinatal photoperiod has lasting effects on the circadian rhythms expressed by clock neurons as well as on mouse behavior, and sets the responsiveness of the biological clock to subsequent changes in photoperiod. These developmental gene × environment interactions tune circadian clock responses to subsequent seasonal photoperiods and may contribute to the influence of season on neurobehavioral disorders in humans.

Environmental factors, particularly light, can markedly influence neural development[1–5]. It is known that seasonal light input can acutely reorganize the mature biological clock located in the suprachiasmatic nuclei (SCN)[6], but whether development under different seasonal photoperiods can imprint the mammalian circadian clock is unknown. We exposed mice to different seasonal developmental photoperiods (short day, light:dark (LD) 8:16; long day, 16:8) until weaning, followed by 4 weeks of a matching or counter-balanced continuation seasonal photoperiod. At approximately 7 weeks of age, we either assayed the properties of their SCN circadian clocks by *ex vivo* imaging of a dynamic fluorescent reporter of circadian gene activity (as in ref. 7, see **Supplementary Methods**) or recorded wheel-running behavior in constant darkness (**Supplementary Fig. 1**). All animal care was conducted in accordance with Vanderbilt University Institutional Animal Care and Use Committee guidelines. In mature mice raised on LD 12:12 light cycles, seasonal photoperiod has been shown to be encoded by altering the relative peak times of individual neuronal electrical or molecular rhythms in the SCN, with long days eliciting more dispersed timing of neuronal rhythms and a broadened overall rhythmic waveform, and short days eliciting an increased degree of neuronal synchrony and a narrowed SCN waveform[8–10]. Analysis of the main effects of the proximal continuation photoperiod with our mouse line and reporter imaging method revealed similar findings, with long days eliciting SCN molecular waveform broadening, primarily as a result of increased variation in the phases of individual clock neurons, but with substantial changes in neuronal waveform and period as well (**Supplementary Results**, **Supplementary Fig. 2** and **Supplementary Table 1**).

To determine whether there were persistent effects of perinatal exposure to seasonal photoperiods on the SCN clock, we analyzed the main effects on SCN and neuronal properties grouped by developmental photoperiod regardless of ensuing continuation photoperiods (**Fig. 1** and **Supplementary Table 2**). In contrast with encoding of proximal photoperiod in mature mice, perinatal exposure to seasonal photoperiods persistently altered the waveform of the SCN ensemble molecular rhythm solely via alterations in the waveforms and periods of individual SCN neurons, rather than by network-level relative timing of neuronal rhythms. SCN from long day–developed mice were imprinted with narrow *Per1* GFP rhythm waveforms ($P = 0.006$; **Fig. 1a**) as compared with those developed on short days, regardless of whether mice had been subsequently maintained on long days or on short days. These developmental effects on SCN molecular waveform occurred exclusively at the level of individual neurons and were a result of waveform narrowing of individual SCN neurons in long day–developed mice ($P = 0.018$; **Fig. 1b**), rather than of any changes in the variance of neuronal peak times ($P = 0.601$; **Fig. 1c**). In addition, SCN neurons in the long day–developed SCN were imprinted with shortened rhythmic period ($P = 0.018$; **Fig. 1d**) and there was a similar trend in the period of SCN at the tissue level ($P = 0.002$;

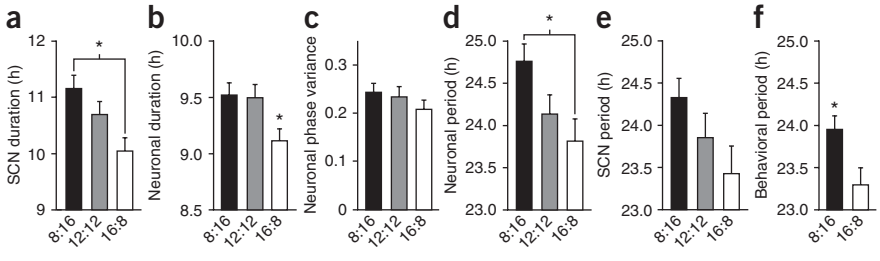

Figure 1 Persistent effects of perinatal seasonal photoperiod on SCN slice rhythms, on SCN neuronal rhythms, and on behavior. (**a**,**b**) Duration of the SCN peak (**a**) and neuronal peak (**b**) in *Per1* GFP expression, measured as the time from 50% maximum on the rising phase to 50% maximum on the falling phase of the molecular circadian rhythm. (**c**) Neuronal phase variance calculated from the peak times of individual SCN neurons using Rayleigh circular statistics. (**d**) Neuronal period calculated on the first full circadian cycle recorded *ex vivo*. (**e**) SCN period calculated from the first full circadian cycle recorded *ex vivo*. (**f**) Behavioral period calculated from the first 3 d in constant darkness. The black bar indicates the short-day developmental photoperiod, the gray bar indicates the equinox developmental photoperiod and the white bar indicates the long-day developmental photoperiod. $^*P < 0.05$, two-way ANOVA, main effect for perinatal photoperiod.

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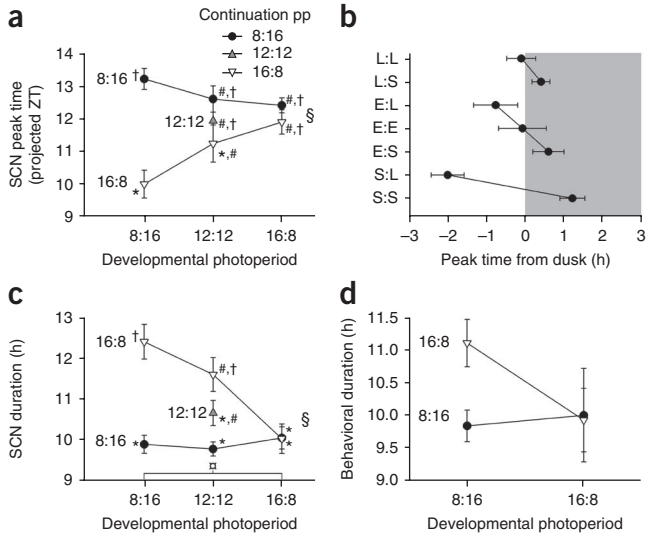

Figure 2 Interactions of perinatal seasonal photoperiod with subsequent seasonal photoperiod. (**a**) Two-way ANOVA interaction plot for peak times of *Per1* GFP expression in SCN. The developmental photoperiod is shown on the *x* axis. The continuation photoperiod (PP) for short day (LD 8:16) is represented by black circles, equinox (LD 12:12) by gray inverted triangles and long day (LD 16:8) by open triangles. (**b**) Timing of SCN molecular rhythm peaks relative to dusk. The white background represents lights-on and the gray background represents lights-off. The photoperiodic procedure is represented as 'developmental:continuation' (L, long-day photoperiod; E, equinox photoperiod; S, short-day photoperiod). (**c**) Two-way ANOVA interaction plot for waveform duration from 50% rise to 50% fall of the first peak *ex vivo*. (**d**) Behavioral duration of activity per circadian cycle in the first 3 d of constant darkness. Error bars represent s.e.m. Significance is indicated by symbols (*, #, †) such that means sharing a symbol are not significantly different ($P > 0.05$), whereas means with different symbols are significantly different ($P < 0.05$). § indicates a significant difference between continuation photoperiods ($P < 0.05$); ¤ indicates a significant main effect of developmental photoperiod ($P < 0.05$).

Fig. 1e). The developmental imprinting of neuronal circadian period was also reflected in circadian behavior; mice that developed in the long photoperiod had a significantly shorter free-running period than mice that developed in the short photoperiod (**Fig. 1f**). Of note, the LD 12:12–developed group showed similar trends in most SCN and neuronal parameters, but with values intermediate between the short day– and long day–developed groups. These results suggest that there are developmental influence of perinatal seasonal photoperiods on circadian neuronal rhythms that persist even in the face of weeks of alternate photoperiodic input and that these effects are manifest in circadian behavior.

This developmental imprinting of clock neurons by seasonal photoperiods markedly altered the functional response of the circadian pacemaker to subsequent photoperiodic stimuli (**Fig. 2** and **Supplementary Table 3**). SCN from long day–developed mice exhibited a stable timing relationship to the nocturnal light/dark transition on both long-day and short-day light cycles, with their molecular rhythms closely tracking dusk on both long-day and short-day continuation photoperiods (**Fig. 2a**,**b**). In contrast, SCN from short day–developed mice exhibited large changes in their timing relative to dusk in different continuation photoperiods, with their molecular rhythm peaking after dusk when continued in short days, but peaking before dusk when continued on long days ($P = 0.007$; **Fig. 2a**,**b**). Similarly, SCN from long day–developed mice were imprinted with a stable, narrow waveform that persisted in either continuation photoperiod, whereas short day–developed SCN exhibited narrow waveforms when continued on short days, but responded to subsequent lengthening of the photoperiod with greatly broadened waveforms ($P < 0.001$; **Fig. 2c**). The duration of behavioral activity on each cycle followed a similar trend ($P = 0.06$; **Fig. 2d** and **Supplementary Table 3**).

These results indicate that there is an environmental imprinting of the mammalian circadian clock and its response to subsequent seasonal change under seasonal light cycles. Perinatal exposure to long days imprints the SCN clock and its constituent neurons with narrowed waveforms of *Per1* promoter activation that manifest as shorter behavioral activity duration in constant darkness. SCN from long day–developed mice also exhibit shortened neuronal circadian period, which was reflected in behavioral free-running period and in a consistent timing relationship to dusk. All of these properties were stable in the face of subsequent exposure to seasonally changing photoperiod. In contrast, perinatal exposure to short days imprinted lengthened neuronal waveforms and circadian periods (neuronal and behavioral), which then resulted in high amplitude changes in SCN rhythm timing and waveform when exposed to subsequent seasonally changing photoperiod. In particular, SCN neuronal waveform changes may manifest as a change in the duration of behavioral activity, depending on the proximal photoperiod. Development in LD 12:12, which has been used in previous studies of SCN seasonal encoding[8–10], induced an intermediate state in which subsequent photoperiods evoked waveform changes of larger amplitude than those evoked in long day–developed mice, but that were smaller amplitude than those evoked in short day–developed mice. Taken together, these results suggest that perinatal seasonal light cycles modulate the neural network encoding of seasonal light cycles by imprinting the molecular pacemaker in individual clock neurons.

The precise molecular mechanisms by which photoperiodic imprinting modifies clock properties are unknown. Our finding that this imprinting occurs at the level of individual clock neuron gene rhythms rather than at the level of neuronal phase variance suggests that the mechanism is cell-autonomous. Although there are many potential control points by which photoperiod could modify molecular circadian rhythms, genetic and pharmacological manipulation of casein kinase 1 isoforms (δ and ε) have been shown to have profound effects on circadian period and waveform[11], and expression of these kinases in the SCN is affected by light[12], making them likely targets for further study. Future studies will be needed to determine whether the imprinting that we found is a result of direct epigenetic modification of clock genes (that is, gene methylation or acetylation) or whether it occurs at another level of organization.

Our results, as well as a recent report on the epigenetic effects of illumination during the development of dopamine neurons in the frog SCN[3], suggest that circadian visual pathways are capable of the kind of developmental plasticity seen in cortical visual pathways[2,4,5]. A limitation of our study is that, although we found that these developmentally induced changes in the SCN and its constituent neurons persisted for weeks and were not reversed by altered photoperiods, we do not know how long they may persist. However, alterations in the circadian pacemaker properties of cockroaches, induced by perinatal exposure to non–24-h days, have been shown to persist for a substantial portion of total lifespan[13].

The seasonal imprinting of the biological clock that we found likely has further implications for circadian-influenced neurobehavioral disorders. Perinatal exposure to winter-like seasonal light cycles identical to our procedure induces persistent elevated depressive and anxiety-like behaviors in rodents[14], and winter-born humans exhibit

enhanced rates and severity of seasonal affective disorder, as well as elevated risk of bipolar disorder and schizophrenia[6]. In addition, individuals with seasonal affective disorder also exhibit enhanced circadian responses to seasonal light cycles[15], similar to the short day–matured mice that we used. An understanding of the mechanisms by which seasonal stimuli shape the circadian system during development may contribute to understanding the neural basis of seasonality and the influence of season on neurobehavioral disorders.

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

C.M.C. and D.G.M. designed the experiments. C.M.C., J.C.A. and B.R.S. performed the experiments and compiled the results. C.M.C. and K.L.G. performed statistical analyses. C.M.C. and D.G.M. wrote the paper.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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