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PHOTOPERIOD MODULATES FAST DELAYED RECTIFIER POTASSIUM CURRENT IN THE MAMMALIAN CIRCADIAN CLOCK

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ABSTRACT

One feature of the mammalian biological clock, situated in the suprachiasmatic nucleus (SCN), is its ability to measure day length and thereby contribute to the seasonal adaptation of physiology and behavior. The phase distribution of the electrical activity pattern of SCN neurons is adjusted according to the photoperiod being broader in long-day and narrower in short-day photoperiod. VIP and GABA play a crucial role in the neuronal network of the SCN, and contribute to the phase distribution. However, little is known about the underlying cellular mechanisms. The present study was aimed to identify cellular mechanisms involved in seasonal encoding by the SCN. Mice were adapted to long-day (LD16:8) and short-day (LD 8:16) photoperiods and membrane properties as well as K^+ currents activity of SCN neurons were measured using patch-clamp recordings in acute slices. The results indicate that the rhythms in firing rate, resting membrane potential and SCN neuron's input resistance were not affected by different photoperiods. Among the voltage-sensitive K^+ currents, the peak values of the slow delayed rectifier and transient A-type K^+ currents measured mid-day and mid-night were not affected by the photoperiod. Remarkably, the fast delayed rectifier current (FDR) was increased during the night in long photoperiod while the peak value of FDR current during the day remained unchanged. This led to an inversed rhythm of FDR current activation with peak values at night exceeding 150% of the ones measured during the day. The FDR current is selectively modulated by photoperiod and we suggest that it may contribute to photoperiodic phase adjustment.

INTRODUCTION

Organisms have developed an endogenous clock to adapt their physiology and behavior with daily and seasonal changes. In mammals the suprachiasmatic nucleus (SCN) of the anterior hypothalamus serves as a central circadian clock, which controls rhythms in other brain areas and in peripheral tissues (van Esseveldt et al., 2000). Most SCN neurons show a sinusoidal pattern in electrical activity rhythm with a peak in the middle of the day. Thus in nocturnal animals, the rhythm of the SCN is opposite to the behavioral activity rhythm (Mason, 1991; Zlomanczuk et al., 1991; Mrugala et al., 2000b; Moore, 2007; Houben et al., 2009). Besides its role as a daily pacemaker, the SCN is able to encode the seasonal change in day length, by adjusting the composite pattern of electrical activity (Mrugala et al., 2000a; Vanderleest et al., 2007; Brown and Piggins, 2009).

Seasonal changes in day length modulate the waveform of the SCN electrical activity rhythm resulting in a broad peak during long summer days and a narrow pattern in short winter days (Sumova et al., 1995; Vanderleest et al., 2007; Brown and Piggins, 2009). The electrical activity is an important output of the SCN which affects behavioral activity patterns. As a consequence of the altered waveform in electrical activity, mice shorten the duration of their behavioral activity in long days and lengthen the duration in short days.

The seasonal change in SCN waveform is based on a phase redistribution of individual neuronal activity rhythms. In short days, the cells are more synchronized in phase while in long days they become desynchronized. This is the case both for SCN single cell electrical activity patterns and for molecular expression profiles (Vanderleest et al., 2007; Naito et al., 2008; Brown and Piggins, 2009). Altered phase distribution of clock genes expression such as period 1, period 2, rev-erb and dbp was observed in long-day and short-day photoperiod (Hazlerigg et al., 2005; Inagaki et al., 2007). At the single cell level no influence was detected on the profile of clock gene expression (Naito et al., 2008) or electrical activity patterns (Vanderleest et al., 2007; Brown and Piggins, 2009). Therefore, the plasticity within the SCN networks account for a narrower phase distribution of neuronal activity and of clock gene expression in short (8h) winter days compared to a broader phase distribution in long (16h) summer days (Rohling et al., 2006; Vanderleest et al., 2007; Brown and Piggins, 2009). There is evidence for a role of VIP as well as for GABA in photoperiodic phase adjustment (Lucassen et al., 2012; Evans et al., 2013). Thus intercellular communication plays a major role in the adjustment of the SCN to photoperiod.

The seasonal adaptation of network properties seems to be based on cellular changes in neurotransmission and receptor function. In the SCN, photoperiodic cues switch the GABAergic function from inhibitory to excitatory by changing the equilibrium potential of GABA (Farajnia et al., 2014). In paraventricular and periventricular nuclei of rats exposed to different photoperiods, a switch between somatostatin and dopamine neurotransmitters has been reported via modification in the number of intracellular storage vesicles as well as changes in post synaptic receptor population (Dulcis et

al., 2013). These findings indicate that cellular changes occur in paraventricular, periventricular and SCN nuclei of hypothalamus under the influence of photoperiod. Despite detailed knowledge of the neurotransmitters and neuropeptides involved in intercellular communication within the SCN, the mechanisms of synchronization and photoperiod-induced phase dispersal are still elusive. Potential changes in neuronal properties - like ion currents - affecting excitability and therefore the degree of synaptic interaction, have not been investigated and are the focus of the present study.

We performed patch-clamp experiments in acutely prepared slices of the SCN from mice which had been entrained to either long-day (16 h) or short-day (8 h) photoperiod. We measured the activity of three different K^+ currents –fast delayed rectifier (FDR), A-type current (I_A) and slow delayed rectifier (SDR)– as well as spike frequency and passive membrane properties during the day and at night. We report an altered circadian modulation of FDR current activity induced by long-day photoperiod with higher amplitude at night compared to short-day photoperiod while no photoperiod-induced differences were observed in the amplitude of I_A and SDR currents.

MATERIAL AND METHODS

Animals and housing

Male C57BL/6 mice (Harlan, Horst, The Netherlands, 100 day-old; n=41) were individually housed in cages with a running wheel under a long-day (16h light/8h dark) and short-day (8h light/16h dark) photoperiod in climate controlled rooms with ad libitum access to food and water. Animals were adjusted to the long-day or short-day photoperiod for a minimal period of 4 weeks which is required to develop the full phenotype (Vanderleest et al., 2007). All experimental procedures were approved by the Committee on Animal Health and Care of the Dutch government (no. 11010).

Slice preparation

On the experimental day animals were anesthetized (2% isofluorane) and killed for slice preparation as described previously (Farajnia et al., 2012). In brief, brain slices containing the SCN were transferred to a recording chamber (RC-26 G; Warner Instruments; Hamden, CT, USA) and placed on the stage of an upright microscope (Axioskop FS-2, Zeiss; Oberkochen, Germany) for the patch-clamp experiments. Recordings were performed at external time 12 ± 3 h and external time 0 ± 3 h. External time 12 is defined as the middle of the day in a given photoperiod (Daan and Merrow, 2002).

Patch-clamp recordings

Micropipettes of 5-7 MΩ were pulled with a commercial puller (PC-10 Narishige; London, UK) from borosilicate tubing and filled with an internal solution (PH: 7.2-7.3; osmolality: 290-300 mOsm) containing (in mM): 112.5 K-gluconate, 1 EGTA, 10 Na^+-

HEPES, 5 MgATP, 1 GTP, 0.1 leupeptin, 10 phosphocreatine, 4 NaCl, 17.5 KCl, 0.5 CaCl2, and 1 MgCl2 (Sigma-Aldrich).

All recordings were performed using a patch amplifier (EPC 10-2; HEKA, Lambrecht/Pfalz, Germany). Firing frequencies and resting membrane potential were measured in current-clamp configuration. Membrane input resistance was obtained from the value provided by the amplifier software which uses holding current, holding voltage and access resistance to calculate the input resistance. Thereafter, fast and slow delayed rectifier (FDR and SDR, respectively) and I_A potassium currents were measured in voltage-clamp configuration. FDR, SDR and I_A currents were isolated as described previously (Farajnia et al., 2012). Briefly, Na^+ and Ca^{2+} currents as well as spontaneous GABAergic currents were eliminated by application of tetrodotoxin (0.5 µM; Tocris bioscience), cadmium (25 µM) and bicuculine (20 µM; Sigma-Aldrich) respectively. Afterward, tetraethylammonium (TEA) in low and high concentrations (1 and 20 mM; Sigma-Aldrich), was applied to block FDR and SDR currents respectively. Current traces were evoked by 400 ms progressively depolarizing voltage pulses (−50 to +60 mV, 10 mV increments), following a prepulse at −100 mV for 100 ms. FDR currents were isolated by subtracting the current traces acquired in the presence of TEA (1mM) from the ones obtained in control solution. In addition, digital subtraction of traces in 20 mM TEA from traces obtained in 1mM TEA (blocking FDR) resulted in isolation of SDR.

To characterize I_A currents, identical voltage protocol (-60 to +60 mv, 150 ms, 10 mV increments) was applied but with two different prepulses to activate (-90 mV, 150 ms) or inactivate (-45 mv, 150 ms) the current. I_A current was isolated by subtracting the traces lacking the I_A current from traces containing the current.

Data analysis

Recordings with series resistance lower than 40 MΩ were considered in the final analysis. Fit master (version 2.67; HEKA, Lambrecht/Pfalz, Germany), Igor Pro (version 6.22A; Wavemetrics, Portland, OR), MiniAnalysis (version 6.0.7; Synaptosoft, Fort Lee, NJ) and SPSS (version 17.0; IBM, Armonk, NY) were used for statistical analysis. All values were tested first for normality of the data and homogeneity of the variances using Shapiro-Wilk and Levene tests respectively. Accordingly, an appropriate statistical test was chosen to evaluate the significant differences. Differences with $P \leq 0.05$ were considered to be significant.

RESULTS

Firing rate, resting membrane potential and input resistance are not affected by photoperiod

The frequency of spontaneously generated action potentials was comparable in long-day and short-day photoperiod both during the day ($t_{12.986} = 1.185$, $P = 0.257$) and

night (t_{38} = -0.259, P = 0.797; **Fig. 1**). Firing rate was significantly decreased at night as compared to the day in cells recorded from animals adjusted to long-day (t_{51} = 2.229, P = 0.03) and short-day photoperiod (t_{26} = 2.682, P = 0.013, independent student *t*-test).

Resting membrane potential exhibited a circadian rhythm in both long-day (t_{52} = 2.473, P = 0.017) and short-day photoperiod ($t_{17.213}$ = 2.607, P = 0.018, independent student *t*-test) with no difference in the amplitude between the photoperiods (**Fig. 2A**). Likewise, membrane input resistance was rhythmically controlled in both long days ($t_{92.833}$ = 2.782, P = 0.007) and short days ($t_{55.794}$ = 2.475, P = 0.016, independent student *t*-test) and values did not differ between photoperiods (**Fig. 2B**).

Fast delayed rectifier K^+ current is enhanced during the night in long photoperiod

The magnitude of the FDR current was higher in the night than in the day in long photoperiod (t_{16} = -2.776, P = 0.0134; **Fig. 3A**). In short photoperiod, significantly higher values were recorded in the day compared to the night ($t_{7.217}$ = 2.386, P = 0.047; **Fig. 3A**). The main difference between long-day and short-day photoperiod was recorded during the night when a larger FDR current was observed in long photoperiod (**Fig. 3B, C**; t_{22} = 2.695, P = 0.0132). During the day no differences in the FDR current magnitude were distinguished between long-day and short-day photoperiod (t_{18} = -0.301, P = 0.8, independent student *t*-test).

SDR and I_A K^+ currents are indistinguishable in long-day and short-day photoperiod

SDR current is not rhythmically controlled in LD 12:12 conditions. Recordings in long-day (t_{13} = -1.956, P = 0.072) and short-day ($t_{18.868}$ = 1.478, P = 0.156) photoperiod did also not reveal a circadian rhythm in the SDR current amplitude (**Fig. 4A**). No difference in the current amplitude was found between short and long photoperiod neither during the day (t_{16} = 1.042, P = 0.313) nor during the night ($t_{12.976}$ = -1.565, P = 0.142, independent student *t*-test).

Consistent with previous findings in LD 12:12 cycle, I_A current amplitude was higher during the day as compared to the night both in long-day (z_{28} = -2.082, P = 0.037) and short-day photoperiod (z_{35} = -2.56, P = 0.010). No significant differences were found between the animals adjusted to long and short photoperiod (day: z_{27} = -0.047, P = 0.962, night: z_{36} = -0.22, P = 0.826, Mann-Whitney test; **Fig. 4B**).

DISCUSSION

To investigate neuronal mechanisms underlying seasonal encoding within the circadian clock, patch-clamp recordings were performed in SCN slices from mice entrained to long-day and short-day photoperiods. Whole-cell recordings showed that the frequency

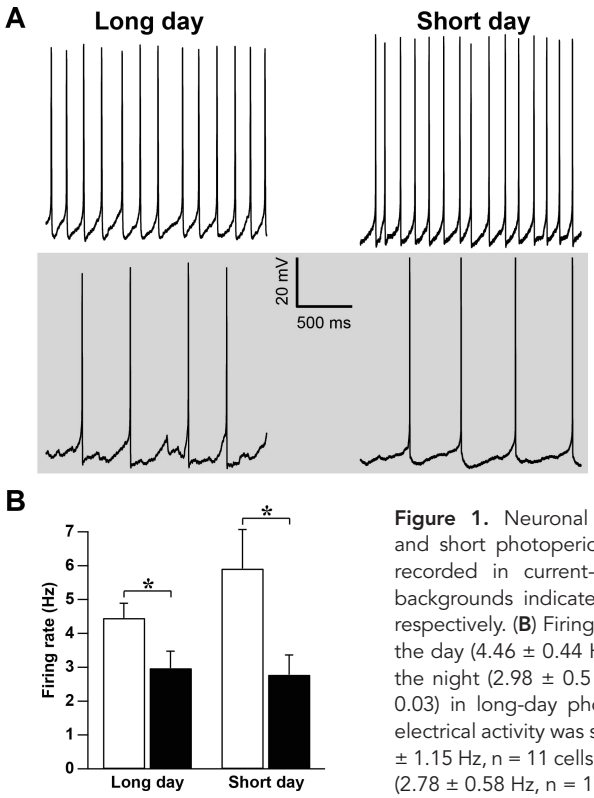

Figure 1. Neuronal electrical activity recorded in long and short photoperiod. (**A**) Examples of neuronal activity recorded in current-clamp mode. The white and gray backgrounds indicate the day- and night-time recordings respectively. (**B**) Firing rate (mean ± SEM) was higher during the day (4.46 ± 0.44 Hz, n = 30 cells from 9 animals) versus the night (2.98 ± 0.5 Hz, n = 23 cells from 8 animals; P = 0.03) in long-day photoperiod. In short-day photoperiod, electrical activity was similarly increased during the day (5.92 ± 1.15 Hz, n = 11 cells from 8 animals) compared to the night (2.78 ± 0.58 Hz, n = 17 cells from 8 animals). * $P < 0.05$.

of spontaneous action potentials is not significantly different in animals adapted to either long days or short days. This result is consistent with previous recordings using extracellular electrophysiological recordings (Vanderleest et al., 2007; Brown and Piggins, 2009). Moreover, we showed that input resistance and resting membrane potential were also comparable in neurons entrained to long-day and short-day photoperiod. In this study we observed an up-regulation of the FDR current during the night in long-day photoperiod, while SDR and I_A currents remained unchanged. Interestingly, the amplitude of the FDR current is even higher during the night than during the day, which indicates a reversal of the circadian modulation as compared to recordings from 12:12 LD conditions (Itri et al., 2005; Farajnia et al., 2012) and as compared to recordings from animals adapted to short days (**Fig. 3**). The results indicate that photoperiodic encoding is accompanied by a selective modulation of the FDR current.

Single cell membrane properties do not contribute in the SCN photoperiodic phase adjustment

It has been shown previously in a 12:12 photoperiod that the electrical activity, membrane potential and input resistance are rhythmically controlled with higher

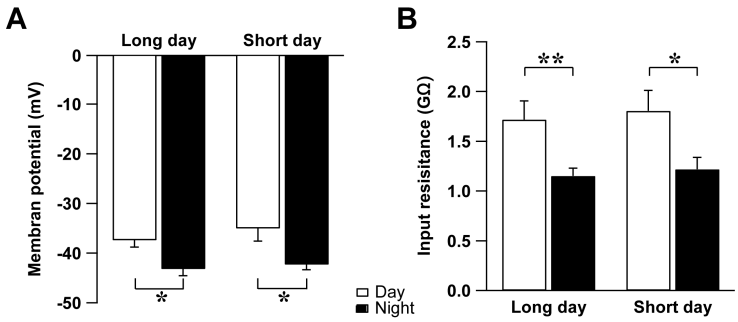

Figure 2. Passive neuronal properties recorded from SCN slices adjusted to long-day and short-day photoperiod. (**A**) Resting membrane potential (mean ± SEM) was rhythmically controlled in both long-day (day: n = 38 cells from 10 animals, night: n = 16 cells from 6 animals) and short-day photoperiod (day: n = 14 cells from 7 animals, night: n = 11 cells from 6 animals). No difference was found between long and short photoperiod neither during the day (t_{50} = -0.848, P = 0.401) nor during the night (t_{25} = -0483, P = 0.633). (**B**) Input resistance (mean ± SEM) showed a circadian rhythm similar to what is seen in 12:12 LD cycles both in long-day (day: n = 71 cells from 11 animals, night: n = 59 cells from 8 animals) and short-day photoperiod (day: n = 35 cells from 11 animals, night: n = 48 cells from 9 animals). There was no difference between the short and long photoperiod (day: t_{104} = -0.288, P = 0.774, night: t_{105} = 0.482, P = 0.631). * $P < 0.05$, ** $P < 0.01$.

values during the day compared to the night. Extracellular recordings of single- and multi-units revealed that individual unit activity patterns are not modified by photoperiod while the waveform of collective electrical activity extends or compresses in long-day and short-day photoperiod respectively (Vanderleest et al., 2007; Brown and Piggins, 2009). Consistent with previous research, our intracellular recording of single SCN neurons shows that neuronal electrical activity remains unchanged in different photoperiods. Resting membrane potential is more depolarized during the day compared to the night and is important for regulation of the firing frequency as it increase the probability of triggering an action potential during the day (Schaap et al., 1999). The membrane potential in SCN neurons is regulated by a yet to be identified K^+ current whose modulation is reflected by a decrease in input resistance during the night as compared to the day (De Jeu et al., 2002; Kuhlman and McMahon, 2004). We found that photoperiod did not affect the circadian modulation or amplitude of either resting membrane potential or input resistance. This implies that K^+ conductances involved in regulation of resting membrane potential, do not contribute to seasonal adaptation, albeit their role in daily rhythms.

FDR current is modulated by photoperiod

The FDR current is the only membrane component among the parameters recorded in the study, which is modulated by photoperiod. In neurons of the SCN, the activity of FDR current is under circadian control and contributes to the rhythm in electrical activity by enhancing repolarization of the action potential and increasing firing rate during the

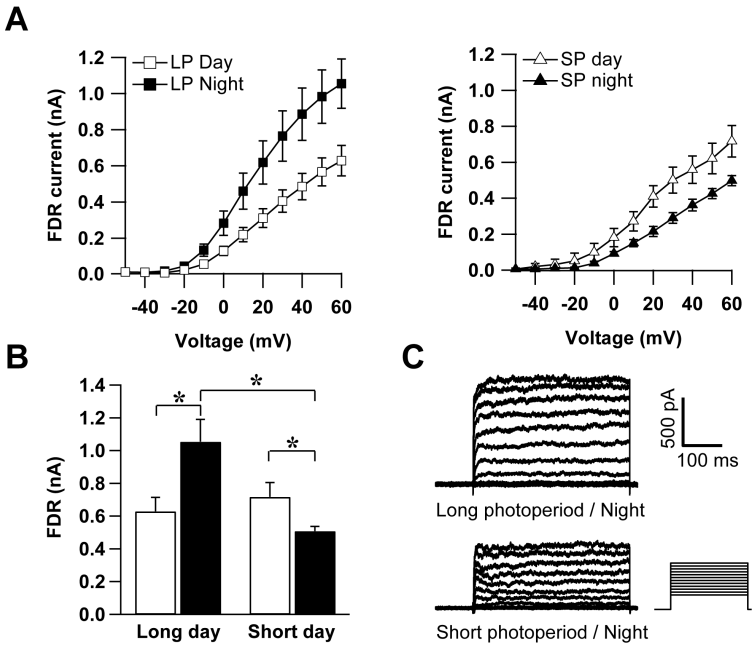

Figure 3. Fast delayed rectifier K^+ current (FDR) recorded from SCN neurons adjusted to long-day and short-day photoperiod. (**A**) FDR current amplitude (mean ± SEM) showed a circadian rhythm in both long-day and short-day photoperiod. This rhythm was reversed in long-day photoperiod as compared to short-day and equinoctial 12:12 photoperiod. LP = long photoperiod and SP = short photoperiod. (**B**) During the day the magnitude of FDR currents did not differ in long (629.05 ± 85.08 pA, n = 12 cells from 4 animals) and short photoperiod (717.28 ± 88.02, n = 8 cells from 6 animals; *P* = 0.8). During the night the FDR current In long photoperiod was significantly increased (1.06 ± 0.14 nA, n = 6 cells from 4 animals) compared to short photoperiod (5.08 ± 2.87 pA, n = 18 cells from 10 animals). (**C**) Examples of FDR current traces elicited by a voltage protocol (right side; -50 mV to +60 mV, 400 ms , from a -100 mV prepulse) in long and short photoperiod during the night.

day (Itri et al., 2005). Thus modification in the amplitude of this current is expected to modify the electrical activity. However in the present study we detected elevated night-time FDR current amplitude whereas the action potential frequency was unchanged. The membrane potential remains hyperpolarized at night in long-day photoperiod, which may prevent the modulation of firing frequency by the increased FDR current. Moreover other ionic conductances responsible for modulating firing rate at night -such as BK currents- may compensate for changes in the FDR current. This raises the question, weather the FDR current plays a different role in photoperiodic encoding in the SCN distinct from regulation of AP frequency. In other brain areas, such as in the auditory system FDR current plays a crucial role for creating sustained high-frequency trains of action potentials with little spike frequency adaptation (Rudy and McBain, 2001). However, FDR channels are also expressed in slow firing neurons, but may serve a different function. For instance in retinal amacrine cells, FDR current provides a voltage dependent shunt that

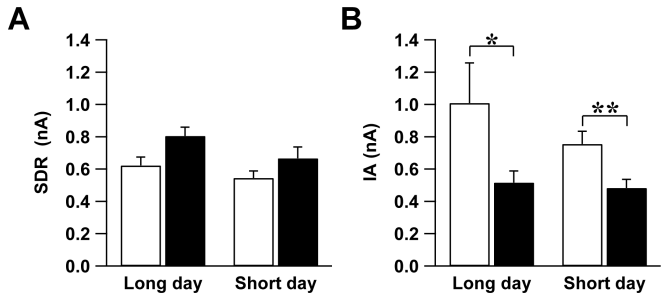

Figure 4. Slow delayed rectifier (SDR) and A-type (I_A) K^+ currents recorded from animals adjusted to long and short photoperiod. (**A**) The amplitude of SDR current (mean ± SEM) was not significantly different between day (n = 11 cells from 4 animals) and night (n = 5 cells from 3 animals) in long photoperiod (P = 0.072). Day (n = 7 cells from 5 animals) and night (n = 14 cells from 6 animals) recordings were also indistinguishable in short photoperiod (P = 0.156). No differences was found between long-day and short-day photoperiod (**B**) I_A current showed a larger amplitude (mean ± SEM) during the day (n = 10 cells from 6 animals) as compared to the night (n = 21 cells from 4 animals) in long photoperiod. In short photoperiod the amplitude of this current was also higher during the day (n = 20 cells from 4 animals) than the night (n = 17 cells from 3 animals). No differences in amplitude of I_A current was found between the two photoperiods neither during the day nor during the night. * $P < 0.05$, ** $P < 0.01$.

controls membrane depolarization (Ozaita et al., 2004). Likewise, in the SCN modulating the electrical rhythm, is not the only function of the FDR current.

FDR current may contribute to photoperiodic regulation in the SCN

FDR channels deficient mice (lacking Kv3.1 and Kv3.2) show a disturbed behavioral phenotype with low amplitude and fragmented circadian patterns in locomotor activity as well as deficits in processing and re-entrainment to photic information (Kudo et al., 2011). FDR currents are therefore considered to be vital to synchronize the SCN to the environmental light cycle. In the context of our data, it is of special interest that FDR current contributes to long-range synchronization between inhibitory interneurons of the neocortex (Harvey et al., 2012). Long-range cell-to-cell connections between ventrolateral and dorsomedial regions of the SCN may play a role in maintaining a narrow phase distribution within the SCN in short-day photoperiod as a simulation study suggested (Bodenstein et al., 2012). Moreover, long-range connections are important for synchronization of the neuronal activity and clock gene expression to photic information between dorsomedial and ventrolateral regions of the SCN (Leak et al., 1999; Welsh et al., 2010). The dorsomedial region of the SCN receives the light input indirectly through the ventrolateral region. Both VIP and GRP -generated in the ventral SCN- increase the FDR current in the dorsal SCN (Gamble et al., 2011; Kudo et al., 2013), suggesting that FDR current contributes to long-range communications in the SCN. If so, the enhancement of the FDR current during the day in 12:12 or short-day photoperiods may

be instrumental to determine the degree of synchronization. The reversal of circadian modulation of FDR in long-day photoperiod may lead to weakening of long-range functional connections and a wider distribution of phases. Therefore, a reversed rhythm in FDR amplitude contributes to plasticity of the SCN network in response to seasonal changes. Conformingly, VIP-deficient animals which have compromised rhythms in FDR current (Farajnia et al., unpublished data) have difficulties to encode different photoperiods in the SCN endogenously (Lucassen et al., 2012).

FDR current in aging and seasonal adaptation

An increase in FDR current amplitude at night has also been found in SCN neurons from mice older than 24 month (Farajnia et al., 2012). There are noticeable resemblances between the aged phenotype and long-day photoperiod (Table 1), such as a short duration of behavioral activity, a low amplitude of multi-unit electrical activity rhythm, an increased phase distribution of neuronal activity and a reduction in phase-shifting capacity (Rohling et al., 2006; Vanderleest et al., 2007; Biello, 2009; Vanderleest et al., 2009; Nakamura et al., 2011; Farajnia et al., 2012). The present study identified another similarity to the previous aging study (Farajnia et al., 2012), which is an elevated FDR current at night. We propose that the reversion in FDR rhythm by long photoperiod is part of the mechanism of day length encoding while, the lack of circadian modulation of FDR in the old SCN may be a consequence of age-related functional decline as also seen for other currents like the I_A (Farajnia et al., 2012) and

Table 1. Aging resembles long photoperiod in some aspects but not in cellular functions

	Aged vs young		Long vs short days		References
Phase-shifting capacity	Reduced		Reduced		1,2
Electrical rhythm amplitude	Low		Low		2,3,4
Behavioral activity duration	Short		Short		2,3
Electrical activity pattern	Anti-phase		Broad		2,3
Membrane potential	-	↑	-	-	3, 9
Input resistance	-	↑	-	-	3, 9
Firing rate	↓	-	-	-	2,3,5,6, 9
I_A current	↓	-	-	-	3, 9
FDR current	-	↑	-	↑	3, 9
SDR current	-	-	-	-	3, 9
GABAergic current	↓	↓	↑	-	3,7,8

↑, increase; ↓, decrease and -, no change in the mentioned property. Aged is compared with young and long-day is compared with short-day. When a cell is divided to white and gray background it indicates the changes during the day or at night respectively. 1: Biello 2009, 2: Vanderleest et al. 2007, 3: Farajnia et al. 2012, 4: Nakamura et al.2011, 5: Aujard et al. 2001, 6: Watanabe et al. 1995, 7: Nygard et al. 2005, 8: Farajnia et al. 2014, 9: current study.

BK (**chapter 4**). The current data suggest that the age-related increase in FDR current during the night may contribute to the desynchronization observed in the aged SCN network. In centenarians, an increase in TEA-sensitive voltage-gated K^+ currents seems to be crucial for longevity (Zironi et al., 2010). Accordingly, amplification of delayed rectifier K^+ currents such as FDR currents may be beneficial for longevity.

In aging, cellular deficiencies change the SCN output and generate a less effective signal. However, it seems that photoperiodic adaptation to long days implies a readjustment of cellular functions to control and support interneuronal communications. For instance, GABAergic signaling, which is important for interneuronal communication, is altered in long-day photoperiod as a consequence of molecular changes in the GABA equilibrium potential (Farajnia et al., 2014). In this study we report a reversed circadian modulation of FDR current which may affect the long-range communication.

In summary we conclude that the circadian modulation of FDR current is subjected to seasonal changes in day length. In addition, FDR currents control or relay photic information in the SCN (Kudo et al., 2011). Therefore, there is a bilateral interaction between photic information and FDR currents. FDR current may contribute in long-range neuronal synchronization in the SCN network. In different physiological and environmental conditions -such as aging and seasonal adaptation- FDR current undergoes changes. As a result, loss or reversal of the rhythm in FDR current may contribute to a broader phase distribution in the SCN network and remains to be elucidated.

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