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Age-related changes in electrophysiological properties of the mouse suprachiasmatic nucleus in vitro

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Abstract

Endogenous biological rhythms are altered at several functional levels during aging. The major pacemaker driving biological rhythms in mammals is the suprachiasmatic nucleus of the hypothalamus. In the present study we used tissue slices from young and old mice to analyze the electrophysiological properties of the retinorecipient ventrolateral part of the suprachiasmatic nucleus. Loose patch and whole-cell recordings were performed during day and night. Both young and old mice displayed a significant variation between day and night in the mean firing rate of suprachiasmatic nucleus neurons. The proportion of cells not firing spontaneous action potentials showed a clear day/night rhythm in young but not in old animals, that had an elevated number of such silent cells during the day compared to young animals. Analysis of firing patterns revealed a more regular spontaneous firing during the day than during the night in the old mice, while there was no difference between day and night in young animals. The frequency of spontaneous inhibitory postsynaptic currents was reduced in ventrolateral suprachiasmatic nucleus neurons in the old animals. Since the inhibitory input to these neurons is mainly derived from within the suprachiasmatic nucleus, this reduction most likely reflects the greater proportion of silent cells found in old animals. The results show that the suprachiasmatic nucleus of old mice is subject to marked electrophysiological changes, which may contribute to physiological and behavioral changes associated with aging.

Keywords: Aging; Circadian; Spontaneous firing; GABA; Biological clock

1. Introduction

The circadian timing system undergoes marked alterations in mammals during aging. These include weakened ability to synchronize with external stimuli, dampening and fragmentation in activity and temperature cycles, and disruptions in sleep patterns [29]. In mammals the suprachiasmatic nucleus (SCN) of the anterior hypothalamus is considered to be the major pacemaker for circadian oscillations. Whether or not age-related alterations in endogenous biological rhythms reflect primary disturbances in the machinery of the SCN remains to be clarified. On one hand, alterations in the cyclic expression of certain neuropeptides [4,6,10,11,22] as well as reductions in amplitudes and occurrence of aberrant peaks in the spontaneous firing activity [3,24,28] have indicated that properties of individual SCN neurons change with aging. On the other hand, in old rats the cycling of the clock genes *Period* and *Cryptochrome* in the SCN remains robust [2,31], and the total number of neurons and mean somatic volume of the SCN are unchanged [17], which suggests that disruptions in circadian organization during aging could reflect changes at other levels of the circadian system. The importance of peripheral, extra-SCN oscillators has recently also come into focus [7] and age-related disturbances in the interactions between circadian oscillators have been suggested [31].

The ventrolateral, or core, part of the SCN receives retinal input, and neurons in this area express vasoactive intestinal polypeptide (VIP) [1]. VIP modulates inhibitory synaptic transmission in the SCN and shows circadian oscillations in its expression [8,9]. Both responses to light [27,32] and day/night oscillations of VIP [4,6,10,11,13] are altered during aging. Electrophysiological studies on the aged SCN have only been made on hamsters and rats previously [24,28].

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In those studies extracellular measurements were performed within the whole SCN, and both studies revealed a reduced amplitude of the rhythm of spontaneous firing. Considering the age-related changes in light responses and VIP expression that occur specifically in the ventrolateral SCN, we wanted to investigate if there are parallel changes in the electrophysiological properties of these neurons. We therefore performed cell attached and whole-cell recordings in the ventrolateral SCN of young and old mice, to study spontaneous firing at the single neuron level as well as synaptic transmission. We report that although the pattern of day/night firing frequencies is similar in the two age groups, there are significant differences at the cellular and synaptic levels.

2. Materials and methods

Male C57B/6 mice (B&K, Sollentuna, Sweden) were maintained on a 12/12 h light/dark cycle for at least 10 days before the experiments. All the animal procedures were conducted under institutional guidelines and local ethical committee approval. The entire research protocol adhered to the guidelines of the European Council Directive (86/609/EEC). Two light/dark regimes were used, one starting at 07:00 for the day measurements (Zeitgeber time (ZT) 4–10), and one starting at 22:00 for the night measurements (ZT 14–20). Mice were either 2–3 ($n = 16$) or 14–21 ($n = 14$) months old. They were anesthetized with fluothane and decapitated during the light phase (ZT 3 for day recordings and ZT 12 for night recordings), the brains were then quickly removed and placed in ice-cold artificial cerebrospinal fluid (aCSF) of the following composition (in mM): 126 NaCl, 2.5 KCl, 2.4 $CaCl_2$, 1.3 $MgCl_2$, 26 $NaHCO_3$, 1.2 NaH_2PO_4 and 6 glucose (osmolarity 295–300 mOsm), and oxygenated with 5% CO_2–95% O_2. Coronal hypothalamic slices (350 μm thick) were cut on a vibratome (H1200 Microcut, Bio-Rad, Hercules, CA, USA) and transferred to a recording chamber (volume 500 μl, manufactured at Karolinska Institutet) and perfused with aCSF (2 ml/min) at room temperature. Slices were held in place with nylon threads attached to a platinum ring and allowed to rest for approximately 1 h. The duration of a recording session was always <6 h. The recording pipettes were prepared from glass microcapillaries (Harvard Apparatus, Edenbridge, Kent, UK) using a two stage puller (PP-830, Narashige, Tokyo, Japan) and filled with (in mM): 140 potassium gluconate, 4 NaCl, 10 HEPES, 5 EGTA, 0.5 $CaCl_2$ and 1 $MgCl_2$ (pH 7.3–7.4; osmolarity 280–285 mOsm). The pipette (resistance 6–10 MΩ) was advanced blindly into the slice with slight positive internal pressure. Recordings were made from the ventrolateral part of the SCN with an Axopatch 200B amplifier (Axon Instruments Inc., Union City, CA, USA), and stored on a personal computer with a digital interface (Digidata 1200, Axon Instruments Inc.). Data acquisition was performed using pClamp 7 software (Axon Instruments Inc.).

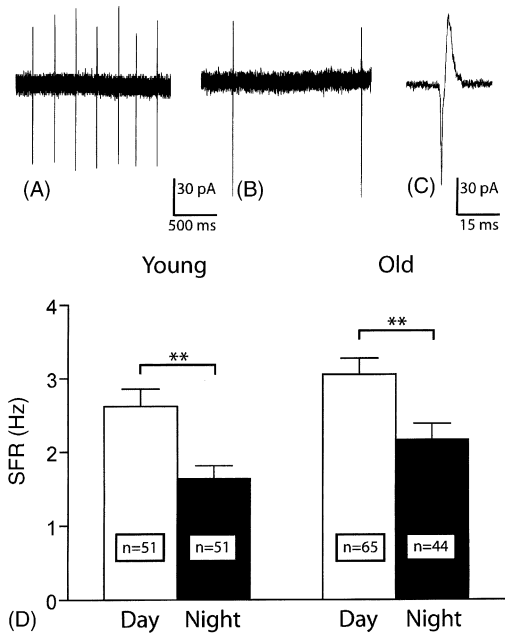

Fig. 1. Representative traces recorded with the loose patch technique during the day (A) and night (B). The characteristic shape of extracellularly recorded spikes could clearly be recognized on an expanded time scale (C). Both young and old animals showed a clear difference in spontaneous firing rate (SFR) between the day (ZT 4–10) and the night (ZT 14–20) (D). The amplitude of the rhythm was somewhat lower in the old animals mainly due to a higher firing rate during the night. The number of cells analyzed in a total of 3–4 slices per group is indicated in each bar. Cells not firing action potentials were not included in this analysis. Each bar represents the mean SFR ± S.E.M. $^{**}p < 0.01$ by one-way ANOVA followed by Bonferroni's post-hoc test.

Single-unit activity was recorded with the loose patch technique [20]. Cell approach and seal formation were achieved by monitoring the changes in current responses to voltage pulses. Seals were obtained with gentle or no suction to produce a loose patch seal with a resistance of less than 50 MΩ. Extracellular currents from spontaneous action potentials were recorded in voltage clamp mode at 0 mV holding potential. Recordings lasted from 30 s for cells firing regularly at high frequency and up to 120 s for cells that were silent or firing at low frequency. Tetrodotoxin (TTX; Sigma, St. Louis, MO, USA) was used (1 μM) to confirm that the observed current transients were action potentials (data not shown). Examples of current traces from cells during the day and night are shown in Fig. 1A–C.

For whole-cell recordings the cells were approached in the same way, but a stronger negative pressure was applied to produce a gigaohm seal. A second negative pressure pulse was then used to break the membrane. When breaking the membrane, a test pulse of −10 mV was delivered to monitor the change in capacitance. The junction potential was calculated to be −15 mV and voltages were corrected for this value off-line. Intracellular recordings lasted 90–120 s. Spontaneous inhibitory postsynaptic currents (sIPSCs) were acquired in voltage clamp mode, and holding potentials were in the range −45 to −70 mV. For correlations between inter-