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De Jeu, M.T.G.

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Chapter 2

Functional characterization of the H-current in SCN neurons in subjective day and night: a whole-cell patch-clamp study in acutely prepared brain slices

Marcel T.G. de Jeu and Cyriel M.A. Pennartz

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2. FUNCTIONAL CHARACTERIZATION OF THE H-CURRENT IN SCN NEURONS IN SUBJECTIVE DAY AND NIGHT: A WHOLE-CELL PATCH-CLAMP STUDY IN ACUTELY PREPARED BRAIN SLICES

2.1 Introduction

It has been repeatedly demonstrated that the suprachiasmatic nucleus (SCN) of the hypothalamus in mammals generates a circadian rhythm [2-4,65,66]. Electrophysiological studies have revealed the existence of circadian rhythmicity in spontaneous firing rate of SCN neurons [24,34-36,67]. Blocking the neuronal firing of SCN neurons does not prevent the circadian clock from running [37,39,40], indicating that these neurons have an intrinsic mechanism for circadian rhythmogenesis. The intracellular mechanism that is responsible for the expression of circadian rhythms in spontaneous firing rate is not yet known.

One prominent feature of many SCN neurons is the presence of a time- and voltage-dependent inward current, \( I_H \) [68,69]. \( I_H \) is a mixed \( \text{Na}^+ / \text{K}^+ \) current, which is activated by membrane hyperpolarization [70-75]. Upon hyperpolarization of the cell, the net influx of positively charged ions makes a slow excitatory contribution to the membrane potential and will rectify it back towards rest [72,73,76,77]. It has been suggested by several authors [72,73,76,78] that the physiological role of this current is to counterbalance transient or prolonged membrane hyperpolarizations in order to keep the membrane potential near the firing threshold. Since the reversal potential of \( I_H \) is estimated to lie between -50 and -20 mV [75], there is a distinct possibility that \( I_H \) contributes positively to the spontaneous firing rate. Results of McCormick and Pape [74,75] indicate that \( I_H \) indeed promotes spontaneous firing in thalamic relay neurons by keeping episodes of hyperpolarization limited in duration. Akasu and coworkers [68] reported that \( I_H \) may contribute to the spontaneous firing mechanism in SCN neurons by shortening the duration of the spike afterhyperpolarization (AHP), an effect which also reduces the interspike interval. This contribution of \( I_H \) to the spontaneous firing rate may vary in a circadian manner and thereby generate the circadian rhythm of spontaneous firing rate in SCN neurons. In this study we reassessed the contribution of \( I_H \) to the spontaneous firing rate of SCN neurons and also compared \( I_H \) across day and night. This reassessment must be considered in the light of the hypothesis that the magnitude and/or kinetics of \( I_H \) may follow a circadian rhythm itself, and may thus underlie the rhythm in spontaneous firing rate. Such a rhythm in \( I_H \) may result from a circadian modulation by a second messenger or from structural changes in the channel protein. The whole-cell patch-clamp technique allows to do single-electrode voltage-clamp experiments in continuous mode and investigate circadian conductance changes in \( I_H \). However, the whole-cell patch-clamp technique has one important disadvantage, namely that the whole-cell configuration will give rise to intracellular dialysis. Thereby this study does not conclusively ad-
dress the possibility of second messenger modulation of I_H. Functional consequences of structural channel modifications, other than those at messenger binding sites, are not subject to this problem.

We first investigated the contribution of I_H to the spontaneous firing rate, resting membrane potential (RMP) and spike AHP of SCN neurons in current-clamp mode during the subjective day phase, which is the period expected to reveal a positive contribution of I_H to the spontaneous firing rate if I_H would underlie the circadian rhythm in spontaneous firing rate. Second, we investigated I_H and its activation kinetics in voltage-clamp mode to obtain more insight in the characteristics of this current. Third, we investigated I_H and its activation characteristics at different circadian time points to determine whether the magnitude and/or kinetics of I_H vary in a circadian manner.

2.2 Material and methods

2.2.1 Slice preparation

Male Wistar rats, weighing 150-300g, were housed in a normal (lights-on 7.00h) or reversed (lights-on 24.00h) 12:12h light:dark cycle for at least 5 weeks before they were used. All rats were anaesthetized intraperitoneally with Nembutal (60 mg/kg sodium pentobarbital and 9 mg/kg benzylalcohol) followed by a transcardial perfusion of 50 ml ice-cold artificial cerebrospinal fluid (ACSF) with a perfusion pressure of 80-100 mm Hg. The ACSF contained (in mM): 124.0 NaCl, 3.5 KCl, 1.0 NaH_2PO_4, 26.2 NaHCO_3, 1.3 MgSO_4, 2.5 CaCl_2 and 11 D(+)glucose and was gassed with a mixture of 95% O_2 and 5% CO_2 (pH 7.4). After the perfusion the rats were decapitated and their brains were removed rapidly and immersed in cooled ACSF. The brains were trimmed to a block containing the hypothalamus and transversal slices of 250 μm were cut with a vibroslicer (Campden Instruments, UK). The slices containing the SCN were transferred to a storage chamber, where they were incubated in ACSF for at least an hour. After this period a slice was placed in the recording chamber through which ACSF (30°C) flowed at 2-3 ml/min. All brain slices were prepared during the light period in order to prevent a phase shift in the circadian rhythm of SCN neurons [79]. Slices that were used for experiments in the subjective day or night period were made at CT 4h and CT 11h, respectively.

2.2.2 Electrophysiological recordings

Patch pipettes were filled with a solution of the following composition (in mM): 135.0 Potassium Gluconate, 10.0 KCl, 10.0 HEPES, 0.5 EGTA, 2 Na_2ATP, 1 MgCl_2, 5 Biocytin. This medium was adjusted to pH 7.3 with 0.5 M NaOH and to an osmolarity between 270 and 275. Electrode resistances varied between 4-9 MΩ and the junction potential was approximately -13 mV [80]. All membrane voltages in this study were corrected for this value. The positioning of the patch electrodes on SCN neurons was performed under visual control by using a microscope (Axioskop, Zeiss) equipped with a 40x water-immersion lens (NA: 0.75) with Hoffman modulation contrast. In order to keep the patch pipette clean during its penetration through the
slice and the approach of a visually selected SCN neuron, constant positive pressure was applied to the pipette [81]. Formation of a gigaseal (>3 GΩ) was accomplished by a suction pulse inside the pipette. Transition to the whole-cell configuration was realized by rupturing the cell membrane with a second suction pulse. Recorded signals were amplified by an Axopatch-1D amplifier (Axon Instruments) and relayed by a Digidata 1200 Interface (Axon Instruments) to a personal computer equipped with pClamp 6.02 and Axotape 2.0.2 (Axon Instruments).

We applied two criteria for accepting neuronal recordings: 1) the RMP was required to be more negative than -45 mV and 2) the action potential amplitude, as assessed with respect to the RMP, should be larger than 60 mV. Only recordings which fulfilled both criteria were used in this paper.

Current clamp traces were recorded at a sampling rate of 5 kHz for the investigation of the depolarizing sag caused by activation of I_H and 20 kHz for the investigation of firing rates and action potentials. Voltage clamp traces were filtered by a -80 dB/decade lowpass Bessel filter with a cut-off frequency of 500 Hz, sampled at 1.0 kHz and averaged 4 times. The maximal voltage-clamp error was estimated to be 3-4 mV. This value is obtained by multiplying the access resistance and the current evoked by a hyperpolarizing voltage step towards -120 mV (R_access*I) [82].

2.2.3 Drugs

Effects of blocking agents used in the present study were tested by bath application. These agents included: Cesium Chloride, Barium Chloride, Nickel Chloride, Tetraethylammonium Chloride, Tetrodotoxin and 4-Aminopyridine. All of these products were obtained from Sigma Chemical Co. (St. Louis, Mo., USA).

2.2.4 Statistical analysis

In order to reveal day-night differences in basic membrane properties and H-current activation parameters, we first evaluated whether the distribution of a given parameter was normal using the Shapiro-Wilk's W test (SW). Because this was generally not the case we used Mann-Whitney's U-test unless noted otherwise. Effects of Cs⁺ on the RMP and spontaneous firing rate were analyzed by Wilcoxon's matched-pairs signed-rank test. Averaged data are presented as mean ± standard error of the mean.

2.3 Results

2.3.1 Basic membrane properties

The data presented in this paper are based on whole-cell patch-clamp recordings from 58 SCN neurons, which were uniformly sampled across the SCN. In 29 neurons, voltage and current clamp recordings were performed between CT7 and CT11; the remaining cells were recorded between CT14 and CT19. The RMP of both groups were -59.0 ± 1.4 mV and -60.2 ± 1.4 mV, respectively (MW U-test: n.s.; both n=29). The average spontaneous firing rate was 2.9 ± 0.5 Hz in the period between CT7-11 and 2.4 ± 0.5 Hz in the period between CT14-19 (MW U-test: n.s.; both n=29).
The input resistance amounted to $1.21 \pm 0.10 \, \Omega$ during CT7-11 and $1.16 \pm 0.10 \, \Omega$ during CT14-19 (MW U-test: n.s.; both $n=29$). Furthermore, no significant differences in cell capacity (CT7-11: $6.4 \pm 0.9 \, \text{pF}$ and CT14-19: $8.6 \pm 0.9 \, \text{pF}$ (MW U-test: n.s.; both $n=29$)) could be detected between these two groups.

2.3.2 The H-current ($I_H$)

Many SCN neurons exhibited a time- and voltage dependent inward rectification. In current-clamp mode this rectification became manifest as a slowly developing depolarizing sag when long hyperpolarizing current pulses (1 s) of sufficient amplitude were injected into the cell (Fig. 2.1A, arrow). Under voltage-clamp conditions a slowly developing inward current was observed when using a holding potential of -50 mV and long voltage steps (1 s) to levels below -70 mV, Fig. 2.1C (arrow). The difference between the instantaneous and steady-state current, which is shown in the IV-plot presented in Fig. 2.1D, represents the H-current of the cell and clearly reveals its voltage dependence. In our study $I_H$ was present in 89.7% of the neurons (52 out of 58 cells). Its probability of occurrence was equal in both circadian time windows examined (26 out of 29 cells for CT7-11 and CT14-19).

In current-clamp mode, bath application of Cs$^+$ (1 mM) almost completely depressed the slow depolarizing sag in response to hyperpolarizing current pulses (Fig. 2.1A). The IV-plot (Fig. 2.1B) showed that the current-voltage relation was linearized by Cs$^+$ and that the neuron was virtually devoid of inward rectification under Cs$^+$ conditions. The voltage-clamp experiments (Fig. 2.1C) confirmed that Cs$^+$ (1 mM) almost completely blocked $I_H$. The application of Cs$^+$ (1 mM) did not have a blocking effect on the instantaneous current responses (Fig. 2.1C), as reported by several other authors investigating $I_H$ in other brain structures [73,78,83]. After washing out Cs$^+$, both $I_H$ studied in voltage clamp and the depolarizing sag studied in current clamp returned to control levels (Fig. 2.1A and 2.1C, $n=5$).

2.3.3 Contribution of $I_H$ to RMP, spontaneous firing rate and spike AHP

The influence of $I_H$ on the RMP, spontaneous firing rate and the shape of the spike AHP was investigated by blocking this current with Cs$^+$ (1 mM). This study was performed in 6 SCN neurons which all expressed a substantial amount of $I_H$ and the recordings were done between CT7 and CT11. Since $I_H$ is an excitatory current, one would expect its contribution to RMP and spontaneous firing rate to be present during this period if it would underlie the circadian rhythm of spontaneous firing. However, bath application of Cs$^+$ (1 mM) did not produce a significant change in the RMP of these neurons (Fig. 2.2E; e.g. Fig. 2.2A and B). Cs$^+$ (1 mM) also had no significant effect on the spontaneous firing rate of these neurons (Fig. 2.2D; e.g. Fig. 2.2A and B). Furthermore, Cs$^+$ (1 mM) did not induce noticeable changes in the shape of the spike AHP in any of these neurons. In the example shown in Fig. 2.2C, Cs$^+$ even caused a slight shortening of the AHP, whereas a prolongation would be expected if $I_H$ plays an active role in the rectification of the AHP. Despite the lack of effect of Cs$^+$ on the spontaneous firing rate (Fig. 2.2D), one might argue that Cs$^+$ might perhaps affect more ionic currents than just $I_H$, resulting in a mixture of...
Fig. 2.1: Activation and blocking of the inward rectifier current ($I_H$) in SCN neurons. A. Hyperpolarizing current pulses (-7.5 to -75 pA for 1s) were applied to evoke the time- and voltage dependent inward rectification, expressed as a slowly developing depolarizing sag (indicated by arrow). Bath application of Cs⁺ (1 mM) blocks the depolarizing sag (adapted current injection: -6 to -60 pA) and after removing Cs⁺ (1 mM) the depolarizing sag recovers again. Asterisks mark some prominent spontaneous postsynaptic potentials. B. Voltage/current plot obtained from data in A. The voltage responses were measured at the end of the current pulses in the control condition (closed circle) and during the Cs⁺ (1 mM) application (open circle). C. In voltage-clamp mode hyperpolarizing steps (-60 to -120mV for 1s) were applied to activate the inward rectifier current ($I_H$; arrow). Bath application of Cs⁺ (1 mM) blocks $I_H$ and after removing Cs⁺ (1 mM) it recovers again. D. Current/voltage plot obtained from data in C. The current responses were fitted with a single exponential function; instantaneous current responses (open circle) and steady-state current responses (closed circle) were estimated from these fitted lines.
effects on the spontaneous firing rate. Indeed, extracellular Cs\(^+\) has been reported to block the K\(^+\)-inward rectifier current \([84]\) and such an effect might counterbalance the I\(_H\)-dependent Cs\(^+\) effect on the spontaneous firing rate. We therefore first applied extracellular Ba\(^{2+}\) (500 \(\mu\)M), a blocker of K\(^+\)-inward rectifier \([84,85]\) and subsequently added Cs\(^+\) (1 mM) to this medium. These experiments (\(n=2\)) showed that Cs\(^+\) even in the absence of a possible inward rectifying K\(^+\) current had no effect on the spontaneous firing rate.

2.3.4 The reversal potential of I\(_H\)

The reversal potential of I\(_H\) was obtained by measuring instantaneous I/V relations under two conditions \([72]\). In the first condition, instantaneous currents were measured when the holding potential was stepped from -50 mV (where I\(_H\) is inactive) to potentials varying from -60 to -120 mV (Fig. 2.3A). These instantaneous currents indicate leak currents without I\(_H\), because the activation of I\(_H\) is a slow process (e.g. Fig. 2.3D). In the second condition, instantaneous currents were measured when the holding potential was stepped from -120 mV (where I\(_H\) is almost fully activated) to potentials varying from -110 to -50 mV (Fig. 2.3B). These instantaneous currents indicate leak currents plus I\(_H\). To minimize contamination of I\(_H\) by other currents, which could be activated under one of these conditions, the cells were exposed to a number of channel blockers including Ni\(^{2+}\) (0.25 mM; blocking T-type Ca\(^{2+}\) channel), 4-AP (1 mM; blocking transient K\(^+\) (A-) current), TEA (20 mM; blocking delayed rectifier K\(^+\) current), TTX (0.5 \(\mu\)M; blocking Na\(^+\) current) and Ba\(^{2+}\) (500 \(\mu\)M; blocking inward rectifier or leak K\(^+\) current). The I/V curves were almost linear (Fig. 2.3C). The difference between the two curves reflects the inward or outward nature of I\(_H\). The extrapolated point of intersection of the two I/V curves represents the reversal potential of I\(_H\) (E\(_H\)) and amounted to -29 ± 3 mV (\(n=4\)). In addition to this method we evaluated the possibility of using the tail current reversal method \([74,86]\) for estimating the reversal potential of I\(_H\). This method could not be applied for our SCN neurons due to masking of the tail current reversal by the activation of an outward current at depolarization levels above -60 mV, despite the use of the blockers mentioned earlier (see also \([72,73]\)).

2.3.5 Activation and deactivation of I\(_H\)

Activation of I\(_H\) was studied by applying hyperpolarizing voltage commands (duration: 1 s) of increasing amplitude from a holding potential of -50 mV. The evoked current responses were measured and fitted with a single exponential function. The time constants of these fitted lines clearly revealed that the activation kinetics of I\(_H\) are voltage-dependent (e.g. Fig. 2.3D): they ranged from a time constant of 107 ± 15 ms at -120 mV to 467 ± 125 ms at -70 mV (\(n=10\)). To make an activation curve of I\(_H\), instantaneous and steady-state currents were estimated from these fitted lines and the amplitude of I\(_H\) was calculated as the difference between the steady state and the instantaneous current. The conductance mediating I\(_H\) (G\(_H\)) was determined by the following equation:
Fig. 2.2: Effect of Cs⁺ (1 mM) on the spontaneous firing rate, resting membrane potential and shape of the spike AHP. A, B Effect of Cs⁺ (1 mM) on spontaneously generated action potentials. C. Effect of Cs⁺ (1 mM) application on the shape of the spike AHP. Spikes were averaged (n=50). D. Effect of Cs⁺ (1 mM) on spontaneous firing rate of SCN neurons which expressed Iₜ. The spontaneous firing rate under Cs⁺ conditions is normalized with respect to the rate under control conditions. The histogram shows the mean and standard error of the mean (n=6). E. Effect of Cs⁺ (1 mM) application on the resting membrane potential of SCN neurons (n=6).
Fig. 2.3: The reversal potential of $I_H$ in SCN neurons. A. Activation of $I_H$ by applying hyperpolarizing steps from -50 mV to -120 mV. B. Deactivation of $I_H$ by applying depolarizing steps starting at -110 mV with 10 mV increments from a potential of -120 mV. Despite the use of several blockers (see text) a small outward current (*) was activated at depolarization levels above -60 mV, masking the deactivation process of $I_H$ at these potentials. C. Current/voltage plot obtained from data in A and B. The current responses of A and B were fitted with a single exponential function and the instantaneous current responses of A (closed circle) and B (open circle) were estimated from these fitted lines. Extrapolation of the linear regression lines reveals the polarity change of $I_H$ in this SCN neuron. The reversal potential of $I_H$ in this neuron was -28 mV. D. Activation (open triangle) and deactivation (closed triangle) kinetics of $I_H$ obtained from data in A and B. The time constants were extracted from the single exponential fitted lines and plotted against the step potential. E. Normalized activation (closed square) and deactivation (open square) curve of $I_H$ obtained from data in A and B. The plots were fitted with the Boltzmann function constrained to values $\leq 1$ (equation 2; activation curve parameters were: $V_1 = -98$ mV and $S = 7.1$, de-activation curve parameters were: $V_1 = -91$ mV and $S = -5.5$). Note that the activation of the additional outward current causes a small excursion above $G_{H,MAX}$ at -70 mV.
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where \( I_H \) represents the H-current amplitude, \( V_C \) the command potential and \( E_H \) the reversal potential of \( I_H \) (-29 mV, see above). In order to investigate the voltage dependency of \( I_H \) activation, conductance/voltage plots were made and fitted with a Boltzmann function:

\[
G_H = \frac{I_H}{(V_C - E_H)}
\]

where \( I_H \) is the H-current amplitude, \( V_C \) the command potential and \( E_H \) the reversal potential of \( I_H \) (-29 mV, see above). In order to investigate the voltage dependency of \( I_H \) activation, conductance/voltage plots were made and fitted with a Boltzmann function:

\[
G_H(V_C) = \frac{G_{H,\text{max}}}{1 + e^{\frac{V_C - V_{\frac{1}{2}}}{S}}}
\]

where \( G_H(V_C) \) is the conductance of the H-current at command potential \( V_C \) and \( G_{H,\text{max}} \) is the maximum conductance of the H-current, respectively. \( V_{\frac{1}{2}} \) is the \( V_C \) at which the H-current is half-activated and \( S \) is a slope factor. The activation curves of \( I_H \), determined in 19 SCN neurons measured between CT7 and CT11, revealed that \( I_H \) started to activate around -55 mV and was fully activated around -120 mV (e.g. Fig. 2.3E). The half-activation potential \( (V_{\frac{1}{2}}) \) was -89 ± 2 mV and the slope factor \( (S) \) was 6.8 ± 0.7 (n=19).

Deactivation of \( I_H \) was assessed using the data we obtained in our measurements of the reversal potential of \( I_H \). We used a voltage protocol, in which the \( V_C \) was first stepped from -50 mV to -120 mV and subsequently to potentials varying from -100 to -50 mV (Fig. 2.3B). These experiments were performed in the presence of a cocktail of blockers to minimize contamination of \( I_H \) (see above). The evoked tail currents were well fitted with a single exponential function and revealed that the deactivation kinetics of \( I_H \) are voltage-dependent (Fig. 2.3D); the \( \tau \) ranged from 516 ± 23 ms at -95 mV to 274 ± 44 ms at -55 mV (n=4). In order to make a de-activation curve of \( I_H \), instantaneous and steady-state currents were estimated from these fitted lines. The difference between the steady-state and instantaneous current represents the amount of \( I_H \) which is deactivated by this voltage change. By using equations (1) we determined the conductance \( (G_H) \) change of \( I_H \) caused by deactivation. The maximum conductance \( (G_{H,\text{MAX}}) \) was determined from the pre-pulse voltage step from -50 to -120 mV and curve fits were made using equation (2). The curve fits revealed that \( I_H \) started to deactivate around -110 mV and was fully deactivated around -60 mV (e.g. Fig. 2.3E). The half-deactivation potential \( (V_{\frac{1}{2}}) \) was -92 ± 2 mV and the slope factor \( (S) \) was -5.4 ± 1.3 (n=4).

2.3.6 Activation kinetics of \( I_H \) in different circadian time domains

Activation kinetics of \( I_H \) were determined in 19 SCN neurons recorded between CT7 and CT11 and in 18 SCN neurons recorded between CT14 and CT19. In the remaining cells, who also showed the presence of \( I_H \) (7 neurons of the CT7-11 group and 8 neurons of the CT14-19 group), the current was too small to accurately determine its
activation kinetics. All of the 37 SCN neurons with large $I_H$ allowed their activation curves to be fitted satisfactorily with the Boltzmann function (equation 2, see above). The parameters ($G_{H,\text{MAX}}$, $V_{\frac{1}{2}}$, and S) of both groups (CT7-11 and CT14-19) were analyzed by Mann-Whitney’s U test in order to compare the activation curves of $I_H$ during the subjective day (CT7-11) and night (CT14-19) (Table 2.1). No significant differences were found in $G_{H,\text{MAX}}$, S and $V_{\frac{1}{2}}$ between the two groups.

### 2.4 Discussion

The results of this study demonstrate that $I_H$ is present in a large majority of SCN neurons and indicate that $I_H$ does not influence the spontaneous firing rate and RMP of the SCN neurons. Furthermore, the magnitude and kinetics of $I_H$ recorded in whole-cell mode are not modulated in a circadian manner. $I_H$ was detected in 89.7% of our SCN neurons and these neurons were uniformly distributed throughout the SCN. The intracellular recordings of Kim and Dudek [13] and Akasu et al. [87] revealed a smaller percentage of SCN neurons displaying $I_H$ (48% and 67% respectively). The smaller fraction found by these two groups may be due to shunting of $I_H$ by current leakage at the site where the sharp electrode penetrates the membrane [88].

The activation curves of $I_H$ were generally well fitted with a Boltzmann function. The mean parameters ($V_{\frac{1}{2}}$ of -89 ± 2 mV and S of 6.8 ± 0.7) of our fits are well within the range of values of these parameters reported in other types of neurons: $V_{\frac{1}{2}}$ of $I_H$ usually lies between -73 and -92 mV and the slope factor (S) between 3.5 and 10 [72-74, 78, 83, 86]. To some extent this large variability may be caused by intracellular messenger systems [78]. In another study on $I_H$ in SCN, Akasu et al. [87] found comparable values for $V_{\frac{1}{2}}$ and S as reported here ($V_{\frac{1}{2}} = -84 ± 7$ mV, S=7.9). Both in the present study and the report by Akasu et al., $I_H$ was activated around -55 mV and reached full activation around -120 mV. The amount of $I_H$ activated around the RMP (-60 mV) is approximately 1-2% of the maximal current. The time course of $I_H$ activation also showed a voltage dependence and revealed that $I_H$ activates very slowly near the RMP (Fig. 2.3D).

The results presented here argue against the hypothesis that $I_H$ plays a significant role in determining the spontaneous firing rate and RMP of SCN neurons. Firstly, blocking $I_H$ by Cs⁺ did not significantly affect the spontaneous firing rate and the RMP of the
SCN neurons during the circadian time period (CT7-11) in which one would expect the largest contribution of $I_{H}$. Cesium blockade of $I_{H}$ did not noticeably prolong the AHP either. Secondly, the activation curve of $I_{H}$ shows that during a spike AHP up to -70 mV only a small amount of $I_{H}$ (about 6% of the maximal current) can be activated. In addition, one needs to take into account the fact that $I_{H}$ is a relatively slow time-dependent current ($\tau_{-70mV} = 467 \pm 125$ ms, $n=10$), while the spike AHP is relatively fast ($\tau_{AHP} = 48 \pm 9$ ms, $n=7$). From these results, it can be understood that the amount of $I_{H}$ activation during spontaneous firing will turn out too small to make a significant contribution to the shape of AHP and to the firing rate, in agreement with our Cs$^+$ experiments in current-clamp mode (Fig. 2.2). This conclusion stands in contrast to that of Akasu et al. [68], who argued in favour of a contribution of $I_{H}$ to spontaneous firing. We can indicate 2 possibilities explaining the discrepancy between the present study and that of Akasu et al [68]. First, Akasu et al., performing sharp electrode recordings with ‘blind’ selection of neurons, may have sampled neurons belonging to a different subgroup than investigated in the current study. At this time it is difficult to verify this possibility because comparative data on the morphology and basic membrane properties are not available. Furthermore, Akasu et al. did not report the percentage of neurons showing a decrease in spontaneous firing rate upon exposure to Cs$^+$ (1 mM), nor did they quantify the absolute or relative decrease in spontaneous firing rate in the cells showing some effect. In short, it cannot be excluded that $I_{H}$ promotes spontaneous firing only in a relatively small subset of SCN neurons. However, in general $I_{H}$ does not seem to influence the spontaneous firing rate of SCN neurons. A second explanation lies in the difference of recording methods. While Akasu et al. [68] used sharp electrodes filled with either 4 M potassium citrate or 3 M potassium chloride, we employed the whole-cell patch-clamp technique. In this technique, cytoplasmic constituents (e.g. cAMP, [75]) can be washed out due to intracellular dialysis with the pipette solution. Thus our results on $I_{H}$ could reflect an unmodulated “null state” of $I_{H}$, whereas intracellular modulation may have been preserved in Akasu’s study. However, it should be recalled that our parameter values of the activation curve of $I_{H}$, $V_{1/2}$ and $S$, were similar to those of Akasu et al. Thus, washout effects of a highly effective modulator of $I_{H}$ does not appear to be likely on account of a comparison of results.

The parameters of the $I_{H}$ activation curve determined in the subjective day and night period did not reveal substantial differences suggesting that $I_{H}$ may not be responsible for the circadian rhythm in spontaneous firing of SCN neurons. Again, the similarity between our activation parameters and those of Akasu et al. [87] argues against the possibility of a modulator of $I_{H}$ being washed out during the recordings. On the other hand, it should be recalled that our whole cell recordings did not reveal a clear day/night difference in spontaneous firing rate of SCN cells. In view of this finding, it is still premature to answer the question whether circadian changes of $I_{H}$ are responsible for the circadian rhythm in spontaneous firing rate and further work will be necessary to elucidate disruptive effects of cell dialysis on this rhythm. Perforated patch recording may be instrumental in a more definitive assessment of this issue [89].

In conclusion, the results show that $I_{H}$, as recorded in whole cell mode, does not
substantially contribute to the spontaneous firing activity of SCN neurons and that $I_H$ is not modulated in a circadian manner under these circumstances. Probably $I_H$ is not an integral part of the electrophysiological machinery underlying the circadian pattern in spontaneous firing rate. Taking into account its activation kinetics a more obvious role for $I_H$ in SCN neurons would be to limit the duration of hyperpolarizing events, as induced by prolonged activation of $K^+$ conductances.