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Mechanism of Irregular Firing of Suprachiasmatic Nucleus Neurons in Rat Hypothalamic Slices

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Kononenko, Nikolai I., and F. Edward Dudek. Mechanism of irregular firing of suprachiasmatic nucleus neurons in rat hypothalamic slices. *J Neurophysiol* 91: 267–273, 2004; 10.1152/jn.00314.2003. The mechanisms of irregular firing of spontaneous action potentials in neurons from the rat suprachiasmatic nucleus (SCN) were studied in hypothalamic slices using cell-attached and whole cell recording. The firing pattern of spontaneous action potentials could be divided into regular and irregular, based on the interspike interval (ISI) histogram and the membrane potential trajectory between action potentials. Similar to previous studies, regular neurons had a firing rate about >3.5 Hz and irregular neurons typically fired about <3.5 Hz. The ISI of irregular-firing neurons was a linear function of the sum of inhibitory postsynaptic potentials (IPSPs) between action potentials. Bicuculline (10–30 μM) suppressed IPSPs and converted an irregular pattern to a more regular firing. Bicuculline also depolarized SCN neurons and induced bursting-like activity in some SCN neurons. Gabazine (20 μM), however, suppressed IPSPs without depolarization, and also converted irregular activity to regular firing. Thus $GABA_A$ receptor–mediated IPSPs appear responsible for irregular firing of SCN neurons in hypothalamic slices.

INTRODUCTION

The suprachiasmatic nucleus (SCN) of the hypothalamus contains the primary circadian pacemaker in mammals (Gillette and Tischkau 1999). Neuronal electrical activity recorded in the SCN shows a circadian rhythm, even after the neurons are isolated (Honma et al. 1998; Liu and Reppert 2000; Welsh et al. 1995). The frequency and pattern of electrical activity of intact individual neurons in slices, however, varies greatly. SCN neurons demonstrate regular (i.e., "beating") or irregular activity, or can be silent (Bos and Mirmiran 1993; Kim and Dudek 1993; Kow and Pfaff 1984; Pennartz et al. 1998). The average firing rate of individual neurons ranges between 0 and 15 Hz, but the instantaneous frequency can be higher. Regular-firing neurons generally have a higher firing rate than that of irregular ones (e.g., Thomson et al. 1984). Applied depolarizing currents are known to convert an irregular pattern to regular firing, and hyperpolarizing currents can convert regular to irregular activity (Kim and Dudek 1993; Thomson and West 1990). The mechanisms responsible for these different patterns of electrical activity and their role in the normal function of the SCN and its targets are presently unknown.

The available data suggest that most (if not all) neurons in SCN are GABAergic (Card and Moore 1984; van den Pol and Gorcs 1986; van den Pol and Tsujimoto 1985). Anatomical studies have revealed that SCN neurons have many local axon collaterals (Card and Moore 1984; van den Pol 1980; van den Pol and Gorcs 1986), and electrophysiological studies support the hypothesis that these collateral axons form a network of local inhibitory circuits (Strecker et al. 1997). The role of interneuronal communication within the SCN is also unclear. Irregular activity could hypothetically be attributable to the synaptic potentials from the numerous inhibitory connections within the SCN. If $GABA_A$ receptor–mediated IPSPs from the local circuits of surrounding SCN neurons inhibited individual neurons in the SCN, and if the underlying firing pattern without synaptic input was regular (i.e., beating) with relatively constant interspike intervals (ISIs), then pharmacological blockade of $GABA_A$ receptors would be expected to convert a slow-irregular pattern to a regular pattern with a higher mean frequency. We investigated the role of $GABA_A$ receptor–mediated IPSPs and the effect of $GABA_A$-receptor antagonists (i.e., bicuculline and gabazine) on the firing pattern of SCN neurons in hypothalamic slices.

METHODS

Preparation of slices

Male adult Harlan Sprague-Dawley rats, 4–6 wk old, were deeply anesthetized with halothane and killed by decapitation at 9:00 to 10:00 A.M. The brain was rapidly removed and the region containing the hypothalamus was dissected free. One slice (350 μm thick), containing the paired SCNs, was cut in the coronal plane with a Vibratome (Lancer) in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 3 KCl, 2.5 $CaCl_2$, 2 $MgSO_4$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 10 dextrose, and continuously aerated with 95% O_2–5% CO_2 to a final pH of 7.4, osmolarity 300–310 mosM. After preparation, the slice was placed in an experimental chamber mounted on the stage of an upright compound microscope (Optiphot, Nikon) and allowed >1 h to recover. Individual SCN neurons could be clearly distinguished using a water-immersion objective (Olympus 40×) with Nomarski differential interference contrast optics and a cooled charge-coupled device camera (Paultek Imaging, Grass Valley, CA). During recovery and recording, the slice was superfused at 1.5–2.5 ml/min (34–35°C). When GABA (100 μM) or TTX (1 μM) were bath applied as a control to evaluate the time required to exchange solutions, complete equilibration of new solutions in the experimental chamber was achieved in 3 min. All experiments were performed at 12:00 to 4:00 P.M.

Preparation of micropipettes

Pipettes for cell-attached and whole cell recordings were prepared from glass microcapillaries (Garner Glass, Claremont, CA; ID = 1.2

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mm, OD = 1.65 mm [KG-33]) filled with (in mM): 120 K^+ gluconate, 10 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), 1 NaCl, 1 $MgCl_2$, 1 $CaCl_2$, 3 KOH (to pH 7.2–7.4), 5 ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA), 2 Na_2ATP, at an osmolarity of 255–260 mosM. Pipette resistance was 2–5 MΩ. Offset potential was zeroed immediately before seal formation. The liquid-junction potential was −15 mV (Neher 1992) and all voltage measurements were corrected off-line for this value. The seal resistance ranged between 2 and 60 GΩ.

Drugs

The $GABA_A$-receptor antagonist, (−)-bicuculline methiodide (Sigma), was used as a 10-mM stock solution in distilled water, and then diluted to its final concentration in ACSF. Gabazine (Sigma), another more specific $GABA_A$-receptor antagonist, was prepared at 2 mM in ACSF, and then diluted to 20 μM before the experiment.

Data acquisition and analysis

Current and voltage data from SCN neurons were recorded with an Axopatch-1D amplifier, low-pass filtered at 2 kHz, and digitized at 44 kHz with a Neuro-Corder (Neurodata) for storage on video tapes for off-line analysis. Electrical signals were transferred to a personal computer by replaying in analog form, filtering at 2 kHz, and sampling at 5 kHz using Axon Instruments software and hardware (Axotape version 2.0, TL-1 DMA A/D interface). Firing frequency, averaged for 10 s, and interspike-interval (ISI) histograms were calculated using pClamp version 6.0 (FETCHAN and pSTAT) and plotted with *SigmaPlot 2000*, version 6.0. For analysis of asymmetry of ISI distributions, experimental plots were fitted by a 3-parameter log normal equation (*SigmaPlot 2000*). The ratio of the area after the maximum of the fitting curve relative to the area before the maximum was used as an index of asymmetry k_a. Examples of fitting curves are presented in Fig. 4*C*. All numerical values in both the text and the figures represent means ± SE.

RESULTS

Spontaneous activity of SCN neurons

Extra- and intracellular recordings of electrical activity were obtained from 84 SCN neurons. After >2 h of recovery from preparation of the coronal hypothalamic slice, cell-attached or whole cell recordings were performed. The cell-attached configuration allowed long-lasting extracellular recording without diffusion of substances from the pipettes; whole cell recording permitted intracellular analysis of action potentials and excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs). The recordings were generally consistent with earlier published data using extracellular (Kow and Pfaff 1984), intracellular (Kim and Dudek 1993), and whole cell recording (Pennartz et al. 1998) of spontaneous action potentials in slice preparations. SCN neurons could display spontaneous activity or be silent. Neurons with rare irregular action potentials (ISI >1 s) and average firing frequency for 1 min at <0.5 Hz were considered essentially to be silent. Spontaneously active neurons could be divided into regular "beating" (about 3.5 Hz or higher) and irregular (0.5–3.5 Hz) activity. Typical examples of sequential extra- and intracellular recordings of regular activity from the same SCN neuron before (*top trace*) and after membrane rupture (*bottom trace*) are shown in Fig. 1*A*. The patterns of electrical activity recorded from individual SCN neurons were generally similar in both whole cell and cell-attached configuration, which suggests that electrical properties were generally preserved in the whole cell configuration. As apparent from the intracellular voltage recordings, regular activity was not associated with visible postsynaptic potentials and showed a smooth depolarizing trajectory between action potentials. Figure 1*B* illustrates an example of irregular activity with extracellular (*top trace*) and intracellular recording (*bottom trace*). A feature of irregular activity was the presence of numerous IPSPs between action potentials, which were clearly visible with intracellular recording and consistent with published data (Kim and Dudek 1992). The present experiments also confirmed that most PSPs in coronal slices are GABAergic IPSPs.

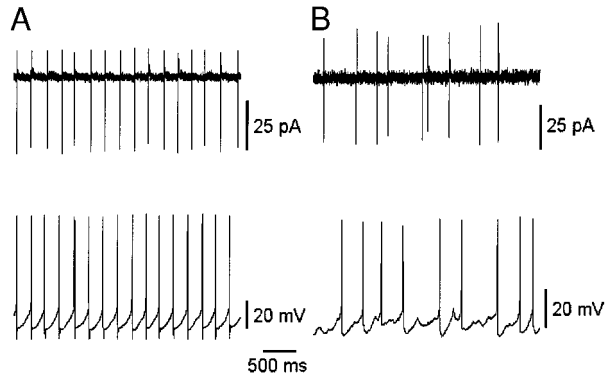

FIG. 1. Regular and irregular firing patterns of suprachiasmatic nucleus (SCN) neurons. *A*: cell-attached (*top*) and whole cell (*bottom*) recording from a neuron with a regular firing pattern. *B*: cell-attached (*top*) and whole cell (*bottom*) recording from a neuron with an irregular firing pattern. Whole cell recordings were obtained 2 min (*A*) and 20 min (*B*) after rupture of patch membrane.

The quantitative expression of differences between regular- and irregular-firing neurons is reflected in ISI histograms of different cells. In Fig. 2, representative examples of ISI distributions are shown for 3 regular-firing neurons (*A*) and 2 irregular ones (*B*). ISI histograms for regular-firing neurons were close to symmetrical with a bell-like shape, a relatively narrow-range of ISIs, and an ISI peak between 80 and 250 ms. In contrast, ISI histograms for irregular-firing neurons were strongly asymmetric with a tail in the histogram reflecting long ISIs (Fig. 2*B*). Figure 2*C* demonstrates the relation between average firing rate and the irregularity of electrical activity, estimated as the asymmetry of the ISI distribution for 18 neurons. The regular- and irregular-firing neurons clearly occupy different positions on graph. In general, regular-firing neurons showed an average firing frequency >3.5 Hz and k_a between 1.00 and 1.35, whereas irregular-firing neurons had an average firing frequency <3.5 Hz and k_a >1.35. The average k_a for regular-firing neurons was 1.18 ± 0.05 and for irregular-firing neurons was 2.16 ± 0.12 (n = 18 cells; $P < 0.001$, Student's *t*-test). Comparison of cumulative ISI distributions between clusters of regular-firing and irregular-firing neurons (Fig. 2*C*) showed highly significant differences ($P < 0.001$, Kolmogorov–Smirnov test).

Neurons with irregular activity may possess the same intrinsic mechanism of generation of spontaneous activity as regular-firing SCN neurons, but receive higher levels of inhibitory synaptic input. This hypothesis was tested by examination of ISIs and IPSPs in regular- and irregular-firing neurons. Three representative records of one neuron with highly irregular activity are presented in Fig. 3*A*, and 5 traces with minimal ISIs

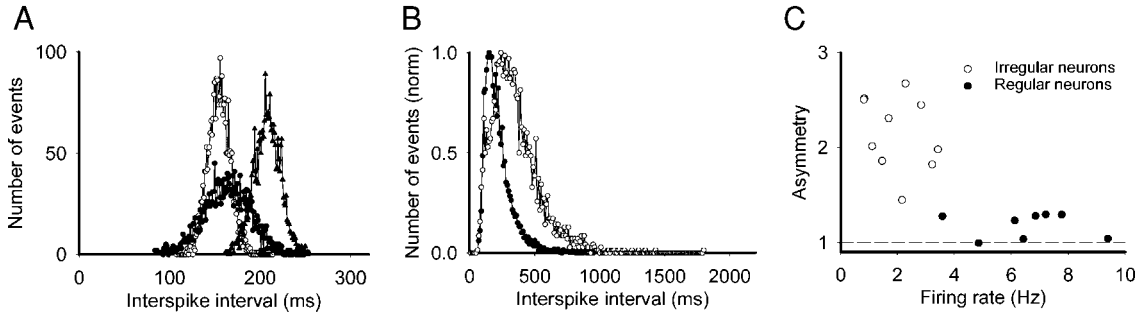

FIG. 2. Interspike interval (ISI) histograms of SCN neurons. *A*: ISI histograms of 3 regular-firing neurons (bin 1 ms). *B*: ISI histograms of 2 irregular-firing neurons (bin 10 ms). *C*: plot of asymmetry of ISI histogram as a function of average firing rate for regular-firing (solid circles) and irregular-firing (open circles) SCN neurons. Data were obtained from extracellular recordings of activity during periods of 3–5 min.

from this neuron are shown in Fig. 3*B*. The duration of each of these short ISIs was similar, and the depolarizing ramp between action potentials was consistently smooth and similar to other regular-firing neurons. Similar observations on ISIs were made with extracellular recording (not shown). Five other ISIs, including both the shortest and longest ones (Fig. 3*A*), are shown in Fig. 3*C*. The IPSPs altered the voltage trajectory between action potentials and prolonged the ISI. The dependency of ISI on the sum of IPSP amplitudes could be fitted by a linear function (Fig. 3*D*, $n = 7$ neurons). The k_i (see legend to Fig. 3*D*), reflecting ISI in the absence of inhibitory synaptic input, was 0.13 ± 0.03 s (range of 0.08 to 0.31 s). The k value, reflecting the sensitivity of ISI to inhibitory synaptic input, was 0.021 ± 0.003 s/mV (range: 0.011 to 0.034 s/mV). These results suggest that irregular activity is mainly attributed to strong inhibitory synaptic input to SCN neurons.

Effect of bicuculline and gabazine

To test the hypothesis that IPSPs are responsible for irregular firing, the effect of bicuculline (10–30 μM) on the electrical activity of irregular-firing neurons was studied (32 neurons). Electrical activity was recorded in both cell-attached and whole cell mode and no obvious differences were found. Representative results are presented in Fig. 4. Application of bicuculline transformed irregular activity into a more regular firing pattern (Fig. 4*A*) and increased both instantaneous frequency (not shown) and the mean frequency of firing (Fig. 4*B*). The change from an irregular to a regular pattern mainly involved the elimination of long ISIs (Fig. 4*C*). The k_a declined from 2.08 ± 0.14 in ACSF to 1.49 ± 0.10 in the presence of bicuculline ($n = 7$ neurons; $P < 0.01$, Student's *t*-test). These changes were observed in 16 of 20 neurons. The average bicuculline-induced increase in firing frequency was from 4.29 ± 0.59 Hz (control; range: 0.8–8.1 Hz) to 6.08 ± 0.59 Hz (in bicuculline; range: 2.4–12.2 Hz) ($n = 16$ neurons; $P < 0.001$, paired Student's *t*-test). In 4 neurons with high-frequency firing (>10 Hz), the effect of bicuculline was minimal or absent. In some SCN neurons ($n = 7$), application of bicuculline evoked oscillations of membrane potential and the appearance of bursting-like activity (Fig. 5*A*). The depolarizing

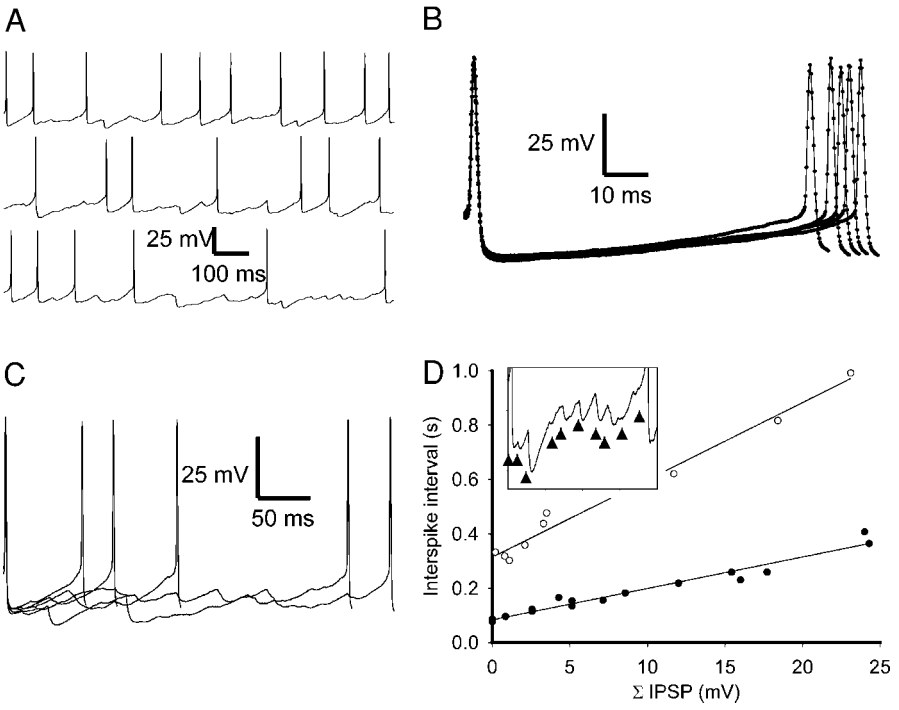

FIG. 3. Dependency of ISI on IPSPs between spikes. *A*: 3 epochs of electrical activity from an irregular-firing neuron, which include examples of both the shortest and the longest ISIs used in the analysis below. *B*: 5 of the shortest ISIs from records in *A*. Note the small difference in the ISIs and the smooth trajectory of the membrane potential between action potentials. *C*: 5 of the ISIs from records in *A*, including both the shortest and longest ISIs. The longest interval was 5 times greater than the shortest one, and had numerous IPSPs. *D*: dependency of the ISI on the sum of the amplitude of the inhibitory postsynaptic potentials (IPSPs) between the action potentials. Data plotted as solid circles were obtained from the records in *A*, and the graph contains all 20 ISIs. *Inset*: expansion of an ISI from *A* (*bottom record*, last interval) demonstrating several IPSPs (triangles), the sum of which was used for one point on the plot in *D*. Data shown as open circles were obtained from another neuron that exhibited the largest ISI in the absence of apparent inhibitory synaptic input. Solid lines are fits by linear equation, $k_i + kx$. Parameters for the solid-circle fitting are $k_i = 0.083$ s, $k = 0.017$ s/mV; for open-circle fitting, $k_i = 0.314$ s, $k = 0.028$ s/mV.

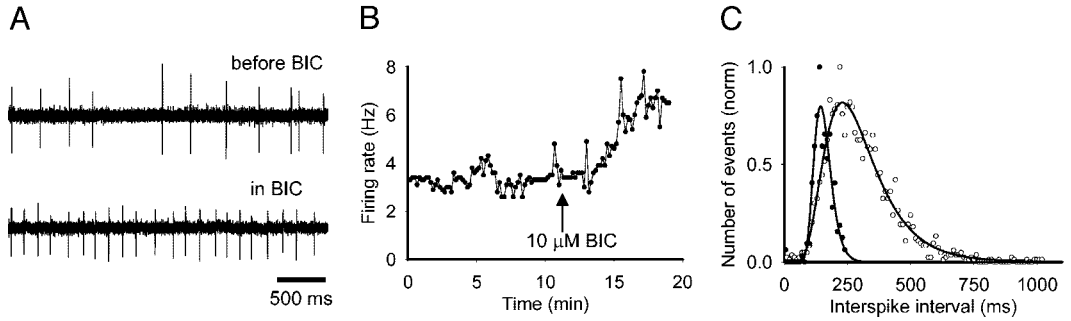

FIG. 4. Effect of bicuculline on electrical activity of irregular-firing SCN neuron. *A*: cell-attached recording of electrical activity before (*top*) and after (*bottom*) application of bicuculline (BIC, 10 μM). *B*: effect of bicuculline on mean frequency of SCN neuron in *A* (interval of averaging was 10 s). *C*: effect of bicuculline on ISI distribution of SCN neuron shown in *A* (bin 10 ms); ISI histograms are shown for the time period before (open circles) and 5 min after (solid circles) application of bicuculline to the SCN neuron in *A*. Solid lines are fits of log normal equation, with k_a values of 1.99 and 1.35 for ISI distributions before and after bicuculline application, respectively.

waves were large and associated with a decrease of action potential amplitude, or even loss of firing on the peak of the depolarizing waves. In the SCN neurons with bicuculline-stimulated bursting-like activity, artificial hyperpolarization blocked the depolarizing waves ($n = 2$ neurons), and voltage clamping ($V_h = -75$ mV) did not reveal oscillations of membrane current that could be responsible for the shifts of membrane potential ($n = 5$ neurons). Because the membrane current did not oscillate in voltage clamp (not shown), in spite of strong oscillations of membrane potential in current clamp, the oscillations of membrane potential were presumably attributable to voltage-dependent mechanisms with an intrinsic origin. Thus nonspecific bicuculline-induced depolarization may hypothetically contribute to or even explain the increase in firing frequency observed in most neurons, and the appearance of bursting-like activity in some SCN neurons after bicuculline application. Bicuculline reversibly produced firing in silent neurons, or those with rare action potentials ($n = 5$; Fig. 5*B*, extracellular recording; Fig. 5*C*, intracellular recording), which further supported this hypothesis. The depolarizing effect of bicuculline, which was present in most SCN neurons, was responsible for the shift in the peak of the ISI distribution to shorter ISI values (Fig. 4*C*), and, besides, k_a in the presence of bicuculline was larger than for typical regular-firing neurons because of the contribution of interburst intervals in general ISI distribution.

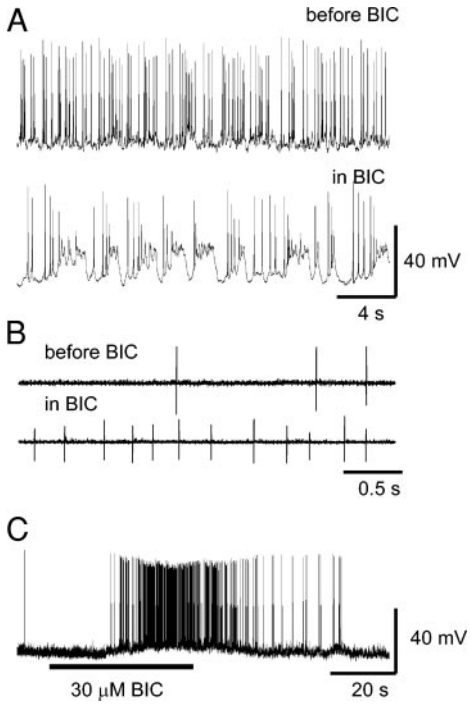

FIG. 5. Diversity of effects of bicuculline on electrical activity of SCN neurons. *A*: bicuculline-induced bursting-like activity in some neurons, as shown in whole cell recordings of electrical activity before (*top*) and after (*bottom*) application of 10 μM bicuculline. *B*: bicuculline (10 μM) caused an increase in electrical activity in an SCN neuron that previously was nearly silent (i.e., fired action potentials infrequently), as shown with cell-attached recording. *C*: bicuculline (30 μM) depolarized an SCN neuron that was nearly silent, as illustrated with whole cell recording.

Gabazine (20 μM, $n = 5$ neurons), another blocker of $GABA_A$ receptors, suppressed IPSPs in irregular-firing neurons (Fig. 6*A*) and eliminated long ISIs in the ISI histogram (Fig. 6*B*). As expected, those neurons with the lowest firing frequency were generally the most affected by the drug. Gabazine appeared to be more selective for $GABA_A$ receptors of SCN neurons than bicuculline because it did not cause a depolarization and burst-like firing while blocking IPSPs. In contrast with bicuculline, gabazine did not cause a visible shift in the peak of the ISI distribution but reduced k_a from 1.80 ± 0.19 to 1.17 ± 0.04 ($n = 5$ neurons; $P < 0.05$, Student's *t*-test), which is comparable to regular-firing neurons (Fig. 2*C*). Therefore these data suggest that IPSPs, which are attributed to activation of $GABA_A$ receptors, are the primary mechanism for the irregularity of SCN neuronal activity in hypothalamic slices.

DISCUSSION

These experiments confirmed the presence of both regular- and irregular-firing patterns, and showed that the long ISIs characteristic of irregular firing were directly dependent on the sum of the IPSPs that occurred between the action potentials. Bicuculline and gabazine blocked the IPSPs and converted irregular to regular firing. The depolarizing (i.e., presumably nonspecific) effect of bicuculline often resulted in burst discharges.

Regular- versus irregular-firing patterns

It is well known that SCN neurons can display regular- or irregular-firing patterns. A regular pattern typically occurs dur-

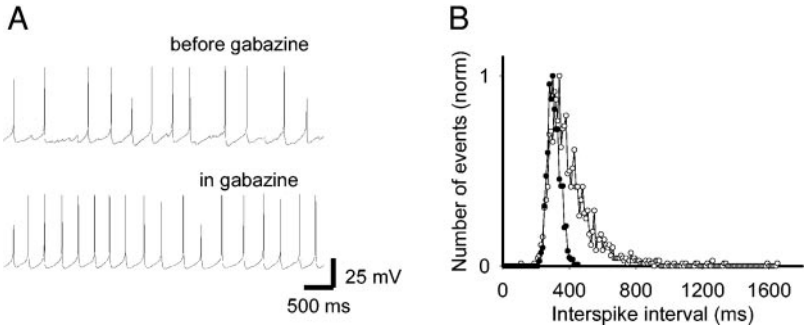

FIG. 6. Effect of gabazine on the electrical activity of irregular-firing SCN neurons. *A*: whole cell recording of electrical activity before (*top*) and after (*bottom*) application of gabazine (20 μM). *B*: ISI histograms (bin 10 ms) before (open circles) and after (solid circles) application of gabazine to the SCN neuron in *A*.

ing high-frequency firing (Bos and Mirmiran 1993; Kim and Dudek 1993; Kow and Pfaff 1984; Pennartz et al. 1998; Thomson et al. 1984), and depolarizing current steps can shift irregular-firing neurons to a regular pattern, and vice versa (Kim and Dudek 1993; Thomson and West 1990). Regular and irregular patterns were observed in the present experiments during both whole cell and cell-attached recording. Regular- and irregular-firing neurons are known to have distinct ISI histograms (Bos and Mirmiran 1993; Kow and Pfaff 1984), and ISI histograms were used in the present study to define the firing patterns (Fig. 2, *A* and *B*). For regular neurons, close to symmetrical bell-shaped curve was typical, with a maximum ISI ranging between 80 and 250 ms. For irregular neurons, a strongly asymmetric shape, with a tail in the direction of long ISIs, was typical. A plot of the asymmetry of the ISI distributions as a function of the average firing rate of the different SCN neurons shows that populations of regular- and irregular-firing neurons cluster in different locations. Regular-firing neurons have a range of firing frequency from 3.5 to 10 Hz (or ISI from 0.1 to about 0.3 s) during extracellular recordings. This frequency range is in good agreement with that for intracellularly recorded activity of irregular-firing neurons, when the data are extrapolated to the minimal ISI (k_i ranged between 0.08 and 0.31 s; Fig. 3*D*), as expected for an absence of synaptic input. These data led to the speculation that the firing rate of regular-firing neurons corresponds to the minimal ISI of irregular-firing neurons, and is defined by an intrinsic mechanism of SCN neurons that is controlled by the circadian clock. Thus these data suggested the hypothesis that an intrinsic mechanism of regular firing is present in the irregular activity of SCN neurons, but the range in irregular patterns of electrical activity is attributed mainly or even exclusively to differences in inhibitory synaptic input. This hypothesis could provide an explanation for the empirical observation that the higher firing rates are usually accompanied by increased regularity, and irregularity occurs in conjunction with low firing rate (e.g., Kim and Dudek 1993; Pennartz et al. 1998; Schaap et al. 1999; Thomson et al. 1984).

Role of GABAergic input in irregular firing

Nearly all SCN neurons display numerous IPSPs resulting from activation of bicuculline-sensitive $GABA_A$ receptors (e.g., Kim and Dudek 1992; Strecker et al. 1997), and spontaneous EPSPs in SCN neurons are minimal in coronal slices (but see Lunkvist et al. 2002 for horizontal slices). Intracellular whole cell recordings of neurons with irregular activity revealed that the shortest ISIs were associated with a smooth depolarizing ramp during the ISIs (Fig. 3*B*), which is typical for neurons with a regular pattern of pacemaker activity. Quantitative analysis of the dependency of ISIs in irregular-firing neurons on the sum of IPSP amplitudes between the two action potentials revealed that it was close to linear (Fig. 3*D*). Both of the blockers of $GABA_A$ receptors (i.e., bicuculline and gabazine) transformed irregular activity to a regular pattern (Figs. 4 and 6). In contrast with gabazine, bicuculline caused depolarization (Fig. 5*C*) and/or development of depolarization-induced burst firing (Fig. 5*A*). Although bicuculline shifted the ISI distribution to shorter ISI values (Fig. 4*C*), the asymmetry factor k_a was larger than typical for regular-firing neurons, presumably because of the burst-firing that was induced.

The nonspecific depolarizing effect of bicuculline on SCN neurons and the associated increase in firing rate were previously reported (Burgoon and Boulant 1998; Kim and Allen 2001; Kim and Dudek 1992). Experiments conducted on other neurons provided evidence that the methyl derivative of bicuculline is not a selective antagonist of $GABA_A$ receptors. Bicuculline also acts on small-conductance Ca^{2+}-activated K^+ channels (Johnson and Seutin 1997; Khavaled et al. 1999; Strobaek et al. 2000). Experiments on SCN neurons with picrotoxin suggested that the small-conductance Ca^{2+}-activated K^+ channel is a target for bicuculline action (Kim and Allen 2001). The Ca^{2+}-activated K^+ channels present in SCN neurons (Huang 1993; Walsh et al. 1995) would provide a hyperpolarizing driving force that would act opposite to the depolarizing drive responsible for spontaneous firing. The bicuculline-induced blockage of Ca^{2+}-activated K^+ channels would thus lead to depolarization, and, at sufficient suppression of Ca^{2+}-activated K^+ channels, to burst-firing. The effects of bicuculline on IPSPs and the firing pattern of SCN neurons obtained in the present experiments are consistent with the hypothesis that IPSPs are the primary mechanism for irregular-firing patterns, but the nonspecific depolarizing effect of bicuculline on membrane potential could possibly serve as an alternative explanation for how bicuculline converts irregular firing to regular activity. Gabazine, on the other hand, transformed irregular activity to a regular firing pattern without changing the short ISIs (Fig. 6), as expected from previous work concerning the selectivity of its action (Bai et al. 2001). Recently, in a study of control and anopthalamic mice, Laemle and coworkers (2002) observed that bicuculline increased the discharge rate and regularized the firing pattern of extracellulary recorded SCN neurons. Therefore $GABA_A$ receptor–mediated IPSPs are responsible for irregular firing patterns in hypothalamic slices because the magnitude of the ISI was a direct function of the sum of the IPSPs and because pharma-

cological blockade of $GABA_A$ receptors converted irregular-firing neurons into regular ones.

Other possible mechanisms for irregular patterns

Although the simplest interpretation of our data is that irregular activity of SCN neurons is mainly attributed to a high level of GABAergic input in these cells, other possible mechanisms that may contribute to irregularity in the firing pattern cannot be excluded. For example, Lovejoy and coworkers (2001) suggested that irregular firing of dopamine neurons in the substantia nigra does not reflect the temporal properties of its inputs, but rather is a consequence of the intrinsic membrane properties of dopamine neurons themselves (see also Elbert et al. 1994). Our pharmacological experiments argue that this explanation of irregularity in SCN neurons would not play a major role, at least compared with the effects of GABAergic synaptic input. We also did not observe an obvious correlation between regularity of firing and the time course of the spike hyperpolarizing afterpotential (monophasic vs. biphasic), which Pennartz and coworkers (1998) associated with regularity. This could be attributable, however, to inadequate sampling in the present work. In our recordings, regular-firing neurons demonstrated both biphasic (Fig. 1*A*, *bottom*) and monophasic (not shown here) hyperpolarizing afterpotentials. Moreover, during some of the longer recordings (e.g., 40–50 min) in regular-firing neurons without obvious inhibitory synaptic input, we observed transformation of biphasic afterpotentials to monophasic ones. Although our data argue for an important role of $GABA_A$ receptor–mediated IPSPs in generating the irregular-firing pattern, they do not rule out a contribution from other hypothetical mechanisms.

Possible role of GABAergic inputs in circadian rhythms

Considerable controversy has centered on the effect of $GABA_A$ receptor–mediated postsynaptic potentials on the firing of SCN neurons. Wagner and colleagues (1997) provided evidence from single-unit extracellular recordings in hypothalamic slices that GABA primarily excited SCN neurons during circadian day and was inhibitory during circadian night (see also Wagner et al. 2001). Other electrophysiological studies have not found a similar diurnal effect of GABA (e.g., Gribkoff et al. 1999, 2003; but also see De Jeu and Pennartz 2002; Ikeda et al. 2003). All of the data in the present study were obtained during the day (i.e., between 12:00 and 4:00 P.M.) in slices obtained from rats maintained in a normal light/dark cycle. Furthermore, although many of the recordings were made with whole cell recordings, where the intracellular concentration of chloride is a function of the pipette concentration, the key results were also obtained with cell-attached recordings, which would not be expected to affect the chloride equilibrium potential. Therefore our data suggest that $GABA_A$ receptor–mediated postsynaptic potentials are inhibitory, and serve to delay action potentials from a regular or "beating" pattern that is presumably generated by a circadian oscillator.

Local inhibitory circuits, irregular firing patterns, and circadian rhythms

One question concerns the physiological consequence of high inhibitory synaptic input in some SCN neurons. We have not addressed that question in our studies, but the average firing frequency in the neuron shown in Fig. 3 was about 6 Hz. The expected firing frequency calculated from the minimal intervals in the putative absence of inhibitory synaptic input, as apparent in Fig. 3, *B* and *D*, would be 12 Hz, a firing frequency that is relatively high for SCN neurons. This high firing frequency is presumably attributable to intracellular mechanisms controlled by an internal circadian oscillator. Therefore the inhibitory connections from local circuits of negative feedback within the SCN may serve as a mechanism to reduce the firing frequency defined by the circadian oscillator.

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DISCLOSURES

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