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# 5-HT$_{1B}$ Receptor-Mediated Presynaptic Inhibition of GABA Release in the Suprachiasmatic Nucleus

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# 5-$HT_{1B}$ Receptor-Mediated Presynaptic Inhibition of GABA Release in the Suprachiasmatic Nucleus

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**Bramley, Jayne R., Patricia J. Sollars, Gary E. Pickard, and F. Edward Dudek.** 5-$HT_{1B}$ receptor-mediated presynaptic inhibition of GABA release in the suprachiasmatic nucleus. *J Neurophysiol* 93: 3157–3164, 2005. First published February 16, 2005; doi:10.1152/jn.00770.2004. The suprachiasmatic nucleus (SCN) receives a dense serotonergic innervation that modulates photic input to the SCN via serotonin 1B (5-$HT_{1B}$) presynaptic receptors on retinal glutamatergic terminals. However, the majority of 5-$HT_{1B}$ binding sites in the SCN are located on nonretinal terminals and most axonal terminals in the SCN are GABAergic. We therefore tested the hypothesis that 5-$HT_{1B}$ receptors might also be located on SCN GABAergic terminals by examining the effects of the highly selective 5-$HT_{1B}$ receptor agonist CP-93,129 on SCN miniature inhibitory postsynaptic currents (mIPSCs). Whole cell patch-clamp recordings of mIPSCs were obtained from rat and mouse SCN neurons in hypothalamic slices. Using CsCl-containing microelectrodes with QX314, we isolated mPSCs that were sensitive to the $GABA_A$ receptor antagonist, bicuculline. Bath application of CP-93,129 (1 $\mu$M) decreased the frequency of mIPSCs by an average of 22% ($n = 7$) in rat SCN neurons and by an average of 30% ($n = 8$) in mouse SCN neurons with no clear effect on mIPSC amplitude. In mice lacking functional 5-$HT_{1B}$ receptors, CP-93,129 (1 $\mu$M) had no clear effect on the frequency or the amplitude of mIPSCs recorded in any of the cells tested ($n = 4$). The decrease in the frequency of mIPSCs of SCN neurons produced by the selective 5-$HT_{1B}$ receptor agonist CP-93,129 is consistent with the interpretation that 5-$HT_{1B}$ receptors are located on GABA terminals in the SCN and that 5-HT inhibits GABA release via a 5-$HT_{1B}$ presynaptic receptor-mediated mechanism.

## INTRODUCTION

The suprachiasmatic nucleus (SCN) controls circadian rhythms and thus functions as the "master clock" of the brain (Klein et al. 1991). The SCN is composed of numerous single-cell oscillators (i.e., "clock cells") that, when synchronized, produce a coordinated circadian output. Modification of SCN output (i.e., firing rate of SCN neurons) ultimately affects physiological and behavioral rhythms.

The SCN receives a direct glutamatergic input from the retina via the retinohypothalamic tract (RHT) that is formed from axons of retinal ganglion cells (Castel et al. 1993). In addition, the SCN receives indirect retinal input from retinorecipient neurons of the intergeniculate leaflet (IGL) via the geniculohypothalamic tract (GHT) (Morin 1994). Both of these sources of photic information modify SCN output and serve to entrain the SCN circadian clock to the 24-h environmental day-night cycle (Hendrickson et al. 1972; Moore and Lenn 1972; Pickard 1982; Pickard et al. 1987). The SCN also receives one of the densest serotonergic innervations in the brain arising from ascending projections of serotonin (5-HT) neurons in the midbrain median raphe nucleus (Meyer-Bernstein and Morin 1996). The terminal fields of retinal, IGL, and serotonergic afferents are coextensive in the ventral region of the SCN, suggesting that 5-HT afferents may modify RHT and/or IGL input to the SCN. Indeed, destruction of 5-HT afferents to the SCN modifies circadian behavioral responses to light (Morin and Blanchard 1991; Smale et al. 1990).

The 5-HT receptor subtype(s) in the SCN and their subcellular distribution remain to be fully elucidated. Of the 14 5-HT receptor subtypes currently described, several have been localized to the SCN, including the 5-$HT_{1A}$, 5-$HT_{1B}$, 5-$HT_{2A}$, 5-$HT_{2C}$, 5-$HT_{5A}$, and 5-$HT_7$ receptor subtypes (Belenky and Pickard 2001; Duncan et al. 1999, 2000; Prosser et al. 1993; Rea and Pickard 2000a; Roca et al. 1993; Smith et al. 2001; Sumner et al. 1992). 5-$HT_{1B}$ receptors are found predominately on axon terminals within the CNS (Boschert et al. 1994). In the SCN, 5-$HT_{1B}$ receptors are located on RHT terminals (Belenky and Pickard 2001; Pickard et al. 1999). There is strong evidence that activation of 5-$HT_{1B}$ receptors in the SCN plays a particularly important role in modifying photic input (Hayashi et al. 2001; Pickard and Rea 1997; Pickard et al. 1996, 1999; Rea and Pickard 2000b; Shimazoe et al. 2004; Smith et al. 2001). However, most 5-$HT_{1B}$ receptors in the SCN are located on nonretinal terminals, as evidenced by the 35% reduction in the number of 5-$HT_{1B}$ binding sites in the SCN after bilateral enucleation (Manrique et al. 1999; Pickard et al. 1996). These nonretinal 5-$HT_{1B}$ receptors might be located on the terminals of other major afferents to the SCN, such as the 5-HT projection from the raphe nucleus (O'Connor and Kruk 1992) or the NPY/GABA projection from the IGL (Manrique et al. 1999). The majority of axonal boutons in the SCN are GABAergic, and they arise from the local GABAergic network, which is extensive (Castel and Morris 2000; Strecker et al. 1997; van den Pol 1986).

Recent studies with 5-$HT_{1B}$ receptor knockout (5-$HT_{1B}$ KO) mice have shown that the lack of functional 5-$HT_{1B}$ receptors produces an attenuated response to light (Ogilvie et al. 2004). If 5-$HT_{1B}$ receptors are located presynaptically on GABA terminals, the reduced response to light in KO mice may be the result of increased GABAergic neural activity in the SCN resulting from the lack of effect of 5-HT on 5-$HT_{1B}$ receptors. In this study, we tested the hypothesis that 5-HT inhibits GABA release in the SCN via activation of 5-$HT_{1B}$ presynaptic receptors on GABA terminals by analyzing the frequency and

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amplitude of $GABA_A$-receptor-mediated miniature inhibitory postsynaptic currents (mIPSCs). We examined the effects of the potent and highly selective 5-$HT_{1B}$ receptor agonist CP-93,129 on mIPSCs of SCN neurons in hypothalamic slices obtained from Sprague-Dawley rats and C57BL/6J wild-type and 5-$HT_{1B}$ KO mice.

## METHODS

### *Hypothalamic slice preparation*

Male Sprague-Dawley rats (Harlan) and C57BL/6J wild-type (Jackson Laboratory, Bar Harbor, ME) and 5-$HT_{1B}$ KO mice on the C57BL/6 background (in-house breeding and originally provided by Dr. René Hen, Columbia University) (Saudou et al. 1994) aged between 4 and 6 wk were maintained under a 12:12 light: dark cycle with lights on at 07:00 h. Food and water were available ad libitum.

Animals were deeply anesthetized by halothane inhalation and killed by decapitation at 10:00–11:00 h. Brains were then rapidly dissected, blocked in the coronal plane, and immersed in oxygenated (95% $O_2$-5% $CO_2$), ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 24 $NaHCO_3$, 3 KCl, 2 $MgSO_4$, 1.25 $NaH_2PO_4$, 2.5 $CaCl_2$, and 10 glucose, pH 7.4. Brain blocks were glued to the stage of a Vibratome and 300-$\mu$m-thick coronal slices containing the bilateral SCN were cut. Slices were placed in a holding chamber and incubated for 1–2 h in oxygenated ACSF at 36°C before being transferred into the recording chamber. All experiments were performed at 12:00–17:00 h.

### *Patch-clamp recording*

Slices were continuously perfused (2 ml/min) with oxygenated ACSF (identical to that used in the dissection) and maintained at a temperature of 35°C. Pipettes were pulled (P-87 Flaming-Brown pipette puller, Sutter Instruments) from borosilicate glass capillaries of 1.65-mm OD and 0.45-mm wall thickness (Garner Glass) and filled with a solution containing (in mM) 130 CsCl, 10 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), 1 NaCl, 1$CaCl_2$, 1 $MgCl_2$, 5 ethylene glycol-bis ($\beta$-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA), 2 ATP, 0.2 GTP, and 5 QX314, and pH adjusted to 7.2–7.3 with CsOH. Open resistance was 4–6 M$\Omega$. Whole cell patch-clamp recordings were made using infrared illumination with differential interference contrast optics (IR-DIC) video microscopy. Seal resistances were typically 1–4 G$\Omega$, and series resistances were <20 M$\Omega$. After the whole cell configuration was established, cells were initially held near the resting membrane potential for 2–5 min to allow equilibration of the extracellular and recording electrode solutions. Whole cell currents were amplified with an Axopatch-Multiclamp 700A amplifier (Axon Instruments) and digitized with a Digidata 1322 (Axon Instruments). Records were collected and stored onto a Pentium III PC using the pCLAMP 8.0 software, (Axon Instruments), and analyzed off-line with Mini-Analysis software (Justin Lee, Synaptosoft). Drug effects were analyzed using the Student's *t*-test. Cumulative plots were compared using the Kolmogorov-Smirnov test (KS-test; 2-tailed). The results of the cumulative plots are presented as the *D* value rather than using the *P* values. The *D* value is defined as the maximum value of the absolute difference between two cumulative distribution functions. Due to the large number of points in each cumulative plot, a very small difference can lead to a significant *P* value (i.e., <0.05), which, when combined with a small difference in the *D* value, can be misleading. It was therefore decided that the most appropriate way to report the results from the cumulative plots was to use the *D* value. All results are reported as ±SE unless stated otherwise.

Miniature IPSCs (mIPSCs) were isolated by using the glutamate AMPA/kainate receptor antagonist, 5,7-dinitroquinoxaline-2,3-dione (DNQX; 10 $\mu$M), the *N*-methyl-D-aspartate (NMDA) receptor antagonist DL-2-amino-5-phosphonopentanoic acid (APV; 50 $\mu$M), and tetrodotoxin (TTX; 1 $\mu$M). Solutions of the $GABA_A$-receptor antagonist bicuculline (30 $\mu$M) were prepared daily. DNQX and APV were obtained from RBI/Sigma (Natick, MA) and TTX was obtained from Alomone Labs. A stock solution of the highly selective 5-$HT_{1B}$ agonist CP-93,129 (1 mM) was prepared in water and subsequently diluted to the final concentration (1 $\mu$M, Tocris Cookson, Ellisville, MO).

## RESULTS

### *Whole cell patch-clamp recordings*

Whole cell patch-clamp recordings were obtained from 7 SCN neurons in coronal hypothalamic slices from rats and 12 SCN neurons from mice (only 1 cell was recorded from each slice). Cells were voltage-clamped at −60 mV. Resting membrane potentials could not be measured due to the pipette recording solution containing CsCl and QX314. However, after establishing the whole cell configuration, a period of 2–5 min was allowed for the equilibration of intracellular and recording solutions before recording began. In all recordings, a stable baseline was achieved, and then the drug was applied followed by a washout period of ≥20 min or for as long as the recording was adequate. $GABA_A$-receptor-mediated mPSCs were pharmacologically isolated by applying a combination of APV (50 $\mu$M) and DNQX (10 $\mu$M) to block NMDA and AMPA/kainate receptors, respectively, and TTX (1 $\mu$M) to block sodium-mediated action potentials and the postsynaptic currents from them. All remaining currents could be reversibly suppressed by the $GABA_A$ receptor antagonist bicuculline (30 $\mu$M), shown in Fig. 1. Due to the high variance in both the frequency and amplitude of mIPSCs between individual cells, effects were best assessed as a percentage of control values. In experiments

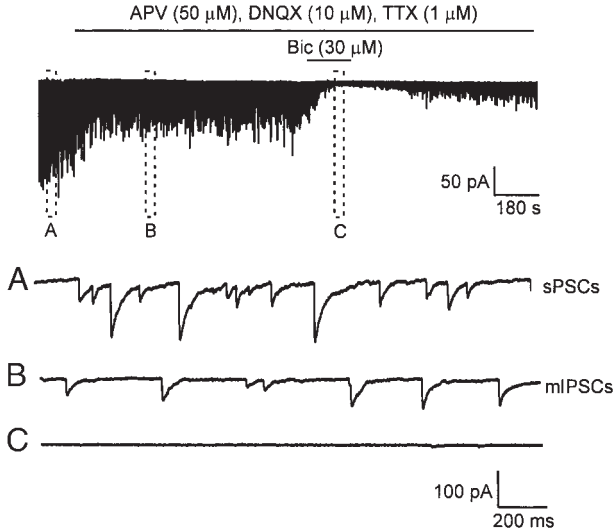


FIG. 1. The pharmacological isolation of $GABA_A$-receptor-mediated miniature inhibitory postsynaptic currents (mIPSCs). *Top*: a 35-min whole cell record of PSCs from a rat suprachiasmatic nucleus (SCN) neuron. *Bottom*: 2-s segments of the recording within the boxed parts are expanded (*A–C*). *A*: combination of both excitatory and inhibitory sPSCs. *B*: isolation of mIPSCs by the addition of 2-amino-5-phosphonopentanoic acid (AP5) and 5,7-dinitroquinoxaline-2,3-dione [DNQX; glutamate receptor antagonists to block excitatory postsynaptic currents (EPSCs)] and TTX (blocks sodium-mediated action potentials and the postsynaptic currents from them). *C*: all remaining currents were reversibly suppressed by the $GABA_A$ receptor antagonist bicuculline (Bic 30 $\mu$M). The IPSCs were inward currents due to a high $Cl^-$ concentration in the patch pipette. The cell was voltage-clamped at −60 mV.

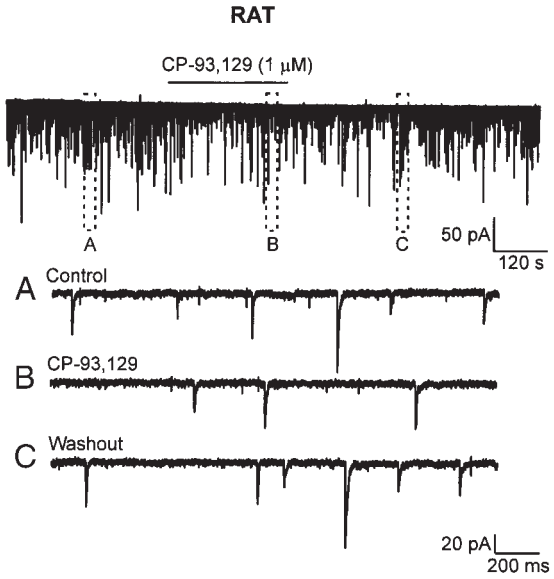


FIG. 2. CP-93,129 reduced the frequency but not the amplitude of $GABA_A$-receptor-mediated mIPSCs in the rat SCN. *Top*: a 20-min recording of mIPSCs. The slice was bathed in AP5 (50 μM), DNQX (10 μM), and TTX (1 μM). *Bottom*: periods of 2 s from within the boxed parts are expanded (*A–C*). *A*: control period before application of CP-93,129. All currents were mIPSCs. *B*: bath application of CP-93,129 (1 μM) reduced the frequency but not the amplitude of mIPSCs. *C*: washout of CP-93,129 to baseline levels.

using mice, the investigator was blind concerning the genotype (i.e., wild-type vs. $5\text{-HT}_{1B}$ KO).

### *Effects of CP-93,129 application in the rat*

CP-93,129 (1 μM) was bath applied, and the slices ($n = 7$) were exposed to the $5\text{-HT}_{1B}$ agonist for 4 min (Fig. 2). Data from dose-dependent responses for CP-93,129 indicate 1 μM to be a maximally effective dose (e.g., Berger and Huynh 2002). In three of the cells tested, mIPSCs showed a decrease in frequency; before exposure to CP-93,129, the average frequency of mIPSCs for these three cells was 3.0 ± 2.0 Hz compared with 1.3 ± 0.6 Hz after exposure to the $5\text{-HT}_{1B}$ agonist (i.e., a 57% reduction). The mIPSC amplitude was reduced from 49.2 ± 10.0 pA in the control period to 41.4 ± 5.9 pA (i.e., 16%) after application of the agonist. The cumulative plot (Fig. 3*C*) of mIPSC amplitude from a representative cell within this group gives a *D* value of 0.1, representing a maximal 10% shift between the two data plots at one point. This indicates a relatively small decrease in mIPSC amplitude, and the KS test showed that this was not significantly different (i.e., $P = 0.1$). Unlike the effect on frequency, this small effect on amplitude did not recover, suggesting that it was due to slight deterioration of the recording rather than an effect of the agonist. Of the three cells that showed the decrease in frequency in response to CP-93,129, the mIPSC frequency of two cells recovered completely to control levels in the washout period, and one recording was lost before complete washout was achieved. The remaining four cells of the seven tested showed no clear change in frequency (7.0 ± 5.2 Hz baseline compared with 6.3 ± 4.7 Hz with $5\text{-HT}_{1B}$ agonist) or amplitude (45.1 ± 2.1 pA compared with 42.4 ± 2.1 pA) of mIPSCs in response to CP-93,129 application. If we consider all seven cells tested as a single population, then a conservative calculation is that CP-93,129 caused a 22% reduction in the frequency of mIPSCs ($P = 0.03$, paired *t*-test), and this is summarized in Fig. 8*A*. That the effect of CP-93,129 was predominately if not exclusively on the frequency of mIPSCs suggests a presynaptic action of the $5\text{-HT}_{1B}$ receptor agonist.

### *Effects of CP-93,129 application in the wild-type mouse*

The same experimental procedure as used for the rat was followed for the mouse. CP-93,129 (1 μM) was bath applied, and cells were exposed to the $5\text{-HT}_{1B}$ agonist for 4 min. In

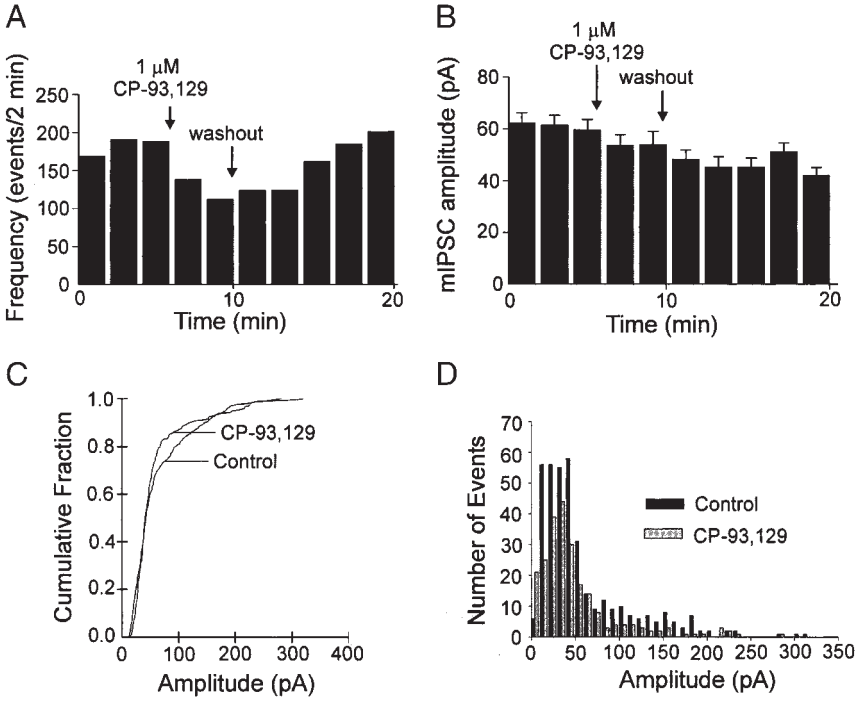


FIG. 3. Analysis of mIPSC frequency and amplitude recorded from a rat SCN neuron. *A*: the frequency of mIPSCs was reduced after application of CP-93,129 and recovered during the washout period. *B*: the amplitude histogram shows a decrease in amplitude during the recording that began before drug application and did not recover during the washout period; therefore these data were not interpreted as a drug effect and may have been caused by gradual deterioration of the recording. Bin size = 120 s and error bars represent SE. *C*: the cumulative plot shows a small apparent decrease (10%) in the amplitude of mIPSCs after application of CP-93,129, but this was not a significant difference ($P = 0.1$). *D*: the amplitude distributions are similar for control and CP-93,129 (bin size = 10 s).

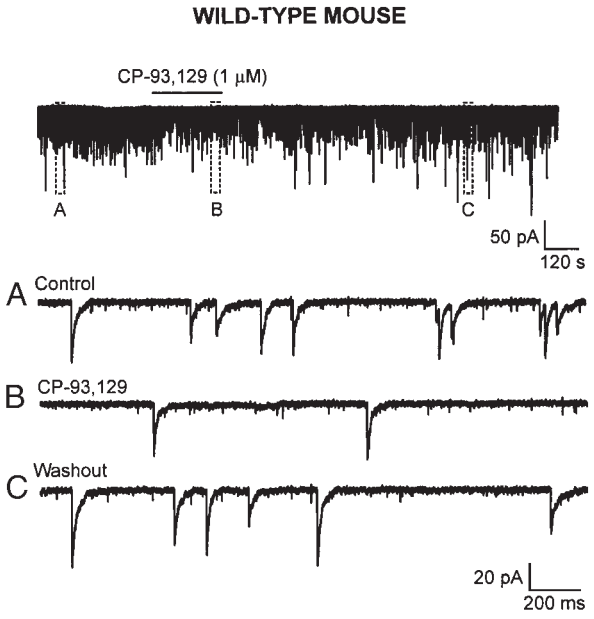


FIG. 4. CP-93,129 reduced the frequency but not the amplitude of $GABA_A$-receptor-mediated mIPSCs in the wild-type mouse SCN. *Top*: a 30-min recording of mIPSCs. The mIPSCs were pharmacologically isolated by bathing the slice in AP5 (50 $\mu$M), DNQX (10 $\mu$M), and TTX (1 $\mu$M). Records *A–C* (2 s in duration) are expanded from within the boxed portions of the top. *A*: control period before application of CP-93,129, all currents are mIPSCs. *B*: bath application of CP-93,129 (1 $\mu$M) reduced the frequency but not the amplitude of mIPSCs. *C*: during washout of CP-93,129, there was a partial recovery of mIPSC frequency to baseline values.

slices prepared from wild-type mice, the effect of CP-93,129 was tested on eight cells. Of the eight cells tested, three showed a large reduction in mIPSC frequency in response to CP-93,129 application. In the control period, the frequency of mIPSCs was 2.3 ± 0.5 Hz compared with 1.1 ± 0.4 Hz during drug exposure, reflecting a 52% reduction. A representative recording from a cell within this group is shown in Fig. 4. An additional three cells showed a more subtle but still clear effect of CP-93,129. In these cells, the frequency of mIPSCs in the control period was 4.8 ± 2.6 Hz and was 3.7 ± 2.1 Hz during drug application, a 23% reduction in frequency. The frequency of mIPSCs did not fully recover to control levels in those cells that showed a more dramatic response to CP-93,129, but there was a definite trend of increasing mIPSC frequency during the washout period. The remaining two cells did not show a clear effect in response to CP-93,129. The cumulative plots of the eight cells show *D* values in the range of 0.02 (2%) and 0.18 (18%), and the cumulative plot for the representative recording from wild-type mice is shown in Fig. 5*C*. However, the amplitude changes were not consistent: in five cells the amplitude of mIPSCs was slightly smaller after the application of CP-93,129, but the amplitudes were larger in the remaining three cells. Overall, the amplitude was 31.1 ± 6.2 pA in the control period compared with 32.9 ± 6.8 pA during exposure to the $5\text{-HT}_{1B}$ receptor agonist. In the eight cells tested, CP-93,129 decreased the frequency (but not the amplitude) of mIPSCs by an overall average of 30% ($P = 0.01$, paired *t*-test), as summarized in Fig. 8*B*.

### *Effects of CP-93,129 application in the 5-HT$_{1B}$ KO mouse*

There was a negligible effect of CP-93,129 application when tested on slices from four $5\text{-HT}_{1B}$ KO animals; one cell was recorded from each animal. In the control period, the frequency of mIPSCs was 2.5 ± 0.5 Hz and the amplitude was 17.8 ± 4.1 pA compared with 2.4 ± 0.5 Hz and 14 ± 3.6 pA during drug exposure. A representative recording of an SCN neuron from a $5\text{-HT}_{1B}$ KO mouse is shown in Fig. 6, and data for all four cells are summarized in Fig. 8*C*; the change in amplitude was not found to be significant ($P = 0.2$). The *D* values from the cumulative plots ranged from 0.1 to 0.2 (Fig. 7), and as with the wild-type mice, the change in mIPSC amplitude was not consistently in one direction (i.e., 50% of the cells showed a small increase in mIPSC amplitude after exposure to CP-

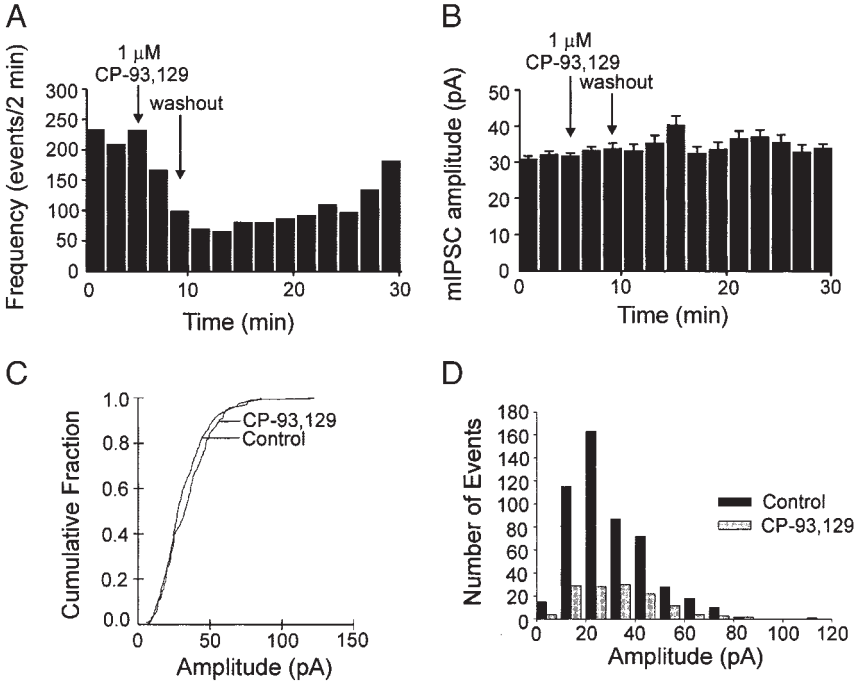


FIG. 5. Analysis of mIPSC frequency and amplitude recorded from a wild-type mouse SCN neuron. *A*: the frequency histogram shows a decrease in mIPSC frequency in response to CP-93,129 that partially recovered during the washout period. *B*: the amplitude histogram clearly shows a lack of effect of the $5\text{-HT}_{1B}$ agonist on mIPSC amplitude. Bin size = 120 s and error bars represent the SE. *C*: the cumulative plot shows a slight decrease (13%) in the amplitude of mIPSCs after application of CP-93,129 ($P = 0.04$). *D*: the amplitude distributions are similar for control and CP-93,129, although the significant decrease in the number of events in each bin reflects the effect on the frequency of mIPSCs (bin size = 10 s).

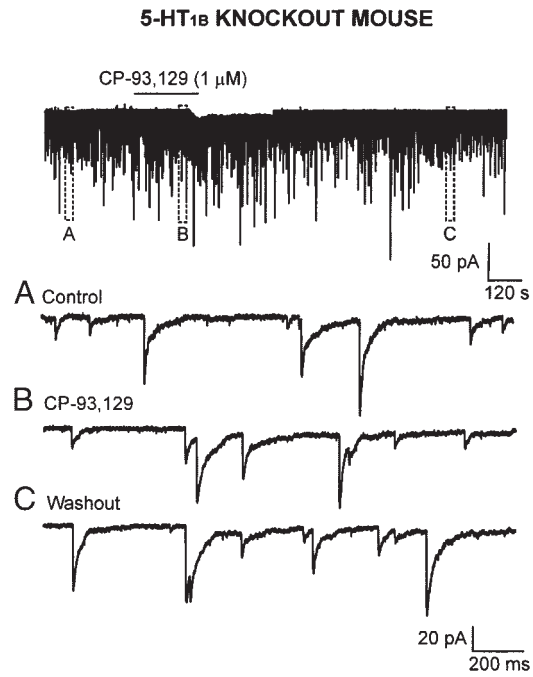


FIG. 6. CP-93,129 had a negligible effect on the frequency and amplitude of $GABA_A$-receptor-mediated mIPSCs in the 5-$HT_{1B}$ KO mouse SCN. *Top*: a 30-min recording of mIPSCs. The mIPSCs were pharmacologically isolated by bathing the slice in AP5 (50 $\mu$M), DNQX (10 $\mu$M), and TTX (1 $\mu$M). Records *A–C* (2 s in duration) are expanded from within the boxed portions of the *top*. *A*: baseline before application of CP-93,129; all currents were mIPSCs. *B*: bath application of CP-93,129 (1 $\mu$M) had no effect on the frequency or the amplitude of mIPSCs. *C*: washout of CP-93,129.

93,129 and 2 cells showed a decrease in amplitude. The effect of CP-93,129 on the frequency of mIPSCs in six of eight cells from wild-type mice was significantly different from the response to CP-93,129 on the frequency of mIPSCs in cells from 5-$HT_{1B}$ KO mice (0 of 4 cells; $P = 0.03$, Fisher's exact test, 1-tailed).

## DISCUSSION

The present study demonstrates that 5-$HT_{1B}$ receptors are located presynaptically on GABA terminals in the rodent SCN and that activation of these receptors in vitro decreases the release of GABA from these terminals. The highly selective 5-$HT_{1B}$ agonist CP-93,129 (Koe et al. 1992a) reduced the frequency but not the amplitude of GABAergic mIPSCs in the majority of cells tested from animals with functional 5-$HT_{1B}$ receptors, indicating a presynaptic site of action. This finding is consistent with the location of this receptor in the CNS predominately on axon terminals (Boschert et al. 1994) and numerous reports demonstrating 5-$HT_{1B}$ receptor-mediated presynaptic inhibition of transmitter release in several different regions of the CNS (Berger and Huynh 2002; Mlinar et al. 2003; Mooney et al. 1994; Pickard et al. 1999; Singer et al. 1996; Smith et al. 2001; Stanford and Lacey 1996; Tanaka and North 1993).

The location of the cell bodies that give rise to the GABAergic axon terminals examined in this study is not known with certainty. GABA terminals in the SCN are known to arise from several sources, including GABAergic neurons in the IGL that project to the SCN via the GHT (Morin 1994). However, most GABAergic axonal boutons in the SCN originate from the extensive GABAergic local network in the SCN (Castel and Morris 2000; Strecker et al. 1997; van den Pol 1986). 5-$HT_{1B}$ mRNA has been reported in the SCN (Roca et al. 1993), consistent with a local site of receptor synthesis. Manrique and colleagues (1999) have provided indirect evidence for 5-$HT_{1B}$ binding sites associated with GHT terminals in the SCN. It may well be the case that not all GABAergic terminals within the SCN express the 5$HT_{1B}$ receptor. This would explain the lack of effect of CP-93,129 on some of the cells recorded, and because we were not able to determine the source of the GABAergic terminals in these experiments, it is difficult to separate the cells into different groups without additional characterization of the recorded cells. Experiments using pro-

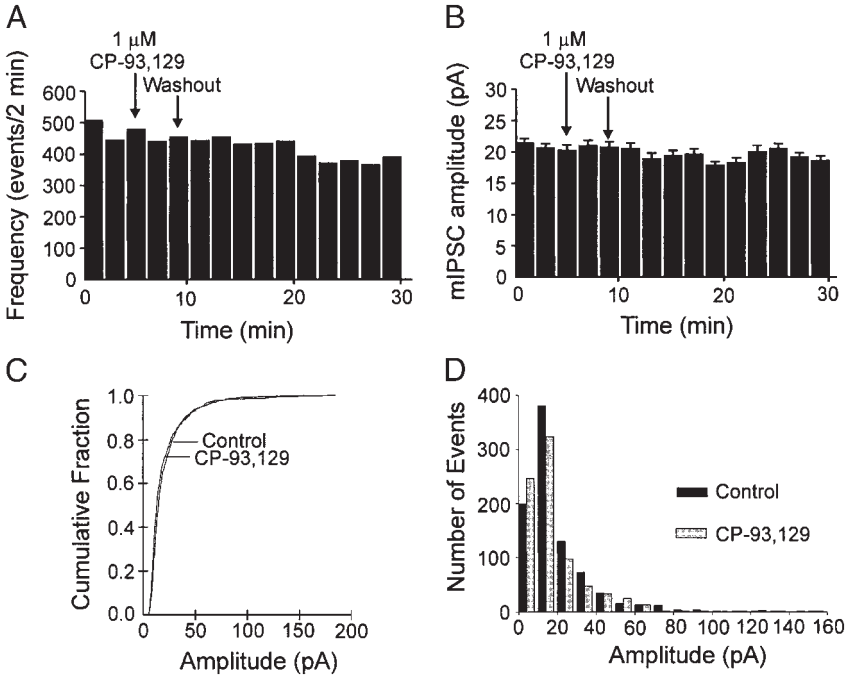


FIG. 7. Analysis of mIPSCs recorded from the KO mouse. *A* and *B*: the frequency and amplitude histograms for this recording show a lack of effect of CP-93,129 on mIPSCs (bin size = 120 s and the error bars represent the SE). *C*: the cumulative plot shows a slight decrease (10%) in the amplitude of mIPSCs after application of CP-93,129. *D*: the amplitude distributions are similar for control and CP-93,129 (bin size = 10 s).

cedures similar to those described in the current study conducted in animals in which the IGL has been ablated may help to resolve this issue.

Although the polar character of CP-93,129 is apparently the reason, it has very low penetrability into the CNS, it is also probably responsible for its selective affinity at 5-$HT_{1B}$ receptors (Koe et al. 1992b). Indeed, at a concentration (1 $\mu$M) shown to be maximally effective at inhibiting transmitter release in vitro in previous studies (Berger and Huynh 2002; Mlinar et al. 2003; Singer et al. 1996), CP-93,129 was ineffective in altering the frequency of mIPSCs in mice lacking functional 5-$HT_{1B}$ receptors. The variability in mIPSC frequency between successive 120-s bins during the baseline period in all recordings was ~8.9 ± 1.1%, so the change of ≤7% that was seen in KO animals is within the inherent variability in the system; therefore CP-93,129 showed negligible effects in the 5-$HT_{1B}$ KO mice. The mIPSC amplitude also varied (6.4 ± 1.3%) between successive 120-s bins during the baseline period. We have shown in previous studies that 5-$HT_{1B}$ KO mice serve as excellent pharmacological controls for examining the specificity of 5-$HT_{1B}$ receptor agonists both in vivo and in vitro (Pickard et al. 1999; Smith et al. 2001). Taken together, these data support the interpretation that the response of the 5-$HT_{1B}$ agonist used in rats and wild-type mice was due to the activation of 5-$HT_{1B}$ receptors (Fig. 8).

Previous work has shown that 5-$HT_{1B}$ receptors on retinal terminals act to inhibit the response of SCN neurons to light stimulation (Pickard et al. 1999; Smith et al. 2001). Thus one might expect that in the 5-$HT_{1B}$ KO mouse there would be an enhanced response of the circadian system to light stimulation. However, this is apparently not the case, as behavioral studies indicate that 5-$HT_{1B}$ KO animals actually exhibit an attenuated response to photic stimulation (Ogilvie et al. 2004). The relative number of GABA terminals in the SCN far outweighs the number of glutamatergic retinal terminals in the SCN, so even with the removal of 5-$HT_{1B}$ inhibition on glutamate release from the retinal terminals, the lack of inhibition of GABA release may have a much greater total effect with an outcome of an attenuated response to light stimulation. It is not understood at present how increased SCN GABA release in 5-$HT_{1B}$ KO mice may alter the response of the SCN circadian system to light. It is possible that excessive GABA release activates $GABA_B$ receptors that are located on RHT terminals (Jiang et al. 1995). Additional work is clearly necessary to reveal the underlying physiological mechanisms responsible for the attenuated response of 5-$HT_{1B}$ KO animals to light.

The relationship between 5-HT and GABA terminals in the SCN has long been recognized. Bosler (1989) described extensive axo-axonal interactions between 5-HT and GABA terminals in the SCN and suggested that "every 5-HT varicos-

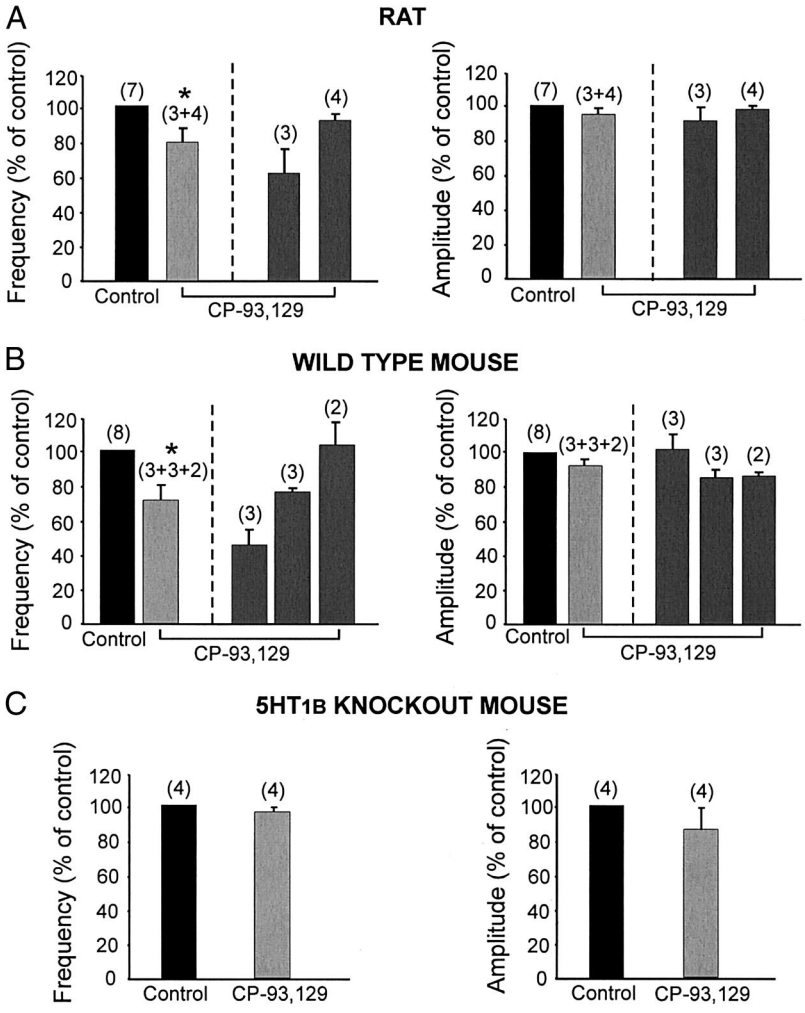


FIG. 8. Frequency and amplitude histograms of the collective data from experiments in the rat, wild-type mouse, and 5-$HT_{1B}$ KO mouse summarize the effects of CP-93,129. *A* and *B*: CP-93,129 reduced the frequency of mIPSCs by 22% ($P$ = 0.03) and 30% ($P$ = 0.01) in the rat and mouse, respectively, when considering all cells recorded as a single population, suggestive of a presynaptic action of CP-93,129. The bars to the right of the dashed line in each histogram show the results when the population is divided into cells that show a strong, subtle or lack of effect to CP-93,129. There were no significant changes in the amplitude of mIPSCs. *C*: CP-93,129 had no effect on the frequency of mIPSCs in all cells recorded from the KO mouse and the change in mIPSC amplitude was not significant ($P$ = 0.2). The numbers of neurons tested are represented by ($n$), and only one neuron was recorded from each slice preparation. Error bars represent the SE.

ity in the SCN might well be apposed to at least one GABA terminal." Indirect evidence from circadian behavioral studies (Mintz et al. 1997) and 5-HT-induced $Ca^{+2}$ transients in the SCN (Flett and Colwell 1999) suggests that 5-HT can act within the SCN to alter GABA activity. Jiang and colleagues (2000) provide evidence from SCN neurons in hypothalamic slices that 5-HT can alter GABA release via a presynaptic mechanism (in 3 of 10 cells); although the 5-HT receptor subtype mediating this response was not specified. $5\text{-HT}_7$ receptors have been described on GABA terminals in the SCN (Belenky and Pickard 2001) and the current study also supports the localization of $5\text{-HT}_{1B}$ receptors on GABA terminals in the SCN. Indeed, the presynaptic interactions between 5-HT and GABA may well be wide spread throughout the CNS (Belenky and Pickard 2001; Johnson et al. 1992; Matsuoka et al. 2004; Stanford and Lacey 1996; van Bockstaele et al. 1996; Yan and Yan 2001).

In summary, the present study provides data from SCN neurons in hypothalamic slices from rat and mouse indicating that $5\text{-HT}_{1B}$ receptors are located presynaptically on GABA axonal terminals and that activation of these receptors inhibits GABA release.

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