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Responsiveness of Suprachiasmatic and Ventral Lateral Geniculate Neurons to Serotonin and Imipramine: A Microiontophoretic Study in Normal and Imipramine-Treated Rats

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MEIJER, J. H. AND G. A. GROOS. *Responsiveness of suprachiasmatic and ventral lateral geniculate neurons to serotonin and imipramine: A microiontophoretic study in normal and imipramine-treated rats.* BRAIN RES BULL **20**(1) 89–96, 1988.—The suprachiasmatic nuclei (SCN) are a major pacemaker of circadian rhythms in mammals. The SCN receive a direct retinal projection and a second optic input via the ventral lateral geniculate nucleus (vLGN). Both visual pathways mediate the entrainment of circadian rhythms, whereas both the SCN and the vLGN receive serotonergic afferents from the raphe nuclei. We investigated the effects of microiontophoretically applied serotonin (5HT) on SCN and vLGN cells in normal rats and rats chronically treated with the 5HT reuptake blocker imipramine (IMI). In the SCN of both groups over 40% of all recorded cells (N=80) responded to 5HT with a dose-dependent suppression of their spontaneous or glutamate-evoked discharge, while twenty percent were tonically light-responsive. Except for one cell with an inconsistent 5HT response, none of the visual SCN neurons were 5HT-sensitive. In the vLGN of normal and IMI-treated rats about 60% of the cells recorded (N=42) were inhibited by 5HT. In IMI-treated rats a few cases of excitation by 5HT were encountered in the vLGN. Visual as well as non-visual vLGN cells were responsive to 5HT. Microiontophoretic application of IMI resulted in suppression of electrical activity in both brain regions and enhanced the response induced by 5HT. Chronic IMI-treatment produced a significant increase in the sensitivity of cells in the SCN and vLGN to iontophoresed 5HT, without affecting the relative magnitude of the inhibition. The recovery from 5HT-induced inhibition was slow in these animals. Interestingly, the spontaneous discharge rate of both 5HT-sensitive and 5HT-insensitive SCN and vLGN cells was significantly lower in the imipramine-treated group. These results indicate that the serotonergic raphe input to the SCN and vLGN is of importance in the afferent control of the circadian pacemaker in the SCN. The functional role of 5HT in this system could be investigated neuropharmacologically with chronic IMI treatment since this tricyclic uptake inhibitor renders SCN and vLGN cells supersensitive to 5HT.

Circadian pacemaker Imipramine Suprachiasmatic nuclei Serotonin
Ventral lateral geniculate nucleus Serotonin-supersensitivity

THE suprachiasmatic nuclei (SCN) of the anterior hypothalamus have been identified as a major pacemaker in the circadian system of various mammalian and avian species [17, 33, 34, 42, 52]. The SCN independently produce circadian cycles of neuronal activity, which are entrained to the environmental light-dark cycles via the retina [16, 18, 27, 33, 35, 42, 52]. Efferent projections from the SCN impose a circadian rhythm on several brain structures as well as the neuroendocrine system [33, 35, 42, 52]. The entrainment of the SCN pacemaker is mediated by photoreceptors in the retina and two central ganglion cell projections. In the rat one of these projections, the retino-hypothalamic pathway (RHP), has its terminal field in the ventral and lateral SCN [18, 27, 35, 41]. The other projection terminates in the ventral portion of the lateral geniculate nucleus (vLGN) from where neuropeptide Y-containing neurons further project to the SCN [10, 18, 35, 41, 48]. The terminal field of this retino-geniculo-suprachiasmatic pathway shows considerable overlap with that of the retino-hypothalamic pathway [10, 35, 41, 48]. Apart from the two optic projections various

[1]This paper reports one of the last investigations in which Gerard Groos participated before his untimely death on March 25, 1985.

other afferents of the SCN have been described [35, 41, 42]. Little information is available on the anatomical, functional and pharmacological organization of these afferents. An exception, however, is the prominent serotonergic projection from the midbrain raphe nuclei to the SCN.

In rodents, the SCN receive an ascending projection from both the median and dorsal raphe nuclei [4, 21, 34, 40, 41, 47]. This projection ends with medium-sized terminals, containing small vesicles, on SCN dendrites, making predominantly asymmetric type synaptic contacts [1, 22, 38]. The raphe terminals contain serotonin (5-hydroxytryptamine, 5HT) and are responsible for the high 5HT content of the SCN [21, 44, 47] while significant activities of the serotonin synthesizing and metabolizing enzymes have been found in the SCN [9,45]. The larger part of the terminal field of the raphe projection in the SCN overlaps with the terminal fields of the RHP and the vLGN projection [10, 34, 47]. Interestingly, the vLGN also receives a serotonergic input from the midbrain raphe nuclei [4,34]. Immuno-electron-microscopic observations have established that at least a portion of the postsynaptic SCN neurones receiving raphe input contain vasoactive intestinal polypeptide [22]. Thus, there is a rather complete characterization of the serotonergic afferent system of the SCN. The function of this serotonergic projection to the SCN, however, remains to be established.

The purpose of this study was to obtain a detailed description of the responsiveness of single, spontaneously active or glutamate-excited SCN neurons in the rat to microiontophoretic application of 5HT. Furthermore, it was assessed whether serotonin-sensitive SCN cells receive an optic input. To study the photic responsiveness of SCN cells, long-duration stimuli illuminating the entire retina bilaterally were employed. Previous investigations have established that such stimuli are particularly effective in altering SCN discharge [15, 18, 27]. Since the tricyclic-antidepressant imipramine blocks presynaptic 5HT reuptake in serotonergic terminals [29, 32, 39], iontophoresis of imipramine was used as a tool to potentiate the response to 5HT. As an extension of this approach altered sensitivity of SCN cells to 5HT following chronic imipramine treatment was studied. The electropharmacological properties of serotonin-sensitive SCN cells were compared to those recorded for vLGN neurons in a separate series of experiments. In this manner the extent to which 5HT has a uniform action on SCN cells and on the ventral geniculate from which afferents arise could be determined.

METHOD

Twenty-four male Wistar rats (270–320 g) were used in this study. The animals were kept in a lighting regimen consisting of 12 hours of light alternating with 12 hours of darkness (LD 12:12). All experiments were carried out during those hours of the day at which the animals would have been exposed to illumination in their home cage. However, depending on the experiment, single cell activity was recorded either in continuous light or continuous darkness or in response to light flash stimulation. Ten of the 24 rats were chronically treated with imipramine before the recording experiment. Imipramine treatment consisted of daily intragastric administration of imipramine hydrochloride (10 $mg \cdot kg^{-1}$) via oral intubation for a period of 3–5 weeks prior to recording. The last dose of imipramine was given at least 24 hours before the start of the recording session.

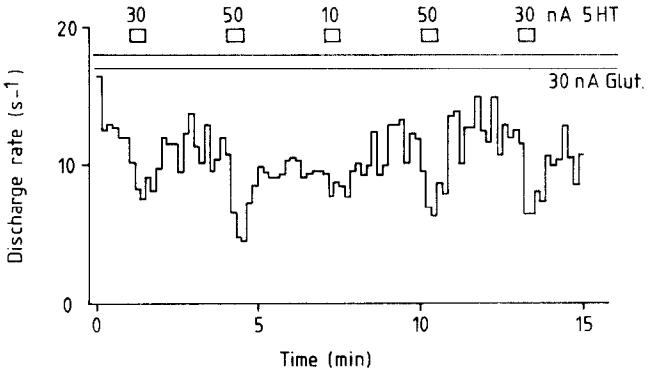

FIG. 1. Responses of a suprachiasmatic cell to various doses of microiontophoretically applied 5HT. The electrical activity of this cell was increased by continuous ejection of glutamate (Glut). The ejection currents are indicated in the figures. The firing rate is indicated as spikes per second calculated as the average of successive 10 sec epochs.

For extracellular single cell recording and microiontophoresis, rats were anaesthetised with urethane (1.3–1.5 $g \cdot kg^{-1}$, IP) and mounted in a stereotaxic frame. Rectal temperature was maintained at 36–37°C with a homeothermic blanket. Craniotomy was performed in the midline to allow for suprachiasmatic nucleus recording in 8 untreated rats and 6 rats pre-treated with imipramine. The sagittal sinus was cauterised in these animals to facilitate vertical stereotaxic placement of multi-barrel pipettes in the SCN. Single cell activity was also recorded in the ventral portion of the lateral geniculate nucleus (vLGN) of 4 imipramine-treated and 6 untreated rats.

For recording and microiontophoresis 3 or 5-barrel micropipettes were constructed from 'kwik-fill' glass capillaries (Clark Electromedical, U.K.) with an overall tip diameter of 4–5 μm. The recording barrel was filled with 0.5 M NaCl containing 5% pontamine sky blue. The drug-barrels were filled with either 20 mM 5-hydroxytryptamine creatinine sulphate (5HT; pH 3.5), 100 mM sodium glutamate (GLUT; pH 8.5) or 10 mM imipramine hydrochloride (IMI; pH 5.5). One barrel containing 4 M NaCl was used for automatic current balancing of the total iontophoretic current. The technique for microiontophoretic delivery of drugs from the drug-barrels was conventional using a FET operational amplifier constant current source [46]. A retaining current of −5 nA was applied to the drug-barrels between ejection periods.

Visual stimulation consisted of whole field retinal illumination with white light. In SCN recordings both eyes were stimulated for periods exceeding one minute, while in vLGN experiments contralateral monocular flash stimulation (1 sec on, 1 sec off) was employed.

Extracellularly recorded action potentials of single SCN or vLGN neurons were amplified and displayed on an oscilloscope. After electronic spike discrimination the discharge rate of each cell was counted in successive 5 second epochs. These counts were continuously printed and plotted on a potentiometric recorder. In the figures, the discharge rates are displayed accordingly unless mentioned otherwise. The sensitivity of cells to 5HT was assessed by measuring the iontophoretic charge ($I.T_{50}$) required to obtain a 50% change from the baseline firing rate [29,30]. A high sensitivity to 5HT was thus reflected by a low $I.T_{50}$ value. Furthermore, the time required to attain 50% recovery of the baseline discharge rate after drug application (RT_{50}) was computed.

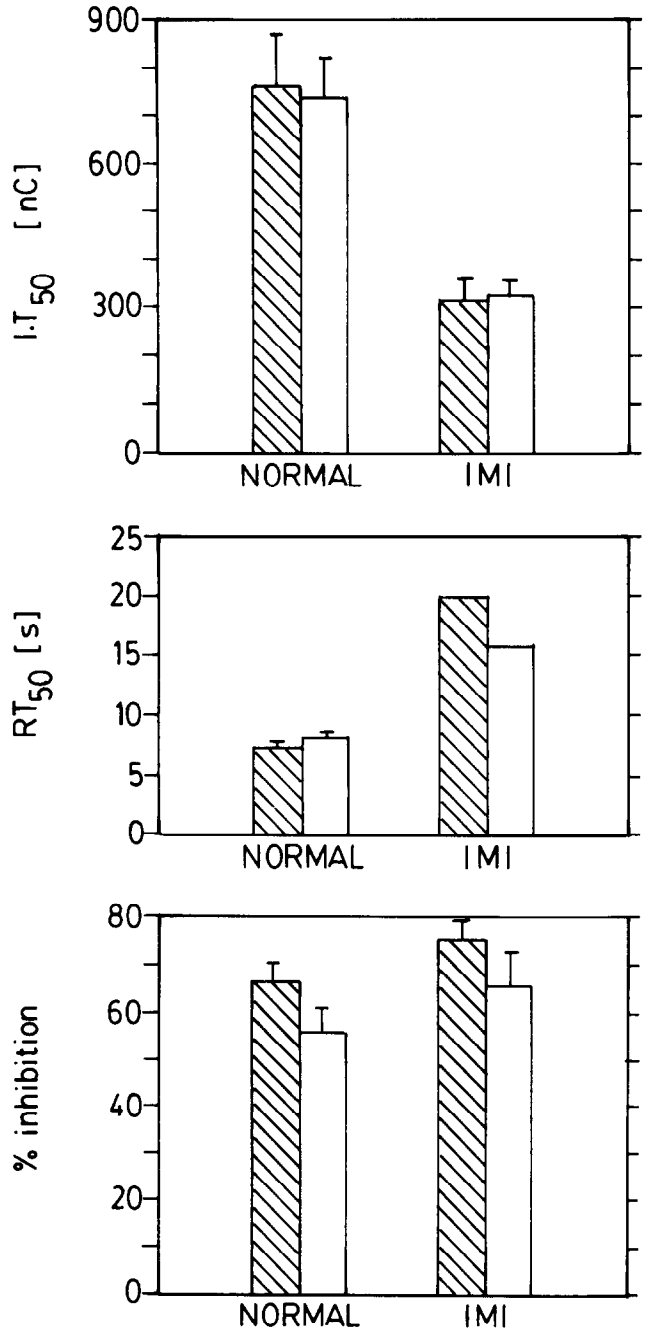

FIG. 2. Effects of illumination and chronic imipramine (IMI) treatment on the $I.T_{50}$, RT_{50} and relative magnitude of inhibition of SCN cell responses to microiontophoresed 5HT. $I.T_{50}$ (nC): ejection current (nA)·time (sec) required to obtain a 50% reduction in discharge rate. RT_{50}: time (sec) required to attain 50% recovery of the baseline discharge rate following drug delivery. The hatched bars represent the measures recorded in darkness, the open bars represent measurements in the light (mean±s.e.m.). In none of these measurements glutamate was employed to stimulate the cells, all other successful measurements are included.

Dose-response effects were obtained for a range of ejection currents (30–70 nA) using 30 sec ejection periods at 3 min intervals. A cell was classified as responsive to 5HT if it consistently responded to at least 5 sequential 5HT ejections at 3 min intervals.

At the end of the experiment the last electrode track was marked by ejecting pontamine sky blue from the recording pipette (20 μA for 5–10 min). The animal was subsequently perfused transcardially with 10% formaline in saline for later histological verification of the recording sites in cresyl violet stained, 50 μm frozen sections of the brain.

RESULTS

Effects of 5HT and Imipramine on SCN Neurons

Histological reconstruction of the SCN recording sites indicated that a total number of 41 spontaneously active cells were studied in the SCN of 8 untreated rats. Iontophoretic microinjection of 5HT consistently resulted in suppression of the discharge rate in 20 SCN cells (49%). The remaining 21 cells (51%) were unresponsive to 5HT ejections. Figure 1 presents a typical example of a response in a serotonin-sensitive SCN neuron. The serotonin-evoked inhibition of discharge found for other SCN cells consistently followed this general pattern. In accordance with reports by other authors [7, 15, 37] the spontaneous discharge rates of our SCN cells were very low, ranging from 0.2–2.2 sec^{-1} (mean 1.03 sec^{-1}). The discharge rate of the 5HT sensitive cells (±s.e.m.) is 1.06±0.15 Hz and of the 5HT insensitive cells 1.00±0.11 Hz. Therefore, four of the 20 serotonin-sensitive cells were activated by continuous glutamate ejection (5–30 nA). This effectively raised their discharge rate to 2–14 sec^{-1}, depending on the glutamate ejection current. When stimulated with 5HT glutamate-activated SCN cells exhibited qualitatively the same response characteristics as the other cells. The mean $I.T_{50}$ value for the glutamate-activated cell responses to 5HT was 574.1 nC, the mean RT_{50} value 11.0 sec and the magnitude of inhibition 65%.

The 5HT ejection current was varied from 30–70 nA. Serotonin was applied for 30 seconds every 3 minutes. In the 8 untreated animals this 3 min recovery interval between each microinjection was sufficient for all 5HT responsive cells to reestablish their baseline discharge rate. The baseline firing frequency was defined as the mean discharge rate during 40 seconds immediately preceding 5HT microiontophoresis. The relative magnitude of the inhibition in 5HT-sensitive SCN cells, expressed as percent inhibition from baseline firing, was found to be dependent on the ejection current; increasing doses of 5HT evoked increasing levels of inhibition.

For each 5HT-sensitive SCN cell the serotonin response was investigated during both retinal illumination and in the dark. The response characteristics (i.e., $I.T_{50}$, RT_{50}, % inhibition) were not affected by the illumination condition (see Fig. 2). The $I.T_{50}$ value in the dark (±s.e.m.) is 764.6±103.5, in the light 741.6±84.6, the RT_{50} value in the dark is 7.4±2.1 and in the light 8.3±1.9. The percentage inhibition in the dark is 66.6±4.0% and in the light 56.4±5.0%. Four visual cells were encountered within the boundaries of the SCN in untreated rats. These cells tonically increased their firing in response to a sustained retinal illumination. In accordance with previous studies [15] these cells were classified as light-activated neurons. Three of these cells were not responsive to iontophoretic administration of 5HT at currents (30–70 nA) that were adequate to depress the non-visual 5HT-sensitive SCN cells. In Fig. 3A visual responsiveness and 5HT-insensitivity are illustrated for one of these visual SCN cells. One of the light-activated cells initially showed a consistent, dose-dependent response to 5HT, but subsequently lost this response to 5HT application.

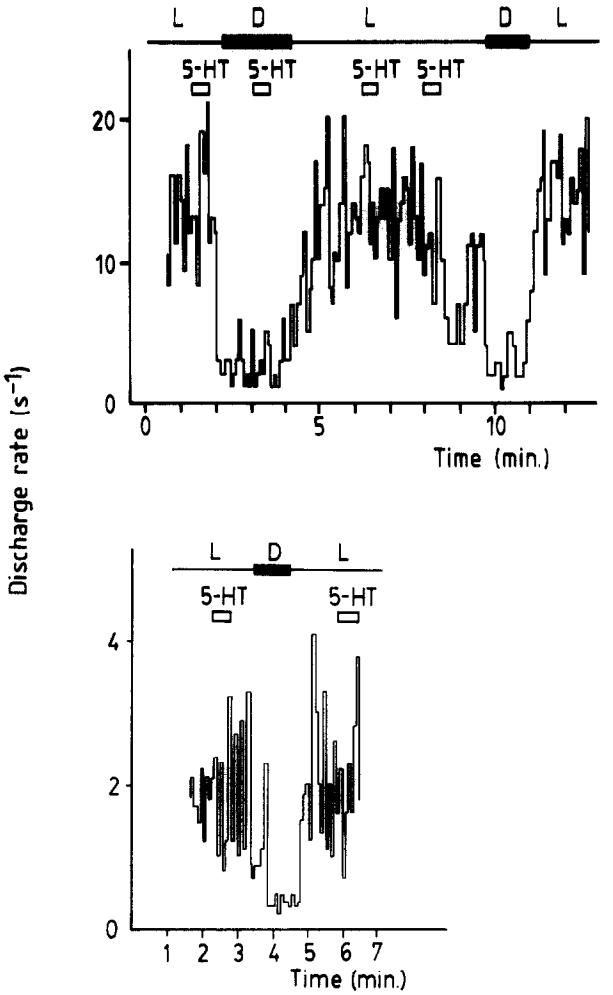

FIG. 3. (A) Absence of 5HT-sensitivity in a light-activated visual SCN cell. The iontophoretic current used was 30, 30, 30 and 50 nA respectively. The discharge rate is displayed over 5 seconds epochs and is expressed in Hz. (B) Absence of 5HT-sensitivity of a light-activated SCN cell of an IMI-treated rat. 5HT was ejected with an iontophoretic current of 70 nA.

The responsiveness of the SCN to acute microiontophoretic administration of imipramine was investigated in two untreated rats. Six out of 8 SCN cells in these animals responded consistently to imipramine with a decrement of their discharge rate in a current-dependent manner (Fig. 4). These IMI-sensitive cells were also sensitive to 5HT, while the remaining two cells were unresponsive to administration of either imipramine or 5HT. Iontophoresis of imipramine simultaneously with or just before a 5HT ejection generally resulted in more pronounced inhibition and a lower $I.T_{50}$ value than when 5HT was applied alone. The effect of imipramine application on the RT_{50} value for 5HT, however, showed a greater variability.

Chronic Imipramine Treatment: Effects on 5HT-Sensitivity in the SCN

In six rats chronically treated with imipramine for periods of 3–5 weeks prior to recording, 40 SCN cells were studied. Fourteen of these cells (35%) consistently responded with a

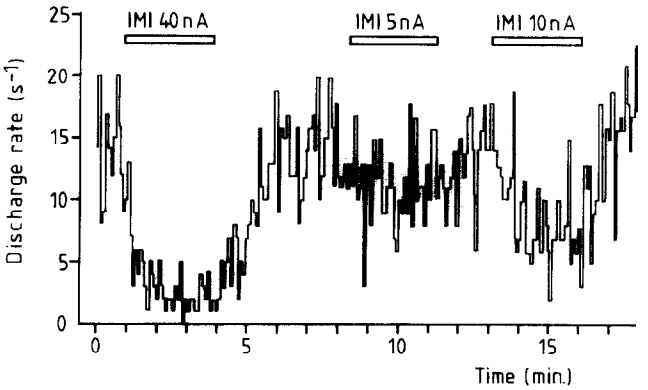

FIG. 4. Responses of a SCN cell to acute microiontophoretic application of imipramine (IMI). Imipramine was ejected for 3 minutes. The iontophoretic currents were 40, 5 and 10 nA respectively.

suppression of their discharge rate to a microiontophoretic ejection of 5HT. Glutamate was not employed to raise the spontaneous discharge activity in these cells. Currents of 30 nA were often sufficient to significantly depress the cells' firing rates (Fig. 5). In the dark the $I.T_{50}$ value (±s.e.m.) is 318.8±42.0, in the light 327.4±31.2, the RT_{50} value in the dark is 20±3.0 and in the light 15.5±5.2 (3 cells that did not recover to 50% of baseline discharge rate were excluded). The percentage inhibition in the dark is 75.3±3.1% and in the light 65.4±6.3%. The $I.T_{50}$ and RT_{50} values and the percentage inhibition of these cells are summarized in Fig. 2 for comparison with the corresponding measures obtained from the SCN of untreated rats. As in the untreated animals, each responsive SCN cell was studied with and without retinal illumination. A significant increase in sensitivity indicated by the decrease in the $I.T_{50}$ value in the imipramine-treated rats as compared to the untreated animals was indicated by a t-test ($p \leq 0.005$) (Fig. 2). The magnitude of the inhibitory response to 5HT (% inhibition) in imipramine-treated animals was not distinct from that of the untreated group. A difference in the RT_{50} values between both groups could not be readily evaluated because some cells in imipramine-treated rats failed to recover to 50% of baseline within the allotted time. Since there was no significant difference between $I.T_{50}$ or RT_{50} values in untreated rats with the eyes illuminated or in darkness (Fig. 2), RT_{50} values for these two light conditions were pooled both in the imipramine-treated and the untreated animals. Application of the median test demonstrated a highly significant increase ($p<0.001$) in RT_{50} in the SCN of imipramine-treated rats compared with the untreated group. The lower $I.T_{50}$ values and the increased recovery time following 5HT application in imipramine-treated rats indicated the development of super-sensitivity to iontophoresed 5HT in these animals. In imipramine-treated rats six light-activated and one light-suppressed SCN cell were encountered. These cells responded with a sustained elevation or depression of their discharge activity when the eyes were illuminated. All of these visual cells were insensitive to 5HT even at the high ejection currents (70 nA) which were more than adequate to inhibit the 5HT-sensitive non-visual SCN cells in these IMI-treated animals (Fig. 3B). A further finding in IMI-treated rats was that the spontaneous discharge found in the dark for all SCN cells in imipramine treated rats was significantly lower than in untreated animals (Table 1). The discharge rate of the 5HT sensitive cells (±s.e.m.) is 0.89±0.20 Hz and of the 5HT insensitive cells 0.55±0.07 Hz.

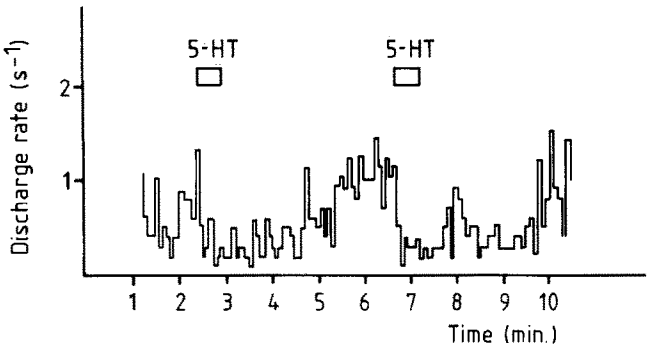

FIG. 5. Serotonin-supersensitivity of a SCN neuron in a chronically imipramine-treated rat. The iontophoretic current used was 30 nA. The firing rate is displayed over 10 second epochs.

TABLE 1

SPONTANEOUS DARK DISCHARGE RATES IN THE SCN AND vLGN OF NORMAL AND IMIPRAMINE-TREATED RATS

	Discharge Rate ± s.e.m. (s^{-1})[1]	
	Normal Rats	Imipramine-Treated Rats
SCN	1.03 ± 0.08 (32)	0.64 ± 0.08 (40)*
vLGN	6.6 ± 0.92 (22)	1.6 ± 0.36 (20)*

[1]Numbers in parentheses indicate number of cells.
*Significantly different from normal value, t-test ($p \leq 0.001$).

Serotonin and Imipramine Responses of the vLGN in Normal and Imipramine-Treated Rats

The response to 5HT was investigated in the vLGN of 4 rats chronically treated with imipramine and in 6 untreated rats. One-second duration light flashes were presented monocularly to the contralateral eye to test the visual responsiveness of the geniculate neurons. Twenty-two vLGN cells were recorded in the untreated rats. Thirteen of these cells (59%) responded to 5HT (5–50 nA) with a suppression of their discharge rate (Fig. 6). Ten of these 5HT-sensitive neurons exhibited a phasic response to light, while 2 cells showed sustained activation in addition to a phasic component in its photic response. Five visual and 4 non-visual cells were insensitive to 5HT. The $I.T_{50}$ values, the percentage inhibition and the RT_{50} values for the 5HT-sensitive vLGN cells are presented in Table 2. The vLGN was found more sensitive to 5HT compared with the SCN since the $I.T_{50}$ values in the SCN were higher than in the vLGN (t-test; $p \leq 0.001$) and the mean recovery time in the SCN was shorter (t-test, $p \leq 0.05$). The relative magnitude of the responses in the two nuclei were not significantly different, although for SCN cells a higher iontophoretic current of 5HT was usually needed to elicit a maximal response than found for vLGN neurons.

Imipramine was administered iontophoretically to 8 vLGN cells. Five neurons which were inhibited by imipramine were also inhibited by 5HT. Three cells did not respond to either imipramine or 5HT. Imipramine enhanced the 5HT response when the two compounds were iontophoresed simultaneously. This effect of imipramine is illustrated in Fig. 7.

Twenty vLGN cells were recorded in animals chronically treated with imipramine. Fifteen cells (75%) responded to

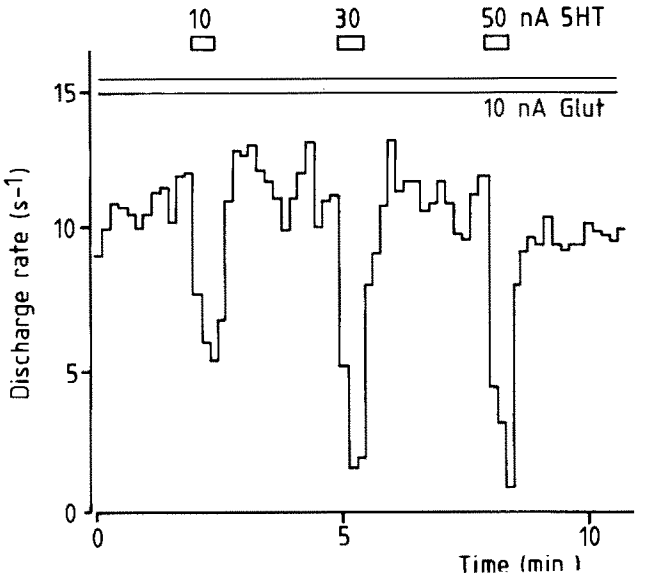

FIG. 6. An example of dose-dependent inhibition of a vLGN cell following 5HT microiontophoresis. The cell was excited by continuous ejection of 10 nA glutamate. The 5HT cell ejection currents were 10, 30 and 50 nA. The discharge rate is displayed over 10 second epochs.

5HT (5–50 nA) with a suppression of the discharge rate, two cells (10%) were excited. The $I.T_{50}$ values following chronic imipramine treatment were significantly reduced compared to control rats (Table 2), while the recovery times were significantly lengthened (median test; $p \leq 0.05$) thus producing a similar supersensitivity to that found for SCN cells of the imipramine treated animals. The relative magnitude of the inhibition, however, was similar in both groups. The mean discharge rate of vLGN cells was significantly decreased in IMI-treated rats (Table 1).

Eleven out of 15 5HT-sensitive vLGN cells in treated animals were phasically responsive to light. The 4 remaining cells were non-visual. All the 5HT-insensitive cells responded to retinal stimulation. Microiontophoretic application of IMI to vLGN cells in the IMI-treated rats resulted in suppression of the electrical activity in 4 out of 5 cells. One IMI-insensitive cell was also unresponsive to 5HT.

DISCUSSION

Employing microiontophoretic injection techniques this study showed that a considerable proportion of SCN cells as well as vLGN cells in the rat are sensitive to serotonin and that this sensitivity can be modified by chronic treatment with the tricyclic-antidepressant imipramine. Over 40% of the cells in the SCN responded to 5HT with a dose-dependent inhibition. No excitatory responses to 5HT were observed in the SCN. This finding is consistent with the observation of Bloom and his coworkers [7] who examined the serotonergic raphe innervation of the cat SCN. Neurons in the medial preoptic area of the rat are similarly inhibited by 5HT [26]. However, Nishino and Koizumi [36,37], studying the rat SCN, reported excitatory as well as inhibitory responses to 5HT, inhibition being more common than excitation. It should be noted that the proportions of excited and inhibited cells reported by Nishino and Koizumi (cf. their Table 1) are inconsistent.

TABLE 2

SEROTONIN-SENSITIVITY OF vLGN CELLS IN NORMAL AND CHRONICALLY IMIPRAMINE-TREATED RATS[1]

	$I.T_{50}$ ± s.e.m. (nC)	RT_{50} ± s.e.m. (s)	% Inhibition
Normal rats (n=6)	327.0 ± 35 (10)	16.5 ± 4.0 (8)	76.3 ± 3.5 (10)
Imipramine rats (n=4)	196.6 ± 18 (8)*	—[2]	81.3 ± 5.1 (8)†

[1]Numbers in parentheses indicate number of cells.

[2]In imipramine-treated rats the majority of 5HT sensitive vLGN cells did not recover their baseline firing rate within 3 minutes after 5HT stimulation.

*Significantly different from normal value; $p \leq 0.005$.

†Not significant.

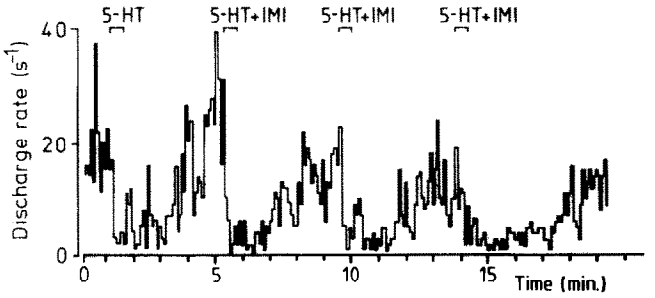

FIG. 7. Serotonin-supersensitivity of a vLGN neuron in a rat chronically treated with imipramine. The iontophoretic current was 20 nA for 5HT. After the first injection with 5HT, the cell recovers in 2 minutes to the baseline discharge rate (21 ap/sec). When 5HT is applied together with IMI (5 nA) the cell does not recover to baseline discharge rate within 3 minutes. A microinjection of imipramine alone (5 nA, not indicated in the figure), did not affect the spontaneous discharge of this cell.

These authors used multibarrel electrodes with the recording electrode tip at a distance of 15–20 μm from the drug pipette tips. In view of the small perikaryal size of the SCN cells and the dense packing of the SCN [33,35] it is possible that excitatory responses have resulted from inhibition of an inhibitory interneuron.

The low spontaneous discharge rate of most SCN cells resulted in obtaining only small absolute magnitudes of inhibition. The relative response magnitude (% inhibition) on the other hand was always considerable. Serotonin-sensitive SCN cells commonly exhibited a depression in discharge activity to 40% of the baseline firing rate. The absolute magnitude of 5HT-evoked inhibition could be increased by experimentally increasing the discharge rate of SCN cells using continuous microiontophoretic administration of glutamate. This procedure did not affect the characteristic pattern of 5HT inhibition.

Over half of the vLGN cells were serotonin-sensitive. The predominant response of vLGN cells to 5HT was inhibition but a small number of vLGN cells recorded in imipramine-treated rats were excited by 5HT. Other authors similarly observed inhibition as the common response to 5HT iontophoresis in the vLGN [30,53] as, more generally, is the case in the forebrain and diencephalon [8, 11, 14, 30, 32, 53]. Accordingly, serotonin was capable of depressing the photic responses of vLGN cells. Thus, the normal visual functions of the vLGN could be modified by the release of 5HT as a result of activation of raphe neurons. In the untreated rats the vLGN was more sensitive to iontophoresed 5HT than the SCN as was evident from the lower $I.T_{50}$ values. Moreover, the RT_{50} values for the vLGN were smaller than those found in the SCN. This indicates that the rate of removal of iontophoresed 5HT in the vLGN may be higher than found in the SCN, i.e., re-uptake, metabolism or diffusion of 5HT in the vLGN may occur at a higher rate.

The increased sensitivity to 5HT and the increase in the percentage of inhibition in both the vLGN and the SCN with simultaneous 5HT and IMI iontophoresis indicate that IMI enhances the effects of 5HT. This enhancement is most readily explained in terms of blocking of presynaptic 5HT uptake by imipramine. It is well-established that tertiary-amine tricyclics like imipramine block preferentially serotonergic uptake [13,32] and that imipramine binding sites are closely associated with the presynaptic terminals of serotonergic fibres [24,39]. Presynaptic uptake blockade also explains the inhibitory effect of iontophoresed imipramine. In this case the spontaneous 5HT release in the presence of imipramine would be expected to result in a higher extracellular concentration of 5HT leading to an increased postsynaptic effect.

Chronic administration of imipramine resulted in a pronounced supersensitivity of SCN and vLGN cells to 5HT. This higher sensitivity is inferred from a decreased $I.T_{50}$ value in treated as compared to untreated animals. Sensitisation following long-term imipramine administration may be the result of either presynaptic or postsynaptic effects of imipramine. A presynaptic effect of imipramine is suggested by the marked prolongation of the recovery time following 5HT application if it is assumed that the RT_{50} value mainly reflects the rate of presynaptic uptake. The development of postsynaptic supersensitivity to iontophoresed 5HT after imipramine treatment has been previously described for the vLGN and the hippocampus [11, 30, 31, 53]. Another effect of chronic treatment with tricyclic anti-depressants is the down regulation of 5HT and norepinephrine receptors [46]. The time course in the development of supersensitivity and in the down regulation of 5HT and norepinephrine receptors both parallel that of the therapeutic action of chronic tricyclic treatment [32,46]. In the present study it was confirmed that imipramine-induced 5HT supersensitivity was present within 3 weeks of treatment. It is likely that presynaptic and postsynaptic effects of chronic imipramine administration work concertedly to produce supersensitivity and long recovery times to 5HT iontophoresis.

A surprising and new finding of our study was the significant decrease of the mean discharge rate in all SCN and vLGN cells in IMI-treated rats, irrespective of whether these cells were 5HT-sensitive or not (Table 1). This effect of chronic treatment has not been described before for either

imipramine or other antidepressants. This observation suggests that the pharmacological effects of chronic imipramine treatment may extend beyond those neurons that belong to the transmitter systems directly affected by the treatment.

The visual SCN cells found in the present experiments were all of the light-activated type described by Groos and Mason [15]. With one exception of a neuron with an inconsistent 5HT response, none of the light-activated SCN cells were sensitive to serotonin. Serotonin responses in visual SCN cells were absent even in rats which were supersensitive to 5HT. This observation is surprising considering the significant overlap of the terminal fields of the optic and the raphe inputs to the SCN.

The role of the serotonergic raphe input for the circadian pacemaker function of the SCN is unclear. The persistence of circadian rhythms following raphe lesions or transsection of the rostral raphe projections [3, 5, 6, 12, 20, 42] suggests that the raphe complex is not essential for the maintenance of circadian rhythmicity. On the other hand, some rather potent effects of raphe lesions on circadian rhythms have recently been demonstrated [25]. According to some reports, neurotoxic lesions of the serotonergic system with parachlorophenylalanine (pCPA) or 5,7-dihydroxytryptamine (5,7-DHT) transiently disrupts neuroendocrine and behavioral rhythms [19, 23, 49, 50]. These effects, however, are inconsistent and can only partly be due to the reduction of the 5HT content of the SCN, since local 5,7-DHT lesions in the SCN less effectively disrupt circadian rhythmicity than general 5HT depletion with pCPA [23]. Yet, the possibility that 5HT is involved in the timing of biological rhythms should not be too easily dismissed. Indirect evidence points to a role for suprachiasmatic serotonin in the timing of adrenocorticotropic hormone secretion and the luteinizing hormone surge preceding ovulation in cycling female rats [23, 28, 29, 50, 51]. The importance of 5HT in the temporal control of ovulation is also suggested by the observation that daily melatonin injections can reinstate the ovulation cycle in rats made anovulatory by constant illumination [43]. This effect of melatonin is counteracted by the 5HT receptor blocker methiothepin or by a tryptophan-deficient diet [43]. This, together with the observations that serotonin turnover in the SCN of cycling female rats precedes the proestrus LH surge [28,29] and that melatonin administration elevates brain serotonin levels [2] demonstrates that the functional importance of this monoamine in the timing of ovulation must not be ignored. The 5HT supersensitivity in the SCN following chronic imipramine treatment found in our study may provide a tool to further investigate this possibility.

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