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CIRCADIAN VARIATION IN SENSITIVITY OF SUPRACHIASMATIC AND LATERAL GENICULATE NEURONES TO 5-HYDROXYTRYPTAMINE IN THE RAT

BY R. MASON

*From the * Department of Physiology and Pharmacology, Medical School, Queens Medical Centre, Clifton Boulevard, Nottingham NG7 2UH and Electropharmacology Laboratory, Department of Pharmacy, University of Nottingham*

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SUMMARY

1. Extracellular single-unit recordings were obtained from neurones in the suprachiasmatic nuclei (s.c.n.) of the rat (a putative circadian pace-maker), the ventral lateral geniculate nucleus (v.l.g.n.) and the hippocampus. These areas receive a 5-hydroxytryptamine (5-HT) innervation from the raphe nuclei. Recording of neuronal activity in the s.c.n., v.l.g.n. and the hippocampus revealed a diurnal variation in the response to the ionophoresis of 5-HT. This variation was manifest as a 2–3-fold increase in post-synaptic sensitivity to 5-HT during the subjective dark (active) phase of the circadian cycle. In contrast there was no apparent circadian variation in the sensitivity of s.c.n., v.l.g.n. or hippocampal neurones to ionophoresed γ-aminobutyric acid (GABA).

2. Neuronal activity recorded in the s.c.n., v.l.g.n. and hippocampus also exhibited a circadian variation in the recovery from 5-HT-induced suppression of firing. This may reflect reuptake processes as recovery can be prolonged by ionophoresis of uptake blockers (imipramine or fluoxetine).

3. Rats ($n = 15$) expressing circadian arrhythmicity in their rest–activity behaviour induced by long-term continuous illumination (150–200 lx) showed no apparent circadian variation in 5-HT sensitivity. This loss was accompanied by either the development of (i) a 5–6-fold subsensitivity to ionophoresed 5-HT (eleven out of fifteen rats) or (ii) a 2–3-fold supersensitivity to ionophoresed 5-HT (four out of fifteen rats).

4. A similar loss of circadian variation and the development of a subsensitivity to ionophoresed 5-HT was also found in three rats sustaining complete electrolytic lesions of the s.c.n. These changes were not found in rats ($n = 4$) with partial s.c.n. lesions.

5. These results implicate the s.c.n., or fibres passing through it, in the circadian modulation of 5-HT sensitivity in neurones both intrinsic to the s.c.n. circadian pace-maker itself and in the hippocampus and lateral geniculate nucleus (regions remote from the s.c.n.).

* Address for correspondence.

INTRODUCTION

A circadian variation in the post-synaptic sensitivity of hippocampal pyramidal cells to ionophoresed 5-hydroxytryptamine (5-HT) has been reported in the rat (Brunel & de Montigny, 1980). This circadian variation may be the result of biochemical events intrinsic to the neurones or alternatively a consequence of drive from a central circadian pace-maker.

The present study was undertaken to investigate the responsiveness of neurones in the suprachiasmatic nuclei of the hypothalamus (s.c.n.), the ventral division of the lateral geniculate nucleus (v.l.g.n.) and the hippocampus to ionophoresed 5-HT throughout the circadian cycle and secondly to examine the significance of the s.c.n. for the reported circadian variation in the sensitivity of hippocampal pyramidal cells to 5-HT (Brunel & de Montigny, 1980). The s.c.n was selected as a recording site as there is compelling evidence that the s.c.n. are central structures for the regulation of circadian rhythmicity in behavioural, endocrine and physiological processes (Rusak & Zucker, 1979; Groos, Mason & Meijer, 1983). The s.c.n. are densely innervated by a serotonergic projection from the raphe nuclei (Aghajanian, Bloom & Sheard, 1969; Ajika & Ochi, 1978; Van de Kar & Lorens, 1979; Pickard, 1982). The v.l.g.n. was included as a representative site of an extra-hypothalamic region in receipt of serotonergic innervation and additionally because of its possible involvement in s.c.n. physiology (Rusak & Zucker, 1979; Groos & Mason, 1980, 1982). A division within the v.l.g.n., the intergeniculate leaflet (i.g.l.), receives a direct retinal projection and in turn projects to the s.c.n. (Groos & Mason, 1978; Card & Moore, 1982; Groos & Rusak, 1982; Pickard, 1985). This geniculo-suprachiasmatic projection is associated with immunoreactivity to neuropeptide-Y (Card & Moore, 1982; Card, Brecha & Moore, 1983). Local infusion of neuropeptide-Y (NYP) into the s.c.n. (Albers & Ferris, 1984) or electrical stimulation of the v.l.g.n. (Meijer, Groos & Rusak, 1984; J. H. Meijer, personal communication) alters the phase of free-running circadian rhythms, suggesting that this pathway may be important in the entrainment of circadian rhythms to the light–dark cycle. Some recordings from the hippocampus were used for comparison with Brunel & de Montigny's report (1980) of diurnal variation to ionophoresed 5-HT. A preliminary report of some of these experiments has been presented to the Physiological Society (Mason, 1984).

METHODS

Male Wistar rats were divided into four experimental groups. One group of rats were kept on a 12 h light: 12 h dark (lights on 07.00 h g.m.t.; $n = 30$) or on a reversed 12 h light: 12 h dark (lights on 19.00 h g.m.t.; $n = 12$) lighting schedule. A second group of rats ($n = 7$) were anaesthetized with 'Hypnorm' (Duphar–Phillips: fluanisone–fentanyl, 1 mg/kg I.M.) and bilateral electrolytic lesions directed at the s.c.n. (using adjusted coordinates according to Pelligrino, Pelligrino & Cushman (1979): A 7·2, L 0·8, V 9·2 mm at an angle of 5 deg to the vertical) using a constant anodal current (0·75 mA) applied for 15–20 s once on each side of the brain. These rats were allowed to recover in a 12 h light: 12 h dark environment and monitored for the development of arrhythmicity in their rest–activity behaviour using capacitance measurement with an 'Animex' monitor (LKB, Sweden). A third group of rats ($n = 6$) was kept on a 12 h: 12 h light–dark cycle and treated daily with *para*-chlorophenylalanine (*p*CPA methyl ester HCl: 300 mg/kg I.P., over 3–7 days). A fourth group of rats ($n = 20$) was kept under constant illumination (150–200 lx at the cage floor) for periods of 6–8 weeks. All of these groups were subsequently used for electrophysiological recording.

Rats were anaesthetized with urethane (1·3–1·5 g/kg, I.P.) and mounted in a stereotaxic frame. Electrophysiological experiments were made throughout the light–dark cycle to allow for any diurnal variation in anaesthetic efficacy. Craniotomies were made appropriate for electrode penetrations into the hippocampus, the v.l.g.n. and/or the s.c.n. (Pelligrino *et al.* 1979). Five-barrel micropipettes, tip diameter 4 μm, were used for recording and ionophoresis. The centre barrel, filled with 5 % Pontamine Sky Blue in 0·5 M-NaCl, was used for extracellular recordings. Outer drug barrels contained: 5-HT creatinine sulphate (20 mM, pH 4·0), γ-aminobutyric acid (GABA: 20 mM, pH 4·0) and sodium glutamate (100 mM, pH 8·5) or acetylcholine chloride (20 mM, pH 4·0). The remaining outer barrel contained 4 M-NaCl and was used for automatic current balancing. The position of the electrodes at one or two recording sites in each penetration was marked by ionophoretic ejection of Pontamine Sky Blue. Action potentials were amplified and filtered (bandpass 100 Hz–10 kHz), single units discriminated and standard pulses fed into an electronic counter and plotted as integrated firing rate histograms, accumulated over successive 2 or 5 s epochs, on a potentiometric recorder (Servoscribe, Smiths Industries, U.K.) and on a counter-printer (Digitec 6100, U.S.A.).

Suprachiasmatic and geniculate neurones were identified according to their response to visual stimuli (Hale & Sefton, 1978; Groos & Mason, 1980; Meijer *et al.* 1984). The responsiveness of neurones to 5-HT was estimated from the ionophoretic charge (i.e. the product of the ejecting current I (nA) and the time T (s), units: nanocoulombs (nC)) required to obtain a 50 % decrease in the firing rate (IT_{50}) (de Montigny & Aghajanian, 1978; Wang, de Montigny, Gold, Roth & Aghajanian, 1979). Ionophoresis of 5-HT and GABA were standardized as 30 s duration ejections repeated every 3–5 min. The recovery time, i.e. the period required by the neurone to recover by 50 % from termination of the ionophoretic ejection (RT_{50}), was used as an index of the efficacy of the transmitter reuptake process (Wang *et al.* 1979). To avoid electrode bias, when possible, the same electrode was used for recording–ionophoresis from the hippocampus, v.l.g.n. and s.c.n. in the same animal, or the same electrode used for recording–ionophoresis in different animals.

Recording sites and the extent of any electrolytic lesions were verified histologically. After completion of a recording session the rats were deeply anaesthetized and perfused with 10 % buffered formaldehyde. Their brains were removed and cut on the following day into 50 μm transverse sections on a freezing microtome, then stained with cresyl violet or neutral red. The location of the Pontamine Sky Blue spots was correlated with the micro-electrode readings made during the recording and used to reconstruct the positions of cells along the electrode penetrations. Only electrophysiological data from cells recorded within the histological boundaries of the s.c.n., v.l.g.n. and hippocampus were used in this report. The extent of the electrolytic lesions of the s.c.n. was also verified in 50 μm frozen sections stained with cresyl violet and only minor damage to the surrounding neuropil was evident in those s.c.n. lesioned rats used for this study. 5-hydroxyindoleamine levels in the s.c.n. were measured by the method of high-pressure liquid chromatography (h.p.l.c.), with electrochemical detection (Lighton, Marsden & Mason, 1982; Mason & Beeston, 1984).

RESULTS

The response of the majority of neurones recorded in the s.c.n., v.l.g.n. and hippocampus sensitive to ionophoresed 5-HT was a suppression of discharge (Groos & Mason, 1982; Mason & Meijer, 1982). At low ejection currents (< 10 nA) the uptake blockers imipramine or fluoxetine prolonged the suppression of firing induced by ionophoresed 5-HT (RT_{50} v.l.g.n.: (control) $21{\cdot}5 \pm 4{\cdot}0$ s; (imipramine) $57{\cdot}8 \pm 16{\cdot}5$ s, $n = 10$ cells, $P < 0{\cdot}001$ paired t test). This prolongation of the RT_{50} index is consistent with the recovery process being at least partially dependent on neuronal uptake of 5-HT (Wang *et al.* 1979). A detailed description of the pharmacology of s.c.n. neurones will be reported elsewhere.

A marked increase in post-synaptic 5-HT responsiveness (indicated by a reduced IT_{50} value) was found for neurones recorded in the v.l.g.n. (Fig. 1; Table 1), hippocampus (Table 1) and s.c.n. (Fig. 2; Table 1) during the subjective active (dark) period when compared with the resting (light) period (Figs. 1 and 2). In contrast, no

significant circadian variation was found in the post-synaptic sensitivity to ionophoresed GABA. This is illustrated in Fig. 1 for a v.l.g.n. cell recorded continuously over 16 h where a higher mean ejecting current for 5-HT (and correspondingly an elevated IT_{50} value) was required during the subjective light phase as compared to the dark phase of the circadian cycle to induce a similar suppression of discharge. The response to GABA remained similar during both the light and dark phases of the circadian cycle. This circadian variation in sensitivity to ionophoresed 5-HT was also found for neurones recorded in the s.c.n. and hippocampus.

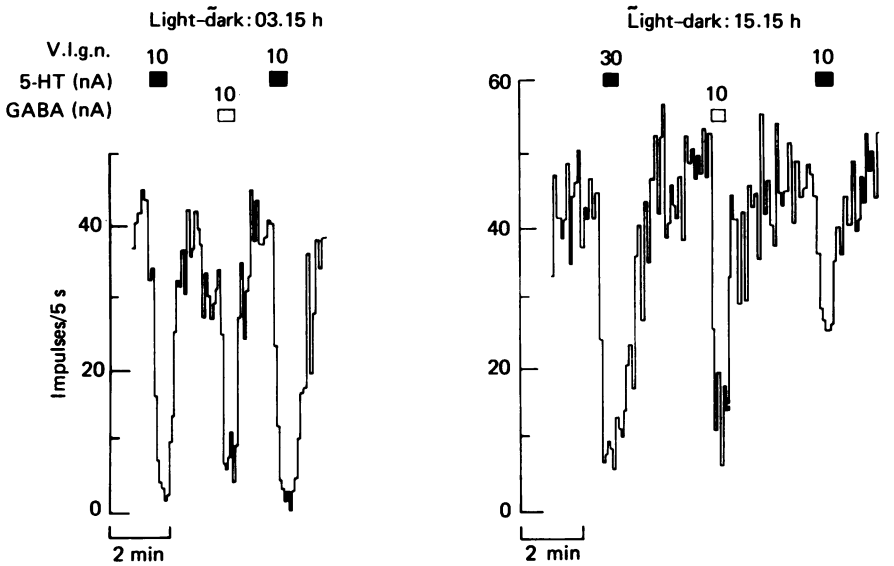

Fig. 1. Integrated firing rate histograms accumulated over successive 5 s epochs for the same v.l.g.n. neurone driven by 1 s duration diffuse light flashes presented every 2·5 s to the contralateral eye. This cell was recorded continuously over a period of 16 h. The left and right Figures illustrate recordings during the subjective active (dark) and resting (light) phases, respectively, of the circadian cycle of a rat kept on a 12 h:12 h light–dark lighting regime. The computed IT_{50} product shows a high responsiveness (IT_{50} product: 265 nC) to ionophoresed 5-HT during the active (dark) phase and a low sensitivity (IT_{50} product: 585 nC) during the resting phase. Recovery from 5-HT-induced suppression was enhanced during the active (dark) phase, as indexed by RT_{50} measurement (dark: 25 s; light: 36 s). The ~ superimposed over the words 'light' or 'dark' in the Figure indicates the subjective time of recording during the light or dark phase.

The distribution of post-synaptic sensitivity of 166 s.c.n. cells to ionophoresed 5-HT is plotted against the extrapolated subjective light or dark period in Fig. 2. Such a plot reveals the circadian variation in sensitivity, which is higher during the subjective dark period.

There also appears to be a circadian variation in the recovery from 5-HT-induced suppressions of firing in both the s.c.n. and v.l.g.n. A prolonged recovery was found during the subjective light phase for recordings from v.l.g.n. neurones (v.l.g.n. RT_{50}: $35{\cdot}8 \pm 13{\cdot}3$ s, $n = 60$ cells; mean $\pm$ S.E. of mean) and s.c.n. neurones (s.c.n. RT_{50}:

28·3 ± 14·1 s, $n = 46$). This contrasted with the enhanced recovery found during the subjective dark phase for recordings from the v.l.g.n. (v.l.g.n. RT_{50}: 24·6 ± 8·9 s, $n = 55$ cells) and s.c.n. (s.c.n. RT_{50}: 17·9 ± 7·5 s, $n = 34$). The circadian variation in recovery appears to parallel the variation in 5-HT sensitivity, i.e. the development of enhanced recovery begins during the last 2–3 h of the light phase and wanes during the latter half of the dark phase of the circadian cycle.

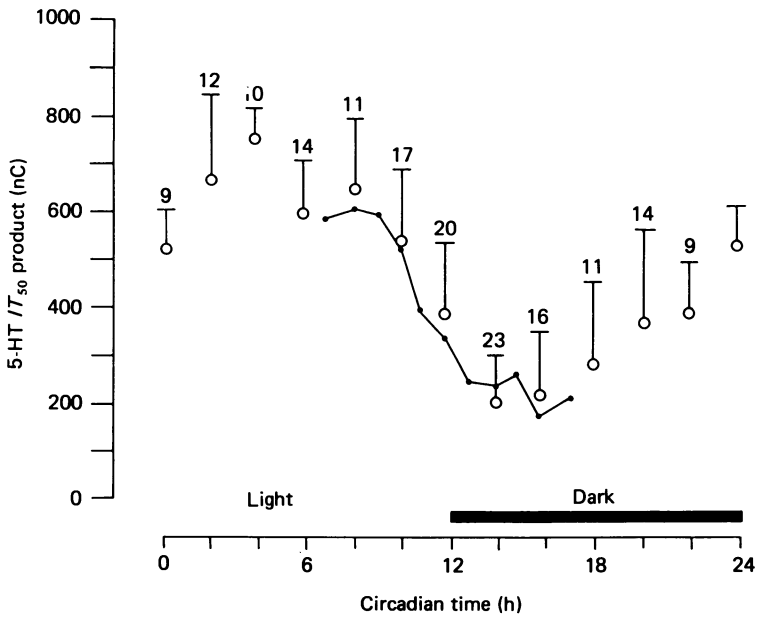

Fig. 2. Distribution of the post-synaptic sensitivity of s.c.n. neurones ($n = 166$) to ionophoresed 5-HT, indexed as IT_{50} products, recorded during the extrapolated subjective resting (light) and active (dark) phases of the rats' circadian cycle. The IT_{50} products are the mean of determinations pooled over 2 h periods during recording sessions. Individual s.c.n. units were recorded over a minimum period of 15 min and up to 11 h duration, on average 1–2 h. The number of cells recorded at each point are indicated above the standard error bars. Open circles: mean 5-HT IT_{50} product ± S.E. of mean. Continuous line: the mean hourly 5-HT IT_{50} products for the longest duration recording (11 h) from a single s.c.n. neurone.

Rats receiving electrolytic lesions directed at the s.c.n. exhibited either normal circadian rest–activity behaviour ($n = 4$) or arrhythmia ($n = 3$), similar to that shown in Fig. 3 for light-induced arrhythmia. Following electrophysiological recording, histological analysis revealed that only those animals showing arrhythmic behaviour had lesions which completely destroyed the s.c.n. It was in these animals that an absence of a circadian variation in both post-synaptic 5-HT sensitivity and in recovery from 5-HT suppressions were found in hippocampal and v.l.g.n. cells. This absence was accompanied by a 3–4-fold decrease in 5-HT sensitivity (Table 1). This decrease in responsiveness, reflected as an elevated mean 5-HT IT_{50} product (Table 1), was mostly attributable to higher threshold ejection currents required to

elicit suppression of neuronal firing. In two animals sustaining only partial lesions to the s.c.n. a circadian variation and a mean sensitivity to 5-HT was found similar to that of control animals (Table 1).

TABLE 1. The mean IT_{50} products (nC) of histologically identified s.c.n., hippocampal and v.l.g.n. neurones to ionophoresed 5-HT recorded during the subjective resting (light) and active (dark) periods of the circadian cycle in rats kept on a 12 h:12 h light–dark photoperiod. Neuronal sample shown in parentheses. Rats with incomplete s.c.n. lesions showed a circadian rhythm in locomotor activity whereas those sustaining complete lesions of the s.c.n. exhibited arrhythmicity

	5-HT IT_{50} (nC ± S.E. of mean)			
	(Light) Resting phase		(Dark) Active phase	
12 h light:12 h dark				
S.c.n.	653 ± 56	(93)	298 ± 74	(73)***
V.l.g.n.	350 ± 38	(34)	186 ± 42	(21)**
Hipppocampus	290 ± 66	(105)	134 ± 47	(74)*
S.c.n. lesion, incomplete				
V.l.g.n.	377 ± 43	(27)	189 ± 49	(19)
Hippocampus	352 ± 61	(22)	175 ± 56	(16)
Chronic *p*CPA				
S.c.n.	682 ± 82	(22)	326 ± 68	(17)
Continuous illumination				
S.c.n. (subsensitive group)	804 ± 77	(33) light†, dark‡		
S.c.n. (supersensitive group)	178 ± 67	(14) light†		
V.l.g.n.	571 ± 73	(53) light†, dark‡		
Hippocampus	397 ± 51	(51) dark‡		
S.c.n. lesion, complete				
V.l.g.n.	601 ± 70	(24) light†, dark‡		
Hippocampus	367 ± 53	(21) dark‡		

Values are mean + S.E. of mean. * $P < 0.1$, ** $P < 0.01$, *** $P < 0.001$, with respect to the light (resting) phase of the circadian cycle. † $P < 0.01$, ‡ $P < 0.001$, with respect to the light or dark phase of the circadian cycle (Student's t test).

Following exposure to long-term (6–8 weeks) continuous illumination rats either showed arrhythmicity in their rest–activity behaviour (fifteen out of twenty-two rats, see Fig. 3), similar to that found for s.c.n. lesioned rats, or a free-running rhythm in rest–activity behaviour ($n = 7$). The development of arrhythmicity under conditions of continuous illumination has been reported by others (Pittendrigh, 1974; Pittendrigh & Daan, 1976; Rusak, 1977).

In rats of the light-exposed group, expressing arrhythmicity, recordings of s.c.n., v.l.g.n. and hippocampal neuronal activity showed no evidence of a circadian variation in post-synaptic sensitivity or in recovery from 5-HT ionophoresis. Cells recorded in the s.c.n., v.l.g.n. and hippocampus from the majority of arrhythmic animals (eleven out of fifteen rats) also showed a pronounced subsensitivity to ionophoresed 5-HT (Fig. 4, Table 1) and to ionophoresed GABA (Fig. 4, Table 2). However, recordings from the s.c.n. in four arrhythmic animals showed the development of a supersensitivity to ionophoresed 5-HT (Table 1). Similar light-

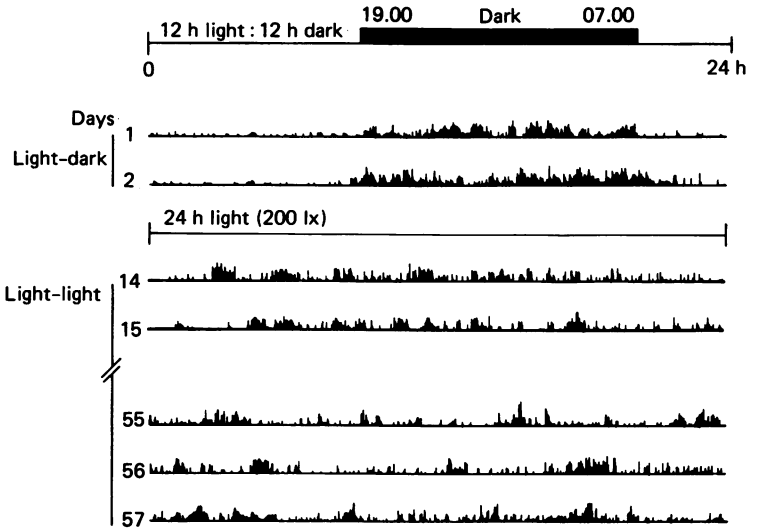

Fig. 3. Rest–activity actograms for the long-term light-exposed rat from which the v.l.g.n. neurone (*B*) shown in Fig. 4 was recorded. This was monitored using an LKB 'Animex' capacitance instrument and plotted directly on to a *Y–T* recorder. Note the arrhythmicity in rest–activity after a period of 5 weeks exposure to constant illumination compared with actograms recorded earlier under 12 h light:12 h dark conditions for the same rat.

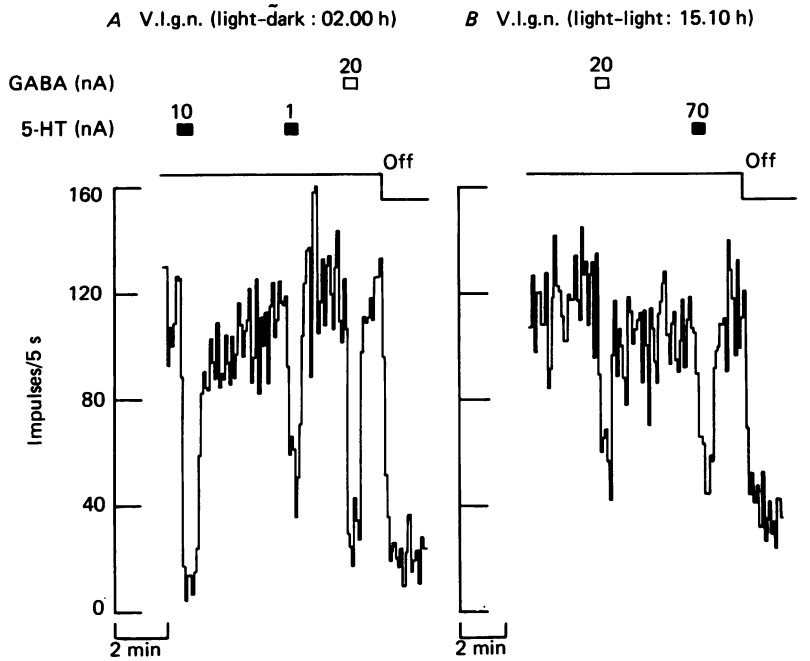

Fig. 4. Integrated firing rate histograms for a v.l.g.n. neurone recorded from a rat exposed to a 12 h light:12 h dark cycle (*A*) and a v.l.g.n. neurone recorded from a rat exposed to continuous illumination for 8 weeks at 200 lx (*B*). The step function in the Figure indicates the period during which visual stimulation, comprising a 1 s duration whole visual field diffuse flash repeated every 2 s, was presented.

induced subsensitivity or supersensitivity to 5-HT, but not GABA, have also been observed in the hippocampus of Long–Evans pigmented rats (C. M. Cox & R. Mason, unpublished observations).

Neurones in both the v.l.g.n. and s.c.n. of light-exposed rats still responded to visual stimulation. This is illustrated by the presence of the visually evoked discharge in v.l.g.n. neurones recorded from a 12 h light:12 h dark-exposed rat (Fig. 4*A*) and from a continuous light-exposed rat (Fig. 4*B*).

TABLE 2. The mean IT_{50} products (nC) of histologically identified s.c.n., v.l.g.n. and hippocampal neurones to ionophoresed GABA recorded during the subjective resting (light) and active (dark) periods of the circadian cycle in rats kept on a 12 h light:12 h dark photoperiod and subjected to continuous illumination at 150–200 lx over 8 weeks. Neuronal sample shown in parentheses

	GABA IT_{50} (nC ± S.E. of mean)			
	(Light) Resting phase		(Dark) Active phase	
12 h light:12 h dark				
S.c.n.	26 ± 5	(40)	35 ± 7	(40) n.s.
V.l.g.n.	21 ± 8	(30)	36 ± 9	(25) n.s.
Hippocampus	58 ± 7	(33)	63 ± 6	(29) n.s.
Continuous illumination				
S.c.n.	95 ± 25	(20) light***, dark**		
V.l.g.n.	105 ± 25	(30) light**, dark*		

Values are mean + S.E. of mean. N.s., not significant with respect to the light (resting) phase of the circadian cycle. * $P < 0.02$, ** $P < 0.01$, *** $P < 0.001$, with respect to the light or dark phase of the circadian cycle (Student's t test).

Depletion of 5-HT using *p*CPA was attempted in order to ascertain the effects of reduced 5-HT levels in the s.c.n. on the circadian variation in 5-HT sensitivity. The group of rats administered *p*CPA for 3–7 days, showed a depletion of 5-HT levels in the s.c.n. by 90–95 % (control 5-HT levels: 28·8 ± 7·3 pmol/mg protein, $n = 6$; *p*CPA treated: 1·7 ± 1·4 pmol/mg protein, ± S.E. of mean, $n = 6$).

*p*CPA induced only a transient disruption of the circadian rest–activity behaviour for 1–3 days, and following recovery, an increase in activity during the light period of the circadian cycle similar to that reported by others (Borbely, Huston & Wise, 1973). The sensitivity to ionophoresed 5-HT in this 5-HT-depleted group showed a normal circadian variation in 5-HT sensitivity and no evidence of any subsensitivity.

DISCUSSION

The circadian variation in sensitivity of central neurones to ionophoresed 5-HT supports and extends Brunel & de Montigny's observation (1980) of a similar diurnal variation in responsiveness to 5-HT for hippocampal neurones. Their report of a circadian variation appeared selective for 5-HT as no marked variation was found for noradrenaline, although GABA sensitivity was reported higher during the subjective light phase for the hippocampus (Brunel & de Montigny, 1980). In the present study while no significant circadian variation in GABA sensitivity was found

in the s.c.n., v.l.g.n. or hippocampus (Table 2), there was a tendency for higher IT_{50} values to be recorded in the s.c.n. and v.l.g.n. during the light phase. A subsensitivity to ionophoresed GABA was also noted in the continuous light exposure group of rats which showed a subsensitivity to 5-HT (Table 2). A circadian variation in a 5-HT receptor-mediated behavioural response induced by the 5-HT agonist 5-methoxy-*N*, *N*-dimethyltryptamine has been reported (Moser & Redfern, 1984); this presumably reflects a diurnal rhythm in central 5-HT receptor function.

The enhanced recovery from 5-HT-induced suppression of neuronal firing, as indexed by a smaller RT_{50} value, peaks near the onset of subjective darkness, with the minimum recovery occurring near the onset of the light phase. This recovery (RT_{50}) from 5-HT-induced suppression of firing is believed to be largely due to reuptake processes (Wang *et al.* 1979) and the circadian variation in recovery presently found for s.c.n. neurones follows closely the circadian variation in 5-HT uptake found biochemically in the s.c.n. (Meyer & Quay, 1976).

These observed circadian variations in both 5-HT sensitivity (IT_{50}) and in recovery (RT_{50}) found for s.c.n. neurones in the present study parallels other physiological parameters associated with s.c.n. function which show a relationship to the light–dark cycle. 5-hydroxyindoleamine levels peak at the beginning of the active phase of the circadian rest–activity cycle (Lighton *et al.* 1982; Faradji, Cespuglio & Jouvet, 1983). Increased activity in 5-HT uptake has been reported in male rats during the onset of the active phase of the circadian cycle (Meyer & Quay, 1976). Similarly there is a peak in imipramine binding to the 5-HT uptake system at this time (Wirz-Justice, Krauchi, Morimasa, Willener & Feer, 1983). These biochemical changes are reflected in electrophysiological observations found for s.c.n. neurones, notably a reduced mean discharge rate (Groos & Hendricks, 1982; Green & Gillette, 1982; Shibata, Oomura, Kita & Hattori, 1982). Concomitant with the lower firing rate is a decrease in glucose utilization (Schwartz, Davidsen & Smith, 1980) in the s.c.n. during the active phase. The circadian variation in sensitivity to ionophoresed 5-HT is also paralleled by a circadian rhythm in adenosine 3′,5′-monophosphate levels in the s.c.n. (Murakami & Takahashi, 1983). Interestingly, these authors also noted a loss of this circadian rhythm in the cyclic nucleotide in rats exposed to continuous light. Whether the effects of 5-HT on s.c.n. neuronal firing are mediated through activation of an adenylate cyclase is at present unknown.

Long-term light exposure results in retinal degeneration accompanied by a loss of visually evoked potentials in the l.g.n. and visual cortex in albino, but not pigmented, strains of rats (O'Steen & Anderson, 1971). In the present investigation visually driven neurones were recorded in the s.c.n., v.l.g.n. and d.l.g.n. of rats subjected to continuous light exposure (150–200 lx), implying some normal retinal function. Indeed, these rats exhibited arrhythmia in rest–activity behaviour, whereas a complete loss of retinal function would be expected to lead to a free-running rhythm as found in enucleated animals (Rusak & Zucker, 1979).

In an electron microscope study Guldner & Ingham (1979) have shown that 14 days of exposure to constant light resulted in a loss of post-synaptic density material and an increase in the relative number of 'symmetrical' (? inhibitory) synaptic appositions in the s.c.n. These structural changes may represent a morphological correlate of the present electrophysiological finding of altered post-synaptic sensitivity found in

light-exposed rats. Further evidence in support of a loss of s.c.n. circadian function following exposure to continuous light is the loss of the circadian rhythm in glucose utilization by the s.c.n. (Schwartz *et al.* 1980) and the absence of the circadian rhythm in s.c.n. neuronal discharge activity (Shibata, Liou, Ueki & Oomura, 1984).

The absence of a diurnal variation in 5-HT responsiveness in rats with completely ablated s.c.n. suggests that the functional integrity of the s.c.n. is essential for this circadian variation in post-synaptic 5-HT sensitivity observed in hippocampal and v.l.g.n. neurones. The sensitivity of neurones within the s.c.n. itself to ionophoresed 5-HT also appears dependent on the functional state of the s.c.n. as a circadian pace-maker as the circadian variation in 5-HT responsiveness is absent in the s.c.n. of rats exhibiting a disruption of circadian rest–activity following exposure to continuous illumination. In addition, the development of subsensitivity to 5-HT following s.c.n. lesions and the 5-HT sensitivity changes following exposure to continuous illumination provides further evidence for the importance of the s.c.n. in the modulation of 5-HT sensitivity. Why continuous light exposure should result in the development of either subsensitivity or supersensitivity to ionophoresed 5-HT in individual arrhythmic animals is puzzling at present and is under further investigation.

Rats pre-treated with *p*CPA exhibited a transient disruption in their circadian rest–activity behaviour concomitant with reduced 5-HT levels in the s.c.n. (Groos *et al.* 1983; Mason & Beeston, 1984; Meyer & Quay, 1976) and recording sessions conducted during this period of transient disruption in circadian behaviour revealed no change in the diurnal variation in their sensitivity and recovery to ionophoresed 5-HT. The absence of altered sensitivity to 5-HT following treatment with *p*CPA has been reported for other forebrain regions (Wang *et al.* 1979; Ferron, Reader & Descarries, 1981). This finding may be associated with the demonstration that *p*CPA depletes 5-HT levels while retaining an intact 5-HT innervation, whereas the use of the specific neurotoxin 5,7-dihydroxytryptamine (5,7-DHT), which produces a similar degree of 5-HT depletion as *p*CPA, causes degeneration of 5-HT terminals and subsequently the development of supersensitivity to 5-HT (Wang *et al.* 1979; Ferron *et al.* 1981). It has been suggested that a factor co-exists with 5-HT in serotonergic terminals which modulates the characteristics of the 5-HT receptor (Ogren, Fuxe, Agnati & Celani, 1985); this factor would be lost following nerve terminal degeneration induced by 5,7-DHT.

The mechanism(s) by which the s.c.n. mediate this circadian modulation in 5-HT sensitivity of neurones both intrinsic to the s.c.n. circadian pace-maker and at extra-suprachiasmatic serotonergic terminal zones remains to be determined. A role for the raphe nuclei in this modulation is difficult to reconcile with reports that raphe lesions or transection of the raphe–s.c.n. projection has little effect on either entrained or free-running rhythms (see references in Groos *et al.* 1983). An altered diurnal sensitivity to 5-HT was only demonstrable in the present study following experimental manipulations which, through presumed actions on the s.c.n., resulted in prolonged disruption of circadian behaviour, i.e. exposure to continuous illumination or following s.c.n. lesions. The circadian variation in 5-HT sensitivity might, however, be explained via a s.c.n.-mediated circadian drive of raphe function. Recordings of raphe discharge activity in freely moving animals show an elevated discharge during

wakefulness and reduced activity during sleep (Trulson & Jacobs, 1983). Thus during the light (sleeping) phase of the circadian cycle raphe discharge and hence 5-HT release will be lower than during the dark (active) phase. This reduced 5-HT transmission might be expected to lead to an adaptive up-regulation in post-synaptic 5-HT sensitivity. Support for this notion is that the s.c.n. regulates circadian sleep–wake rhythms (Ibuka, Inouye & Kawamura, 1977; Rusak & Zucker, 1979; Groos, 1983). S.c.n. lesions disrupt the sleep–wake cycle and thus presumably raphe neuronal discharge patterns associated with sleep–wakefulness. This disruption of raphe activity would in turn not allow for the circadian adaptive changes in post-synaptic 5-HT sensitivity to develop. Indeed, it can be predicted also to lead to a down-regulation of 5-HT sensitivity, as found in the present report. Continuous light exposure, leading to rest–activity dysrhythmia, is similarly explained by this model. Alternatively, variations in sensitivity to 5-HT may be mediated via an endogenous 'neuromodulator' or hormonal system, which in turn is under the influence of circadian drive from the s.c.n.

The present experimental observations of a circadian modulation of sensitivity to 5-HT and of reuptake processes, coupled with the demonstration that imposed disturbances on the circadian system (s.c.n.) cause dysrhythmia in circadian rest–activity behaviour and altered neurotransmission (as observed by the development of subsensitivity or supersensitivity to 5-HT and a loss of the diurnal variation in 5-HT responsiveness) may have significance for the notion implicating the circadian system in the aetiology of manic-depressive disorders (Wehr, Sack, Rosenthal, Duncan & Gillin, 1983). Interestingly, chronic antidepressant administration induces supersensitivity to ionophoresed 5-HT in the forebrain, including the s.c.n. (de Montigny & Aghajanian, 1978; Mason & Meijer, 1982) and alters circadian behaviour (Hill, Mason & Reffin, 1985). Should the circadian system contribute to the pathophysiology of manic-depressive illness then it would be of interest to see if manic-depressive patients showed an altered circadian variation and sensitivity to 5-HT, and whether this was modified by antidepressant treatments.

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