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# INVOLVEMENT OF THE RETINOHYPOTHALAMIC TRACT IN THE PHOTIC-LIKE EFFECTS OF THE SEROTONIN AGONIST QUIPAZINE IN THE RAT

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**Abstract—Light is the major synchronizer of the mammalian circadian pacemaker located in the suprachiasmatic nucleus. Photic information is perceived by the retina and conveyed to the suprachiasmatic nucleus either directly by the retinohypothalamic tract or indirectly by the intergeniculate leaflet and the geniculohypothalamic tract. In addition, serotonin has been shown to affect the suprachiasmatic nucleus by both direct and indirect serotonin projections from the raphe nuclei. Indeed, systemic as well as local administrations of the serotonin agonist quipazine in the region of the suprachiasmatic nucleus mimic the effects of light on the circadian system of rats, i.e. they induce phase-advances of the locomotor activity rhythm as well as c-FOS expression in the suprachiasmatic nucleus during late subjective night. The aim of this study was to localize the site(s) of action mediating those effects. Phase shifts of the locomotor activity rhythm as well as c-FOS expression in the suprachiasmatic nucleus after s.c. injection of quipazine (10 mg/kg) were assessed in Lewis rats, which had received either radio-frequency lesions of the intergeniculate leaflet or infusions of the serotonin neurotoxin 5,7-dihydroxytryptamine into the suprachiasmatic nucleus (25 μg) or bilateral enucleation. Lesions of intergeniculate leaflet and serotonin afferents to the suprachiasmatic nucleus did not reduce the photic-like effects of quipazine, whereas bilateral enucleation and the subsequent degeneration of the retinohypothalamic tract abolished both the phase-shifting and the FOS-inducing effects of quipazine. The results indicate that photic-like effects of quipazine are mediated via the retinohypothalamic tract. © 2005 Published by Elsevier Ltd on behalf of IBRO.**

**Key words: suprachiasmatic nucleus, 5-HT, afferences, retinohypothalamic tract, locomotor activity, c-FOS.**

In mammals, the suprachiasmatic nuclei (SCN) of the hypothalamus contain the major pacemaker, which generates and regulates circadian rhythms (Moore, 1983; Morin, 1994). The most effective synchronizer of the circadian clock in the SCN is the light/dark (LD) cycle that resets the pacemaker every day (Pittendrigh and Daan, 1976). However, the circadian system can also be reset and entrained by various non-photic stimuli such as access to a running wheel (Mrosovsky, 1989) or arousal due to daily injections of triazolam, a benzodiazepine (Van Reeth and Turek, 1989).

According to the current state of knowledge (Moga and Moore, 1997; Morin, 1999), there are three major inputs to the SCN: (i) the retinohypothalamic tract (RHT), a direct projection from the retina to the SCN (Moore and Lenn, 1972), which releases glutamate (Ebling, 1996) and pituitary adenylate cyclase-activating polypeptide (PACAP) (Hannibal et al., 1997) as neurotransmitters, (ii) the geniculohypothalamic tract (GHT), an indirect projection originating in the retinorecipient intergeniculate leaflet (IGL) (Card and Moore, 1989), which releases neuropeptide Y (NPY) and GABA (Moore and Speh, 1993) and (iii) a dense serotonergic input both directly from the median raphe nucleus (MRN) to the SCN and indirectly from the dorsal raphe nucleus (DRN) to the IGL (Azmitia and Segal, 1978; Moga and Moore, 1997; Hay-Schmidt et al., 2003). Furthermore, a tract connecting the retina with the DRN has been demonstrated in rats (Kawano et al., 1996). Although this pathway has not been detected in golden hamster (Morin, 1994), it indicates that, in the rat, photic information reaches the DRN and consequently may affect the circadian clock through the serotonergic inputs to the SCN and/or the IGL.

Data obtained in hamsters and rats indicate that the role of the serotonin (5-HT) system is species dependent (Kohler et al., 2000). In hamsters, for example, systemic injections of 5-HT agonists such as 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT, a 5-$HT_{1A/7}$ agonist) induce phase advances during the subjective day (Tominaga et al., 1992; Cutrera et al., 1996). Furthermore, phase advances induced by triazolam injections and

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*Abbreviations:* ANOVA, analysis of variance; CT, circadian time; DD, constant darkness; DLG, dorsal lateral geniculate; DOI, (±)-1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane; DRN, dorsal raphe nucleus; GHT, geniculohypothalamic tract; IGL, intergeniculate leaflet; IGL-$X_P$, partially IGL-lesioned; IGL-$X_W$, wholly IGL-lesioned; ir, immunoreactive; LD, light/dark; mCPP, 1-(3-chlorophenyl)-piperazine HCl; MRN, median raphe nucleus; NAN-190, 1-(2-methoxyphenyl)-4(4-(2-phtalimido)butyl)piperazine; NMDA, N-methyl D-aspartate; NPY, neuropeptide Y; OX, optic chiasm; PACAP, pituitary adenylate cyclase-activating polypeptide; PBS, phosphate-buffered saline; PBST, phosphate-buffered saline containing 0.1% Triton X-100; RHT, retinohypothalamic tract; RO 60-0175, (s)-2-(6-chloro-5fluoroindol-1-yl)-1-methylethylamine; SB-242084, (6-chloro-5-methyl-1-[[2-methylpyrid-3-yloxy)pyrid-5-yl]carbamoyl]indoline; SCN, suprachiasmatic nucleus; TFMPP, *N*-(3-trifluoromethylphenyl)-piperazine HCl; VGL, ventral lateral geniculate; WAY 100635, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl-cyclohexanecarboxamine; 3V, third ventricle; 5-HT, serotonin; 5-HT-$X_P$, partially 5-HT lesioned; 5-HT-$X_W$, wholly 5-HT lesioned; 5,7-DHT, 5,7-dihydroxytryptamine; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin.

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arousal due to saline injections, but not novel-wheel running, are reduced by depletion or blockade of serotonergic transmission (Meyer-Bernstein and Morin, 1998). These results demonstrate the involvement of the 5-HT system in the phase resetting effects of non-photic stimuli observed during subjective day. Moreover, during subjective night, 5-HT has been reported to reduce photic effects on the hamster circadian system, including light-induced phase shifts of locomotor activity rhythm (Rea et al., 1994), increase in cellular firing (Ying and Rusak, 1994) and stimulation of c-FOS protein immunoreactivity (ir) in SCN cells (Selim et al., 1993).

In rats, on the other hand, 5-HT enhances photic responses. Systemic injections of 5-HT agonists such as quipazine or the more specific 5-$HT_{2C}$ agonists (±)-1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane (DOI) and 1-(3-chlorophenyl)-piperazine HCl (mCPP) have been shown to mimic the phase-shifting effects of light on the circadian rhythms of locomotor activity (Kohler et al., 1999) and melatonin (Kennaway et al., 1996; Kennaway and Moyer, 1998, 1999). Furthermore, it has been shown that similar to light, quipazine and other 5-HT agonists injected in the periphery or in the SCN region induce c-FOS expression in the ventrolateral area of the SCN during subjective night, but not during subjective day (Moyer et al., 1997; Kalkowski et al., 1999; Kennaway and Moyer, 1998, 1999; Kohler et al., 1999; Varcoe et al., 2003).

However, in *in vitro* studies on rat SCN slices, no photic-like effects have been observed. On the contrary, non-photic effects of 5-HT agonists have been demonstrated (Prosser et al., 1990, 1993). In addition, quipazine, *in vitro*, failed to induce *c-fos* mRNA during subjective night (Prosser et al., 1994). These differences between the *in vivo* and *in vitro* data open questions concerning the pathway(s) involved in the photic-like effects of quipazine and other 5-HT agonists.

The photic-like effects of quipazine and other 5-HT agonists could be due to a direct action on the SCN neurons through postsynaptic 5-HT receptors, the 5-$HT_{2C}$ receptor being the most likely candidate (Kennaway et al., 2001). An alternative explanation would be that 5-HT affects the SCN at a presynaptic level. Possible targets include 5-HT receptors located on fibers (either RHT, GHT or 5-HT fibers from raphe nuclei) ending on SCN cells. The main focus of this study was thus to solve this issue by analyzing the photic-like effects of quipazine (phase-shifting effect and c-FOS protein expression) in animals bearing lesions of either the RHT, the GHT, or specific destruction of the serotonergic input to the SCN.

## EXPERIMENTAL PROCEDURES

### Animals

Male rats of the inbred strain LEW/Ztm (albino) were housed individually in plastic cages (35×55×15 cm) equipped with a running wheel (diameter 35 cm, width 10 cm) under standard laboratory conditions (12-h LD, lights on at 07:00 h, 20±1 °C, 50±5% relative humidity) with food and water *ad libitum* for at least 2 weeks before the start of the experiments. Running wheel revolutions were recorded by a computer and collected in 5-min bins. All experiments with animals were performed in accordance with European Communities Council Directive of November 24, 1986 (86/609/EEC) as well as in accordance with the German law. All efforts were made to minimize the number of animals used and any potential suffering.

### Surgical procedures

*Bilateral IGL lesions.* Bilateral IGL lesions were performed on animals weighing 332±4 g. Ten minutes prior to surgery the animals received an i.p. injection of atropine sulfate (0.1 mg/kg, Wirtschaftsgenossenschaft Deutscher Tierärzte; Garbsen, Germany) in order to diminish the mucous pulmonary secretion and avoid severe hypoxemia. The animals were then anesthetized (100 mg/kg ketamine hydrochloride, CeVa Tiergesundheit GmbH, Düsseldorf, Germany, and 4 mg/kg xylazine, Bayer, Leverkusen, Germany, i.m.) and placed in a Kopf stereotaxic instrument with the incisor bar set at −3.5 mm below the interaural line (Challet et al., 1996). IGL lesions were made by applying a 500 kHz radiofrequency current for 1 min (first group of $n=9$ animals) or 2 min (second group of $n=7$ animals) through the tip (diameter= 0.25 mm) of a temperature-monitoring electrode (Radionics model RFG-4A Research RF, Radionics, Burlington, MA, USA) with the temperature at the electrode tip set to 80 °C. In the first group, bilateral IGL lesions were made at two rostro-caudal levels, i.e. 4.1 and 4.7 mm posterior to bregma, 4.4 mm lateral to the midline and 4.9 mm below the dura. In the second group an additional lesion was aimed at 4.7 mm posterior to bregma, 3.7 mm lateral to the midline and 5.5 mm below the dura. A similar procedure was used in eight sham-operated rats, except that the electrode was lowered only to 2.9 mm below the dura and no current was passed through the electrode.

*5-HT depletion.* Lesions of the 5-HT terminals within the SCN were performed following the technique developed by Cutrera et al. (1994), on nine animals weighing 261±8 g. The animals received an i.p. injection of desmethylimipramine (20 mg/kg, Sigma, Steinheim, Germany) 30 min prior to surgery to protect catecholaminergic terminals (Björklund et al., 1975) followed by an i.p. injection of atropine sulfate 20 min later. Surgery was conducted stereotaxically under deep anesthesia (100 mg/kg ketamine hydrochloride and 1 mg/kg diazepam, Ratiopharm GmbH & Co, Ulm, Germany) with the incisor bar set at +5 mm above the interaural line. Microinjections of the 5-HT specific neurotoxin 5,7-dihydroxytryptamine creatinine sulfate (5,7-DHT, Sigma) or vehicle (0.5% ascorbic acid, Merck, Rathway, NJ, USA) were delivered bilaterally into the SCN (25 μg 5,7-DHT in 0.2 μl vehicle per side) via a 1 ml Hamilton syringe. Serotonergic lesions of the SCN were made with the syringe positioned at a 2° angle at two rostro-caudal levels: 2.0 mm and 2.4 mm anterior to bregma, 0.7 mm lateral to midline, and 8.9 mm ventral to dura. Microinjections were made over a 1 min period and the cannula was left in place for 5–10 min to reduce neurotoxin leakage into the needle tract. Sham-operated rats ($n=4$) received bilateral microinjections of vehicle only.

*Biocular enucleation.* Five rats were subjected to bilateral enucleations under deep anesthesia (100 mg/kg ketamine hydrochloride and 1 mg/kg diazepam).

### Experimental protocol

After post-surgical recovery, the animals were returned to their running-wheel cages and transferred to constant darkness (DD) for at least 10 days before receiving the first injection. IGL-lesioned and 5-HT-lesioned animals first received an injection of quipazine (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany; 10 mg/kg s.c.) and 3 weeks after, an injection of vehicle (saline). The last quipazine injection, 3 weeks after the saline injection, was followed by a transcardiac perfusion. A first group of enucleated