npg

Increased wakefulness, motor activity and decreased theta activity after blockade of the 5-HT$_{2B}$ receptor by the subtype-selective antagonist SB-215505

[1]Sandor Kantor, [1]Rita Jakus, [1]Brigitta Balogh, [1]Anita Benko & *,[1,2]Gyorgy Bagdy

[1]Laboratory of Neurochemistry and Experimental Medicine, Semmelweis University National Institute of Psychiatry and Neurology, Budapest, Huvosvolgyi ut 116, H-1021, Hungary and [2]Department of Vascular Neurology, Semmelweis University National Institute of Psychiatry and Neurology, Budapest, Huvosvolgyi ut 116, H-1021, Hungary

1 Serotonin-2 receptor antagonists, like ritanserin, greatly enhance deep slow wave sleep (SWS-2) and low-frequency EEG power in humans and rodents. 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors may be involved in these effects, but the role of the 5-HT$_{2B}$ receptor is still unclear.

2 To investigate the role of the 5-HT$_{2B}$ receptor in regulation of the sleep–wake cycle, the subtype-selective antagonist SB-215505 (0.1, 0.3 and 1.0 mg kg^{-1} i.p.) was administered to Sprague–Dawley rats at light onset (beginning of passive phase). EEG, EMG and motor activity were recorded during the subsequent 8 h.

3 SB-215505 dose-dependently increased wakefulness (W) at the expense of the intermediate stage of sleep, paradoxical sleep (PS) and SWS-2 in the first hour. Parallel to increased W, significantly increased motor activity was found. Spectral analysis of the EEG in W showed a dose-dependent decrease in power density in the 3–8 Hz frequency range (maximum effect at 6 Hz). In light slow wave sleep and SWS-2, the drug reduced low-frequency ($<$8 Hz) EEG power, suggesting decreased sleep intensity after SB-215505 treatment. In PS, the drug dose-dependently decreased EEG power solely in the theta (6–9 Hz) band, primarily affecting the peak power value (7 Hz).

4 The well-known SWS-2 enhancing effect of 5-HT$_2$ receptor antagonists is mediated by 5-HT$_{2A}$ and/or 5-HT$_{2C}$ receptors. In contrast, blockade of 5-HT$_{2B}$ receptors increases motor activity and W along with decreased theta activity during W and PS. Activation of 5-HT$_{2B}$ receptors may contribute to initiation of sleep and to theta generation during W and PS under physiological conditions.
British Journal of Pharmacology (2004) **142**, 1332–1342. doi:10.1038/sj.bjp.0705887

Keywords: Serotonin; 5-HT$_{2B}$ receptor; SB-215505; spectral analysis; motor activity; theta activity; sleep; vigilance; fast Fourier transformation; EEG analysis

Abbreviations: DHN, dorsal hypothalamic nucleus; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; IS, intermediate stage of sleep; LS, lateral septum; *m*-CPP, 1-(3-chlorophenyl)piperazine; MS/DBv, medial septum/vertical limb of the diagonal band nucleus; PHA, posterior hypothalamic area; PS, paradoxical sleep; SB-215505, 6-chloro-5-methyl-1-(5-quinolylcarbamoyl) indoline; SWS-1, light slow wave sleep; SWS-2, deep slow wave sleep; W, wakefulness

Introduction

Serotonin (5-HT) was one of the first neurotransmitters associated with physiological regulation of the sleep–wake cycle (Jouvet, 1999). There is overwhelming evidence that 5-HT$_2$ receptor antagonists like ritanserin greatly enhance slow wave sleep (stages 3 and 4) in humans (Idzikowski *et al.*, 1986; 1987; 1991; Clarenbach *et al.*, 1988; Dijk *et al.*, 1989; Sharpley *et al.*, 1990; 1994) and increase deep slow wave sleep (SWS-2) in rats (Dugovic & Wauquier, 1987; Dugovic *et al.*, 1989a, b; Silhol *et al.*, 1992; Stutzmann *et al.*, 1992; Detari *et al.*, 1999; Kantor *et al.*, 2000; 2003). The 5-HT$_2$ agonists, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and 1-(3-chlorophenyl)piperazine (*m*-CPP), have opposite sleep–waking effects from 5-HT$_2$ receptor antagonists: they increase wakefulness (W), decrease slow wave sleep and REM sleep (Monti *et al.*, 1990; Kantor *et al.*, 2000). In addition to the sleep–

waking effects, ritanserin and seganserin increase low-frequency EEG activity administered at the beginning of the passive phase in rats (Borbely *et al.*, 1988; Kantor *et al.*, 2002) and humans (Dijk *et al.*, 1989). Ritanserin, which binds selectively to 5-HT$_2$ receptors (Leysen *et al.*, 1985), cannot differentiate among subtypes of the 5-HT$_2$ receptor family, namely 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors (Bonhaus *et al.*, 1997). In a recent study (Sebban *et al.*, 2002), M100907, a subtype-selective 5-HT$_{2A}$ receptor antagonist, caused similar EEG effects to ritanserin. In another study (Smith *et al.*, 2002), the 5-HT$_{2C}$ antagonist 6-chloro-5-methyl-1-(5-quinolylcarbamoyl) indoline (SB-243213) was found to produce similar sleep–waking effects to ritanserin, namely increased SWS-2 and decreased paradoxical sleep (PS) in rats. However, the role of 5-HT$_{2B}$ receptors in sleep and EEG processes has not been described. To investigate the possible role of the 5-HT$_{2B}$ receptor in regulation of the sleep–wake cycle, we analysed vigilance states and motor activity in rats and examined EEG

---

*Author for correspondence; E-mail: bag13638@mail.iif.hu

spectra in different vigilance states after a selective blockade of this receptor subtype by the 5-HT$_{2B}$ antagonist SB-215505.

Methods

Animals and surgery

All animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978). Permission was given by the local ethical committee.

Male Sprague–Dawley rats (Crl:CD^{R}BR, Charles River, Hungary), weighing 230–260 g at implantation, were used in the experiments. Rats were kept in single cages with food and water available ad libitum, and maintained in a 12 h light/dark cycle (lights on from 0900 to 21 00 hours, daylight type fluorescent tubes, 18 W, approximately 300 lx), at an ambient temperature of 21°C. Animals were equipped with EEG and EMG electrodes as described earlier (Kantor *et al.*, 2000; 2002; Filakovszky *et al.*, 2001). Briefly, stainless steel screw electrodes were implanted epidurally over the left frontal cortex (L: 2.0 mm and A: 2 mm to bregma) and over the left parietal cortex (L: 2.0 mm and A: 2.0 mm to lambda) for fronto-parietal EEG recordings. The ground electrode was placed over the cerebellum. In addition, EMG electrodes (stainless steel spring electrodes embedded in silicon rubber, Plastics One Inc., Roanoke, U.S.A.) were placed in the muscles of the neck. Surgery was performed under halothane (2%) anaesthesia (Fluotec 3) using a Kopf stereotaxic instrument. After a 10-day recovery period, the rats were attached to the polygraph by a flexible recording cable and an electric swivel, fixed above the cages, permitting free movement of the animals. In order to habituate the animals to the recording conditions, the rats were attached to the polygraph and received intraperitoneal (i.p.) injections of physiological saline for 5 days before the experiments. The animals remained connected to the recording cables throughout the study.

Data acquisition

EEG, EMG and motor activity were recorded for 8 h, starting at light onset. The signals were amplified (amplification factors approximately 5000 for EEG and motor activity, 20,000 for EMG), conditioned by analogue filters (filtering below 0.53 Hz and above 30 Hz at 6 dB octave^{-1}) and subjected to analogue to digital conversion with a sampling rate of 64 Hz. The digitized signals were displayed on a monitor and stored on the computer for further analysis.

Scoring of vigilance states

The vigilance states were scored visually for 4 s periods over 1–5 h as follows: W, the EEG is characterized by low-amplitude activity at beta (14–30 Hz) and alpha (8–13 Hz) frequencies accompanied by high EMG and motor activity; light slow wave sleep (SWS-1), high-voltage slow cortical waves (0.5–4 Hz) interrupted by low-voltage fast EEG activity (spindles, 6–15 Hz) accompanied by reduced EMG and motor activity; SWS-2, continuous high-amplitude slow cortical waves (0.5–4 Hz) with reduced EMG and motor activity; intermediate stage of sleep (IS), a brief stage (mean 3 s) just prior to PS and sometimes just after it characterized by unusual association of high-amplitude spindles (mean 12.5 Hz) and low-frequency (5.4 Hz) theta rhythm; PS, low-amplitude and high-frequency EEG activity with regular theta waves (6–9 Hz) accompanied by silent EMG and motor activity with occasional twitching (Gottesmann, 1992; Kantor *et al.*, 2000; 2002; Filakovszky *et al.*, 2001).

In addition to the visual scoring, 4 s periods of the polygraphic recordings were classified using sleep analysis software (SleepSign for Animal, Kissei Comtec America Inc., U.S.A.) for 8 h. The results obtained from this software were compared to those obtained by visual analysis: six animals, 4 × 30 min each (from 0000 to 0030 , 0100 to 0130, 0230 to 0300 and 0430 to 0500 hours). The values obtained by the software yielded strong correlations with those obtained by visual inspection (W, $r = 0.972$; SWS-1, $r = 0.806$; SWS-2, $r = 0.933$; IS, $r = 0.716$; PS, $r = 0.841$; $P < 0.01$ for all parameters, respectively).

EEG power spectral analysis

EEG power spectra were computed for consecutive 4 s epochs in the frequency range 0.25–30 Hz (fast Fourier transformation routine, Hanning window; frequency resolution 0.25 Hz). Epochs with artefacts were discarded on the basis of the polygraph records. Adjacent 0.25 Hz bins were summed into 1 Hz bins, and those above 30 Hz were omitted. Bins are marked by their upper limits; thus, 2 Hz refers to 1.25–2.00 Hz. The values of consecutive 4 s EEG epochs in W, SWS-1, SWS-2 and PS, respectively, were averaged in the first hour after treatment to obtain the power density values for these vigilance states.

Analysis of motor activity

To assess motor activity, the potentials generated in electromagnetic transducers activated by the movements of the recording cable were used and averaged for consecutive 4 s epochs (Svensson *et al.*, 1987). The absolute value or full-wave rectified signal was derived from the original motor activity signal. The unwanted baseline noise was removed by shifting the rectified signal below zero reference (submerging the band of noise under zero), re-establishing zero at the baseline, and then taking the positive half-wave rectification of the re-zeroed signal. After this, the periods above the zero reference line were quantified, obtaining the time of motor activity. The values obtained by this method yielded very strong correlations with those obtained by visual inspection and scoring (12 × 2 h intervals, $r = 0.93$, $P < 0.01$).

Drugs

All animals (10 rats) received vehicle and two doses of SB-215505 (0.1, 0.3 and 1.0 mg kg^{-1}, i.p.) treatment in a random order (4 days between treatments). The solutions were administered i.p. in the first 5 min at the beginning of the light period. SB-215505 (kindly donated by Dr Guy A. Kennett) was dissolved in 40% 2-hydroxypropyl-β-cyclodextrin (Research Biochemicals International, Natick, MA, U.S.A.) solution and administered in a volume of 1 ml kg^{-1}.

Statistical analysis

To analyse statistically the time course of changes in each vigilance state, data from each hour were submitted to multivariate analysis of variance (MANOVA) for repeated measures with two main factors: treatment (vehicle and SB-215505 $1.0\,mg\,kg^{-1}$) and time (1–5 h). When the effects of different treatments (vehicle, SB-215505 0.1, 0.3 and $1.0\,mg\,kg^{-1}$) on sleep/W were compared, MANOVA for repeated measures was used. Values of motor activity were also evaluated by MANOVA for repeated measures. The EEG power spectral values were submitted to MANOVA for repeated measures with two main factors: treatment (vehicle, SB-215505 0.1, 0.3 and $1.0\,mg\,kg^{-1}$) and frequency bins (1–30 Hz). Tukey's honest significant difference test was used for *post hoc* comparisons.

Results

Vigilance states and motor activity

SB-215505 (0.1, 0.3 and $1.0\,mg\,kg^{-1}$ i.p.) dose-dependently increased W ($F_{3,12} = 4.288$; $P < 0.05$) decreased IS ($F_{3,12} = 6.798$; $P < 0.01$) and PS ($F_{(3,12)} = 3.689$; $P < 0.05$) compared to vehicle (Figure 2b, e and f). The drug significantly decreased SWS-2 relative to controls ($F_{1,5} = 6.857$, $P < 0.05$; Figure 2d) at the highest dose ($1.0\,mg\,kg^{-1}$ i.p.). Duration of SWS-1 was not significantly affected by SB-215505 (Figures 1c and 2c). The effect of the drug was restricted to the first hour, with maximal effects between 15 and 45 min (Figure 1a). The time × treatment interaction was significant for W ($F_{4,20} = 3.128$, $P < 0.001$; Figure 1b) and a strong trend was found for SWS-2 ($F_{4,20} = 2.714$, $P = 0.059$; Figure 1d). No significant changes were found in vigilance states at later time points up to 8 h analysed by the SleepSign software.

Parallel to the increased W, we found a dose-dependent increase in motor activity ($F_{3,12} = 3.613$, $P < 0.05$; Figure 2a).

EEG power in different vigilance states

Effects of SB-215505 on EEG power were analysed separately in W, SWS-1, SWS-2 and PS. In W, the drug dose-dependently decreased EEG power mainly in the frequency range of 3–8 Hz (Figure 3). A significant treatment × frequency interaction was found ($F_{29,116} = 1.716$, $P < 0.05$; $F_{29,116} = 3.827$, $P < 0.01$; $F_{29,116} = 3.545$, $P < 0.01$) for 0.1, 0.3 and $1\,mg\,kg^{-1}$ i.p., respectively. The most prominent decrease was observed at 6 Hz; this was already present at the lowest dose ($0.1\,mg\,kg^{-1}$ i.p.) of the drug (Figure 3a and b).

In SWS-1, the drug reduced low-frequency ($< 8\,Hz$) EEG power with a maximal effect at 2–4 Hz (Figure 4e and f). Significant treatment × frequency interactions were found ($F_{29,145} = 6.371$, $P < 0.01$; $F_{29,145} = 3.236$, $P < 0.01$; $F_{29,145} = 8.331$, $P < 0.01$) for 0.1, 0.3 and $1\,mg\,kg^{-1}$ i.p., respectively.

Spectral analysis of the EEG in SWS-2 after SB-215505 treatment showed a dose-dependent decrease in power density mainly at 2–3 Hz compared to control treatment (Figure 5). The treatment × frequency interaction was highly significant for all three doses ($F_{29,116} = 13.499$, $P < 0.01$; $F_{29,116} = 2.126$, $P < 0.01$; $F_{29,145} = 6.200$, $P < 0.01$).

In PS, the drug decreased EEG power only in the theta band (6–9 Hz) in a dose-dependent manner (Figure 6). A highly significant treatment × frequency interaction was found at all three doses ($F_{29,116} = 3.340$, $P < 0.01$; $F_{29,116} = 3.917$, $P < 0.01$; $F_{29,116} = 8.241$, $P < 0.01$). The drug decreased most prominently the frequency with the highest power value (7 Hz) during this stage, and this effect was already present at the lowest dose ($0.1\,mg\,kg^{-1}$ i.p.) of the drug (Figure 6a and b).

Discussion and conclusions

The central role of the 5-HT$_{2B}$ receptor has been a controversial topic for a long time due to lack of evidence for the presence of 5-HT$_{2B}$ receptors in the CNS (Kursar *et al.*, 1992; Pompeiano *et al.*, 1994). Low levels of the 5-HT$_{2B}$ receptor mRNA have been detected recently in mouse (Loric *et al.*, 1992), rat (Pompeiano *et al.*, 1994) and human brains (Kursar *et al.*, 1994; Schmuck *et al.*, 1994; Bonhaus *et al.*, 1995). By performing immunohistochemistry with antiserum raised against the 5-HT$_{2B}$ receptor protein, the expression of the receptor has been identified in both mouse (Choi & Maroteaux, 1996) and rat (Duxon *et al.*, 1997) brains. In the mouse, a lower level of immunoreactivity was also found in the cerebrum (Choi & Maroteaux, 1996), while in the rat the presence of the 5-HT$_{2B}$ receptor has been demonstrated in discrete nuclei in the cerebellum, lateral septum (LS), dorsal hypothalamic nucleus (DHN) and medial amygdala (Duxon *et al.*, 1997).

The function of the 5-HT$_{2B}$ receptor is unclear due to the lack of selective drugs available. In rodent studies, stimulation of 5-HT$_{2B}$ receptors has been reported to have a modest anxiolytic effect (Kennett *et al.*, 1996). In addition, the 5-HT$_{2B}$ receptor agonist BW 723C86 caused hyperphagia and reduced grooming (Kennett *et al.*, 1997).

The 5-HT$_{2B}$ receptor antagonist SB-215505 exhibits at least 95-fold selectivity for 5-HT$_{2B}$ over 5-HT$_{2C}$ in the rat (Kennett *et al.*, 1998b). Pretreatment with SB-215505 (1 and $3\,mg\,kg^{-1}$ p.o.) effectively opposed the antipunishment effect of the 5-HT$_{2B}$ receptor agonist BW 723C86 in the rat Vogel conflict test (Kennett *et al.*, 1998a). Furthermore, SB-215505 ($1\,mg\,kg^{-1}$ i.p.) failed to affect *m*-CPP-induced behavioural effects mediated by 5-HT$_{2C}$ receptors (Graf *et al.*, 2003). In contrast, pretreatment with SB-242084 (0.1 and $0.3\,mg\,kg^{-1}$ i.p.), a subtype-selective 5-HT$_{2C}$ receptor antagonist, dose-dependently inhibited these effects of *m*-CPP (Graf *et al.*, 2003).

In our study, SB-215505 dose-dependently increased W at the expense of IS, PS and SWS-2. Thus, the blockade of 5-HT$_{2B}$ receptors resulted in the opposite sleep/waking effect than the previously reported 5-HT$_2$ antagonists like ritanserin (Dugovic *et al.*, 1989b; Detari *et al.*, 1999; Kantor *et al.*, 2000; 2002) or the 5-HT$_{2C}$ receptor antagonist SB-243213 (Smith *et al.*, 2002). This may be the consequence of different CNS distribution and/or cellular localization of the 5-HT$_2$ receptor subtypes.

A large body of experimental data has confirmed the key role played by the posterior hypothalamic area (PHA) in neocortical activation (or desynchronization) and behavioural arousal (Moruzzi, 1972; Vanni-Mercier *et al.*, 2003). The presence of 5-HT$_{2B}$ receptors has been described on multipolar and bipolar soma in the DHN (Duxon *et al.*, 1997), which forms the extreme rostral border of the PHA
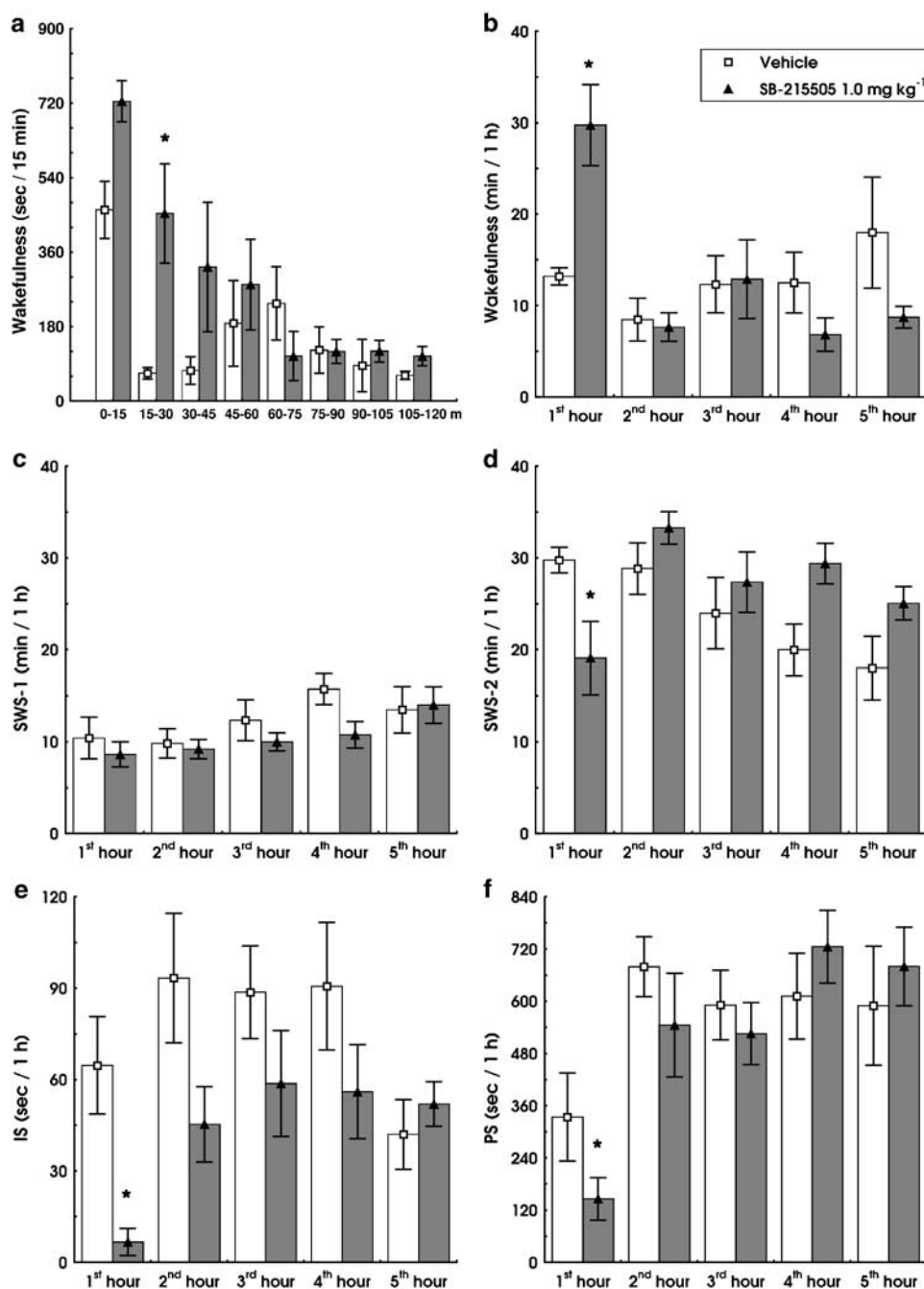
Figure 1 Effect of SB-215505 (1.0 mg kg^{-1} i.p.) on different vigilance states in conscious, freely moving rats. Time of W in each 15 min period within the first 2 h after treatment (a). Time of W (b), SWS-1 (c), SWS-2 (d), IS (e) and PS (f) per hour within the first 5 h after treatment. All columns represent mean values ($\pm$s.e.m., $n = 6$). *Significant effect of SB-215505 compared to vehicle, $P < 0.05$.

(Abrahamson & Moore, 2001). In addition, a large population of GABA- and GAD-immunoreactive neurons is present at the border between the PHA and dorsomedial hypothalamic nucleus (Abrahamson & Moore, 2001). Therefore, it is interesting to speculate that 5-HT$_{2B}$ receptors are localized on GABA-ergic neurons in the DHN and these neurons send their inhibitory projections to the waking centre of the PHA. Thus, under physiological conditions, 5-HT may increase GABA-ergic inhibition of the waking centre throughout 5-

HT$_{2B}$ receptors. Consequently, blockade of these receptors may stop inhibition of the waking centre followed by an increase in W.

Although SB-215505 did not change the amount of SWS-1, we found a depressed low-frequency EEG activity (mainly between 2 and 4 Hz) during this stage. Similar to SWS-1, the drug dose-dependently decreased EEG power in SWS-2 mainly at 2–3 Hz compared to control treatment. The decrease in low-frequency EEG activity (primarily in the
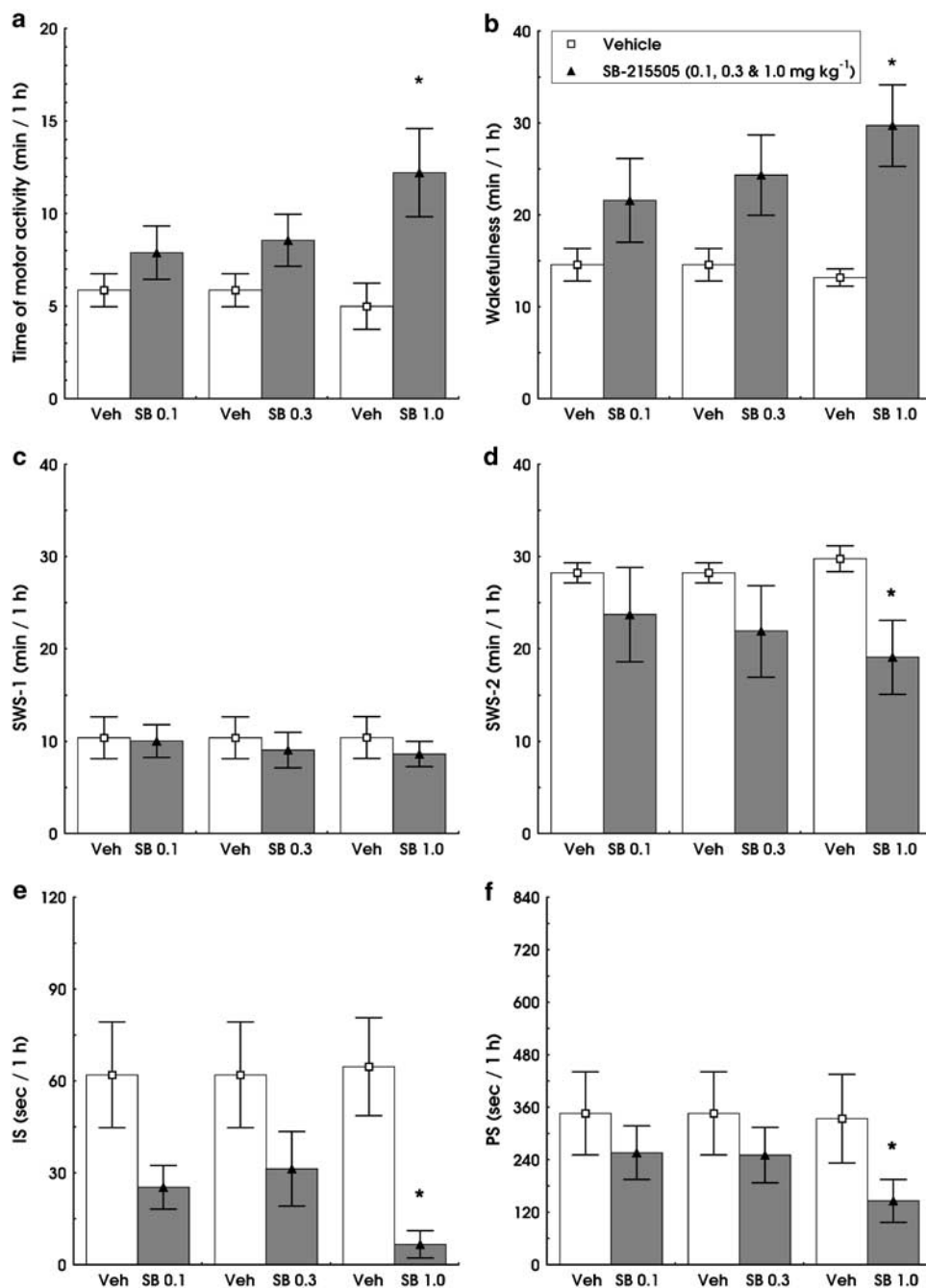
Figure 2 Dose–response effects of SB-215505 (0.1, 0.3 and $1.0 \, \text{mg kg}^{-1}$ i.p.) on motor activity (a) and different vigilance states in conscious, freely moving rats in the first hour after treatment. W (b), SWS-1 (c), SWS-2 (d), IS (e) and PS (f). All columns represent mean values ($\pm$ s.e.m., $n = 6$). *Significant effect of SB-215505 compared to vehicle, $P < 0.05$.

delta range) both in SWS-1 and SWS-2 reflects a reduced sleep intensity that may be a consequence of a fragmentation of sleep. These EEG changes are in contrast to those produced by 5-HT_2 receptor antagonists (Borbely *et al.*, 1988; Dijk *et al.*, 1989; Kantor *et al.*, 2002) or the subtype-selective 5-HT_{2A} receptor antagonist M100907 (Sebban *et al.*, 2002). Therefore, the decreased delta power after selective blockade of the 5-HT_{2B} receptor implies that ritanserin-induced increase in delta activity (Borbely *et al.*, 1988; Kantor *et al.*, 2002) may be a result of 5-HT_{2A} receptor blockade. However, the

role of 5-HT_{2C} receptors cannot be excluded (Smith *et al.*, 2002). The selective 5-HT_{2A} receptor antagonist M100907 at low doses (0.01 and $0.05 \, \text{mg kg}^{-1}$ s.c.) increased EEG power density in the low-frequency range (Sebban *et al.*, 2002) and the rate of this increase was two-fold higher than that caused by ritanserin (Borbely *et al.*, 1988; Kantor *et al.*, 2002; Sebban *et al.*, 2002). Consequently, the smaller increase in slow wave activity after ritanserin treatment may be a consequence of a parallel 5-HT_{2B} receptor blockade.
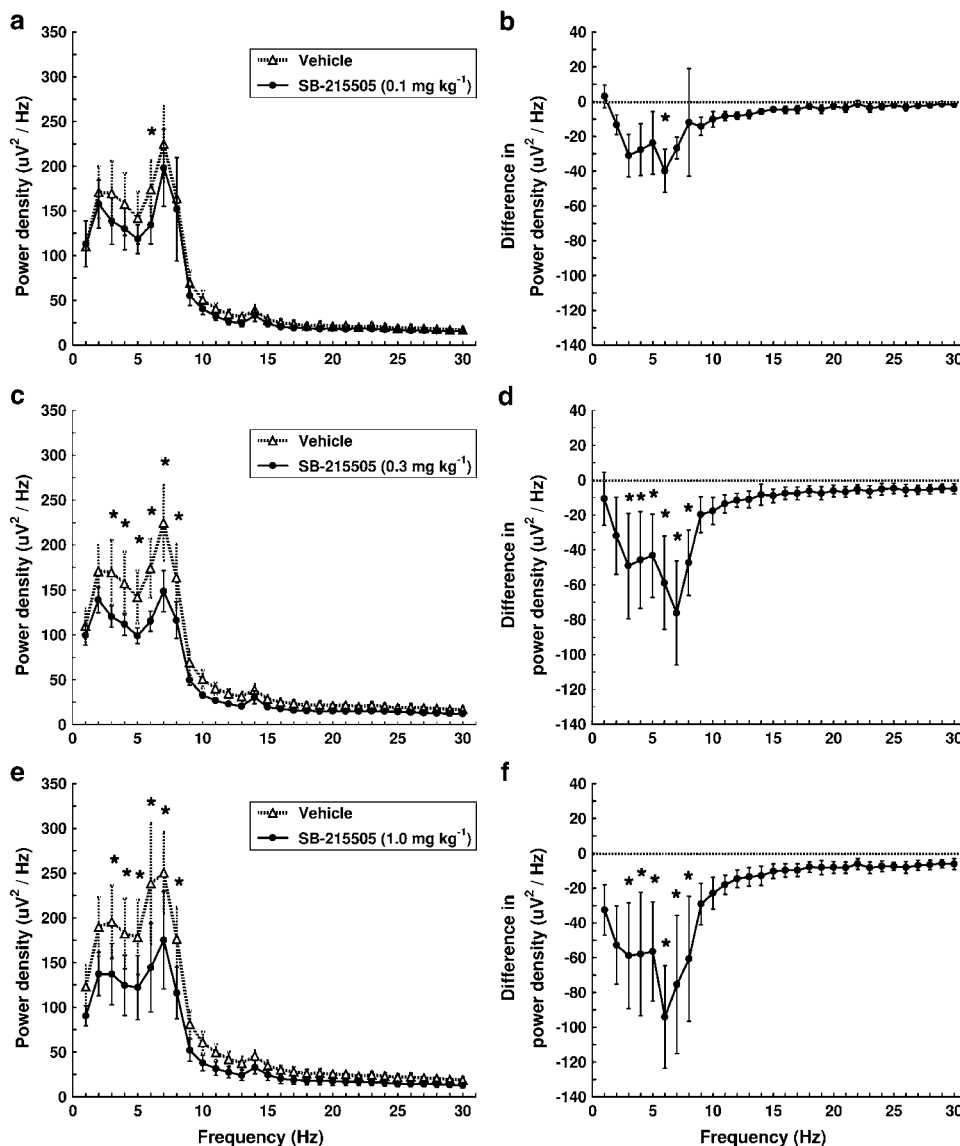
Wakefulness (W)

Figure 3 Dose–response effect of SB-215505 on EEG power spectra in W in conscious, freely moving rats in the first hour after treatment. Each point represents mean values ($\pm$s.e.m.) of 1 Hz bins in a 1 h recording period. The curves connect absolute power density values after vehicle and drug treatment (a, c, e) or the differences in drug treatment compared to vehicle treatment (dashed line) (b, d, f). *Significant effect of SB-215505 compared to vehicle, $P < 0.05$. (a, b) 0.1 mg kg^{-1} i.p. ($n = 5$); (c, d) 0.3 mg kg^{-1} i.p. ($n = 5$); (e, f) 1.0 mg kg^{-1} i.p. ($n = 5$).

Spectral analysis of the EEG in W showed a dose-dependent decrease in EEG power in the frequency range of 3–8 Hz with a maximal effect at 6 Hz after SB-215505 treatment. In addition, the drug selectively decreased neocortical theta activity (6–9 Hz) during PS, affecting most prominently the frequency with the highest power value (7 Hz) during this stage. It has been shown that the neocortical theta rhythm of rats is passively spread from the underlying hippocampus (Gerbrandt *et al.*, 1978). Thus, the theta reducing effect of the 5-HT$_{2B}$ receptor blockade described here can be explained by the distribution of this receptor in the CNS. The 5-HT$_{2B}$ receptor was identified on the neurons of the LS (Duxon *et al.*, 1997) and it is generally accepted that the rhythm generator

(i.e. pacemaker) of hippocampal-enthorinal theta oscillation is the septal complex (Stewart & Fox, 1990; Lee *et al.*, 1994; Leranth & Kiss, 1996). It is well established that the rhythmically discharging cells of the medial septum/vertical limb of the diagonal band nucleus (MS/DBv) that fire synchronously with the theta rhythm are responsible for its generation in hippocampal formation (Vertes & Kocsis, 1997). Furthermore, it has been proposed that the LS, which receives the most important afferents from the hippocampus, contributes to theta rhythm generation by giving a feedback input from the hippocampus to the MS/DBv (Raisman, 1966; Risold & Swanson, 1997a, b; Pedemonte *et al.*, 1998). Moreover, infusion of carbachol, a cholinergic agonist, into the LS

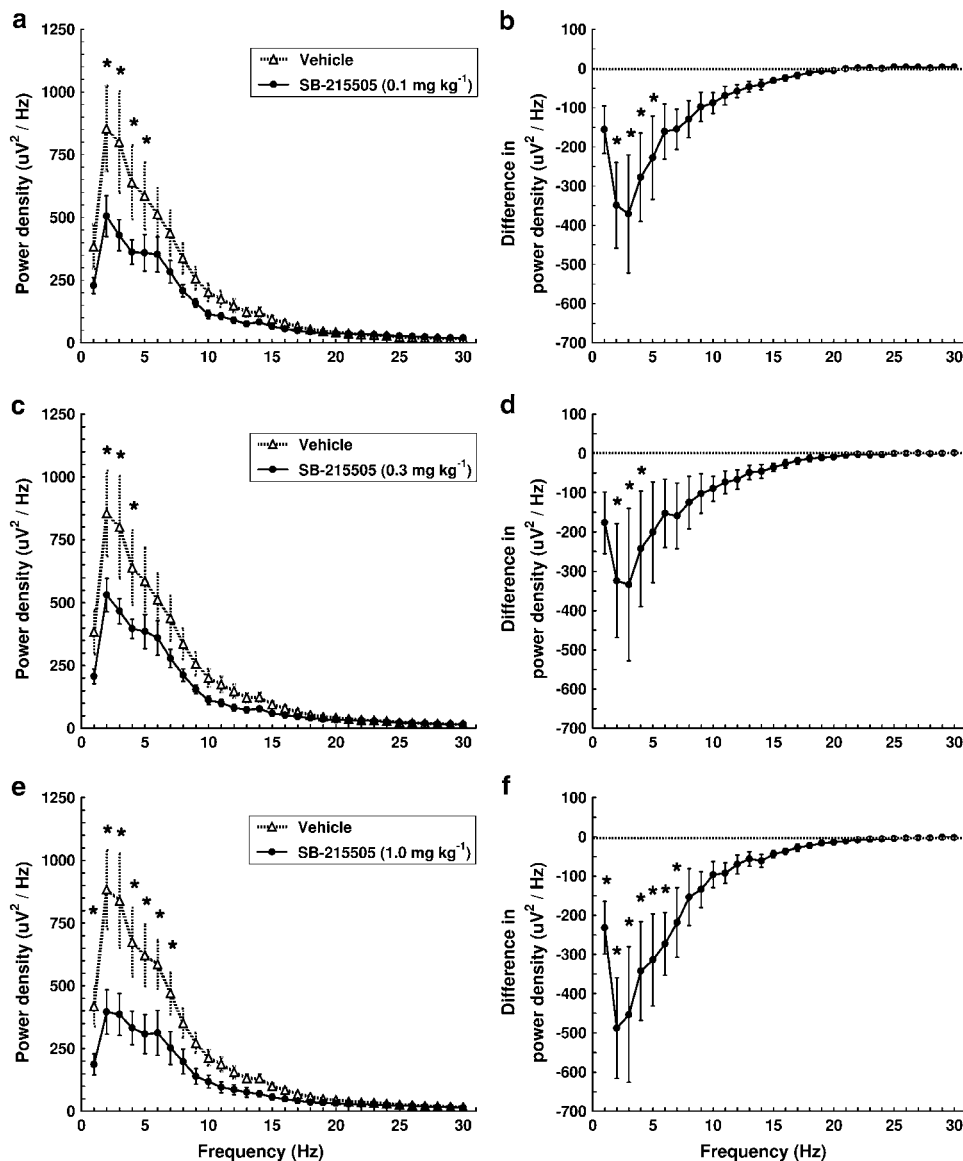## Light slow-wave sleep (SWS-1)

Figure 4 Dose–response effect of SB-215505 on EEG power spectra in SWS-1 in conscious, freely moving rats in the first hour after treatment. Each point represents mean values ($\pm$ s.e.m.) of 1 Hz bins in a 1 h recording period. The curves connect absolute power density values after vehicle and drug treatment (a, c, e) or the differences in drug treatment compared to vehicle treatment (dashed line) (b, d, f). *Significant effect of SB-215505 compared to vehicle, $P < 0.05$. (a, b) 0.1 mg kg^{-1} i.p. ($n = 6$); (c, d) 0.3 mg kg^{-1} i.p. ($n = 6$); (e, f) 1.0 mg kg^{-1} i.p. ($n = 6$).

induced theta rhythm in the hippocampus (Monmaur *et al.*, 1993) while ibotenic lesion of the LS significantly attenuated the hippocampal theta rhythm about 50% bilaterally, at both surface and deep electrodes (Leung *et al.*, 1994). LS receives dense serotonergic input from several parts of the raphe nuclei including dorsal raphe (Dinopoulos *et al.*, 1993; Risold & Swanson, 1997a, b; Leger *et al.*, 2001) and it has been demonstrated that approximately 55% of dorsal raphe neurons discharge synchronously with the theta rhythm of the hippocampus during awake-moving or rapid eye movement sleep (Kocsis & Vertes, 1992). Accordingly, these findings and our present data suggest that serotonin, acting

through 5-HT$_{2B}$ receptors of the LS, plays an important role in theta rhythm generation under physiological conditions.

Parallel to sleep and EEG changes, we found increased motor activity after the highest dose of SB-215505. In addition, a slight but not significant increase in time of self-grooming and number of grooming bouts was observed at the 1 mg kg^{-1} dose (Graf *et al.*, 2003). SB-215505 did not induce penile erections but there were some signs of restlessness noted by the observer; this was not scored (Graf *et al.*, 2003). These data are consistent with the effect of the 5-HT$_{2B}$ receptor agonist BW 723C86 described previously, namely the drug tended to suppress locomotor activity and rearing at higher doses

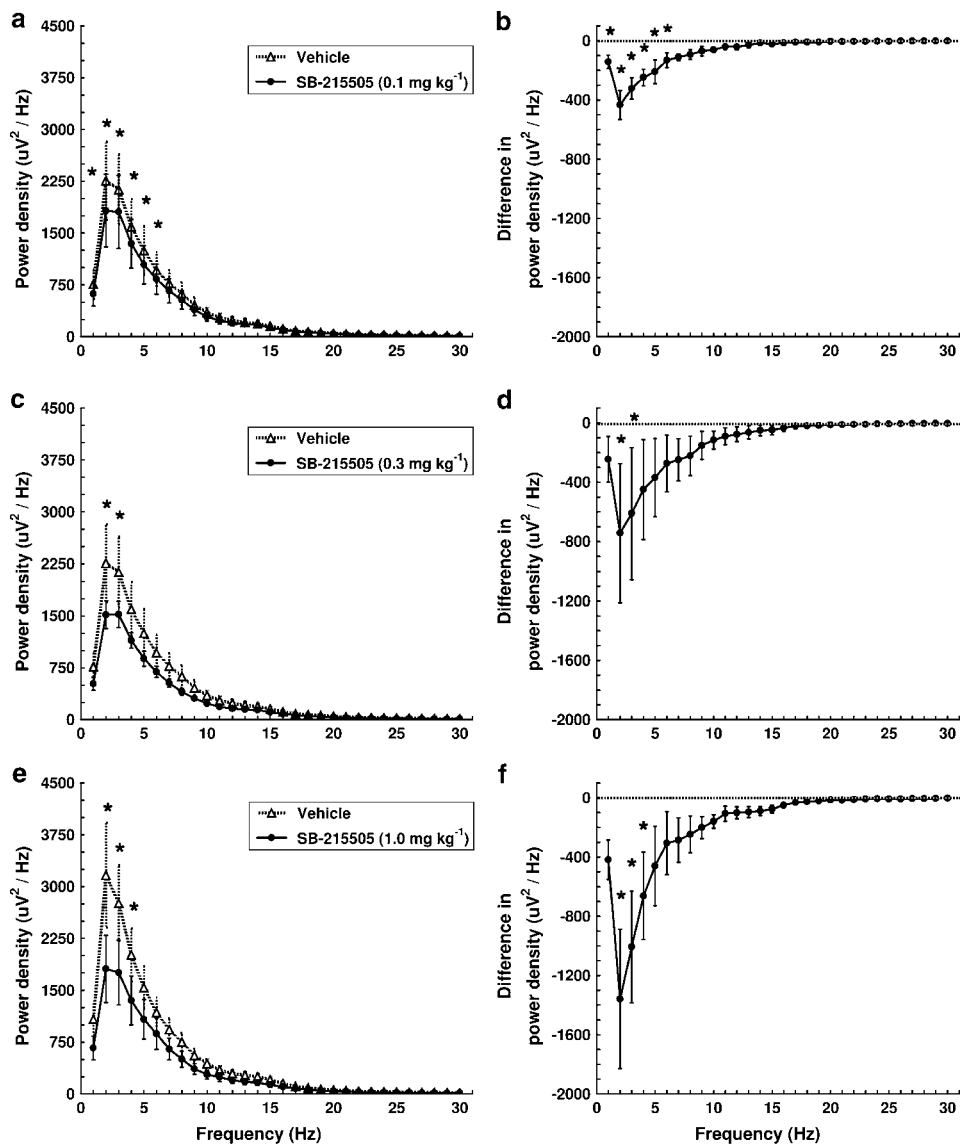## Deep slow-wave sleep (SWS-2)

Figure 5 Dose–response effect of SB-215505 on EEG power spectra in SWS-2 in conscious, freely moving rats in the first hour after treatment. Each point represents mean values ($\pm$s.e.m.) of 1 Hz bins in a 1 h recording period. The curves connect absolute power density values after vehicle and drug treatment (a, c, e) or the differences in drug treatment compared to vehicle treatment (dashed line) (b, d, f). *Significant effect of SB-215505 compared to vehicle, $P < 0.05$. (a, b) 0.1 mg kg^{-1} i.p. ($n = 5$); (c, d) 0.3 mg kg^{-1} i.p. ($n = 5$); (e, f) 1.0 mg kg^{-1} i.p. ($n = 6$).

(Kennett *et al.*, 1997). Thus, it is possible that the 5-HT$_{2B}$ receptor plays an important role in the regulation of motor activity. Expression of 5-HT$_{2B}$ receptors on cerebellar Purkinje cells provides further evidence to support this hypothesis (Duxon *et al.*, 1997).

In conclusion, our studies show that the selective 5-HT$_{2B}$ receptor antagonist SB-215505 administered at light onset dose-dependently increases W and motor activity, and decreases IS, PS and SWS-2 in the first hour after treatment. Spectral analysis of the EEG in SWS-1 and SWS-2 shows a dose-dependent decrease in low-frequency EEG power (<8 Hz), reflecting reduced sleep intensity after the treat-

ment. In addition, the drug significantly reduced theta activity in W and PS in a dose-dependent manner, suggesting an important role of the 5-HT$_{2B}$ receptor in theta rhythm generation.

These data indicate that the well-known SWS-2 enhancing effect of 5-HT$_2$ receptor antagonists is mediated by 5-HT$_{2A}$ and/or 5-HT$_{2C}$ receptors. In contrast, blockade of 5-HT$_{2B}$ receptors may increase W along with a decreased theta activity during W and PS. The results suggest that, under physiological conditions, activation of 5-HT$_{2B}$ receptors may contribute to initiation of sleep as well as to theta generation during W and PS.
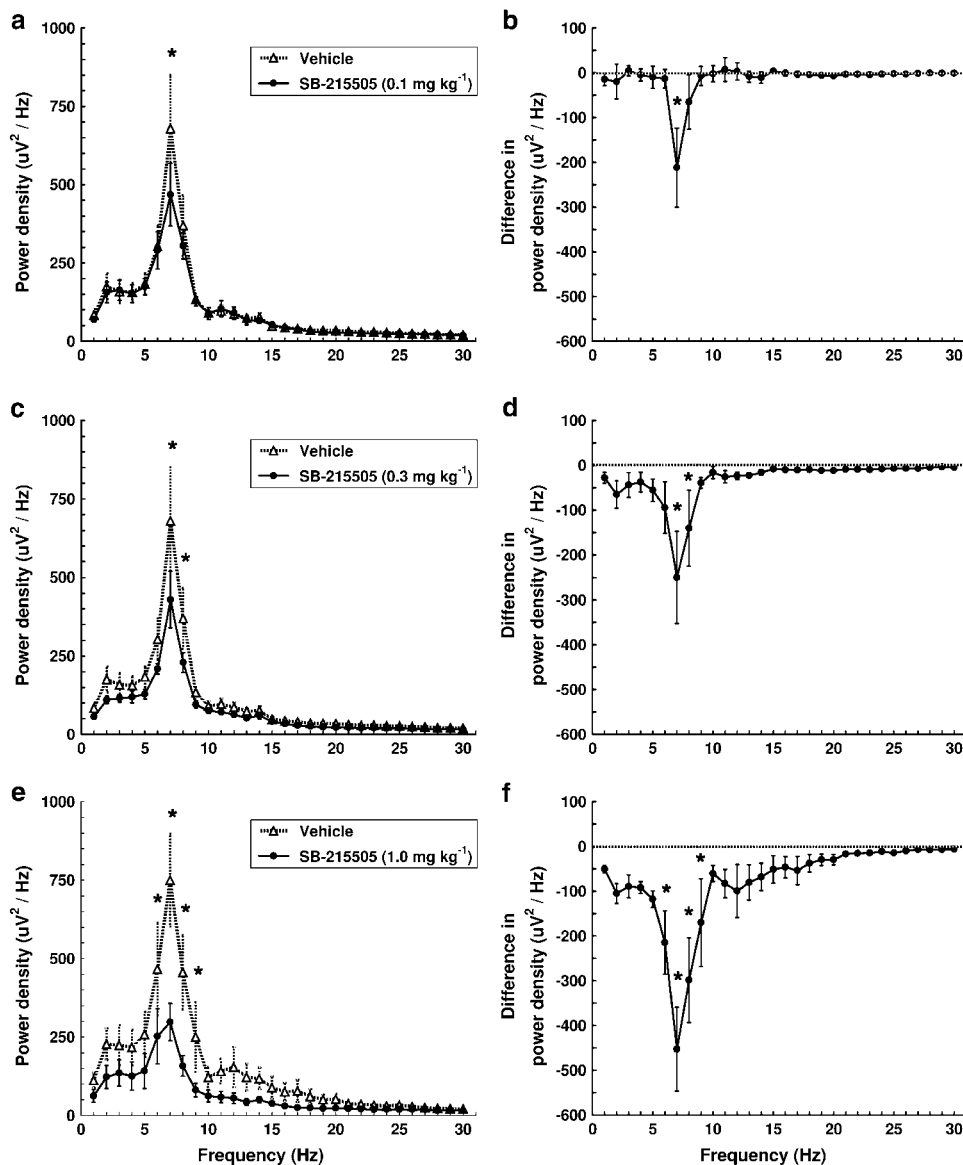
Paradoxical sleep (PS)

Figure 6 Dose–response effect of SB-2715505 on EEG power spectra in PS in conscious, freely moving rats in the first hour after treatment. Each point represents mean values ($\pm$s.e.m.) of 1 Hz bins in a 1 h recording period. The curves connect absolute power density values after vehicle and drug treatment (a, c, e) or the differences in drug treatment compared to vehicle treatment (dashed line) (b, d, f). *Significant effect of SB-215505 compared to vehicle, $P < 0.05$. (a, b) 0.1 mg kg^{-1} i.p. ($n = 5$); (c, d) 0.3 mg kg^{-1} i.p. ($n = 5$); (e, f) 1.0 mg kg^{-1} i.p. ($n = 5$).

References

ABRAHAMSON, E.E. & MOORE, R.Y. (2001). The posterior hypothalamic area: chemoarchitecture and afferent connections. *Brain Res.*, **889**, 1–22.

BONHAUS, D.W., BACH, C., DESOUZA, A., SALAZAR, F.H., MATSUOKA, B.D., ZUPPAN, P., CHAN, H.W. & EGLEN, R.M. (1995). The pharmacology and distribution of human 5-hydroxytryptamine2B (5-HT$_{2B}$) receptor gene products: comparison with 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors. *Br. J. Pharmacol.*, **115**, 622–628.

BONHAUS, D.W., WEINHARDT, K.K., TAYLOR, M., DESOUZA, A., MCNEELEY, P.M., SZCZEPANSKI, K., FONTANA, D.J., TRINH, J., ROCHA, C.L., DAWSON, M.W., FLIPPIN, L.A. & EGLEN, R.M. (1997). RS-102221: a novel high affinity and selective, 5-HT$_{2C}$ receptor antagonist. *Neuropharmacology*, **36**, 621–629.

BORBELY, A.A., TRACHSEL, L. & TOBLER, I. (1988). Effect of ritanserin on sleep stages and sleep EEG in the rat. *Eur. J. Pharmacol.*, **156**, 275–278.

CHOI, D.S. & MAROTEAUX, L. (1996). Immunohistochemical localisation of the serotonin 5-HT$_{2B}$ receptor in mouse gut, cardiovascular system, and brain. *FEBS Lett.,* **391,** 45–51.

CLARENBACH, P., BIRMANNS, B. & JAURSCH-HANCKE, C. (1988). The effect of ritanserin on sleep and hormones in man. In: *Sleep '86,* ed. Koella, W.P., Obal, F., Schultz, H. & Visser, P. pp. 355–358. Stuttgart: Gustav Fischer Verlag.

DETARI, L., SZENTGYORGYI, V., HAJNIK, T., SZENASI, G., GACSALYI, I. & KUKORELLI, T. (1999). Differential EEG effects of the anxiolytic drugs, deramciclane (EGIS-3886), ritanserin and chlordiazepoxide in rats. *Psychopharmacology (Berl.),* **142,** 318–326.

DIJK, D.J., BEERSMA, D.G., DAAN, S. & VAN DEN HOOFDAKKER, R.H. (1989). Effects of seganserin, a 5-HT$_2$ antagonist, and temazepam on human sleep stages and EEG power spectra. *Eur. J. Pharmacol.,* **171,** 207–218.

DINOPOULOS, A., DORI, I. & PARNAVELAS, J.G. (1993). Serotonergic innervation of the mature and developing lateral septum of the rat: a light and electron microscopic immunocytochemical analysis. *Neuroscience,* **55,** 209–222.

DUGOVIC, C., LEYSEN, J.E., JANSSEN, P.F. & WAUQUIER, A. (1989a). The light–dark cycle modulates the effects of ritanserin on sleep–wakefulness patterns in the rat. *Pharmacol. Biochem. Behav.,* **34,** 533–537.

DUGOVIC, C. & WAUQUIER, A. (1987). 5-HT$_2$ receptors could be primarily involved in the regulation of slow-wave sleep in the rat. *Eur. J. Pharmacol.,* **137,** 145–146.

DUGOVIC, C., WAUQUIER, A., LEYSEN, J.E., MARRANNES, R. & JANSSEN, P.A. (1989b). Functional role of 5-HT$_2$ receptors in the regulation of sleep and wakefulness in the rat. *Psychopharmacology (Berl.),* **97,** 436–442.

DUXON, M.S., FLANIGAN, T.P., REAVLEY, A.C., BAXTER, G.S., BLACKBURN, T.P. & FONE, K.C. (1997). Evidence for expression of the 5-hydroxytryptamine-2B receptor protein in the rat central nervous system. *Neuroscience,* **76,** 323–329.

FILAKOVSZKY, J., KANTOR, S., HALASZ, P. & BAGDY, G. (2001). 8-OH-DPAT and MK-801 affect epileptic activity independently of vigilance. *Neurochem. Int.,* **38,** 551–556.

GERBRANDT, L.K., LAWRENCE, J.C., ECKARDT, M.J. & LLOYD, R.L. (1978). Origin of the neocortically monitored theta rhythm in the curarized rat. *Electroencephalogr. Clin. Neurophysiol.,* **45,** 454–467.

GOTTESMANN, C. (1992). Detection of seven sleep–waking stages in the rat. *Neurosci. Biobehav. Rev.,* **16,** 31–38.

GRAF, M., KANTOR, S., ANHEUER, Z.E., MODOS, E.A. & BAGDY, G. (2003). m-CPP-induced self-grooming is mediated by 5-HT$_{2C}$ receptors. *Behav. Brain Res.,* **142,** 175–179.

IDZIKOWSKI, C., COWEN, P.J., NUTT, D. & MILLS, F.J. (1987). The effects of chronic ritanserin treatment on sleep and the neuroendocrine response to L-tryptophan. *Psychopharmacology (Berl.),* **93,** 416–420.

IDZIKOWSKI, C., MILLS, F.J. & GLENNARD, R. (1986). 5-Hydroxytryptamine-2 antagonist increases human slow wave sleep. *Brain Res.,* **378,** 164–168.

IDZIKOWSKI, C., MILLS, F.J. & JAMES, R.J. (1991). A dose–response study examining the effects of ritanserin on human slow wave sleep. *Br. J. Clin. Pharmacol.,* **31,** 193–196.

JOUVET, M. (1999). Sleep and serotonin: an unfinished story. *Neuropsychopharmacology,* **21,** 24S–27S.

KANTOR, S., GERBER, K., HALASZ, P. & BAGDY, G. (2000). The role of 5-HT$_{2A}$, 5-HT$_{2C}$ and 5HT$_3$ serotonin receptors in the regulation of sleep. *J. Physiol.,* **526,** 66P–67P.

KANTOR, S., JAKUS, R., BODIZS, R., HALASZ, P. & BAGDY, G. (2002). Acute and long-term effects of the 5-HT$_2$ receptor antagonist ritanserin on EEG power spectra, motor activity, and sleep: changes at the light–dark phase shift. *Brain Res.,* **943,** 105–111.

KENNETT, G.A., AINSWORTH, K., TRAIL, B. & BLACKBURN, T.P. (1997). BW 723C86, a 5-HT$_{2B}$ receptor agonist, causes hyperphagia and reduced grooming in rats. *Neuropharmacology,* **36,** 233–239.

KENNETT, G.A., BRIGHT, F., TRAIL, B., BAXTER, G.S. & BLACKBURN, T.P. (1996). Effects of the 5-HT$_{2B}$ receptor agonist, BW 723C86, on three rat models of anxiety. *Br. J. Pharmacol.,* **117,** 1443–1448.

KENNETT, G.A., TRAIL, B. & BRIGHT, F. (1998a). Anxiolytic-like actions of BW 723C86 in the rat Vogel conflict test are 5-HT2B receptor mediated. *Neuropharmacology,* **37,** 1603–1610.

KENNETT, G.A., TRAIL, B., RILEY, G., BICKERDIKE, M.J., RANSON, J., FORBES, I.T., PRICE, G.W., BAXTER, G.S. & HUNTER, A.J. (1998b). SB 215505, a selective 5-HT2B receptor antagonist in rats. *Soc. Neurosci. Abstr.,* **24,** 541.12.

KOCSIS, B. & VERTES, R.P. (1992). Dorsal raphe neurons: synchronous discharge with the theta rhythm of the hippocampus in the freely behaving rat. *J. Neurophysiol.,* **68,** 1463–1467.

KURSAR, J.D., NELSON, D.L., WAINSCOTT, D.B. & BAEZ, M. (1994). Molecular cloning, functional expression, and mRNA tissue distribution of the human 5-hydroxytryptamine2B receptor. *Mol. Pharmacol.,* **46,** 227–234.

KURSAR, J.D., NELSON, D.L., WAINSCOTT, D.B., COHEN, M.L. & BAEZ, M. (1992). Molecular cloning, functional expression, and pharmacological characterization of a novel serotonin receptor (5-hydroxytryptamine2F) from rat stomach fundus. *Mol. Pharmacol.,* **42,** 549–557.

LEE, M.G., CHROBAK, J.J., SIK, A., WILEY, R.G. & BUZSAKI, G. (1994). Hippocampal theta activity following selective lesion of the septal cholinergic system. *Neuroscience,* **62,** 1033–1047.

LEGER, L., CHARNAY, Y., HOF, P.R., BOURAS, C. & CESPUGLIO, R. (2001). Anatomical distribution of serotonin-containing neurons and axons in the central nervous system of the cat. *J. Comp. Neurol.,* **433,** 157–182.

LERANTH, C. & KISS, J. (1996). A population of supramammillary area calretinin neurons terminating on medial septal area cholinergic and lateral septal area calbindin-containing cells are aspartate/glutamatergic. *J. Neurosci.,* **16,** 7699–7710.

LEUNG, L.S., MARTIN, L.A. & STEWART, D.J. (1994). Hippocampal theta rhythm in behaving rats following ibotenic acid lesion of the septum. *Hippocampus,* **4,** 136–147.

LEYSEN, J.E., GOMMEREN, W., VAN GOMPEL, P., WYNANTS, J., JANSSEN, P.F. & LADURON, P.M. (1985). Receptor-binding properties *in vitro* and *in vivo* of ritanserin: a very potent and long acting serotonin-S2 antagonist. *Mol. Pharmacol.,* **27,** 600–611.

LORIC, S., LAUNAY, J.M., COLAS, J.F. & MAROTEAUX, L. (1992). New mouse 5-HT$_2$-like receptor. Expression in brain, heart and intestine. *FEBS Lett.,* **312,** 203–207.

MONMAUR, P., AYADI, K. & BRETON, P. (1993). Hippocampal EEG responses induced by carbachol and atropine infusions into the septum and the hippocampus in the urethane-anaesthetized rat. *Brain Res.,* **631,** 317–324.

MONTI, J.M., PINEYRO, G., ORELLANA, C., BOUSSARD, M., JANTOS, H., LABRAGA, P., OLIVERA, S. & ALVARINO, F. (1990). 5-HT receptor agonists 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and 8-OH-DPAT increase wakefulness in the rat. *Biog. Amines,* **7,** 145–151.

MORUZZI, G. (1972). The sleep–waking cycle. *Ergeb. Physiol.,* **64,** 1–165.

PEDEMONTE, M., BARRENECHEA, C., NUNEZ, A., GAMBINI, J.P. & GARCIA-AUSTT, E. (1998). Membrane and circuit properties of lateral septum neurons: relationships with hippocampal rhythms. *Brain Res.,* **800,** 145–153.

POMPEIANO, M., PALACIOS, J.M. & MENGOD, G. (1994). Distribution of the serotonin 5-HT$_2$ receptor family mRNAs: comparison between 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors. *Brain Res. Mol. Brain Res.,* **23,** 163–178.

RAISMAN, G. (1966). The connexions of the septum. *Brain,* **89,** 317–348.

RISOLD, P.Y. & SWANSON, L.W. (1997a). Chemoarchitecture of the rat lateral septal nucleus. *Brain Res. Brain Res. Rev.,* **24,** 91–113.

RISOLD, P.Y. & SWANSON, L.W. (1997b). Connections of the rat lateral septal complex. *Brain Res. Brain Res. Rev.,* **24,** 115–195.

SCHMUCK, K., ULLMER, C., ENGELS, P. & LUBBERT, H. (1994). Cloning and functional characterization of the human 5-HT$_{2B}$ serotonin receptor. *FEBS Lett.,* **342,** 85–90.

SEBBAN, C., TESOLIN-DECROS, B., CIPRIAN-OLLIVIER, J., PERRET, L. & SPEDDING, M. (2002). Effects of phencyclidine (PCP) and MK 801 on the EEGq in the prefrontal cortex of conscious rats; antagonism by clozapine, and antagonists of AMPA-, α_1- and 5-HT$_{2A}$-receptors. *Br. J. Pharmacol.,* **135,** 65–78.

SHARPLEY, A.L., ELLIOTT, J.M., ATTENBURROW, M.J. & COWEN, P.J. (1994). Slow wave sleep in humans: role of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors. *Neuropharmacology,* **33,** 467–471.

SHARPLEY, A.L., SOLOMON, R.A., FERNANDO, A.I., DA ROZA DAVIS, J.M. & COWEN, P.J. (1990). Dose-related effects of selective 5-HT$_2$ receptor antagonists on slow wave sleep in humans. *Psychopharmacology (Berl.),* **101,** 568–569.

SILHOL, S., GLIN, L. & GOTTESMANN, C. (1992). Study of the 5-HT$_2$ antagonist ritanserin on sleep–walking cycle in the rat. *Pharmacol. Biochem. Behav.,* **41,** 241–243.

SMITH, M.I., PIPER, D.C., DUXON, M.S. & UPTON, N. (2002). Effect of SB-243213, a selective 5-HT(2C) receptor antagonist, on the rat sleep profile: a comparison to paroxetine. *Pharmacol. Biochem. Behav.,* **71,** 599–605.

STEWART, M. & FOX, S.E. (1990). Firing relations of lateral septal neurons to the hippocampal theta rhythm in urethane anesthetized rats. *Exp. Brain Res.,* **79,** 92–96.

STUTZMANN, J.M., EON, B., LUCAS, M., BLANCHARD, J.C. & LADURON, P.M. (1992). RP 62203, a 5-hydroxytryptamine2 antagonist, enhances deep NREM sleep in rats. *Sleep,* **15,** 119–124.

SVENSSON, K., ALFOLDI, P., HAJOS, M., RUBICSEK, G., JOHANSSON, A.M., CARLSSON, A. & OBAL JR, F. (1987). Dopamine autoreceptor antagonists: effects on sleep–wake activity in the rat. *Pharmacol. Biochem. Behav.,* **26,** 123–129.

VANNI-MERCIER, G., GIGOUT, S., DEBILLY, G. & LIN, J.S. (2003). Waking selective neurons in the posterior hypothalamus and their response to histamine H3-receptor ligands: an electrophysiological study in freely moving cats. *Behav. Brain Res.,* **144,** 227–241.

VERTES, R.P. & KOCSIS, B. (1997). Brainstem-diencephalo-septohippocampal systems controlling the theta rhythm of the hippocampus. *Neuroscience,* **81,** 893–926.