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Depression and poor sleep: The effect of monoaminergic antidepressants in a pre-clinical model in rats

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Abstract

The effects of five antidepressants (escitalopram, paroxetine, duloxetine, venlafaxine, and reboxetine) on the sleep architecture were investigated in freely moving rats in the light phase of a 12:12 h light:dark cycle following a single i.p. dose of antidepressant. Overall, paroxetine and escitalopram exhibited the least sleep disruptive profiles, whereas duloxetine, venlafaxine, and reboxetine increased the time spent awake and suppressed paradoxical sleep. Analysis of the EEG at 1 h intervals revealed only subtle differences from the overall picture. The effect of venlafaxine on disruption of sleep architecture could not be readily explained by its *in vitro* serotonin (5-HT) and noradrenaline (NA) reuptake inhibitory potencies. *In vivo* microdialysis experiments in the ventral hippocampus of freely moving rats revealed that venlafaxine affected the 5-HT and NA systems equally at the doses tested. Duloxetine (7.7 mg/kg) induced maximal blockade of the 5-HT transporter and duloxetine 7.7 mg/kg also modulated the noradrenaline system. Thus, in this animal model, the enhancement of noradrenergic activity is more disruptive on the sleep architecture than enhancement of serotonergic activity.

Keywords: Depression; Sleep; Sleep disruption; EEG; Escitalopram

1. Introduction

Depression is one of the most frequently occurring disorders, affecting about 10–15% of the population each year, and a disturbed sleep pattern is a prominent feature of the depressive symptomatology affecting more than 80% of depressed patients (Reynolds and Kupfer, 1987; Armitage and Hoffmann, 2001). The neuronal pathways and the neurotransmitters and receptors involved in the regulation of sleep are not fully understood, and the neurotransmitter systems involved in disturbed sleep remain to be investigated in greater detail (Delgado, 2000; Adrien, 2002; Gottesmann, 2004). Furthermore, there is a marked overlap between the neuronal pathways involved in sleep/wake regulation and depression, but the causal relationship remains unclear (Lustberg and Reynolds, 2000; Morawetz, 2003). The sleep pathway is modulated by serotonergic as well as noradrenergic input; these are the two main neurotransmitter systems on which current antidepressant drugs exert their pharmacological effect (Lin, 2000; Winokur et al., 2001; Ursin, 2002; Brunello et al., 2003). It is, therefore, not surprising that medication for the treatment of depressive episodes can also affect, and sometimes improve, accompanying sleep disturbances (Lader et al., 2005).

Antidepressant drugs are usually divided into several classes depending on their *in vitro* binding profiles. The selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors (SSRIs) specifically inhibit the 5-HT transporter (5-HTT), whereas the selective noradrenaline (NA) reuptake inhibitors (NRIs) inhibit the NA transporter (NAT). SNRIs inhibit both 5-HTT and NAT (e.g., venlafaxine and duloxetine) with different ratios between their effects on the two neurotransmitter systems.

In vivo studies in rats and mice have shown that dosing with SSRIs results in an increase in brain 5-HT levels due to blocking reuptake of 5-HT (Bymaster et al., 2002; Felton et al., 2003; Sánchez et al., 2003a). The most consistent effect of SSRIs on

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overall sleep architecture in rodents is a moderate increase in time spent awake, an increase in light slow-wave sleep, and a decrease in deep slow-wave sleep and paradoxical sleep (which corresponds to REM sleep in humans) (Neckelmann et al., 1996; Gervasoni et al., 2002; Monaca et al., 2003).

The NRIs increase the level of brain NA in rodents by blocking reuptake of this neurotransmitter (Sacchetti et al., 1999; Page, 2003; Benmansour et al., 2004; Linnér et al., 2004). To our knowledge, no reports have been published on their overall effect on sleep architecture in rodents.

The SNRIs generally produce an increase in both 5-HT and NA by blocking reuptake of both transmitters (Engleman et al., 1995; Wikell et al., 2001; Karpa et al., 2002; Koch et al., 2003). Dosing rodents with these compounds led to an increase in time awake, and a decrease in slow-wave sleep and paradoxical sleep (Katoh et al., 1995; Salín-Pascual and Moro-Lopez, 1997).

The aim of the present study was to investigate the roles of 5-HT and NA under identical test conditions in the modulation of sleep architecture in rats using radiotelemetry methodology. The study investigated antidepressants with a range of 5-HTT/NAT antagonistic activity, from the most selective 5-HT uptake inhibitor, escitalopram (Owens et al., 2001), paroxetine, and the SNRIs, duloxetine and venlafaxine, to the NRI, reboxetine. Furthermore, the *in vitro* uptake inhibiting potencies were determined under identical assay conditions, as were extracellular levels of 5-HT and NA in the ventral hippocampus of freely moving rats dosed with duloxetine and venlafaxine, in order to compare the effects of the different antidepressants.

2. Materials and methods

2.1. In vitro inhibition of monoamine uptake in rat brain synaptosomes

2.1.1. Tissue preparation

Synaptosomes were prepared from male Wistar rats (125–225 g), as described by Hyttel (1978). Synaptosomes for [^{3}H]-5-HT uptake were prepared from whole brain (without cerebellum), whereas synaptosomes for [^{3}H]-NA uptake were prepared from occipital, temporal and parietal cortices. Briefly, rats were decapitated and the relevant tissue was rapidly removed and homogenised in 40 vol (w/v) ice-cold 0.32 M sucrose solution containing 1 mM nialamide. The homogenate was centrifuged at 600 ×*g* for 10 min and the supernatant was then centrifuged twice at 20,000 ×*g* for 55 min. The final pellet was resuspended in modified Krebs–Ringer phosphate buffer (122 mM NaCl, 5 mM KCl, 972 μM $CaCl_2$, 1.2 mM $MgSO_4$, 10 mM glucose, 1.1 mM ascorbic acid, 161 μM EDTA, 12.66 mM Na_2HPO_4, 2.77 mM $NaHPO_4$, pH 7.4).

2.1.2. [^{3}H]-5-HT uptake in rat synaptosomes

Freshly prepared synaptosomes were incubated with varying concentrations of drug and 10 nM [^{3}H]-5-HT for 5 min. Samples were then rapidly filtered and radioactivity trapped on the filters was counted in a liquid scintillation counter. Non-specific binding and passive transport were determined by adding 10 μM citalopram to the reaction mixture.

2.1.3. [^{3}H]-NA uptake in rat synaptosomes

Freshly prepared synaptosomes were incubated with varying concentrations of drug and 10 nM [^{3}H]-NA for 5 min at room temperature. Samples were then rapidly filtered and radioactivity trapped on the filters was counted in a liquid scintillation counter. Non-specific binding and passive transport were determined by adding 10 μM talsupram to the reaction mixture.

2.2. In vivo studies

2.2.1. Animals

The animal welfare committee, appointed by the Ministry of Danish Justice, granted ethical permission for the study. All animal procedures were carried out in compliance with the EC Directive 86/609/EEC and with Danish law regulating experiments on animals.

Adult male Sprague Dawley rats [200–250 g (sleep studies) or 275–300 g (microdialysis studies); Møllegaard, Denmark] were housed in pairs (one with implanted transmitter) and had free access to standard laboratory chow and water. Room temperature (21±2 °C), relative humidity (55±5%), and air exchange (16 times per hour) were automatically controlled and animals were housed under a 12:12 h light:dark cycle (lights on at 06:00 h).

2.2.2. Radiotelemetry surgery

Surgery was performed after anaesthetising the rats with a subcutaneous (s.c.) injection of one part fentanyl-fluanisone (Hypnorm®, Janssen Inc., USA), one part midazolam (Dormicom®, Roche Ltd., Switzerland) and two parts sterilized water (0.02 ml/10 g). The transmitter (TL10M3-F50-EEE implant, Data Sciences International, USA) was implanted in the peritoneum (i.p.) of the rat as described by Vogel et al. (2002). Briefly, the shaved and iodine swabbed abdomen was opened through an incision of the *linea abla*, and the transmitter placed in the peritoneum. The leads were fed s.c. through a trochar from the abdomen and out through a small incision made between the scapulae and further s.c. up to the exposed skull. A total of four holes (0.5 mm) were drilled in the skull, two for the parietal electrodes (2 mm anterior to lambda and 2 mm on either side of the midline), and two for the frontal electrodes (2 mm anterior to bregma and 2 mm on either side of the midline). The four EEG leads were placed subdurally and the common leads were placed over the anterior sinus and were held in place with dental reinforced glass ionome luting cement (GC Corporation, Japan). The EMG leads were placed in either side of the *musculus cervicoauricularis* and were sutured in place. The animals were allowed to recover one week post-surgery. Each animal received antibiotic [Baytril Vet™ (enrofloxacin; 10 mg/kg)] and analgesic [Rimadyl® (carprofen; 0.1 ml/100 g) once daily subcutaneously (s.c.)] during the recovery period.

2.2.3. Drug administration, sleep recording and visual EEG scoring

Dataquest A.R.T. Gold 2.2 electrodes were used to record EEG and EMG simultaneously. EEG recording took place in the

rat's home cage. EEG recordings were initiated around 8 a.m. and continued for 5 h after dosing. The drugs were administered s.c. after approximately 30 min baseline EEG recording. A total of 32 telemetrised rats were used in the study. The animals were used for multiple testing and randomly assigned to different treatment groups after a minimum washout period of 3 days between tests. A total of 8 rats were included in each treatment group except the vehicle control group where $n=20$. The 5 antidepressants were tested in two rounds. Each round consisted of five trials with three treatment groups, a high and low dose of the drug ($n=4$ per group) plus a vehicle group ($n=2$).

The EEG data were scored as wake (W), light slow-wave sleep (SWS-1), deep slow-wave sleep (SWS-2), or paradoxical sleep (PS) based on visual analysis of EEG frequency and amplitude characteristics and EMG activity (Neckelmann and Ursin, 1993). The EEG recordings for each animal were manually scored under blinded conditions in 10 s epochs. Data were analysed using the sleep analysis software program Somnologica 3 (Flaga^{hf} Medical Devices, Iceland).

Statistical analysis was carried out using analysis of variance. For each EEG measure: 2-way ANOVA of the overall effect over the 5 h test interval and for each treatment group a 2-way repeated measure ANOVA *versus* vehicle were carried out and followed by post-hoc analysis using the Student Newman−Keuls test to confirm significant differences between treatments. Differences with $p<0.05$ were considered statistically significant.

2.2.4. Microdialysis surgery

Rats were anaesthetised with Hypnorm/Dormicum; 2 ml/kg (fentanyl citrate, 0.079 mg/ml; fluanisone 2.5 mg/ml; midazolam, 1.25 mg/ml) and intracerebral guide cannulas (CMA/12) were stereotaxically implanted into the hippocampus, positioning the dialysis probe tip in the ventral hippocampus (co-ordinates: 5.6 mm posterior to bregma, lateral −5.0 mm, 7.0 mm ventral to dura) (Paxinos et al., 1980). Anchor screws and acrylic cement were used to fix the guide cannulas. The body temperature of the animals was monitored by rectal probe and maintained at 37 °C. The rats were allowed to recover from surgery for 2 days, housed singly in cages.

2.2.5. Microdialysis experiments

Experiments were conducted in the morning. On the day of the experiment, a microdialysis probe (CMA/12, 0.5 mm diameter, 3 mm length) was inserted through the guide cannula. The probes were connected via a dual channel swivel to a microinjection pump. Perfusion of the microdialysis probe with filtered Ringer solution (145 mM NaCl, 3 mM KCl, 1 mM $MgCl_2$, 1.2 mM $CaCl_2$) was begun shortly before insertion of the probe into the brain and continued for the duration of the experiment at a constant flow of 1.3 μl/min. After 180 min of stabilization, the experiments were initiated. Dialysates were collected every 30 min. After the experiments the animals were sacrificed, their brains removed, frozen and sliced for verification of probe placement.

2.2.6. Analysis of dialysates for 5-HT and NA

Concentration of monoamines in the dialysates was determined by means of HPLC with electrochemical detection. The monoamines were separated by reverse phase liquid chromatography (ODS 150×3 mm, 3 μM). 5-HT: Mobile phase consisting of 75 mM NaH_2PO_4, 150 ml/l sodium octanesulfonic acid, 100 μl/l triethylamine and 10% acetonitrile (pH 3.0) at a flow rate of 0.4 ml/min. NA: The mobile phase consisted of 75 mM NaH_2PO_4, 800 mg/l sodium 1-heptanesulfonic acid, 100 μl/l triethylamine and 8% acetonitrile (pH 4.7) at a flow rate of 0.17 ml/min. Electrochemical detection used a coulometric detector; potential set at 250 mV (guard cell at 350 mV) (Coulochem II, ESA). The mean value of 3 consecutive 5-HT/NA samples immediately preceding compound administration served as the basal level for each experiment and data were converted to percentage of basal (mean basal pre-injection values normalised to 100%).

2.2.7. Drugs

For the *in vitro* studies, drugs were dissolved in dimethyl sulfoxide and diluted in incubation buffer. 5-hydroxy-[^{3}H]-tryptamine trifluoroacetate (spec. act. 91 Ci/mmol) and 1-[7,8-^{3}H]-noradrenaline (spec. act. 37 Ci/mmol) were obtained from Amersham, UK.

In the *in vivo* studies, all compounds were dissolved in 0.9% NaCl except duloxetine, which was dissolved in 5% hydroxypropyl-β-cyclodextrin. The compounds were injected intraperitoneally (i.p.) (EEG studies) or subcutaneously (s.c.) (microdialysis studies) in a volume of 5 ml/kg body weight. All doses are expressed as mg/kg of the base. Escitalopram (oxalate) was synthesised by the Department of Medicinal Chemistry, H. Lundbeck A/S and all other compounds were obtained from ordinary commercial sources.

3. Results

Escitalopram had the highest selectivity for the 5-HTT relative to the NAT (Table 1), with greater than 1000-fold higher potency in inhibiting 5-HT transport *versus* NA transport. Paroxetine showed an approximately 60-fold difference in potency for the 5-HTT *versus* the NAT. Duloxetine and venlafaxine both had a slightly higher selectivity for the 5-HTT (5-HTT/NAT ratios of 0.61 and 0.19, respectively (Table 1). Reboxetine was selective for NAT, with approximately 84 times higher potency in inhibiting NA *versus* 5-HT uptake.

The overall sleep pattern data for all compounds tested is shown in Fig. 1. During the 5 h observation period, escitalopram 2.0 mg/kg significantly increased the time spent in light slow-wave sleep (SWS-1) compared to vehicle, whereas

Table 1
In vitro 5-HT and NA uptake IC_{50} values for selected antidepressants in rat synaptosomes

	5-HTT IC_{50} (nM)	NAT IC_{50} (nM)	5-HTT/NAT ratio
Escitalopram	2.1	2800	<0.001
Paroxetine	1.3	80	0.016
Duloxetine	1.9	3.1	0.61
Venlafaxine	82	440	0.19
Reboxetine	270	3.2	84

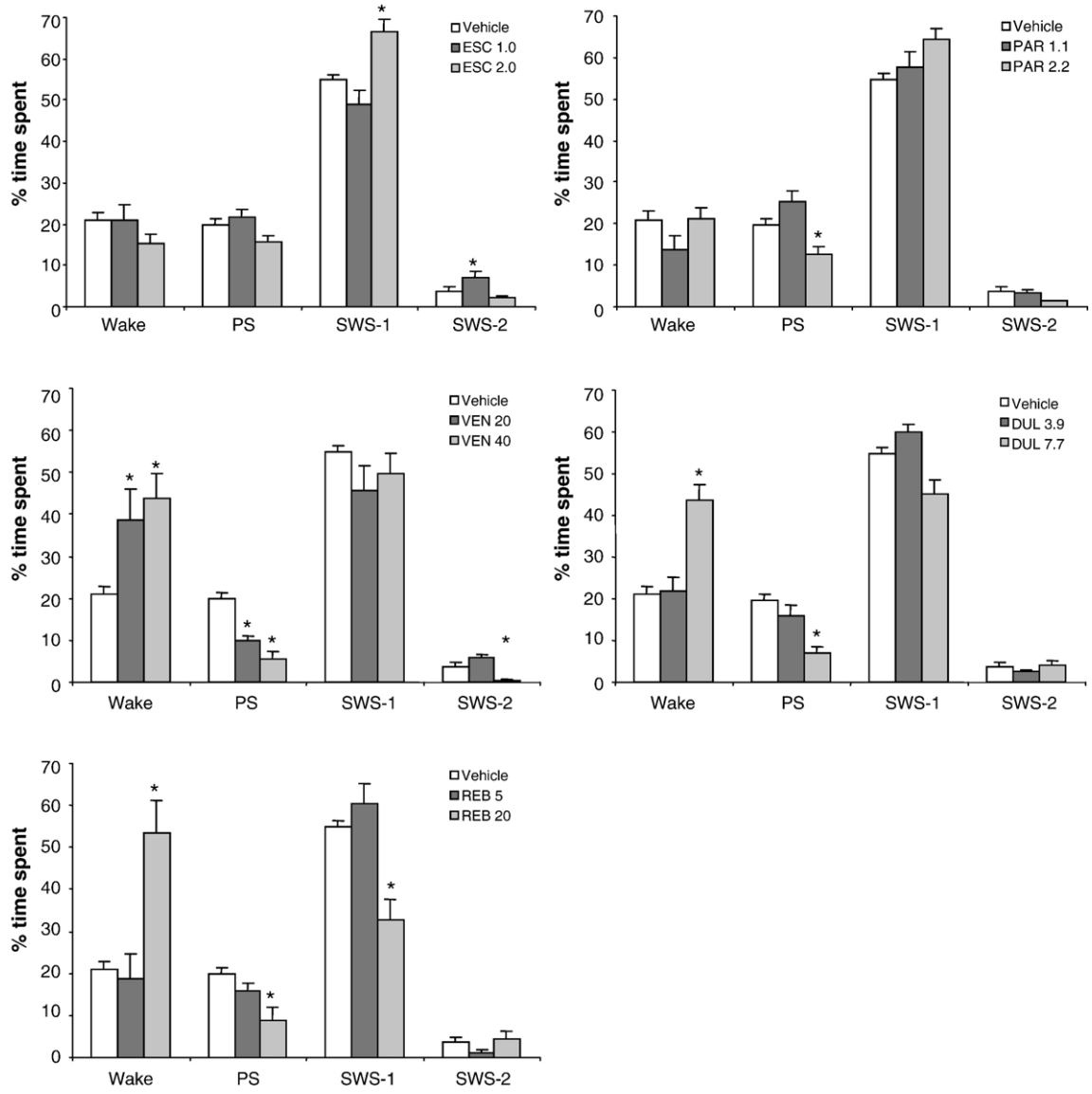

Fig. 1. Overall effect of single doses of vehicle, escitalopram (ESC; 1.0 and 2.0 mg/kg), paroxetine (PAR; 1.1 and 2.2 mg/kg), duloxetine (DUL; 3.9 and 7.7 mg/kg), venlafaxine (VEN; 20 and 40 mg/kg) and reboxetine (REB; 5 and 20 mg/kg) on sleep EEG recorded 0–5 h after i.p. administration (mean ± SEM). W = wake, PS = paradoxical sleep, SWS-1 = light slow-wave sleep, SWS-2 = deep slow-wave sleep. *$p<0.05$ *versus* vehicle (2-way ANOVA followed by Newman–Keuls test). A total of 8 rats were included in each treatment group, except the vehicle control where $n=20$.

escitalopram 1.0 mg/kg significantly increased the time spent in deep slow-wave sleep (SWS-2). Neither dose of escitalopram had any significant effect on time awake or paradoxical sleep (PS). Paroxetine 2.2 mg/kg produced a significant decrease in paradoxical sleep, whereas paroxetine 1.1 mg/kg produced no significant change in the overall EEG pattern. Duloxetine 7.7 mg/kg significantly increased time spent awake and decreased the time in paradoxical sleep, whereas duloxetine 3.9 mg/kg showed no significant change from vehicle. Venlafaxine 20 mg/kg produced a change similar to the one seen for duloxetine 7.7 mg/kg, significantly increasing the time spent awake and decreasing the time in paradoxical sleep. In addition to the effect seen for venlafaxine 20 mg/kg, venlafaxine 40 mg/kg significantly decreased the time spent in deep slow-wave sleep. Reboxetine 20 mg/kg significantly increased the time spent awake, and decreased light slow-wave sleep and paradoxical sleep, whereas no significant effects were seen for reboxetine 5 mg/kg.

A further analysis at 1 h intervals revealed more subtle differences. In general, escitalopram and paroxetine had little effect on time awake. Duloxetine showed a tendency towards more time awake. Venlafaxine 40 mg/kg and reboxetine 20 mg/kg both resulted in more time awake, especially early in the observation period with venlafaxine (Fig. 2).

Escitalopram 1.0 mg/kg had no significant effects on the time spent in paradoxical sleep. A weak decrease in the early observation period was seen for 2.0 mg/kg escitalopram. Paroxetine 1.1 mg/kg produced an increase from 2 h onwards, whereas paroxetine 2.2 mg/kg decreased the time spent in paradoxical sleep in the early observation period. A marked

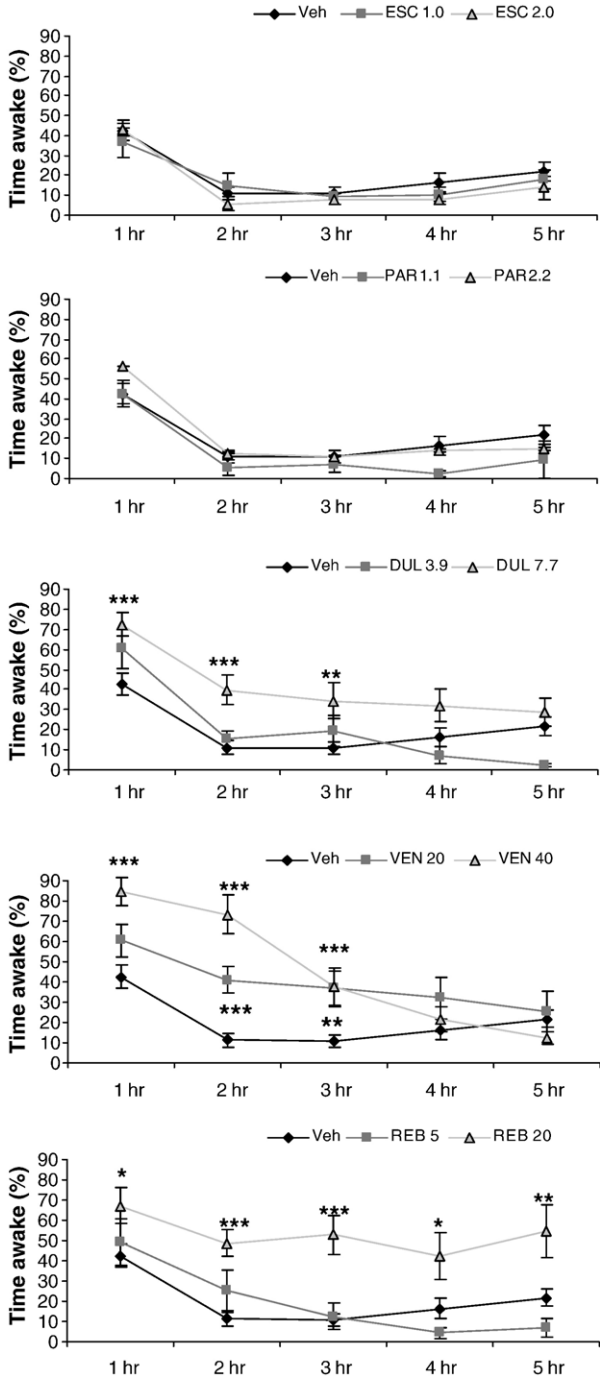

Fig. 2. Effects of single doses (i.p.) of vehicle, escitalopram (ESC; 1.0 and 2.0 mg/kg), paroxetine (PAR; 1.1 and 2.2 mg/kg), duloxetine (DUL; 3.9 and 7.7 mg/kg), venlafaxine (VEN; 20 and 40 mg/kg) and reboxetine (REB; 5 and 20 mg/kg) on time awake per hour over a 5 h recording interval (mean±SEM). *$p<0.05$ *versus* vehicle, **$p<0.01$ *versus* vehicle, ***$p<0.001$ *versus* vehicle (for each treatment group a 2-way repeated measure ANOVA *versus* vehicle followed by Newman–Keuls test). A total of 8 rats were included in each treatment group, except the vehicle control where $n=20$.

detrimental effect was seen throughout the observation period for all doses of venlafaxine, duloxetine, and reboxetine, with a slightly lesser effect for duloxetine 3.9 mg/kg (Fig. 3).

Escitalopram and paroxetine both generally increased light slow-wave sleep, with the exception of escitalopram 1.0 mg/kg, which resulted in a slight decrease in the middle of the observation period. Duloxetine 3.9 mg/kg and reboxetine 5.0 mg/kg increased light slow-wave sleep at the late observation period, whereas higher doses of the same compounds suppressed it. Venlafaxine 20 mg/kg produced a

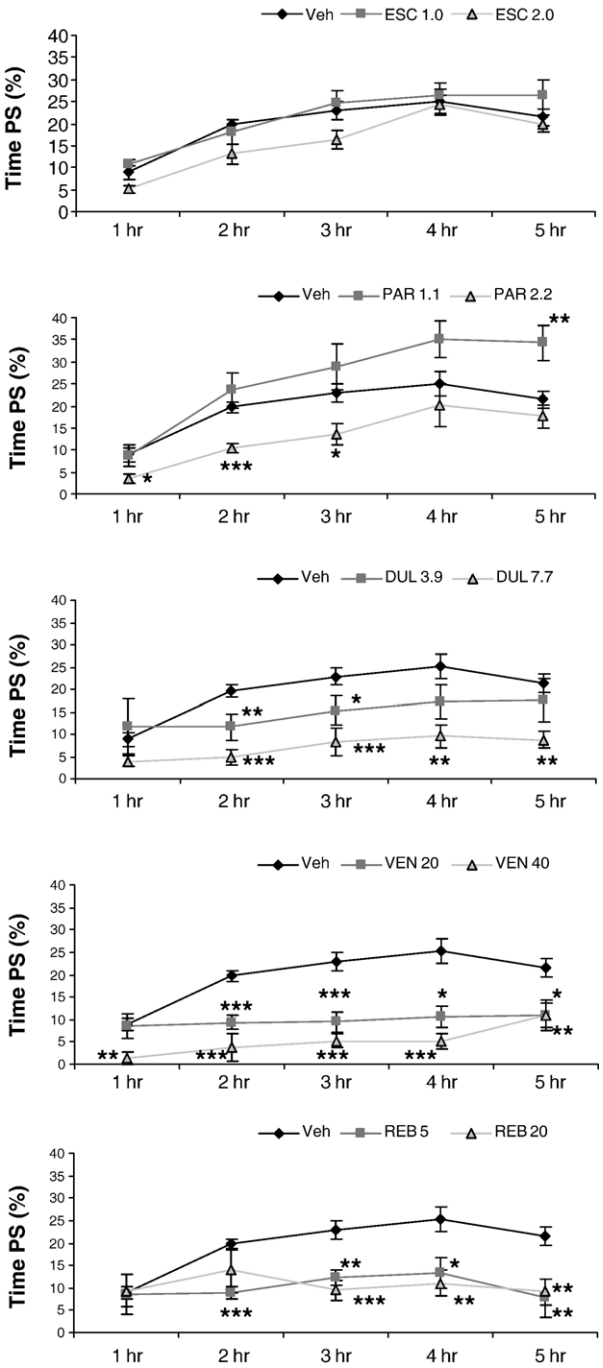

Fig. 3. Effect of single doses (i.p.) of vehicle, escitalopram (ESC; 1.0 and 2.0 mg/kg), paroxetine (PAR; 1.1 and 2.2 mg/kg), duloxetine (DUL; 3.9 and 7.7 mg/kg), venlafaxine (VEN; 20 and 40 mg/kg) and reboxetine (REB; 5 and 20 mg/kg) on time in paradoxical sleep per hour over a 5 h recording interval (mean±SEM). *$p<0.05$ *versus* vehicle, **$p<0.01$ *versus* vehicle, ***$p<0.001$ *versus* vehicle (for each treatment group a 2-way repeated measure ANOVA *versus* vehicle followed by Newman–Keuls test). A total of 8 rats were included in each treatment group, except the vehicle control where $n=20$.

sustained suppression, whereas venlafaxine 40 mg/kg initially suppressed light slow-wave sleep, but towards the second half of the observation period this was reversed to an enhancing effect (Fig. 4).

For deep slow-wave sleep, escitalopram 1.0 mg/kg had an enhancing effect, whereas paroxetine 2.2 mg/kg suppressed deep slow-wave sleep. Duloxetine 3.9 mg/kg, venlafaxine 40 mg/kg and reboxetine 5 mg/kg generally had a suppressing effect, whereas duloxetine 7.7 mg/kg and venlafaxine 20 mg/kg enhanced deep slow-wave sleep, towards the end of the observation period. Virtually no effect was observed with reboxetine 20 mg/kg (Fig. 5).

To further elucidate the reuptake activity of the two SNRIs, duloxetine (3.8, 7.7 and 15.3 mg/kg) and venlafaxine (5, 10 and 20 mg/kg), at the 5-HTT and NAT, a series of 2 h microdialysis experiments in the ventral hippocampus of freely moving rats was performed.

Duloxetine, at doses of 3.8, 7.7, and 15.3 mg/kg, induced an increase in extracellular levels of both 5-HT and NA. Maximal increase of 5-HT release was achieved already at the lower doses, whilst increasing doses induced a robust enhancement of extracellular levels of NA. Thus, at the lowest doses, duloxetine may recruit the 5-HT system, whereas activation of the NA system requires higher doses. Venlafaxine (5, 10, and 20 mg/kg) preferentially increased extracellular 5-HT over NA levels in the beginning of the experiment whereas the 5-HT and NA systems appeared to be equally affected at the later time points (90 and 120 min). The highest venlafaxine dose used in the EEG studies (40 mg/kg) was not measured in microdialysis experiments (Fig. 6).

4. Discussion

All of the antidepressants tested had an overall effect on sleep architecture, as well as on the hourly sleep patterns in rats during the period when they normally sleep (the light phase of the diurnal cycle). Furthermore, the effects were dose-dependent, with higher doses generally being more disruptive to sleep patterns than lower doses.

The doses used in the present study were chosen based on their pharmacological activity in behavioural models of depression and anxiety in the rat, using comparable routes of administration and vehicles. Thus, in the forced swim test in rats at doses similar to those of the present study, venlafaxine, duloxetine, and reboxetine significantly reduced immobility time compared to vehicle (Rénéric and Lucki, 1998; Connor et al., 1999). In addition, escitalopram and paroxetine demonstrated pharmacological activity in doses of approximately 1 and 2 mg/kg in ultrasonic vocalisation and conditioned fear tests in the rat (Sánchez and Meier, 1997; Sánchez, 2003; Sánchez et al., 2003a,b).

The greatest disruption in overall and hourly sleep patterns was seen with the SNRIs and the NRI, indicating that the enhanced NA levels confer a disruptive effect on the sleep pattern. Our results with duloxetine are similar to those reported by Katoh et al. (1995), and those with venlafaxine are similar to those reported by Salín-Pascual and Moro-Lopez (1997).

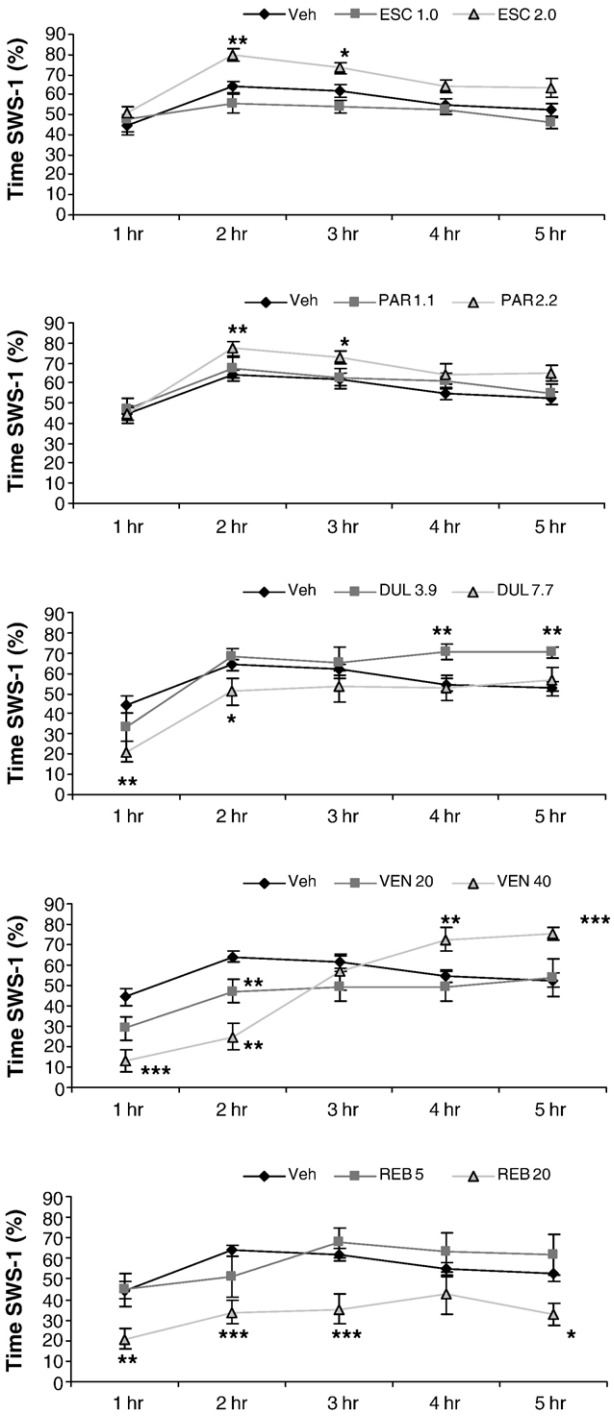

Fig. 4. Effects of single doses (i.p.) of vehicle, escitalopram (ESC; 1.0 and 2.0 mg/kg), paroxetine (PAR; 1.1 and 2.2 mg/kg), duloxetine (DUL; 3.9 and 7.7 mg/kg), venlafaxine (VEN; 20 and 40 mg/kg) and reboxetine (REB; 5 and 20 mg/kg) on time in light slow-wave sleep (SWS-1) per hour over a 5 h recording interval (mean±SEM). *$p<0.05$ *versus* vehicle, **$p<0.01$ *versus* vehicle, ***$p<0.001$ *versus* vehicle (for each treatment group a 2-way repeated measure ANOVA *versus* vehicle followed by Newman–Keuls test). A total of 8 rats were included in each treatment group, except the vehicle control where $n=20$.

Our *in vitro* uptake data from rat synaptosomes revealed that venlafaxine and duloxetine have 5-HTT/NAT selectivity ratios of approximately 0.19 and 0.61, respectively, suggesting that

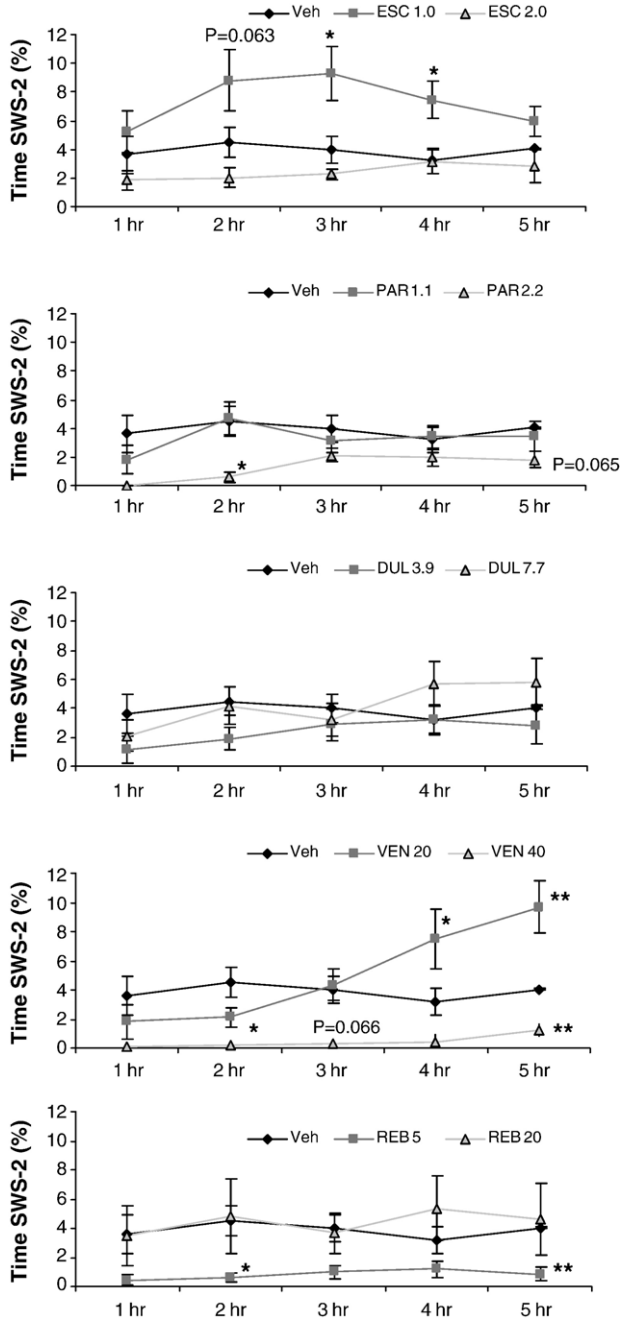

Fig. 5. Effects of single doses (i.p.) of vehicle, escitalopram (ESC; 1.0 and 2.0 mg/kg), paroxetine (PAR; 1.1 and 2.2 mg/kg), duloxetine (DUL; 3.9 and 7.7 mg/kg), venlafaxine (VEN; 20 and 40 mg/kg) and reboxetine (REB; 5 and 20 mg/kg) on time in deep slow-wave sleep (SWS-2) per hour over a 5 h recording interval (mean±SEM). *$p<0.05$ *versus* vehicle, **$p<0.01$ *versus* vehicle, ***$p<0.001$ *versus* vehicle (for each treatment group a 2-way repeated measure ANOVA *versus* vehicle followed by Newman–Keuls test). A total of 8 rats were included in each treatment group, except the vehicle control where $n=20$.

venlafaxine is preferentially a 5-HT reuptake inhibitor at low doses. However, the venlafaxine (20 but not 10 mg/kg, i.p. (unpublished observation)) and duloxetine (7.7 but not 3.9 mg/kg, i.p.) doses related to disrupted sleep architecture are likely to produce markedly increased NA release, e.g. approximately 500% increase 2 h after venlafaxine and duloxetine 20 and 7.7 mg/kg, s.c., respectively. Even though a complete 5 h time course was not measured, the microdialysis data support at least at the qualitative level the notion that the NA component (activating component) of the SNRIs accounts for the sleep disruptive effects of this drug class. Thus duloxetine 3.9 and 7.7 mg/kg increased NA release approximately 250 and 500% (after 1 h) and 300 and 500% (after 2 h), respectively, compared to baseline. Only duloxetine, 7.7 mg/kg, increased time awake significantly at the two time points. This may suggest a concentration response relationship between NA release and wake promoting effect. At the same time points 5-HT releases were of the same order of magnitude for high and low doses of duloxetine. The microdialysis data for venlafaxine may suggest that the enhanced NA level is longer lasting than the 5-HT level and it may be speculated whether this could account for the long lasting effects of venlafaxine on the sleep architecture.

Compared to vehicle, escitalopram and paroxetine generally showed less disruption in both overall and hourly sleep patterns than did the SNRIs and the NRI. Compared to paroxetine, escitalopram appeared to be less disruptive to sleep architecture. One explanation for this observation may lie in the different

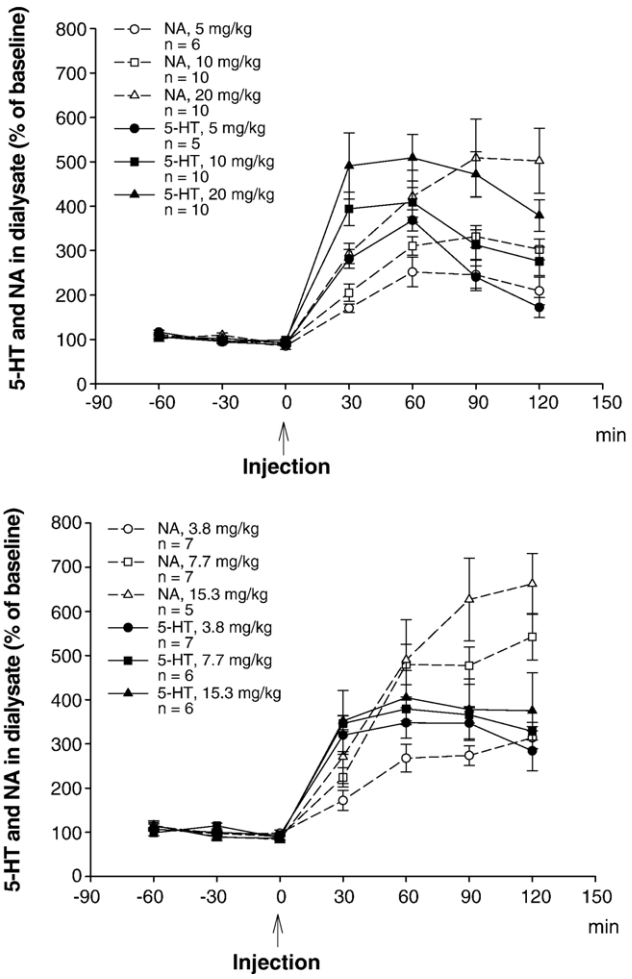

Fig. 6. Effects of single injections (s.c.) of duloxetine (DUL; 3.8, 7.7 and 15.3 mg/kg) and venlafaxine (VEN; 5, 10 and 20 mg/kg) on extracellular 5-HT and NA in the ventral hippocampus of freely moving rats. Data given as mean±SEM (mean basal pre-injection values normalised to 100%).

levels of selectivity. Although paroxetine is classified as a selective 5-HT reuptake inhibitor, our data reveal a substantial blockade of the NAT, paroxetine being far less selective for 5-HTT than is escitalopram. This is in agreement with the *in vivo* findings by Owens et al. (2000). In addition, paroxetine weakly inhibits dopamine reuptake and has weak affinity for muscarinic cholinergic receptors, whereas escitalopram has negligible affinity for these targets (Owens et al., 2001). Alternatively, since escitalopram is a serotonin dual action antidepressant that binds to both the primary site on the serotonin transporter as well as to the allosteric site of the 5-HTT, its mechanism of action is slightly different from that of paroxetine (Chen et al., 2005) and this may underlie the differential effects on sleep EEG.

It may be speculated whether the results of the present study share any similarity to the findings in the clinic with its limitation of being acute studies in a non-disease-state animal model. Effects on sleep architecture similar to those described here for rats have been found in humans for some of the drugs. Studies with paroxetine have revealed that sleep efficiency is decreased in early treatment, and a marked suppression of REM sleep is seen (Röschke et al., 1997; Schlösser et al., 1998; Nowell et al., 1999; Silvestri et al., 2001; Hicks et al., 2002). A study of sertraline in patients with depression also documented a suppressing effect on REM sleep (Jindal et al., 2003). Venlafaxine has been shown to increase REM latency time and to decrease total REM time in patients with major depressive disorder and healthy volunteers. Furthermore, venlafaxine impaired the sleep continuity pattern without significantly affecting SWS duration (Luthringer et al., 1996; Salín-Pascual et al., 1997). This is consistent with the overall picture from the present study. For reboxetine, preliminary results indicate a prolonged REM latency combined with a REM suppression effect (Farina et al., 2002). Polysomnograph data from citalopram (Wilson et al., 2004) showed an increase in REM latency time and a decrease in total REM time in healthy subjects. There are currently no published sleep EEG studies with escitalopram, although *post-hoc* analysis of the sleep item on the Montgomery–Åsberg Depression Rating Scale suggest that escitalopram has a significant beneficial effect compared with placebo or citalopram in reducing sleep disturbance in patients suffering from major depressive disorder (Lader et al., 2005).

It has been suggested that the REM suppressive effect of antidepressant in part may account for the efficacy of the compounds (Vogel et al., 1990). However, although there has been a certain degree of consensus about the hypothesis, recent polysomnograph data have challenged this statement, since data from novel antidepressants that have proven efficacious in the treatment of depression showed no marked REM suppression (Hicks et al., 2002; Suzuki et al., 2002).

Taking the pre-clinical and the clinical data together, it may be hypothesised that some antidepressants may produce less disturbance in sleep architecture and hence potentially aid in the restoration of normal sleep patterns in depressed patients, whereas the disruptive effects on sleep of venlafaxine, duloxetine, and reboxetine could result in a slower and/or more incomplete recovery of normal sleep patterns in patients treated with these compounds. However, only further sleep EEG studies with antidepressants in disease-state animal models, healthy humans, and depressed patients can further elucidate these points. In addition, it would be of interest to investigate the role of long-term treatment, since many adaptive mechanisms are known to come into play with the chronic administration of antidepressants.

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