Chronic agomelatine treatment corrects the abnormalities in the circadian rhythm of motor activity and sleep/wake cycle induced by prenatal restraint stress in adult rats

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Abstract

Agomelatine is a novel antidepressant acting as an MT1/MT2 melatonin receptor agonist/5-HT2C serotonin receptor antagonist. Because of its peculiar pharmacological profile, this drug caters the potential to correct the abnormalities of circadian rhythms associated with mood disorders, including abnormalities of the sleep/wake cycle. Here, we examined the effect of chronic agomelatine treatment on sleep architecture and circadian rhythms of motor activity using the rat model of prenatal restraint stress (PRS) as a putative ‘aetiological’ model of depression. PRS was delivered to the mothers during the last 10 d of pregnancy. The adult progeny (‘PRS rats’) showed a reduced duration of slow wave sleep, an increased duration of rapid eye movement (REM) sleep, an increased number of REM sleep events and an increase in motor activity before the beginning of the dark phase of the light/dark cycle. In addition, adult PRS rats showed an increased expression of the transcript of the primary response gene, c-Fos, in the hippocampus just prior to the beginning of the dark phase. All these changes were reversed by a chronic oral treatment with agomelatine (2000 ppm in the diet). The effect of agomelatine on sleep was largely attenuated by treatment with the MT1/MT2 melatonin receptor antagonist, S22153, which caused PRS-like sleep disturbances on its own. These data provide the first evidence that agomelatine corrects sleep architecture and restores circadian homeostasis in a preclinical model of depression and supports the value of agomelatine as a novel antidepressant that resynchronizes circadian rhythms under pathological conditions.

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Introduction

The circadian system plays a major physiological role to ensure optimal functioning of the organism and its adaptation to predicted environmental changes (Gerstner & Yin, 2010). Disruption of circadian rhythms including the sleep–wake cycle is considered as a core symptom and therapeutic target in mood disorders (Fountoulakis, 2010; Kennaway, 2010; Wulff et al. 2010). Depressive patients show a lower threshold for awakening, rapid eye movement (REM) sleep disorders and loss of delta wave-related sleep, which represents the deepest stage of non-REM (NREM) sleep (Akiskal & McKinney, 1975; McClung, 2007). The relationship between sleep abnormalities...
and mood disorders is strengthened by the evidence that the sleep/wake cycle critically regulates synaptic plasticity in the hippocampus and cerebral cortex, with wakefulness and sleep being associated with synaptic potentiation and depression, respectively (Vyazovskiy et al. 2008). Remarkably, synaptic homeostasis in the hippocampus is dysregulated in mood disorders and restored by antidepressant drugs (Berton & Nestler, 2006; Duman et al. 2007).

Thus, an 'optimal' antidepressant drug should behave as a resynchronizer of circadian rhythms, including the sleep/wake rhythm, yet most classical antidepressants only partially meet this requirement (Fountoulakis, 2010; Kennaway, 2010; Pandi-Perumal et al. 2009). Agomelatine, a recent drug marketed in Europe for the treatment of major depression (de Bodinat et al. 2010), can be considered the potential to resynchronize circadian rhythms because it activates MT₁ and MT₂ melatonergic receptors (Audinot et al. 2003) and also blocks 5-HT₂C receptors (Millan et al. 2003). A single injection of agomelatine has been shown to influence the sleep/wake cycle architecture in 'healthy' rats (Descamps et al. 2009; Tobler et al. 1994) and to restore sleep synchronization in rats infected by Trypanosome brucei (Grassi-Zucconi et al. 1996). In addition, a chronic treatment with agomelatine corrects abnormalities of circadian rhythms in a rat model of the delayed sleep-phase syndrome (Armstrong et al. 1993). However, there are no available data on the effect of agomelatine on sleep/wake cycle abnormalities in animal models of mood disorders.

Here, we used rats subjected to prenatal restraint stress (PRS), which can be considered as a model endowed with 'construct validity', i.e. a model that incorporates factors implicated in the aetiology of major depression (Krishnan & Nestler, 2010; Nestler & Hyman, 2010). PRS rats show a generalized disorganization of circadian rhythms and sleep/wake cycle (Dugovic et al. 1999; van Reeth et al. 2000, 2007), a long-term impairment in the feedback regulation of the hypothalamic-pituitary-adrenal axis (Maccari et al. 1995), increased anxiety-like behaviour (Vallee et al. 1997; Zuena et al. 2008), reduced active coping and social play (Morley-Fletcher et al. 2003), an age-dependent impairment in spatial learning (Darnaudery et al. 2006), a reduced adult neurogenesis in the hippocampal dentate gyrus (Lemaire et al. 2000; Morley-Fletcher et al. 2011; Zuena et al. 2008) and biochemical changes that reflect abnormalities of synaptic transmission and plasticity in the hippocampus (Mairesse et al. 2007; Zuena et al. 2008).

We now report that chronic agomelatine treatment corrects abnormalities in the circadian rhythms of motor activity and sleep architecture in PRS rat.

Material and method

Animals

Sprague–Dawley nulliparous female rats (250 g) were purchased from a commercial breeder (Harlan, Italy). Animals were kept at a constant temperature (22 ± 2°C), with a regular 12-h light/dark cycle (lights on 08:00 hours). Water and food were available ad libitum. After arrival, females were group housed (four per cage) to coordinate their oestrous cycle for 1 wk before being placed with a sexually experienced male for a night. The following day, designated as day 0 of gestation, females were individually housed in Plexiglas cages (30 × 20 × 15 cm). Pregnant females were then randomly assigned to PRS or control (Con) groups (n = 18 in each group).

Stress procedure

PRS was carried out according to our standard protocol (Maccari et al. 1995): from day 11 of pregnancy until delivery, pregnant female rats were subjected daily to three stress sessions starting at 09:00, 12:00 and 17:00 hours, during which they were placed in plastic transparent cylinders (diameter = 7 cm; length = 19 cm), under a bright light for 45 min. Con pregnant females were left undisturbed. Offspring were weaned 21 d after birth and only male offspring from litters containing 10–14 pups with equivaled sex ratio were used in the present study. After weaning, male rats from each experimental group (Con or PRS) were housed per three until experiments started (age 2 months). All experiments followed the rules of the European Communities Council Directive 86/609/EEC.

See Fig. 1 for experimental protocol and time line.

Agomelatine and S22153 treatment

Agomelatine was orally administered (Ago group) at 2000 ppm in a mixed diet available ad libitum for 5 wk in expt 1 (circadian running wheel activity) and for 3 wk in expt 2 (sleep, in-situ hybridization) and expt 3 (sleep, S22153). The dose of 2000 ppm was determined based on the effects of agomelatine on circadian rhythms in a previous study (van Reeth et al. 2001; Weibel et al. 2000). PRS and Con vehicle-treated animals were fed with virgin mixed diet (Veh groups).
Fig. 1. Experimental design. We used three different experimental conditions. Prenatal restraint stress (PRS) and control (Con) rats (aged 2 months) were assigned to the first, second or third experimental procedure. In expt 1, circadian running wheel activity was recorded after feeding with virgin dust food and then after feeding with dust food containing agomelatine (2000 ppm). In expt 2, sleep was recorded after feeding with dust food containing or not containing agomelatine (2000 ppm). At the end of the sleep recording procedure, animals were killed and brains were processed for in-situ hybridization analysis of c-Fos mRNA. In expt 3, the MT$_1$ receptors antagonist (S22153, 10 mg/kg i.p.) or its vehicle (1% hydroxyethylcellulose) were administered 1 h before the beginning of the dark phase of the light/dark cycle to Con and PRS rats receiving or not receiving agomelatine in the food (2000 ppm). EEG, electroencephalography; EMG, electromyography.
For expt 3, the MT$_{1/2}$ receptors antagonist (S22153, 10 mg/kg i.p.) or its vehicle (1% hydroxyethylcellulose) was administrated 1 h before the beginning of the dark phase of the light/dark cycle to Con and PRS rats receiving or not receiving agomelatine in the food (2000 ppm).

Running wheel activity

In expt 1, PRS and Con male rats ($n=10/group$) were individually housed in cages equipped with a 14-inch running wheel to allow continuous recording of locomotor activity through an electrical switch connected to a computer (Chronobiology Kit software package; Stanford Software Systems, USA; Fig. 1). During the course of the experiments, a powder diet and water were provided ad libitum, room temperature (22°C) and humidity (60%) were kept constant and light intensity was set at 40–50 lx at cage floor level (van Reeth et al. 2001). Animals were treated with vehicle and maintained under these conditions for 3–4 wk before showing a uniform and regular pattern of circadian running activity. After 4 wk of habituation, running activity was recorded continuously for 8 d. The rats were then treated for 5 wk with agomelatine and the rhythms of activity were individually analysed over the last 8 d. The onset of activity was identified with a 5-min resolution and was defined as the first time-point at which the mean intensity of activity was >10% of the maximum and remained above that point for at least 50% of the time during the following 30 min. The reversed procedure was used for the cessation (offset) of activity (first time-point <10% of maximum and activity remained below that point for at least 50% of the time during the following 30 min). The time elapsed between the onset and offset of activity was defined as the total time of activity ($\tau$).

Experimental procedure for sleep recording

Surgery

PRS and Con rats (aged 2 months; expt 2: $n=6/group$; expt 3: $n=4/group$) were anaesthetized with ketamine/xylazine (75/10 mg/kg i.m.). Electrodes for the assessment of sleep–wake parameters were chronically implanted under aseptic conditions. Three stainless steel screw electrodes were threaded through the skull bilaterally over the frontal and parietal cortex to record electroencephalography (EEG). One screw electrode threaded through the midline of the frontal bone was used as ground. Teflon-coated multi-stranded stainless steel wires with 2 mm exposed at the tips (Goodfellow Sarl, France) were placed in the dorsal neck muscles to record electromyography (EMG). EEG and EMG leads were attached to small connectors (MS363; Plastics One Inc., Germany) and fixed to the skull with dental acrylic. Lidocaine (0.5%) was topically administered.

Recording

After surgery, rats were individually housed in Plexiglas cages (30 cm diameter, 40 cm high) and left undisturbed for a post-surgery recovery period of 2 wk. During the second week of recovery, rats were habituated to virgin dust food. Animals were then habituated to the sleep recording procedure for the following 3 wk, during which they received agomelatine (2000 ppm in dust food) or vehicle (virgin food). Habituation consisted of two recording sessions of 8 h and two sessions of 24 h. At the end of the habituation and treatment period, sleep was continuously recorded for 24 h. For registration, the electrodes were connected to a preamplifier (8213; Pinnacle Technology Inc., USA) through the plastic connector placed on the animal’s head. The preamplifier was connected through a rotating swivel to the EEG/EMG Data Conditioning & Acquisition System (8206; Pinnacle Technology Inc.). Signal acquisition was performed using the Sirena acquisition suite (Pinnacle Technology). EEG and EMG were recorded in 10-s bouts at a frequency of 400 Hz. Both EEG channels were high- and low-pass filtered at 0.5–40 Hz. EMG was high-pass filtered at 10 Hz and subjected to a 100 Hz low-pass cut-off.

At the end of the sleep registration procedure (expt 2), rats were killed, in accordance with the rules of the European Communities Council and French Directive, by a short exposure to CO$_2$ followed by decapitation. Brains were rapidly dissected and stored at −80°C until cryostat slicing. This method limits non-specific changes in c-Fos mRNA expression caused by long CO$_2$ exposure.

Vigilance state analysis

Vigilance states were visually scored offline using Sirena score suite (Pinnacle Technology), by a researcher blind to treatment. Scoring was performed on 10-s epochs as being awake (low-voltage, high-frequency EEG with high-amplitude EMG), NREM sleep (high-voltage, low-frequency EEG with low-amplitude EMG) or REM sleep (prominent 0 activity in EEG channels and low EMG). The amount of time spent in the three vigilance states and number and duration of episodes for each state were recorded. Sleep–wake parameters were analysed over 1–12 h...
intervals, as well as over the total 24 h. The time spent in the different states of vigilance was expressed in min.

In situ hybridization

Cryostat sections obtained from five animals per group of the six rats used in the sleep study were processed for in situ hybridization. Riboprobes ([35]cRNA) of c-Fos (coding region 583–1250 of rat c-Fos) were radioactively labelled by using RNA polymerases (Roche Diagnostic, Germany) in the presence of both [35]SCTP and [35]SUTP. Serial coronal brain sections (18 μm thick) were cut on a cryostat (Microm, Germany) and hybridized at 60 °C for 16 h in a buffer containing [35]cRNA (75 × 10^4 dpm/ml, for c-Fos), 10% dextran sulfate, 50% formamide, 1 × Denhardt’s solution, 100 μg/ml denatured salmon sperm DNA, 0.15 mg/ml tRNA and 40 m M dithiothreitol. After hybridization, the sections were exposed to Kodak Biomax film (Sigma Aldrich, Italy) for 48 h. The specificity of the hybridization signal was ascertained by hybridization of the same sections labelled with corresponding sense probes.

Densitometric analyses

 Autoradiography films were scanned at high resolution (1200 dpi) and a brain atlas (Paxinos & Watson, 2005) was used to define localization of brain structures. Quantitative analyses of hybridized signals were performed using Scion Image software (Scion Corporation, USA). Optical density (OD) values were converted into radioactivity concentrations by densitometric analysis of ^14C-microscale standards (American Radiolabeled Chemicals, USA), so as to create for each film a calibration curve with a linear coefficient \( r^2 > 0.9 \). In every brain section, the OD of the corpus callosum was used as background and an integrated OD value was calculated as radioactivity per extension of hybridized area. Measurements were obtained for at least eight consecutive tissue sections containing the target structure for each animal (Gaetani et al. 2010).

Statistical analysis

Data from expt 1 were analysed using two-way analysis of variance (ANOVA) with repeated measures [group as between variable (Con and PRS) × measures before and after agomelatine treatment as within variable]. Data from expt 2 were analysed using two-way ANOVA [two groups (Con and PRS) × two treatments (Veh or agomelatine)]. Data from expt 3 were analysed using three-way ANOVA [two groups (Con and PRS) × two treatments in food (Veh or agomelatine) × two treatments injected i.p. (Veh or S22153)]. The ANOVA analyses were always followed by Newman–Keuls post-hoc comparisons. The level of significance was set at \( p < 0.05 \). Correlations between c-Fos expression and sleep parameters were tested using Pearson’s correlation analysis.

Results

Chronic agomelatine reversed the alterations of the circadian running wheel activity induced by PRS

In expt 1 (Fig. 1), we studied the circadian rhythm of running wheel activity under a regular 12 h (08:00–20:00 hours) light/dark cycle over eight consecutive days in PRS and Con rats fed with virgin food (Veh; Figs. 1, 2). PRS induced a phase advance for both the onset and offset of the running wheel activity without altering the duration of the period of activity (Fig. 2a, b) (activity onset: ANOVA for group effect; \( F_{1,18} = 4.38, p < 0.05 \); Newman–Keuls post-hoc test, PRS/Veh vs. Con/Veh, \( p < 0.05 \); activity offset, group effect; \( F_{1,18} = 5.47, p < 0.05 \); PRS/Veh vs. Con/Veh, \( p < 0.05 \)). In addition, PRS rats showed an increase in total activity (total number of wheel revolutions) per day (Fig. 2c; group × treatment interaction: \( F_{1,18} = 4.60, p < 0.05 \), PRS/Veh vs. Con/Veh, \( p < 0.05 \)).

We also examined the effect of chronic agomelatine treatment on the circadian rhythm of running wheel activity in Con and PRS rats. Agomelatine was delivered in the food (2000 ppm mixed in virgin food) to avoid stress by manipulation/injection during the recording of motor activity. The amount of agomelatine consumed with the food roughly corresponds to a daily dose of 20 mg/kg (Weibel et al. 2000). Daily food consumption and body weight gain in the 3 wk of treatment did not differ between Con and PRS rats and were not influenced by the presence of agomelatine in the food (data not shown).

The analysis over eight consecutive days of the circadian rhythm of running wheels of PRS and Con animals after 5 wk of agomelatine treatment revealed a reversal effect of agomelatine on some of the chronobiological alterations induced by PRS (Figs. 1, 2). After agomelatine treatment, the phase advance of the onset of activity induced by PRS was less pronounced. Agomelatine treatment tended to reverse the phase advance in the onset of activity (group × treatment interaction: \( F_{1,18} = 3.91, p = 0.07 \), PRS/Veh vs. PRS/Ago, \( p = 0.08 \)) and completely reversed the increase of total activity per day observed in PRS rats (group × treatment interaction: \( F_{1,18} = 4.60, p < 0.05 \), PRS/Veh vs. PRS/Ago, \( p = 0.08 \)).
In Con rats, the onset of activity and the total activity per day were not affected by agomelatine treatment. Finally, in both PRS and Con rats, agomelatine treatment induced an anticipation of the offset of activity (Fig. 2b; treatment effect: $F_{1,18}=12.44$, $p<0.002$), thus leading to a reduced duration of the period of activity ($\alpha$, group effect: $F_{1,18}=19.89$, $p<0.0003$).

**Chronic agomelatine reversed the alterations of the 24-h sleep/wake pattern induced by PRS**

In expt 2 (Fig. 1), we studied the characteristics of the wake, NREM sleep and REM sleep of PRS and Con rats during a complete 12 h/12 h light/dark cycle after 3 wk of chronic treatment with dust food containing or not containing agomelatine (Figs. 1, 3–5, Table 1). Analysis of the animals treated with the virgin food (Veh) revealed that PRS altered both REM and NREM sleep (see also Dugovic et al. 1999). PRS increased REM sleep duration (Fig. 3; ANOVA for group effect: $F_{1,20}=5.83$, $p<0.05$; Newman–Keuls post-hoc test: PRS/Veh vs. Con/Veh, $p<0.05$), increased the number of episodes of REM sleep (Fig. 3; group effect: $F_{1,20}=5.22$, $p<0.05$; PRS/Veh vs. Con/Veh, $p<0.05$) and reduced the duration of NREM sleep (Fig. 4; group effect: $F_{1,20}=4.32$, $p<0.05$; PRS/Veh vs. Con/Veh, $p<0.05$). PRS had no effect on wake (Fig. 5).
Agomelatine treatment reversed the effect of PRS by reducing the duration of REM sleep (Fig. 3; group \( \times \) treatment interaction: \( F_{1,28} = 5.48, p < 0.05 \); PRS/Ago vs. PRS/Veh, \( p < 0.05 \)), by decreasing the number of REM sleep episodes (Fig. 3; treatment effect: \( F_{1,28} = 13.27, p < 0.005 \); PRS/Ago vs. PRS/Veh, \( p < 0.05 \)) and by increasing the duration of NREM sleep (Fig. 4; group \( \times \) treatment interaction: \( F_{1,28} = 4.97, p < 0.05 \); PRS/Ago vs. PRS/Veh, \( p < 0.05 \)). Agomelatine treatment did not affect the sleep/wake architecture in Con rats (Fig. 5).

### Chronic agomelatine had opposite effects in Con and PRS rats on hippocampal and cortical c-Fos expression

We measured the expression of c-Fos mRNA by in situ hybridization at the end of sleep registration, 1 h before switching off the lights (Figs 1, 6). In rats fed virgin food, PRS increased the expression of c-Fos mRNA in the hippocampal CA3 region (Fig. 6b; ANOVA for group \( \times \) treatment interaction: \( F_{1,18} = 18.92, p < 0.0005 \); Newman–Keuls post-hoc test: PRS/Veh vs. Con/Veh, \( p < 0.05 \)) and dentate gyrus (Fig. 6c; group \( \times \) treatment interaction: \( F_{1,18} = 29.26, p < 0.0001 \), PRS/Veh vs. Con/Veh, \( p < 0.05 \)). This is consistent with the increased Fos protein expression seen in the CA3 region and dentate gyrus of PRS rats (Viltart et al. 2006). Agomelatine reversed the effect of PRS animals by reducing c-Fos mRNA expression in both the CA3 region (PRS/Ago vs. PRS/Veh, \( p < 0.05 \)) and dentate gyrus (PRS/Ago vs. PRS/Veh, \( p < 0.05 \)). Interestingly, agomelatine treatment induced opposite changes in Con rats by increasing c-Fos mRNA expression in both the CA3 region (Con/Ago vs. Con/Veh, \( p < 0.05 \)) and dentate gyrus (Con/Ago vs. Con/Veh, \( p < 0.05 \)).

**Correlation between c-Fos expression in the CA3 region of the hippocampus and duration of REM sleep**

A growing body of evidence suggests the involvement of hippocampal activity in the regulation of REM sleep (Best et al. 2007; Hegde et al. 2008; Karlsson \\& Blumberg, 2003; Staba et al. 2002). Hence, we examined the correlation between hippocampal c-Fos mRNA expression and duration of REM sleep in our experimental groups. Expression of c-Fos mRNA was measured at the end of the light period, when there was a higher frequency of REM episodes. We found a strong correlation when we pooled data obtained in Con and PRS rats treated with vehicle, although Con and PRS data were grouped at the opposite sides of the correlation line (Fig. 7a; \( r = 0.82, p = 0.004 \)). The correlation was still present after adding the data obtained in ‘pathological’ PRS rats treated with agomelatine (Fig. 7b; \( r = 0.7, p = 0.004 \)). Agomelatine treatment moved PRS data close to the data obtained in Con rats treated with vehicle, evidence that supports the restorative action of agomelatine in PRS rats. Interestingly, we lost the correlation after adding data obtained in Con rats treated with agomelatine, in which the increase in c-Fos mRNA was not associated with changes in REM sleep (Fig. 7c). There was no correlation between NREM sleep duration and Fos expression in the hippocampus or cerebral cortex (data not shown).

**MT1/MT2 melatonin receptor blockade caused alterations in the 24-h sleep/wake pattern in Con rats and counterbalanced the action of agomelatine in PRS rats**

In expt 3, we studied the 24-h sleep/wake pattern in Con and PRS rats receiving virgin food or

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**Table 1.** Wake, non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep durations during the light phase and the dark phase of the light/dark cycle in control (Con) and prenatal restraint stress (PRS) rats treated by vehicle or agomelatine (expt 2)

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Agomelatine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light</td>
<td>Dark</td>
</tr>
<tr>
<td>Wake</td>
<td>Con 275.8 ± 22.2</td>
<td>241.4 ± 16.8*</td>
</tr>
<tr>
<td>PRS</td>
<td>274.5 ± 15.9</td>
<td>455.0 ± 24.7*</td>
</tr>
<tr>
<td>NREM</td>
<td>Con 393.8 ± 16.6</td>
<td>260.1 ± 15.9*</td>
</tr>
<tr>
<td>PRS</td>
<td>372.1 ± 15.1</td>
<td>214.2 ± 17.8*</td>
</tr>
</tbody>
</table>

| REM        | Con 51.4 ± 4.7 | 37.5 ± 5.1* | 50.2 ± 7.6 | 28.7 ± 4.6* |
| PRS        | 73.3 ± 8.7 | 50.8 ± 8.3* | 46.8 ± 3.6 | 31.1 ± 5.6* |

Values are means (min) per 12 h of recording \( \pm \) S.E.M. Student’s t test: * \( p < 0.05 \), dark phase vs. light phase.
agomelatine-containing food as described above and injected daily i.p. with either vehicle or the MT₁/MT₂ melatonin receptor antagonist, S22153 (10 mg/kg) for 3 wk. Rats were injected always 1 h prior to the dark phase of the light/dark cycle (Fig. 1). Daily food consumption and body weight gain was not affected by S22153 treatment (data not shown). Data of REM duration, NREM duration and wake duration in Con and PRS rats receiving virgin or agomelatine-containing food and injected with vehicle were identical to those obtained in expt 2. Treatment with S22153 in Con rats receiving virgin food (indicated as ‘Veh/S22153’ in Fig. 8) increased the duration of REM sleep and reduced the duration of NREM sleep (three-way ANOVA for group × food treatment × i.p. treatment interaction. REM sleep duration: $F_{1,24} = 7.52$, $p < 0.01$; Newman–Keuls post-hoc test: Con/Veh/Veh vs. Con/Veh/S22153; NREM sleep duration: $F_{1,24} = 5.95$, $p < 0.05$; Con/Veh/Veh vs. Con/Veh/S22153, $p < 0.01$; Fig. 8). In Con rats treated with both agomelatine and S22153, values of REM duration were lower than those obtained with S22153 alone (Con/Ago/S22153 vs.
Con/Veh/S22153, \( p < 0.05 \). The effect of treatment with S22153 on REM sleep duration occluded the effect of PRS or vice versa. In addition, treatment with S22153 largely attenuated the rescuing effect of agomelatine on REM sleep duration in PRS rats (PRS/Ago/Veh vs. PRS/Ago/S22153, \( p < 0.01 \)). A clear-cut antagonism between S22153 and agomelatine in PRS rats was also seen by measuring NREM sleep duration (PRS/Ago/Veh vs. PRS/Ago/S22153, \( p < 0.05 \), Fig. 8).

Data of expt 3 on the number of REM sleep episodes, NREM sleep episodes and wake episodes (Fig. 8d-f) only partially overlapped with data obtained in expt 2. Con rats receiving virgin food and vehicle injection (Con/Veh/Veh) in expt 3 showed a lower number of REM sleep, NREM sleep and wake episodes than Con rats receiving virgin food (with no injections) in expt 2 (no statistics were applied here because the two groups of rats are not part of the same experimental setting). In expt 3, PRS substantially increased the number of REM sleep, NREM sleep and wake episodes (three-way ANOVA for group \( \times \) food treatment \( \times \) i.p. treatment interaction, number of REM
sleep episodes: $F_{1,24} = 14.4$, $p < 0.001$; post-hoc test: Con/Veh/Veh vs. PRS/Veh/Veh, $p < 0.0005$; number of NREM sleep episodes: $F_{1,24} = 12.09$, $p < 0.005$; Con/Veh/Veh vs. PRS/Veh/Veh, $p < 0.005$; number of wake episodes: $F_{1,24} = 13.42$, $p < 0.005$; Con/Veh/Veh vs. PRS/Veh/Veh, $p < 0.05$, Fig. 8). Agomelatine treatment reversed all these effects of PRS ($p < 0.001$, $p < 0.01$ and $p < 0.03$ for number of REM, NREM and wake episodes, respectively). In Con rats receiving virgin food, treatment with S22153 mimicked the effect of PRS in increasing the number of REM, NREM and wake episodes (Con/Veh/S22153 vs. Con/Veh/Veh, $p < 0.005$, $p < 0.001$, $p < 0.0001$, respectively). Again, values with agomelatine + S22153 were generally higher than values with agomelatine + vehicle injection and lower than values with virgin food + S22153 injection (number of REM sleep episodes: Con/Ago/S22153 vs. Con/Veh/S22153, $p < 0.01$; number of NREM sleep episodes: Con/Ago/S22153 vs. Con/Veh/S22153, $p < 0.001$; Con/Ago/S22153 vs. Con/Veh/S22153, $p < 0.001$; Con/Ago/S22153 vs. Con/Veh/S22153, $p < 0.001$).

Fig. 5. Wake profile over 24 h. (a) Wake circadian profile. (b) Wake duration per day. (c) Wake episode numbers per day. Values are means ± S.E.M. of six rats per group. * $p < 0.05$, prenatal restraint stress (PRS) vs. control (Con) rats; † $p < 0.05$, agomelatine (Ago) vs. virgin food (Veh).


**Discussion**

Adult PRS rats show an anxious/depressive phenotype that likely reflects a pathological epigenetic programming triggered by early life stress and, therefore, they represent a valuable ‘aetiological model’ of stress-related disorders including major depression (see Introduction and references therein). Here, we found that chronic agomelatine treatment corrected circadian abnormalities, including the sleep/wake cycle in PRS rats, thus offering evidence that agomelatine behaves as a resynchronizer of circadian rhythms in an animal model of mood disorders. Moreover, this is the first evidence that any antidepressant drug restores the circadian homeostasis disrupted in PRS rats (Dugovic et al. 1999; van Reeth et al. 2000).

Agomelatine was combined with food, which is mainly consumed by rats during the dark phase of the light/dark cycle, when animals are awake and active. In humans, agomelatine is administered *per os* in the late evening before sleeping (de Bodinat et al. 2010). We have applied this particular protocol of agomelatine administration because, in both rats and humans, melatonin peaks during the dark phase (Forsling, 2000) and agomelatine is able to activate both MT₁ and MT₂ melatonin receptors (Audinot et al. 2003).

There are numerous reports on the effect of agomelatine on circadian rhythms in Con animals. Agomelatine showed efficacy in entraining circadian rhythms of rats free-running in constant darkness (Martinet et al. 1996) and enhanced the duration of REM sleep and slow-wave sleep (SWS) when acutely administered shortly before the dark phase of the light/dark cycle in Con rats (Descamps et al. 2009). A chronobiotic effect of agomelatine was shown in old hamsters (Weibel et al. 2000) and in the diurnal rodent *Arvicanthis mordax* (van Reeth et al. 1998). In both models, agomelatine accelerated resynchronization of the circadian clock after phase shift of the light/dark cycle (van Reeth et al. 1998; Weibel et al. 2000). Agomelatine also corrected the abnormalities in circadian activity in a rat model of the delayed sleep-phase syndrome, in which the onset of nocturnal
Fig. 7. Correlation between c-Fos expression in the CA3 region of the hippocampus and duration of rapid eye movement (REM) sleep. A significant correlation was found when we pooled data obtained in control (Con) and prenatal restraint stress (PRS) rats treated with vehicle (Veh) with (a) or without (b) data of PRS rats treated with agomelatine (Ago). Correlation was lost after adding data of Con rats treated with Ago (c). (r: coefficient of Pearson’s correlation analysis; Con: open symbols; PRS: full symbols; Veh rats, square; Ago rats, triangles).

activity was delayed by several hours (Armstrong et al. 1993). Here, we have found that chronic agomelatine treatment corrected the reduction of SWS duration, the increase in REM sleep duration and the increase in REM sleep fragmentation seen in undisturbed (non-injected) PRS rats (see exp 2). In addition, chronic agomelatine treatment restored the normal circadian pattern of motor activity in PRS rats. These last data are in line with recent evidence that agomelatine restored the disturbed circadian rhythm of locomotor activity in the chronic corticosterone model of depression (Rainer et al. 2011). It can be argued that motor activity at the wheel might have interfered with the depressive phenotype of PRS rats, introducing a bias in our experiment. However, this is unlikely because PRS rats continue to show alteration in the circadian pattern of activity after several weeks of exercise in the wheel (see also van Reeth et al. 2000).

Thus, agomelatine can correct the abnormalities of circadian rhythms in two different animal models of depression. Agomelatine behaves as a mixed MT₁/MT₂ melatonin receptor agonist and 5-HT₂c receptor antagonist. To examine the involvement of melatonin receptors in the restoring effect of agomelatine on sleep architecture in PRS rats, we used the potent and brain-permeant MT₁/MT₂ receptor antagonist, S22153 (Kopp et al. 1999). This drug has already been used to dissect the role of melatonin receptors in the effect of agomelatine on anhedonia induced by chronic mild stress in rats (Papp et al. 2003). It was technically impossible to combine S22153 with agomelatine in the food. Thus, we decided to inject the drug i.p. (Papp et al. 2003) 1 h prior to the dark phase of the light/dark cycle. The injection procedure per se caused changes in sleep architecture and unraveled an effect of PRS on the frequency of NREM and wake episodes that was not seen in undisturbed rats. We found that treatment with S22153 per sc caused changes in sleep architecture that were similar to, and non-additive with, those induced by PRS. This nicely demonstrates that endogenous activation of melatonin receptors critically regulates sleep architecture and suggests that an impairment of the melatonergic system contributes to the disruptive effect of PRS on sleep. Remarkably, treatment with S22153 consistently attenuated or abolished the rescued effect of agomelatine on sleep architecture in PRS rats, suggesting that most, if not all, of the effects of agomelatine were mediated by the activation of MT₁/MT₂ melatonin receptors. However, these data do not rule out the involvement of 5-HT₂c receptors in the rescuing effect of agomelatine on sleep (for example, MT₁/MT₂ receptor activation and 5-HT₂c receptor blockade may be necessary but not sufficient for the action of agomelatine on sleep). The strong anorexigenic effect of 5-HT₂c receptor agonists (Halldor et al. 2007) has prevented the use of these drugs combined with food-containing agomelatine in our study.

Agomelatine shows positive chronobiotic effects in the clinic. Depressed patients show severe impairments in the sleep/wake cycle and in circadian rhythms in general (Srinivasan et al. 2006). In depressed patients, agomelatine treatment reduces sleep
complaints, increases the duration of SWS and normalizes sleep structure (Lopes et al. 2007; Quera-Salva et al. 2007). Moreover, agomelatine shows a favourable effect on the relative amplitude of circadian rest–activity and sleep/wake cycle (Kasper et al. 2010). The use of a self-rating scale for the assessment of sleep and circadian rhythm disturbances has shown a good efficacy of agomelatine in patients with seasonal affective disorder (Pjrek et al. 2007).

A positive effect of agomelatine on sleep architecture might be particularly relevant to the overall therapeutic activity in depressed patients, because changes of NREM sleep induced by agomelatine precede the improvement of depressive symptoms (Zupancic & Guilleminault, 2006). In PRS rats, agomelatine treatment not only corrects abnormalities in the sleep/wake cycle and circadian rhythm of motor activity (see present data), but can also reverse all biochemical, cellular and behavioural changes associated with the anxious/depressive phenotype (Morley-Fletcher et al. 2011). These data support the importance of the regulation of circadian rhythms in the treatment of mood disorders and highlight the value of agomelatine as a novel antidepressant drug (Millan, 2006; Pandi-Perumal et al. 2009; Quera-Salva et al. 2010).

While agomelatine promotes NREM sleep both in PRS animal and depressed patients, agomelatine does not affect REM sleep in humans as opposed to what is seen in PRS rats (Quera-Salva et al. 2007). Moreover, agomelatine differs from other antidepressants, including SSRIs (Wilson & Argyropoulos, 2005), since it does not produce REM sleep inhibition. However, this divergence between rats and humans can be explained because of the different sleep architecture between both species. What is important here is that agomelatine was able to correct sleep abnormalities in PRS rats, which included both changes in REM and NREM sleep.
Searching for a mechanism underlying the chronobiotic effect of agomelatine in PRS rats, we measured the expression of c-Fos mRNA in the hippocampus 1 h prior to the end of the light phase, when PRS rats showed the anticipated onset of motor activity. We measured c-Fos expression as a non-specific marker of neuronal activation because of the established correlation between stress-induced changes in sleep and neuronal activity in the hippocampus (Best et al. 2007; Hegde et al. 2008; Karlsson & Blumberg, 2003; Staba et al. 2002). Our PRS rats showed an increased c-Fos mRNA expression in the hippocampal CA3 region and dentate gyrus (see also Viltart et al. 2006), which was normalized by agomelatine treatment. In this line, a 21-d treatment with agomelatine has been shown to normalize the reduction in hippocampal c-Fos expression induced by daily foot shock stress in the hippocampal dentate gyrus (Dagyte et al. 2010). Globally, our data suggest that the action of agomelatine on neuronal activity is ‘disease-dependent’, i.e. the drug acts as a ‘normalizer’ of neuronal activity under pathological conditions. In the motor and cingulate cortex, PRS had no effect on c-Fos mRNA expression and agomelatine increased c-Fos mRNA expression in Con rats but not in PRS rats. These findings suggest that the effect of agomelatine on c-Fos in PRS rats is region-specific, with the drug acting primarily in the hippocampus and not in the cingulate cortex.

We found a strong correlation between hippocampal changes in c-Fos mRNA and changes in REM sleep when we pooled data obtained in Con and PRS rats treated with vehicle and PRS rats treated with agomelatine. This strengthens the evidence that agomelatine has restorative effects on changes in REM sleep induced by PRS. We lost the correlation when we pooled all groups of animals because agomelatine increased c-Fos mRNA levels but had no effect on REM sleep in Con rats. Perhaps in Con rats agomelatine predominantly activates hippocampal neurons that are not involved in the regulation of REM sleep.

We wish to highlight that a reciprocal relationship exists between sleep and neuronal activity in the hippocampus. On the one hand, the pattern of neuronal firing in the hippocampus is tightly associated with the sleep phases (particularly with REM sleep; Best et al. 2007; Hegde et al. 2008; Karlsson & Blumberg, 2003; Staba et al. 2002). On the other hand, the sleep/wake cycle regulates synaptic plasticity in the hippocampus, with biochemical changes associated with synaptic potentiation and depression being predominant during wakefulness and sleep, respectively (Vyazovskiy et al. 2008). Perhaps PRS and agomelatine primarily affect the sleep/wake cycle, leading to secondary changes in neuronal plasticity in the hippocampus (Morley-Fletcher et al. 2011; Soumier et al. 2009; Zuena et al. 2008). This interesting hypothesis warrants further investigations.

In conclusion, we have shown that agomelatine restores sleep architecture and corrects some abnormalities in the circadian rhythm of motor activity in a rat model of depression. This supports the value of agomelatine as a novel antidepressant that acts by resynchronizing circadian rhythms and is therefore able to increase the quality of life in depressed patients (Millan, 2006; Pandi-Perumal et al. 2009; Quera-Salva et al. 2010).

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Statement of Interest

Cecilia Gabriel and Elisabeth Mocaër are employed by Servier. The other authors have no conflict of interest.

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