Beneficial behavioural and neurogenic effects of agomelatine in a model of depression/anxiety

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Abstract
Agomelatine (S20098) is a novel antidepressant drug with melatonergic agonist and 5-HT2C receptor antagonist properties, displaying antidepressant/anxiolytic-like properties in animal models and in humans. In a depression/anxiety-like mouse model in which the response of the HPA axis is blunted, we investigated whether agomelatine could reverse behavioural deficits related to depression/anxiety compared to the classical selective serotonin reuptake inhibitor, fluoxetine. Adult mice were treated for 8 wk with either vehicle or corticosterone (35 μg/ml.d) via drinking water. During the final 4 wk, animals were treated with vehicle, agomelatine (10 or 40 mg/kg i.p.) or fluoxetine (18 mg/kg i.p.) and tested in several behavioural paradigms and also evaluated for home-cage activity. Our results showed that the depressive/anxiety-like phenotype induced by corticosterone treatment is reversed by either chronic agomelatine or fluoxetine treatment. Moreover, agomelatine increased the dark/light ratio of home-cage activity in vehicle-treated mice and reversed the alterations in this ratio induced by chronic corticosterone, suggesting a normalization of disturbed circadian rhythms. Finally, we investigated the effects of this new antidepressant on neurogenesis. Agomelatine reversed the decreased cell proliferation in the whole hippocampus in corticosterone-treated mice and increased maturation of newborn neurons in both vehicle- and corticosterone-treated mice. Overall, the present study suggests that agomelatine, with its distinct mechanism of action based on the synergy between the melatonergic agonist and 5-HT2C antagonist properties, provides a distinct antidepressant/anxiolytic spectrum including circadian rhythm normalization.

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Introduction
Depression and anxiety, two distinct psychiatric disorders with high comorbidity, are major causes of disability worldwide. The major shortcomings in treating these disorders with selective serotonin reuptake inhibitors (SSRIs) is that the therapeutic response develops slowly (3–4 wk), that side-effects often occur and that there is a significant percentage (30%) of non-responders (Wong & Licinio, 2001). Thus, it is necessary to identify and develop alternative therapeutic options for the treatment of depression and anxiety disorders (Millan, 2006).

The development of antidepressant drugs with melatonergic agonist and 5-HT2C antagonist properties may be promising given that affective disorders are characterized by abnormal circadian rhythms (Germain & Kupfer, 2008). Agomelatine, acting as an agonist at melatonergic receptors and antagonist at 5-HT2C receptors (De Bodinat et al. 2010), has demonstrated efficacy in major depressive disorders in several clinical trials (Kennedy & Emsley, 2006; Loo et al. 2002; Olié & Kasper, 2007). Possibly because of this novel receptor profile, one reason agomelatine
is efficacious in depression is through the rhythmic circadian rhythms (for review see, Gorwood, 2010; Kasper et al. 2010). It is noteworthy that agomelatine also displays robust antidepressant- and anxiolytic-like activities in several animal paradigms (Barden et al. 2006; Bertaina-Anglade et al. 2006; Bourin et al. 2004; Millan et al. 2005; Papp et al. 2003, 2006; Tuma et al. 2005).

Recently, we developed a new mouse model of a depressive/ anxiety-like state induced by long-term exposure of exogenous corticosterone (4-pregnen-11b-diol-3,20-dione 21-hemisuccinate) in rodents. In this model, we showed that chronic fluoxetine treatment reversed the inhibition of hippocampal neurogenesis and the behavioural dysfunction induced by chronic corticosterone in several behavioural paradigms, but was ineffective in reversing the flattened circadian rhythm (David et al. 2009).

Since agomelatine has a different mechanism of action from currently available antidepressants, we first assessed its antidepressant/anxiety-like activity in the chronic corticosterone animal model of a depressive/ anxiety-like state. We compared the behavioural consequences of either chronic agomelatine (10 or 40 mg/kg.d) or fluoxetine (18 mg/kg.d) treatment in various paradigms such as the open-field paradigm (OF), novelty suppressed feeding (NSF), the splash test (ST), the forced swim test (FST) as well as the coat state. Moreover, considering that desynchronization of circadian rhythms plays a key role in mood disorders, we also enquired whether agomelatine reversed the flattened circadian rhythm in chronic corticosterone-treated mice.

Finally, recent preclinical data also demonstrated that agomelatine, similar to other antidepressants such as SSRIs and tricyclics, increases cell proliferation in the dentate gyrus of non-stressed adult rats (Banasr et al. 2006; Soumier et al. 2009). Furthermore, chronic agomelatine reversed the decreased neurogenesis in the glucocorticoid receptor-impaired mice (GR-i mice) model of depression (Païzanis et al. 2010). Interestingly, Banasr et al. (2006) and Soumier et al. (2009) showed that this effect was specifically seen in the ventral hippocampus. This is of particular interest, since the hippocampus may be functionally segregated along its dorsal/ventral axis, with the dorsal hippocampus more responsible for spatial memory and the ventral hippocampus implicated in anxiety and mood regulation (Bannerman et al. 2004; Fanselow & Dong, 2010; Moser & Moser, 1998). Thus, since the selective effect of agomelatine on ventral hippocampus neurogenesis may have implications for its mechanism of action, we assessed the effects of agomelatine on neurogenesis in both the ventral and dorsal hippocampus.

**Experimental procedures**

An extensive description of methods is provided in Supplementary Material (available online).

**Animals**

Adult male C57BL/6Ntac mice were purchased from Taconic Farms (Germantown, USA; Lille Skensved, Denmark). All corticosterone-treated mice were aged 7–8 wk and weighed 20–24 g at the beginning of the treatment, and were maintained on a 12-h light/dark cycle (lights on at 06:00 hours). Mice were housed in groups of five. Food and water were available ad libitum. Behavioural testing occurred during the light phase between 09:00 and 17:00 hours for the OF, NSF, FST and ST. Separated groups were used to assess home-cage activity. All testing was conducted in compliance with protocols approved by the Institutional Animal Care and Use Committee (Council directive no. 87–848, 19 October 1987, Ministère de l’Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale, permissions no. 92–256 to D. J. David).

**Drugs**

The dose and duration of corticosterone treatment was selected based on previous study (David et al. 2009). Corticosterone (35 μg/mLd, equivalent to about 5 mg/kg.d) or vehicle (0.45% β-cyclodextrine, β-CD), was available ad libitum in the drinking water in opaque bottles to protect it from light. Corticosterone-treated water was changed every 3 d to prevent any possible degradation. Thereafter, while administration with β-CD or corticosterone continued, mice were treated intraperitoneally (i.p.) with vehicle [hydroxyethylcellulose (HEC) 1%], agomelatine (S20098) (10 or 40 mg/kg) or fluoxetine (18 mg/kg) for 28 d (see timeline, Supplementary Fig. 1, available online). Fluoxetine served as a positive control in these experiments. Drug administration occurred daily at 05:00 hours; 1 h before the start of the dark phase.

**Behavioural testing**

**OF paradigm**

We used the procedure described previously (David et al. 2009). Motor activity was quantified in four Plexiglas open-field boxes 43 × 43 cm² (Med Associates, France). Total time in the centre and number of entries into the centre over a 30-min test period were
recorded. The total ambulatory distance was also measured to ensure the absence of any locomotor effects of the treatment (Supplementary Fig. 2). This test was performed 23 d after the beginning of treatment, in the morning (~16 h after drug administration on day 22).

**NSF paradigm**

The NSF paradigm is a conflict test that elicits competing motivations between the drive to eat and the fear of venturing into the centre of a brightly lit arena. Latency to begin eating is used as an index of depressive/anxiety-like behaviour. The NSF test was performed during a 10-min period as previously described (David et al. 2009). This test was performed 26 d after the beginning of treatment, in the morning (~16 h after drug administration on day 25).

**Changes in coat state**

The state of the coat was assessed 27 d after the beginning of treatment in the morning (~16 h after drug administration on day 26). The total score resulted from the sum of the score of five different body parts: head, neck, dorsal/ventral coat, tail, fore/hindpaws. For each of the five body areas, a score of 0 was given for a well-groomed coat and 1 for an unkempt coat (Santarelli et al. 2003).

**Splash test**

This test consisted of squirting 200 µl of a 10% sucrose solution on the mouse’s snout (Ducottet & Belzung, 2004). The grooming frequency was recorded. This test was performed 28 d after the beginning of treatment, in the morning (~16 h after drug administration on day 27).

**FST**

The FST procedure was modified to enhance the sensitivity for detecting the putative antidepressant-like activity of drugs (Holick et al. 2008; Porsolt et al. 1977). Briefly, mice were placed into clear plastic buckets, 20 cm in diameter and 23 cm deep, filled up to two thirds with water at 23–25 °C. Automated scoring was done using the automated X’PERT FST (Bioseb, France). Dependent variables were mobility, swimming and climbing duration. This test was performed 29 d after the beginning of treatment, in the morning (~16 h after the last administration of drug).

**Home-cage activity**

Home-cage activity was quantified using the ActiV-Meter (Bioseb) over a 24-h period during the last week of treatment. During the experiment, food and water were available ad libitum. To assess any effects of chronic antidepressant treatment in reversing the flattened circadian rhythm induced by corticosterone (indicated by a reduction in home-cage activity), total ambulatory activity over a 24-h period, and total activity during the light and dark phase were measured. Finally, the ratio of ambulatory distance during the dark phase over the light phase was calculated.

**Immunohistochemistry**

5-bromo-2-deoxyuridine (BrdU) labelling for proliferation study

Mice were administered BrdU (150 mg/kg i.p.) 2 h before sacrifice. We then proceeded as described previously in David et al. (2009). BrdU-positive (BrdU+) cells were counted using an Olympus BX51 microscope (Germany).

Doublecortin (DCX) labelling for maturation index study

Histo-immunochemistry protocol was adapted from David et al. (2009). DCX-positive (DCX+) cells were subcategorized according to their dendritic morphology: DCX+ cells and DCX+ cells with tertiary (or higher order) dendrites. The maturation index was defined as the ratio of DCX+ cells possessing tertiary dendrites over the total DCX+ cells.

**Data analysis and statistics**

Results from data analyses were expressed as mean ± S.E.M. Data were analysed using StatView 5.0 software (SAS Institute, USA). For all experiments a two-way ANOVA with repeated measures was applied to the data as appropriate. Significant main effects and/or interactions were resolved by Student Newman–Keuls post-hoc ANOVA analysis or post-hoc unpaired t test as appropriate.

In the NSF test, we used also the Kaplan–Meier survival analysis because of the lack of normal distribution of the data. Animals that did not eat during the 10-min test period were statistically censored. The Mantel–Cox log-rank test was used to evaluate differences between experimental groups.

**Results**

**Chronic agomelatine reversed depressive/anxiety-related behaviours induced by chronic corticosterone treatment.**

We first assessed the effect of 4-wk treatment with agomelatine (10 or 40 mg/kg, d) or fluoxetine
Fig. 1. Chronic agomelatine reversed anxiety/depressive-related behaviours induced by chronic corticosterone treatment. Effects of 4-wk antidepressant treatment (fluoxetine 18 mg/kg.d or agomelatine 10 and 40 mg/kg.d), started after a 4-wk corticosterone regimen (35 µg/ml.d), on anxiety behaviours in the open-field (OF) or the novelty suppressed feeding (NSF) tests and on depressive-like behaviour on coat state degradation over days or in the splash test (ST). In the OF paradigm, anxiety is
(18 mg/kg.d), in our model of corticosterone-induced depression/anxiety-like behaviour in C57BL/6NAct mice in the OF paradigm. A two-way ANOVA on the time spent in the centre revealed a significant effect of pretreatment \( [F(1, 125) = 24.5, p < 0.01] \) (Fig. 1a) and a significant pretreatment \( \times \) treatment interaction \( [F(3, 125) = 3.3, p < 0.05] \). Similar to the time spent in centre, a two-way ANOVA on the entries in the centre showed a significant effect of pretreatment \( [F(1, 125) = 8.7, p < 0.01] \) (Fig. 1b) and a significant pretreatment \( \times \) treatment interaction \( [F(3, 125) = 4.3, p < 0.05] \). In the OF test, chronic exogenous corticosterone had a marked effect on all anxiety parameters, resulting in decreased time spent (Fig. 1a) and decreased number of entries (Fig. 1b) with no change in the ambulatory distance (Supplementary Fig. 2). Interestingly, this anxiety phenotype in the OF paradigm was reversed by chronic fluoxetine or agomelatine treatment (10 mg/kg.d) for all the parameters tested (Fig. 1a, b).

In the NSF test, another anxiety-related behavioural paradigm (Fig. 1c, d), we explored whether agomelatine and fluoxetine were able to reverse the depressive/anxiety-like state observed in NSF (Fig. 1c; Kaplan–Meier survival analysis, Mantel–Cox log-rank test, \( p < 0.01 \)). A two-way ANOVA revealed a significant effect of pretreatment \( [F(1, 115) = 29.5, p < 0.01] \) on the latency to feed (Fig. 1d). Overall, agomelatine, at both doses, and fluoxetine prevented the increase in the latency to feed without affecting the home food consumption (Supplementary Table 1), suggesting that both drugs modulate the depressive/anxiety-like state.

We next assessed the coat state of the animals, a well-validated index of a depressed-like state (Santarelli et al. 2003). Long-term glucocorticoid exposure, similar to chronic stress (Surget et al. 2008), induced physical changes including deterioration of the coat state. A two-way ANOVA on the coat state indicated a significant effect of pretreatment \( [F(1, 111) = 194.46, p < 0.01] \), treatment \( [F(3, 111) = 4.57, p < 0.01] \) and a significant pretreatment \( \times \) treatment interaction \( [F(3, 111) = 4.51, p < 0.01] \). Interestingly, both doses of agomelatine, and fluoxetine, reversed the deterioration of the coat state (Fig. 1c).

We next investigated whether the deterioration of the coat state was linked to changes in grooming behaviour by squirting a 10% sucrose solution on the mouse’s snout (Fig. 1f). A two-way ANOVA revealed a significant effect of pretreatment \( [F(1, 109) = 33.0, p < 0.01] \) and a significant pretreatment \( \times \) treatment interaction \( [F(3, 109) = 6.6, p < 0.01] \). A 4-wk agomelatine regimen at 10 or 40 mg/kg.d, and fluoxetine reversed the decrease in grooming activity induced by corticosterone treatment in this sucrose splash test.

### Chronic agomelatine treatment induced antidepressant-like activity in the mouse FST by increasing swimming and climbing behaviour

Since the FST is well recognized as a screening test for antidepressant action, we explored the behavioural effects of chronic corticosterone treatment in the presence of either agomelatine or fluoxetine. We also tested mice treated with antidepressants but not subjected to chronic corticosterone. A two-way ANOVA on mobility duration showed a significant effect of treatment \( [F(3, 112) = 15.8, p < 0.01] \). In both corticosterone- and non-corticosterone-treated animals, either dose of agomelatine (10 or 40 mg/kg.d) and fluoxetine increased mobility duration (Fig. 2a). Interestingly, a two-way ANOVA on swimming duration revealed a significant effect of treatment \( [F(3, 112) = 12.7, p < 0.01] \) and a significant pretreatment \( \times \) treatment interaction \( [F(3, 112) = 2.6, p < 0.05] \). Agomelatine at 10 or 40 mg/kg.d or fluoxetine increased swimming duration in corticosterone- and non-corticosterone-treated animals (Fig. 2b). Regarding the climbing duration, a two-way ANOVA showed a significant effect of treatment \( [F(3, 112) = 8.1, p < 0.01] \). In non-corticosterone- and corticosterone-treated animals, only agomelatine at 10 mg/kg.d induced a significant increase in climbing duration (Fig. 2c). Finally, a chronic fluoxetine treatment increased the climbing duration in corticosterone-treated animals (Fig. 2c).

Taken together, these results suggest through multiple behavioural readouts that chronic agomelatine and fluoxetine treatments were effective in reversing a depression/anxiety-like phenotype induced by excess of glucocorticoids.
Chronic agomelatine normalized disturbances of circadian rhythm

Since agomelatine induces a phase advance in animals and resynchronization of circadian rhythms (Redman et al. 1995, Van Reeth et al. 2001), a phase advance of circadian rhythms in healthy human volunteers (Krauchi et al. 1997; Leproult et al. 2005) and depressed patients (Quera-Salva et al. 2007), as well as resynchronization of circadian rhythms in depressed patients (Kasper et al. 2010) we wanted to assess whether it could reverse the flattened circadian rhythm in our animal model. We measured the ratio activity of the ambulatory distance during the dark phase over the light phase (Fig. 3a) and total ambulatory activity over a period of 24 h (Fig. 3b). We also showed the segregation of the total activity during the light phase (Fig. 3c) and the dark phase (Fig. 3d). A two-way ANOVA revealed an effect of the main treatment factor on the ratio for activity in the home cage [F(3, 89) = 9.6, p < 0.01]. A planned comparison showed that chronic corticosterone treatment induced a reduction in home-cage activity, decreasing the ratio of ambulatory distance during night over day by >20%. Agomelatine, at both doses studied, increased this parameter in both vehicle- (from +30%) and corticosterone-treated mice (from +40%) (Fig. 3a) indicating an increase in the amplitude of the rhythm. In addition, a two-way ANOVA showed an effect of both pretreatment [F(1, 89) = 34.7, p < 0.01] and treatment [F(3, 89) = 5.2, p < 0.01] on the total activity over 24 h (Fig. 3b). A reduction of 35% and 30%, respectively, of the total activity was observed in agomelatine (10 mg/kg.d) and fluoxetine groups with corticosterone pretreatment compared to corticosterone/vehicle-treated mice. Mice that were not exposed to corticosterone did not have any differences with antidepressant treatment. Regarding activity during the light phase

Fig. 2. Chronic agomelatine treatment induced antidepressant-like activity in the mouse forced swim test (FST) by increasing swimming and climbing behaviour. Effects of 4-wk antidepressant treatment (fluoxetine 18 mg/kg.d or agomelatine 10 and 40 mg/kg.d), started after a 4-wk corticosterone regimen (35 µg/ml.d), on the FST. Results are expressed as the total mobility duration (a), the swimming duration (b) and the climbing duration (c) in seconds. Values plotted are mean ± S.E.M. (n = 14–17 per group). * p < 0.05, ** p < 0.01, * p < 0.05, ## p < 0.01, vs. control group and corticosterone/vehicle group, respectively.
a two-way ANOVA revealed an effect of treatment \( F(3, 89) = 16.1, p < 0.01 \). All antidepressant treatments decreased light phase activity in both vehicle- and corticosterone-treated mice by around 30%. A two-way ANOVA for activity during the dark phase (Fig. 3c) revealed an effect of pretreatment \( F(1, 89) = 39.5, p < 0.01 \) and treatment \( F(3, 89) = 4.5, p < 0.01 \). Indeed, corticosterone decreases activity during the dark phase, an effect that was actually enhanced by 20% with fluoxetine treatment compared to the control group.

### Chronic agomelatine treatment after long-term corticosterone exposure affects proliferation and maturation of adult hippocampal neurogenesis

To investigate the potential cellular mechanisms underlying the behavioural effects of agomelatine, we next evaluated changes in adult hippocampal neurogenesis. A two-way ANOVA on the number of BrdU\(^+\) cells showed a significant effect of pretreatment \( F(1, 59) = 4.3, p < 0.05 \), treatment \( F(3, 59) = 5.6, p < 0.01 \) and a significant pretreatment \( \times \) treatment interaction \( F(3, 59) = 9.3, p < 0.01 \). As previously described, chronic corticosterone treatment mimicked the effects of chronic stress on cell proliferation by decreasing the number of proliferating (BrdU\(^+\)) cells in the dentate gyrus (Fig. 4a) of the adult mouse hippocampus. This change in proliferation was completely reversed by antidepressant treatment, either with agomelatine (10 or 40 mg/kg/d) or fluoxetine. No change in BrdU\(^+\) cells was observed in non-stressed mice.

We next explored whether chronic corticosterone in the presence or absence of antidepressant treatment had any selective influence on neurogenesis along the dorsal/ventral axis of the hippocampus. A two-way ANOVA on BrdU\(^+\) cells in the dorsal hippocampus revealed a significant pretreatment \( \times \) treatment interaction \( F(3, 59) = 5.1, p < 0.01 \). In the
ventral hippocampus, a two-way ANOVA also revealed a significant effect of pretreatment \( F(1, 59) = 9.0, p < 0.01 \), treatment \( F(3, 59) = 9.2, p < 0.01 \) and a significant interaction \( F(3, 59) = 10.3, p < 0.01 \). Therefore, the effects of agomelatine or fluoxetine on cell proliferation in corticosterone-treated animals were similar between the dorsal and the ventral hippocampus.

Furthermore, we assessed the effects of agomelatine or fluoxetine on dendritic maturation in our animal model (Fig. 5). To this end, we examined the dendritic morphology of cells that express DCX. A two-way ANOVA revealed a significant effect of treatment \( F(3, 43) = 21.9, p < 0.01 \) and pretreatment × treatment interaction \( F(3, 43) = 3.4, p < 0.05 \) on DCX+ cells with tertiary dendrites. Indeed, both doses of agomelatine induced an increase in the number of DCX+ cells with tertiary dendrites in control and corticosterone-treated animals. Moreover, chronic antidepressant treatment induced an increase in the maturation index for newborn neurons in the dentate gyrus of the hippocampus [effect of treatment: \( F(3, 43) = 11.9, p < 0.01 \], in both basal and chronic corticosterone conditions.

Fluoxetine, as previously published, significantly
increased the number of DCX+ cells with tertiary dendrites (Wang et al. 2008).

So far the regional influence of agomelatine on dendritic morphology has not been studied in stressed or non-stressed animals. In the dorsal part of the hippocampus, a two-way ANOVA indicated a significant effect of treatment \( F(3, 43) = 10.2, p < 0.01 \) and a pretreatment \times treatment interaction \( F(3, 43) = 3.7, p < 0.05 \) on DCX+ cells with tertiary dendrites and significant effects of treatment \( F(3, 43) = 6.7, p < 0.01 \) and pretreatment \times treatment interaction \( F(3, 43) = 2.9, p < 0.05 \) on maturation index. Moreover, in the ventral part of the hippocampus, a two-way ANOVA indicated a significant effect of treatment \( F(3, 43) = 26.4, p < 0.01 \) on DCX+ cells with tertiary dendrites and a significant effect of treatment \( F(3, 43) = 12.9, p < 0.01 \) on maturation index. Thus, in contrast to chronic glucocorticoid treatment, both antidepressants modified the maturation index. Interestingly, the number of DCX+ cells with tertiary dendrites is increased with agomelatine (10 or 40 mg/kg/d) in corticosterone-treated animals only in the ventral hippocampus.

**Discussion**

The present study aimed to investigate the antidepressant/anxiolytic-like and neurogenic activities of agomelatine, a novel antidepressant with melanergic agonist and 5-HT2C receptor antagonist properties in a model of depression/anxiety based on elevation of glucocorticoid levels.

Enhanced activity of the HPA axis involving elevated glucocorticoid levels is considered a key neurobiological alteration in major depression. In depressed patients, many studies have shown that successful
antidepressant therapies are associated with normalization of impaired HPA axis negative feedback (Greden et al. 1983; Heuser et al. 1996; Holsboer-Trachsler et al. 1991; Linkowski et al. 1987). Consistent with previous findings, the present results demonstrate that an elevation of glucocorticoid levels is sufficient to induce a depressive/anxiety-like state in C57BL/6Ntac mice in various paradigms including OF (Fig. 1a, b), NSF (Fig. 1c, d), coat state (Fig. 1e), and the sucrose splash test (Fig. 1f). As previously shown these deficits were reversed by fluoxetine (David et al. 2009). Here we show that the new antidepressant agomelatine is also capable of reversing these deficits.

Overall, in the OF, the NSF, the sucrose splash test and the coat state, the magnitude of agomelatine-induced effects was comparable to that of fluoxetine. Indeed, agomelatine exerted antidepressant-like properties in several animal models of depression and anxiety (Bertaina-Anglade et al. 2006; Fuchs et al. 2006; Millan et al. 2005; Papp et al. 2003, 2004; Tuma et al. 2005). The mechanism underlying the antidepressant/ anxiolytic-like activity of agomelatine appears to involve both its melatonergic receptor agonist and its 5-HT2C receptor antagonist properties (Bertaina-Anglade et al. 2006; Papp et al. 2003, 2004). Interestingly, melatonergic and 5-HT2C receptors are expressed in the suprachiasmatic nuclei and in other brain areas possibly involved in the pathophysiology of depression, such as the cerebral cortex, hippocampus, amygdala and thalamus (Clemett et al. 2002; Holmes et al. 1995; Weaver et al. 1989).

In the FST, our present data are consistent with the conclusion of recent studies including ours, showing that a chronic corticosterone regimen did not change swimming behaviour in mice (David et al. 2009; Murray et al. 2008). Agomelatine, and fluoxetine, also induced antidepressant-like effects in ‘depressed/ anxious’ and normal ‘non-depressed/anxious’ animals. Previous data with agomelatine suggest that its efficacy in the FST involves both its melatonergic receptor agonist and 5-HT2C receptor antagonist properties (Bourin et al. 2004). When analysing mice in the FST, we explored swimming, climbing and mobility duration. Swimming behaviour relies on the serotonergic system, and climbing behaviour on the noradrenergic system in rats (Page et al. 1999).

Interestingly, we provided evidence that changes in mobility duration after chronic agomelatine treatment at the higher dose is mainly related to a change in swimming activity, whereas at the lower dose (10 mg/kg.d), both swimming and climbing duration were increased. This effect on climbing duration could be linked to the enhancement of the extracellular levels of noradrenaline in the frontal cortex and dorsal hippocampus (Millan et al. 2003, 2005). With regard to swimming behaviour, even though agomelatine failed to change extracellular levels of 5-HT in the dorsal hippocampus or the frontal cortex (Millan et al. 2005), or the levels of 5-HT1A receptors (Hanoun et al. 2004), it has been demonstrated that reciprocal interactions among norepinephrine, dopamine and serotonin exist (Guiard et al. 2010).

Moreover, a number of studies have demonstrated chronobiotic activity of agomelatine (Martinet et al. 1996). This component of the pharmacological activity of agomelatine is important since abnormal circadian rhythms including modifications of active/sleep state have been described in depression as a major symptom (Germain & Kupfer, 2008; Healy, 1987; Mendelson et al. 1987; Perlis et al. 1997). Our animal model is particularly attractive for assessing compounds with chronobiotic and antidepressant properties, since a flattened circadian rhythm with reduction in home-cage activity was observed with depression/ anxiety-like state in corticosterone-treated animals (Fig. 3). Indeed, agomelatine at 10 or 40 mg/kg.d normalized the disturbance of circadian rhythms in corticosterone-treated animals, increasing the dark/light ratio (Fig. 3a). This change in the ratio is a consequence of decreased activity during the light period (Fig. 3c), since no change was observed during the dark period. Overall, these data are consistent with the well-known effects of agomelatine on normalization of circadian rhythm disturbance in several animal models (Amstrong et al. 1993; Martinet et al. 1996; Pitrosky et al. 1999; Redman et al. 1995) as well as in depressed patients (see de Bodinat et al. 2010 for review; Kasper et al. 2010). Interestingly, an increase in dark/light home-cage activity ratio is also observed in vehicle-treated mice confirming circadian effects of agomelatine in both baseline and pathological situations. The absence of an increase in the total activity rules out a stimulant-like effect of agomelatine administration (Fig. 3b). As mentioned above, this change is a consequence of decreased activity during the light phase only (Fig. 3c).

In order to investigate the potential cellular mechanisms underlying the behavioural effects of agomelatine, we next evaluated changes in adult hippocampal neurogenesis hypothesized to be relevant for antidepressant action (Airan et al. 2007; David et al. 2007, 2009; Santarelli et al. 2003; Wang et al. 2008). In agreement with previous findings (David et al. 2009; Murray et al. 2008; Qiu et al. 2007), we found a reduction in the proliferation of progenitor cells after chronic corticosterone treatment was observed (Fig. 5),
emphasizing a role for glucocorticoids in the regulation of the proliferation stage of the neurogenic process.

Here, chronic administration of agomelatine or fluoxetine reversed the decrease in cell proliferation in the hippocampus without any discrimination between the dorsal and the ventral hippocampus. Previous data obtained in stress paradigms with fluoxetine in mice (David et al. 2009) or with agomelatine in rats (Dagyte et al. 2010) corroborate our results. No regional differences in the effects of agomelatine on cell proliferation were observed in our model, suggesting that this effect may be prominent only in unstressed rodents (Banasr et al. 2006; Païzanis et al. 2010; Soumier et al. 2009). Furthermore, another report demonstrated that effects of chronic agomelatine treatment on cell proliferation appeared throughout the dentate gyrus in mice with decreased expression of glucocorticoids in the brain (Païzanis et al. 2010). Interestingly, neither chronic agomelatine nor fluoxetine administration affected hippocampal cell proliferation in non-corticosterone-treated C57BL/6Ntac mice. In contrast, some other studies have shown that 3-wk treatment with agomelatine promoted cell proliferation in the ventral hippocampus of non-stressed Wistar rats (Banasr et al. 2006; Soumier et al. 2009), wild-type GR-i mice (Païzanis et al. 2010), or the dorsal part of the hippocampus of Sprague–Dawley rats (AlAhmed & Herbert, 2010). Overall, strains and species differences (rats vs. mice) in hippocampal adult cell proliferation are more likely to account for these differences (David et al. 2010 for review) than changes in the experimental protocols as suggested by Dagyte & colleagues (2010). Indeed, as previously demonstrated, various antidepressants do not have any effects on strains with high basal hippocampal neurogenesis levels such as non-stressed C57BL/6 (David et al. 2009; Navailles et al. 2008; Schauwecker, 2006). The facilitation of cell proliferation in the dentate gyrus by agomelatine could reflect, at least in part, its 5-HT_{1C} receptor antagonist properties (Soumier et al. 2009; AlAhmed & Herbert, 2010).

The characterization of the neurogenic effects of new compounds requires an understanding of the effects on maturation of young neurons in addition to proliferation effects. Maturation is a crucial step for the functional integration of young neurons into neural circuits. In the adult dentate gyrus, DCX is exclusively expressed in immature neurons from age 1 d to 4 wk (Couillard-Despres et al. 2005) and thus has been widely used as a reliable marker of immature neurons that reflects the level of neurogenesis and its modulation. While about 20% of DCX^+ cells are still in the cell cycle, 70% are post-mitotic and represent young immature neurons; the remaining 10% are progressing from late neuroblasts to immature neurons. Studies done in rats demonstrated that agomelatine, or other antidepressants such as fluoxetine, promote maturation of post-mitotic neurons (Banasr et al. 2006; Dagyte et al. 2010; Soumier et al. 2009, Wang et al. 2008). In a recent study, agomelatine appeared to induce an early acceleration of cell maturation at 8 d development (Soumier et al. 2009). To date, no data with an earlier time-point than 21 d examined the effects of fluoxetine on cell maturation (Wang et al. 2008). However, it is unlikely that SSRIs have an effect on maturation at 8 d since it has been well documented that fluoxetine does not induce any changes in proliferation (Santarelli et al. 2003) or survival (Wang et al. 2008) during the first week of treatment. The latest data obtained by Daszuta’s group might therefore suggest that agomelatine, in comparison to other monoaminergic antidepressants, has a more rapid effect on cell maturation in non-stressed conditions Soumier et al. 2009).

We investigated the effect of chronic agomelatine and fluoxetine treatment on cell maturation in corticosterone-treated animals. Agomelatine and fluoxetine induced an increase in the dendritic arborization complexity of newborn neurons in the hippocampus. Indeed, a larger fraction of DCX^+ cells possessed tertiary dendrites after chronic agomelatine treatment in both corticosterone- and non-corticosterone-treated animals suggesting that agomelatine facilitates maturation. These results could be linked to previous data where agomelatine increased the proportion of mature vs. immature neurons after 15 d treatment in rats, a time-point when newborn cells start to develop dendritic arborization, extend axon terminals for establishing synapses with their targets, and become integrated into hippocampal circuitry. In keeping with these results, agomelatine also increased dendritic length and branching points in vitro in postnatal hippocampal cultures at 8 d in vitro (Soumier et al. 2009). Finally, the effects on maturation observed in previous studies under basal or stressed conditions (Banasr et al. 2006; Dagyte et al. 2010; Soumier et al. 2009) in rats were confirmed with the increase in the maturation index induced by chronic agomelatine treatment.

Our study distinguishes for the time, the effects of antidepressants on neuronal maturation between the dorsal and the ventral hippocampus. Interestingly, in contrast to the non-selective increase in dendritic maturation with fluoxetine, agomelatine-induced increase in maturation of newborn neurons was primarily observed in the ventral hippocampus of
corticosterone-treated mice. The ventral hippocampus is of particular interest due to the anatomical and functional differences between the ventral and dorsal hippocampus (Bannerman et al. 2004; Fanselow & Dong, 2010; Moser & Moser, 1998). The projections of the ventral hippocampus to the prefrontal cortex and amygdala support the view that the ventral hippocampus is particularly involved in ‘emotional circuitry’, especially in the control of anxiety/depressive states (Bannerman et al. 2004; Engin & Treit, 2007). This regional effect of agomelatine could be related to its 5-HT$_{2C}$ properties since this receptor seems to be located in the ventral hippocampus (Alves et al. 2004). However, a joint action of agomelatine on cell maturation through its action on melatonergic and 5-HT$_{2C}$ receptors cannot be ruled out as suggested by Soumier et al. (2009).

It should be also noted that the effects of agomelatine on neurogenesis (proliferation and maturation) both dorsally and ventrally are slightly lower than those of fluoxetine particularly in chronic corticosterone-treated animals. On the other hand, comparable behavioural responses are observed for both drugs in tests of antidepressant and anxiolytic activity.

Conclusion

Our work shows that agomelatine induces an antidepressant-like effect in a model of anxiety/depression that is comparable to that of fluoxetine, a classical SSRI. However, agomelatine also induced a change in circadian rhythm, which may contribute to its distinct profile of antidepressant action. Agomelatine is therefore a strong candidate for a new approach to treating depression and anxiety due to an innovative mechanism of action based on melatonin agonist and 5-HT$_{2C}$ antagonistic properties.

Note

Supplementary material accompanies this paper on the Journal’s website (http://journals.cambridge.org/pnp).

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Statement of Interest

None.

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