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Agomelatine suppresses locomotor hyperactivity in olfactory bulbectomised rats: A comparison to melatonin and to the 5-HT_{2c} antagonist, S32006

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ABSTRACT

The novel melatonergic agonist/5-HT_{2C} antagonist agomelatine displays robust antidepressant properties in humans and is active in pre-clinical models predictive of antidepressant effects. In this study, we investigated its potential influence on the locomotor hyperactivity displayed by olfactory bulbectomised rats, a putative measure of potential antidepressant activity. In addition, we compared the actions of agomelatine to those of melatonin and S32006, a selective antagonist at 5-HT_{2C} receptors. Vehicle, agomelatine (10 and 50 mg/kg), melatonin (10 and 50 mg/kg), S32006 (0.16 mg/kg to 10 mg/kg) and the prototypical tricyclic antidepressant, imipramine (10 mg/kg), were administered by intraperitoneal injection for 14 days to male, Sprague–Dawley sham-operated and bulbectomised rats. In agreement with previous studies, imipramine was active in the model and both the lower and higher doses of agomelatine also significantly and markedly reversed the bulbectomy-induced hyperactivity to a level comparable to that seen in sham operated animals, in which agomelatine exerted no effect. Similarly the 5-HT_{2C} antagonist, S32006, dose-dependently and significantly attenuated hyperactivity of bulbectomised animals, albeit with a maximal effect somewhat less marked than that of agomelatine. On the other hand, melatonin did not affect the locomotor behaviour of bulbectomised rats. The activity of agomelatine in the model is consistent with its known antidepressant properties in the clinic.

1. Introduction

Depression is a chronic, recurrent disorder (Hirschfeld and Schatzberg, 1994; Millan, 2006). While two thirds of depressed patients may respond to initial antidepressant therapy, 10-15% will remain resistant to current treatments (Burrows et al., 1994). Non-compliance with treatment is a short coming of current medications (Zajecka, 2000). Most pharmacological interventions focus on altering serotonergic and noradrenergic neurotransmission (Norman and Olver, 2010; Olver et al., 2001). Novel approaches to the treatment of depression are needed to address some of the apparent problems of current agents (Möller, 2008; Norman, 2006).

Disruption to circadian rhythms has been proposed as an aetiological factor in depressive disorders (Bunney and Potkin, 2008; Monteleone and Maj, 2008; Norman, 2010). Cardinal features of depression (e.g., sleep disturbances, alterations in diurnal body temperature, motor activity) favour a circadian hypothesis (Duncan, 1996; Parry et al., 1989). This is supported by a positive association between the degree of disruption in the timing of sleep-wake cycles and severity of depressive symptoms (Emens et al., 2009). Re-entrainment of disturbed circadian rhythms has been found to be useful in the treatment of some depressive states (Lewy and Sack, 1989; Lewy et al., 2006).

Agomelatine is an agonist at the melatonergic MT_1 and MT_2 receptor (Audinot et al., 2003), and an antagonist at 5 HT_{2C} receptors (Millan et al., 2003, 2010). It can re-synchronise experimentally disrupted circadian rhythms (Redman et al., 1995). Agomelatine restores the circadian rest-activity cycle in depressed patients (Kasper et al., 2010). Agomelatine increases noradrenaline and dopamine in the frontal cortex, without modifications of serotonin levels (Millan et al., 2003, 2005).

In pre-clinical tests agomelatine displays activity indicative of antidepressant potential under chronic conditions. Thus in the forced swim test (FST) (Bourin et al., 2004), the learned helplessness task (Bertaina-Anglade et al., 2006), the chronic mild stress paradigm (Papp et al., 2003) and in a transgenic mouse model (Barden et al., 2005; Païzanis et al., 2010) agomelatine is active. Clinical trials have confirmed antidepressant efficacy superior to placebo (Goodwin et al., 2009; Loo et al., 2002) and at least comparable to other antidepressants (e.g., Hale et al., 2010; Kasper et al., 2010). Preclinical studies have suggested a potential synergy between the melatonin agonist and the 5-HT_{2C} antagonist properties contributing to the antidepressant activity

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(Bertaina-Anglade et al., 2006; De Bodinat et al., 2010; Papp et al., 2003).

This study examined the potential antidepressant activity of agomelatine compared to S32006, a 5-HT_{2C} antagonist (Dekeyne et al., 2008) and melatonin using the olfactory bulbectomised rat model of depression. The bulbectomy model is recognised to possess high face and predictive validity. Removal of the bulbs results in behavioural changes (e.g., irritability, impaired learning, hyperactivity in an open field) (Cairncross et al., 1979; Kelly et al., 1996)), relevant to several major clinical dimensions of depression (Marazziti et al., 2010; Millan, 2006; Song and Leonard, 1995). Changes in immune function have also been reported (Kelly et al., 1997). Behavioural changes can be reversed by chronic treatment with antidepressants (Jancsar and Leonard, 1984a,b; McGrath and Norman, 1998).

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (SPF Laboratories, Perth, Western Australia.) weighing 200-250 g at the start of the experiments were used for all studies described. Rats were housed two per cage in a 12:12 light dark cycle (lights on 7 AM, lights off 7 PM). Food and water were available *ad libitum*. All experimental procedures were performed in accordance with guidelines set down by the National Health and Medical Research Council of Australia. The studies were approved by the Austin Hospital Animal Ethics Committee (Approval Number A2000/00888 and A2005/02254).

2.2. Surgery

Following a one week acclimatisation period, during which rats were handled daily, bilateral olfactory bulbectomy was performed on rats anaesthetised with a mixture of ketamine (90 mg/kg) and xylazine (10 mg/kg). Surgery was performed as described by Cairncross et al. (1979). The head was shaved and a midline sagittal incision made extending 1 cm rostral to bregma. Two drill holes of 2 mm diameter were made in the skull, 5 mm rostral to bregma and 2 mm lateral to the midline. For sham operated animals, the dura was pierced and the wound closed. For bulbectomised animals, the olfactory bulbs were aspirated using a water suction pump, care being taken not to damage the frontal cortex. The wound was sealed with haemostatic sponge, sprinkled with oxytetracycline dusting powder (to prevent infection) and closed with Michel wound clips. (In previous studies carried out in our laboratory using this technique no infections have been observed following surgical intervention). The integrity of the surgery was confirmed at the end of the study when all animals were euthanased and the brain examined.

2.2.1. Post-operative care

Following surgery the animals were placed in clean bedding in their home cages and closely monitored until recovery from anaesthesia (usually within about 30 minutes of the surgery). The animals were maintained at approximately 35 °C for 1 hour during the recovery period to ensure no loss of body heat and a reduction in mortality from the procedure. Animals were handled daily during a two week recovery period prior to treatment with test substances.

2.3. Drugs and chemicals

Agomelatine and S32006 (N-pyridin-3-yl-1,2-dihydro-3H-benzo[e] indole-carboxamide) used in this study were supplied by Institut de Recherches Internationales Servier (I.R.I.S) and were used as received. Melatonin, imipramine hydrochloride and hydroxy ethyl cellulose (HEC) were purchased from Sigma-Aldrich (Sydney, Australia).

2.4. Drug administration

Two weeks after surgery the animals were randomly assigned to their treatment groups: Agomelatine (10 mg/kg and 50 mg/kg), imipramine (10 mg/kg) and vehicle in the first experiment; melatonin (10 mg/kg and 50 mg/kg), imipramine (10 mg/kg) and vehicle in the second experiment; S32006 (0.16 mg/kg, 0.63 mg/kg, 2.5 mg/kg and 10 mg/kg), imipramine (10 mg/kg) and vehicle in the third experiment. Drugs were suspended in 1% HEC in an injection volume of 1 ml/kg and administered by intraperitoneal (i.p.) injection daily 2 h before lights off (i.e., at 5 PM) using 26 G X ½" (0.45 X 13 mm) needles for 14 days. The choice of agomelatine, melatonin and S32006 doses was made on the basis of their activity at these doses in other animal models of depression and anxiety (Dekeyne et al., 2008; Papp et al., 2003, 2006).

2.5. Behavioural testing

The open field is essentially similar to that described by Gray and Lalljee (1974). It consists of a white circular base (90 cm diameter) which is divided into 10 cm squares by black lines. The wall (75 cm in height) surrounding the base is made of aluminium sheeting. The sole source of illumination for the field was provided by a 60 W bulb positioned 90 cm above the floor of the open field apparatus. In order to prevent shadows falling across the apparatus all testing was performed with the normal room lighting turned off. Behaviour in the field was assessed in the morning (15-17 h after the last drug administration on day 14). The test apparatus was carefully cleaned with a damp cloth after each animal was tested.

Each animal was placed in the centre of the open field and the following parameters were measured over a 3-minute testing period: Ambulation (the number of squares crossed by each rat); Rearing (the number of times a rat simultaneously raised both forepaws off the floor of the apparatus); Grooming (the number of times the animal stopped and cleaned itself); Defecation (the number of faecal boli).

2.6. Statistical analysis

Statistical tests were performed using the PRISM program (Version 5, GraphPAD Software Inc, USA). Each behavioural measure was analysed by two-way ANOVA with "Surgery" and "Drug" as between factors, followed by Student-Newman-Keuls tests.

A crude calculation of 'relative antidepressant activity' was estimated from the effect of the drugs on ambulation scores. Thus, if X is the difference in ambulation scores between mean bulbectomised vehicle and mean sham vehicle treated animals and Y is the difference between individual bulbectomised drug and mean sham drug scores then crude antidepressant activity can be obtained from (1-Y/X) * 100%. These data were analysed by one-way ANOVA followed by Dunnett's test

3. Results

3.1. Agomelatine experiment

As shown in Fig. 1, bulbectomised / vehicle rats displayed significantly higher ambulation score than sham / vehicle rats. In bulbectomised rats, 14-days chronic administration of agomelatine at the doses of 10 and 50 mg/kg significantly reduced locomotor hyperactivity to mean scores comparable to their sham treated counterparts. Imipramine (10 mg/kg) also elicited a reduction of ambulation score in bulbectomised rats. Neither agomelatine nor imipramine exerted an effect on ambulation in sham animals different from vehicle treated animals.

There was no overall effect of surgery or drug treatment or a significant interaction on rearing and grooming behaviour (data not shown). Defecation scores were significantly higher in bulbectomised

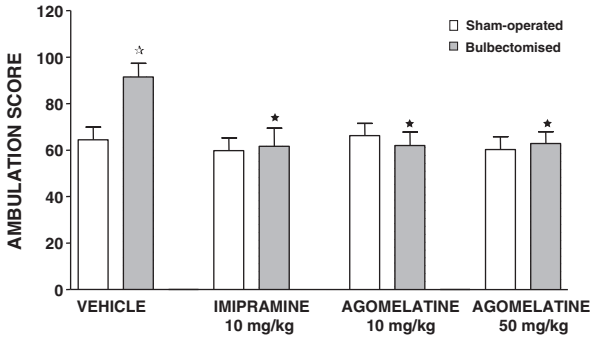

Fig. 1. Influence of chronic treatment with agomelatine as compared to imipramine on ambulation scores in the open field for olfactory bulbectomised (OB) and sham operated (SO) rats. Data are means ± S.E.M. number of squares crossed over a 3-min testing period. N = 8–16 per value. Two-way ANOVA as follows: Surgery, $F(1,89) = 2.6$, $P > 0.05$; Drug, $F(3,89) = 3.7$, $P < 0.05$ and Interaction, $F(3,89) = 2.7$, $P < 0.05$. ☆ indicates the significance of difference of OB/vehicle *versus* SO/vehicle values ($P < 0.05$) and ★ indicates significance of difference of OB/drug *versus* OB/vehicle values ($P < 0.05$) in Newman-Keuls test.

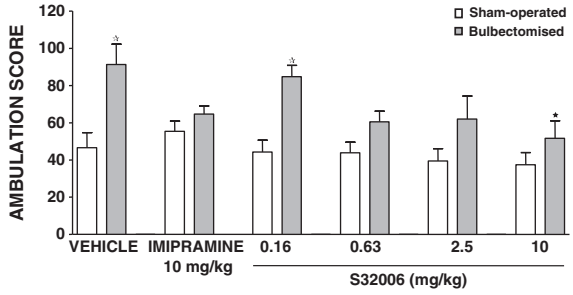

Fig. 3. Influence of chronic treatment with S32006 as compared to imipramine on ambulation scores in the open field for olfactory bulbectomised (OB) and sham operated (SO) rats. Data are means ± S.E.M. number of squares crossed over a 3-min testing period. N = 8–16 per value. Two-way ANOVA as follows: Surgery, $F(1,115) = 32.1$, $P < 0.001$; Drug, $F(5,115) = 3.0$, $P < 0.05$ and Interaction, $F(5,115) = 1.9$, $P > 0.05$. ☆ indicates the significance of difference of OB or *versus* SO values for each drug treatment condition ($P < 0.05$) and ★ indicates significance of difference of OB/drug *versus* OB/vehicle values ($P < 0.05$) in Newman-Keuls test.

rats than in sham animals although *post-hoc* testing did not show evidence of any significant difference between bulbectomised and sham rats for each drug treatment (data not shown).

3.2. Melatonin experiment

As shown in Fig. 2, bulbectomised / vehicle rats displayed significantly higher ambulation score than sham / vehicle rats. Administration of imipramine (10 mg/kg) for 14-days reduced hyperactivity in the bulbectomised rats to levels comparable to that of similarly treated sham operated animals. Melatonin at doses of 10 and 50 mg/kg did not reverse the increased ambulation scores in bulbectomised rats to levels equivalent to that of similarly treated sham operated animals.

There was no overall effect of surgery or drug treatment or a significant interaction effect on grooming behaviour (data not shown). Rearing and defecation scores were significantly higher in bulbectomised rats than in sham operated animals although *post hoc* testing did not reveal any evidence of significant differences between bulbectomised and sham rats for each drug treatment (data not shown).

3.3. S32006 experiment

As shown in Fig. 3, bulbectomised / vehicle rats displayed a significantly higher mean ambulation score than sham / vehicle rats. In bulbectomised rats, 14-days chronic administration of S32006 (0.16 to 10 mg/kg) dose-dependently reduced locomotor activity in such a way that bulbectomised rats receiving the maximally tested dose (10 mg/kg) of S32006 displayed a significantly lower mean score than bulbectomised / vehicle rats. In this experiment, as compared to bulbectomised / vehicle rats, the decrease in ambulation score observed in bulbectomised rats receiving imipramine (10 mg/kg) failed to reach statistical significance. Neither S32006 nor imipramine exerted a significant reduction in activity in sham operated animals.

There was no overall effect of surgery or drug treatment or a significant interaction effect on grooming behaviour (data not shown). Rearing and defecation scores were significantly higher in bulbectomised rats than in sham animals although *post hoc* testing did not show statistically significant differences between bulbectomised and sham rats for each drug treatment. A significant main effect of drug on rearing behaviour was observed, but *post-hoc* testing did not show any significant difference for bulbectomised / drug *versus* bulbectomised / vehicle values or sham / drug *versus* sham / vehicle values (data not shown).

The crude calculation of "relative antidepressant activity" of imipramine, agomelatine, melatonin and S32006 on ambulation scores is presented in Table 1. Agomelatine (10 and 50 mg/kg) significantly reduced ambulation by 116% and 90% respectively compared to the respective vehicle treated pairs, an effect similar to that of imipramine which induced a significant reduction of 93% in this experiment. On the other hand, melatonin (10 and 50 mg/kg) only tended to

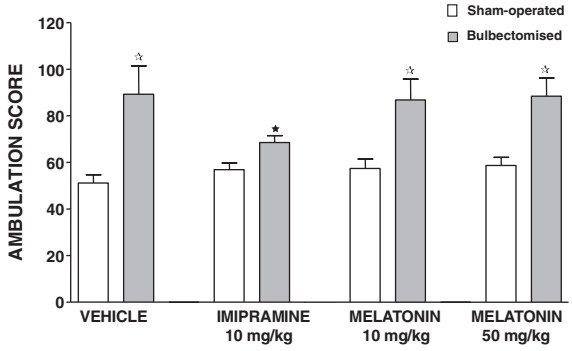

Fig. 2. Influence of chronic treatment with melatonin as compared to imipramine on ambulation scores in the open field for olfactory bulbectomised (OB) and sham operated (SO) rats. Data are means ± S.E.M. number of squares crossed over a 3-min testing period. N = 8–11 per value. Two-way ANOVA as follows: Surgery, $F(1,67) = 37.7$, $P < 0.001$; Drug, $F(3,67) = 1.2$, $P > 0.05$ and Interaction, $F(3,67) = 1.6$, $P > 0.05$. ☆ indicates the significance of difference of OB or *versus* SO values for each drug treatment condition ($P < 0.05$) and ★ indicates significance of difference of OB/drug *versus* OB/vehicle values ($P < 0.05$) in Newman-Keuls test.

Table 1
Relative antidepressant activity (%) of agomelatine, melatonin and S32006 on ambulation scores in OB rats.

Treatment	Relative anti-hyperlocomotive effect (%)
Vehicle (n = 12)	0 ± 22
Imipramine 10 mg/kg (n = 8)	93 ± 29 [a]
Agomelatine 10 mg/kg (n = 11)	116 ± 21 [a]
Agomelatine 50 mg/kg (n = 9)	90 ± 19 [a]
Vehicle (n = 9)	0 ± 32
Imipramine 10 mg/kg (n = 9)	70 ± 8
Melatonin 10 mg/kg (n = 9)	23 ± 23
Melatonin 50 mg/kg (n = 8)	22 ± 20
Vehicle (n = 9)	0 ± 25
Imipramine 10 mg/kg (n = 9)	79 ± 10 [a]
S32006 0.16 mg/kg (n = 10)	9 ± 13
S32006 0.63 mg/kg (n = 8)	63 ± 13
S32006 2.5 mg/kg (n = 9)	50 ± 28
S32006 10 mg/kg (n = 9)	68 ± 21

Data are mean ± S.E.M. One-way ANOVAs as follows: Experiment with agomelatine: $F(3,36) = 5.8$, $P < 0.01$; Melatonin, $F(3,31) = 1.7$, $P > 0.05$ and S32006, $F(5,48) = 2.9$, $P < 0.05$. [a] indicates significance difference *versus* vehicle values ($P < 0.05$) in Dunnett's test.

reduce hyperactivity (23 and 22%, respectively), in an experiment in which imipramine displayed 70% "antidepressant activity"; this score failed to reach statistical significance and is lower than those observed in the two other experiments. S32006 showed a dose-related reduction of ambulation (from 9% at 0.16 mg/kg to 68% at 10 mg/kg), but this failed to reach statistical significance even at the highest dose tested and was slightly lower that observed with imipramine (79% in this experiment).

4. Discussion

4.1. Locomotor hyperactivity induced by olfactory bulbectomy and reversal by imipramine

For the 'open field' evaluation the effect on ambulation score has been extensively validated as the principal outcome measure and as an index of putative antidepressant activity (Cairncross et al., 1979; Kelly et al., 1996; Song and Leonard, 2005).Thus, a statistically significant increase in ambulation (locomotor activity) was systematically observed in vehicle treated animals following bilateral olfactory bulbectomy compared to sham operated control animals. This result is consistent with numerous other studies of bulbectomy in the rat and other species (see Jancsar and Leonard, 1984a,b; McGrath and Norman, 1998). Further, the increase in ambulation in bulbectomised *versus* sham rats was associated with an overall increase of rearing (experiments with melatonin and S32006) and defecation (all experiments), which has been taken to indicate changes in 'emotionality' (Hall, 1934; Steiniger and Kretschmer, 2004; Walsh and Cummins, 1976). However, suggesting less robust reliability for these alternative measures *versus* locomotor hyperactivity, there were no significant differences between sham and bulbectomised pairs for any of the treatments in any experiment. Grooming has been reported to have low reliability as a behavioural measure for characterization of the bulbectomised model (Song and Leonard, 2005), and in line with this assessment, grooming was not significantly affected by surgery. Hyper-ambulation in the open field was reversed by the chronic administration of the clinically effective antidepressant imipramine as has been consistently reported in this model (Jancsar and Leonard, 1984a; Kelly et al., 1997). The effect of imipramine upon ambulation scores failed to reach significance in the "S32006" experiment. Statistically significant differences were demonstrated however, after transformation of the raw data into percent relative antidepressant activity.

Overall, these observations support previous works indicating that locomotor hyperactivity is the most reliable, robust and relevant measure of behaviour disrupted by olfactory bulbectomy and the most pertinent for demonstrating a putative antidepressant activity.

4.2. Effects of agomelatine on ambulation in olfactory bulbectomised rats

The ability of chronic agomelatine (10 and 50 mg/kg) to attenuate increased locomotor activity in the open field supports studies in other models in rats or mice, using this dose range, showing that the compound may possess antidepressant-like activity (Barden et al., 2005; Bertaina-Anglade et al., 2006; Bourin, et al., 2004; Païzanis et al., 2010; Papp, et al., 2003; Rainer et al., 2011).

Although in the present experimental conditions, the mechanism involved in agomelatine's action has not been explored it can be argued that the well documented effect on hippocampal neurogenesis, as well as on growth factors such as Brain Derived Neurotrophic Factor (BDNF) (Banasr et al., 2006; Calabrese et al., 2011; Dagytė et al., 2010, 2011; Païzanis et al., 2010; Soumier et al., 2009) possibly plays a role in the behavioural changes observed in olfactory bulbectomised rats treated with the drug. The data is in line with emerging hypotheses supporting an impairment of adult hippocampal neurogenesis in neuropsychiatric disorders including depression (Duman, 2004; Kempermann, 2008; Sahay and Hen, 2007), and the notion that the therapeutic effects of antidepressants maybe, at least partially, related to increased neurogenesis and growth factor expression in the brain (Castren et al., 2007; Duman and Monteggia, 2006). In addition, since S32006 is also active under these experimental conditions, it can be assumed that blockade of 5-HT_{2C} receptors contributes to the effect of agomelatine on locomotor hyperactivity in the bulbectomy model. However, this remains to be formally demonstrated.

4.3. Effects of S32006 on ambulation in olfactory bulbectomised rats

Similarly the 5-HT_{2C} antagonist, S32006, attenuated hyperactivity of bulbectomised rats in the open field and provides evidence for a potential antidepressant-like effect. These data are of special interest in as much as this is the first demonstration of positive effects of a selective 5-HT_{2C} antagonist with the bulbectomy procedure. Surprisingly, it has been suggested that the 5-HT_{2C} agonist, WAY-163909 is active in this model (Rosenzweig-Lipson et al., 2007). As discussed elsewhere (Millan, 2005) it is possible that both agonist and antagonist actions of 5-HT_{2C} ligands at different populations of 5-HT_{2C} receptor may yield antidepressant-like actions. Further, it is possible that actions of WAY163909 at different receptor populations may account for the "antidepressant-like" effects in the bulbectomy and other animal models. This issue requires further study but in any event the present results are consistent with 1), robust effects of S32006 in other models of "antidepressant-like" activity such as the forced swim test and chronic mild stress procedures (Dekeyne et al., 2008); 2) actions of other 5-HT_{2C} antagonists in diverse putative animal models of depression (Millan, 2005b and 2003); 3)the reversal of the behavioural hyperactivity of bulbectomised rats by the antidepressants, mianserin, its enantiomers and mirtazapine, all of which behave as potent 5-HT_{2C} antagonists (Chanrion et al., 2008; Dekeyne and Millan, 2009; Jancsar and Leonard, 1984b; Leonard and O'Connor, 1987; Millan et al., 2000; O'Connor and Leonard, 1986).

Since 5-HT_{2C} receptor antagonism *per se* does not enhance locomotor activity (Giorgetti and Tecott, 2004; Millan, 2005), this is consistent with the lack of an influence of S32006 upon ambulation in sham operated animals in the present study. Thus the reduction by S32006 (and agomelatine) of the hyperactivity in bulbectomised rats cannot be attributed to a "motor" effect, but is more convincingly related to its putative antidepressant-like profile.

4.4. Effects of melatonin on ambulation in olfactory bulbectomised rats

Neither dose of melatonin (10 and 50 mg/kg) used in this study demonstrated a statistically significant antidepressant-like effect in the bulbectomy model. This result is in contrast to several reports showing an effect of melatonin in the chronic mild stress model in rats, where evening administration dose-dependently reversed anhedonia (reduction of consumption of sucrose induced by stress) (Papp et al., 2003); in the mouse chronic mild stress model (Detanico et al., 2009) as well as in the forced swim test (Mantovani et al., 2003; Micale et al., 2006; Overstreet et al., 1998; Ramirez-Rodriguez et al., 2009). On the other hand, melatonin up to 50 mg/kg was inactive in a learned helplessness model of depression (Bertaina-Anglade et al., 2006). There are currently no published studies examining the effects of melatonin in bulbectomised rats. The current study is in agreement with the fact that melatonin is not active in all animal models of depression. Furthermore, no antidepressant effect of melatonin has been demonstrated in clinical trials with depressed patients (De Wries and Peeters, 1997; Dolberg et al., 1998; Srinivasan et al., 2006).

Overall the open field results with S32006 are consistent with studies in other models and suggest that the drug may possess antidepressant-like properties (Dekeyne et al., 2008). Agomelatine on the other hand has established efficacy in the clinic (Goodwin et al., 2009). In common with other clinically active antidepressants, both compounds were able to reverse the behavioural hyperactivity

displayed in the open field by bilaterally bulbectomised rats. The results of the study imply that the antidepressant effect of agomelatine is likely due to its antagonist effects at 5-HT_{2C} receptors, as the selective 5-HT_{2C} antagonist S32006 is also active in the test. However assuming the crude comparisons of 'relative antidepressant' activity are valid then S32006 is a less active agent than agomelatine at the same dose. Indeed S32006 at 10 mg/kg only partially antagonises the hyperactivity (68%) compared to agomelatine at 10 mg/kg which exerts a full antagonism (116%). Thus 5-HT_{2C} activity may not account entirely for agomelatine's effects on hyper-locomotion in the open field. Whether the putative melatonergic activity of the drug contributes to this effect is a moot point. Data obtained from other pre-clinical studies suggest that melatonergic activity contributes at least partially to the behavioural effects observed in these models. Thus data obtained by Papp et al. (2003) and Bertaina-Anglade et al. (2006) strongly support the notion that the antidepressant-like effects of agomelatine depend on either the combination of or the synergy between melatonergic agonist and 5-HT_{2C} antagonist properties. Moreover, recently Soumier et al. (2009) demonstrated that the effects of agomelatine on hippocampal cell survival maybe due to a combination of both properties reinforcing the idea that melatonergic agonism and $5HT_{2C}$ antagonism are needed to obtain the full antidepressant-like activity of the drug. Examination of the activity of other selective 5-HT_{2C} antagonists as well as selective melatonin agonists or antagonists, alone or co-administered in this and other behavioural models is clearly of interest to further illuminate the mechanism of action of agomelatine.

In conclusion, chronic treatment with agomelatine blunts the behavioural hyperactivity displayed by bulbectomised rats, further suggesting an antidepressant-like profile.

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