Evidence for antimanic efficacy of glycogen synthase kinase-3 (GSK3) inhibitors in a strain-specific model of acute mania

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Abstract
There is a growing body of evidence suggesting that animal models can be developed to probe the specific domains of bipolar disorder (BD) using the endophenotype approach. Here we tested clinically active antimanic drugs to validate amphetamine-induced hyperactivity in Black Swiss mice as a putative model of the manic phase of BD. We also co-administered a mood stabilizer and an atypical antipsychotic drug in a manner akin to the clinical treatment regimens. Since lithium has been shown to potentially act through glycogen synthase kinase-3 (GSK3) inhibition, we evaluated the efficacy of selective GSK3 inhibitors in this model. Habituated animals were pretreated with a compound of interest before being challenged with amphetamine (2.0 mg/kg) and returned to activity cages for an additional 1.5 h. We tested lithium, sodium valproate, carbamazepine, olanzapine, ziprasidone as well as co-administered lithium and olanzapine at sub-efficacious doses. The GSK3 inhibitors tested included indirubin, alsterpaullone, TDZD-8, AR-A014418, SB-216763, and SB-627772. All mood stabilizers and antipsychotic drugs reduced hyperactivity without affecting spontaneous locomotion. While subactive doses of lithium and olanzapine were without effect, their co-administration produced robust reductions in hyperactivity. All GSK3 inhibitors were active in the model, producing selective inhibition of rearing hyperactivity. These data support the predictive validity of the model for the acute manic phase of BD and may have utility as an in-vivo model for identifying novel antimanic therapeutics.

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Key words: Antimanic drugs, antipsychotic drugs, bipolar disorder, hyperactivity, mood stabilizers.

Introduction
One of the key obstacles in bringing recent discoveries and improved understanding of the basic biological processes of the disease into the psychiatric clinic has been the lack of appropriate preclinical animal models (Agid et al. 2007). Developing a model for bipolar disorder (BD) which is characterized by cycling between episodes of pathologically elevated mood (mania and hypomania) and depression (known as bipolar depression) has been particularly challenging (Gould & Einat, 2007). One of the strategies for overcoming some of the difficulties associated with modelling BD in animals has been the endophenotype approach (Einat, 2006; Flaisher-Grinberg et al. 2010; Glahn et al. 2004; Hasler et al. 2006). Endophenotypes are inherent, stable, and quantifiable measures of function that can be used to investigate pathology of complex diseases (Gottesman & Shields, 1973; Gottesman & Gould, 2003). Psychostimulants are known to induce ‘mania-like’ symptoms in healthy individuals and precipitate the onset of mania in patients with BD (Anand et al. 2000; Mamelak, 1978; Peet & Peters, 1995). There is some evidence that lithium can attenuate or prevent the behavioural or functional effects of these drugs (Bell et al. 2005; Silverstone et al. 1998; Van Kammen & Murphy, 1975; Willson et al. 2005). Based on these findings it has been suggested that sensitivity to psychostimulants can be (1) used as a clinical endophenotype of bipolar mania (Hasler et al. 2006) and (2) aspects of this endophenotype can be explored in animal models (Gould et al. 2007).
There has been a growing awareness of the differences in behavioural patterns and sensitivity to drug challenges among mouse strains (Einat, 2007; Flaisher-Grinberg & Einat, 2010; Gould et al. 2007; Kalinichev et al. 2008), in particular in the context of modelling aspects of BD. For example, compared to several other strains of mice, Black Swiss mice exhibit increased activity in a novel environment (Flaisher-Grinberg & Einat, 2010) and have slower habituation rates when re-exposed to a novel environment (M. Kalinichev, unpublished data). Moreover, Black Swiss mice are more sensitive to low doses of amphetamine than C57BL6/J, CBA/J, or A/J mice (Flaisher-Grinberg & Einat, 2010; Hiscock et al. 2007). In addition, Black Swiss mice have increased saccharin preference and show markedly reduced immobility in the forced swim test compared to C57BL6, A/J or CBA/J mice (Flaisher-Grinberg et al. 2009; Flaisher-Grinberg & Einat, 2010). These behaviours have face validity for facets of acute mania, such as hyperactivity, increased hedonistic drive, sensitivity to psychostimulants and pathologically elevated mood and behavioural disinhibition. Furthermore, most of these behaviours have been shown to be sensitive to the prototypic mood stabilizers lithium and valproate, but not to the tricyclic antidepressant imipramine (Flaisher-Grinberg & Einat, 2010; Hiscock et al. 2007) suggesting predictive validity of these models. Thus, these behaviours deserve further investigation as potential models of the facets of BD.

The goal of the present study was to further characterize and validate amphetamine-induced hyperactivity in Black Swiss mice as a putative model of acute bipolar mania. It is important to clarify that we propose increased sensitivity to amphetamine of Black Swiss mice as a putative model for some aspects of bipolar mania. We do not consider Black Swiss mice as ‘manic mice’ as highlighted by others (Flaisher-Grinberg et al. 2009). To this end initial studies evaluated clinically active antimanic agents, such as the mood stabilizer lithium, anticonvulsant drugs (valproate, carbamazepine) and atypical antipsychotics (olanzapine, ziprasidone) in this model. There is growing clinical evidence that combination drug treatment involving a mood stabilizer and an antipsychotic results in better therapeutic outcome than monotherapy involving a mood stabilizer alone (Smith et al. 2007). Therefore, we evaluated effects of such co-therapies using the atypical antipsychotic olanzapine and lithium in this animal model.

Second, we have evaluated the efficacy of several glycogen synthase kinase-3 (GSK3) inhibitors in this model. GSK3, expressed in two isoforms GSK3α and GSK3β, is a ubiquitous, constitutively active enzyme involved in multiple intracellular signalling pathways. There is accumulating evidence suggesting the role of GSK3 in psychopathology of several CNS disorders, such as BD, schizophrenia, attention deficit hyperactivity disorder (ADHD) and Alzheimer’s disease (Beaulieu & Caron, 2008; Beaulieu et al. 2009; Catapano & Manji, 2008; Emamian et al. 2004; Gould, 2006; Gould et al. 2004, 2006; Lovestone et al. 2007; Medina & Castro, 2008). GSK3 has been found to be a target for lithium, therefore receiving particular attention as a possible mediator of therapeutic effects of lithium (Klein & Melton, 1996; Stambolic et al. 1996). GSK3 has also been identified as a common target for selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants, and antipsychotic drugs suggesting a more central role of this enzyme in the psychopharmacology of these agents (Beaulieu, 2007; Li et al. 2004, 2007). Regulation of several dopamine- and serotonin-mediated behaviours in rodents was found to involve GSK3 activity (Beaulieu et al. 2004, 2008). Furthermore, behaviours of mice over-expressing GSK3β exhibit signs of mania (Prickaerts et al. 2006), and those of GSK3β haplo-insufficient mice mimic effects of lithium treatment (O’Brien et al. 2004). Hence we evaluated a range of selective, structurally dissimilar and potent GSK3 inhibitors, namely indirubin, alsterpaullone, thiadiazolidinone (TDZD-8), AR-A014418, SB216763, and SB627772 in this model. Indirubin, alsterpaullone, AR-A014418, SB216763, and SB627772 are ATP-competitive inhibitors of GSK3, whereas TDZD-8 is an ATP-non-competitive inhibitor (Medina & Castro, 2008).

Materials and methods

Animals

Subjects were adult male Black Swiss mice (24–30 g, Taconic Farms, USA). Animals were singly housed and maintained on a 12-h light/dark cycle (lights on 06:00 hours) under constant temperature (21 ± 1 °C) and humidity (50–58%) conditions. Food (Harlan Tekland 2014, Harlan UK Ltd, UK) and water were available ad libitum. Animals were acclimated for at least 5 d before experimentation. Studies were conducted in full compliance with the Home Office Guidance on the operation of the UK Animals (Scientific Procedures) Act 1986, the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health (USA) and were approved by the GlaxoSmithKline Ethical Procedures Review Panel.
Activity monitoring

Locomotor activity (horizontal activity and rearing) was monitored using 24 perspex boxes (42 × 21 × 21 cm) in conjunction with ‘AM Logger’ AM1052 activity monitors. These monitors were equipped with lower and upper sets of infrared photobeam interruption sensors (spaced 25 mm apart) for detection of horizontal activity and rearing, respectively, and a computerized analysis system (Linton Instruments, UK).

Procedures

For testing compounds in amphetamine-induced hyperactivity in Black Swiss mice, we modified the protocol developed by Gould et al. (2007) based on a series of preliminary experiments. Animals were allowed to habituate individually to the activity cage for 1.5–2 h in order to minimize locomotor activity stimulated by the novelty. Following administration of the drug, animals were returned to their cages for an additional 0.5–1 h, depending on the pretreatment time of the compound. The total time of habituation, including pretreatment time of the compound was always 2.5 h. At the end of the habituation period animals were challenged (i.p.) with either saline or amphetamine (2.0 mg/kg) and returned to their cages for additional 1.5 h. Each drug was tested in a separate experiment with its own appropriate control groups.

Since we previously demonstrated that activity in mice differs significantly between morning and afternoon hours (Kalinichev et al. 2008) each experiment was conducted during the morning hours (between 08:00 and 12:00 hours) during the light phase of the light/dark cycle over a 2-d period (counterbalanced across days for each drug).

Data analysis

In each experiment involving testing antimanic drugs and GSK3 inhibitors, the total horizontal and rearing counts during the habituation phase and following amphetamine stimulation (i.e. hyperactivity phase) were analysed using a repeated-measures ANOVA with dose as a between factor and phase (habituation and hyperactivity) as a within factor. All experiments produced a significant phase × treatment interaction, thus the follow-up analysis involved one-way ANOVAs for each phase followed by planned comparisons.

The data obtained from the ziprasidone experiment were log-transformed to stabilize the variance before being analysed as described above. The alpha level chosen was p < 0.05. The statistical package used was Statistica 9.0 (StatSoft Inc., USA).

Results

Study I

In Black Swiss mice lithium (50, 100 mg/kg i.p.) and valproate (50, 100 mg/kg i.p.) had no effect on spontaneous locomotion, but attenuated amphetamine-stimulated activity with statistically significant effects on both, horizontal counts \( F(5, 42) = 118.1, p < 0.001 \) and rearing \( F(5, 42) = 8.9, p < 0.001 \) (Fig. 1). Lithium (50 mg/kg i.p.) reduced amphetamine-stimulated rearing by ~50% \( (p < 0.01) \), whereas 100 mg/kg lithium reduced both horizontal hyperactivity and rearing by 40% \( (p < 0.01) \) and 60% \( (p < 0.001) \), respectively (Fig. 1a, c, d, f). Valproate had no effect on horizontal hyperactivity, but at 100 mg/kg reduced amphetamine-stimulated rearing by 40% \( (p < 0.05) \), Fig. 1e, f).

Similarly, carbamazepine (10, 20, 40 mg/kg i.p.) had no effect on spontaneous locomotion, but

Drugs

Lithium chloride, sodium valproate, carbamazepine, alsterpaullone, TDZD-8, AR-A014418, and amphetamine were purchased from Sigma-Aldrich (UK). Indirubin and SB216763 were purchased from Tocris Bioscience (UK). Olanzapine, ziprasidone and SB627772 were synthesized by Medicinal Chemistry, GlaxoSmithKline (UK). Dosing material was freshly prepared by Medicinal Chemistry, GlaxoSmithKline (UK). Dosing material was freshly prepared by Medicinal Chemistry, GlaxoSmithKline (UK). Dosing material was freshly prepared by Medicinal Chemistry, GlaxoSmithKline (UK). Dosing material was freshly prepared by Medicinal Chemistry, GlaxoSmithKline (UK). Dosing material was freshly prepared by Medicinal Chemistry, GlaxoSmithKline (UK). Dosing material was freshly prepared by Medicinal Chemistry, GlaxoSmithKline (UK). Dosing material was freshly prepared by Medicinal Chemistry, GlaxoSmithKline (UK).
Fig. 1. Horizontal activity (a–c) and rearing (d–f) of male Black Swiss mice pretreated (i.p.) with either vehicle (1% methylcellulose i.p.), lithium (50, 100 mg/kg i.p.) or valproate (50, 100 mg/kg i.p.) during 30 min of spontaneous (Spont) activity and 90 min following challenge (i.p.) with either vehicle (saline) or amphetamine (2.0 mg/kg, Amph hyperactivity). The data are expressed as time-courses (a, b, d, e) or as total counts (c, f) of 30 min of spontaneous locomotion and 60 min of hyperactivity (see text). Each point represents the observed mean (±S.E.M.). * p < 0.05, ** p < 0.01, *** p < 0.001 compared to corresponding reference (ref) groups (n = 7–9 per group).
attenuated amphetamine-stimulated rearing \([F(4, 35) = 5.9, \ p < 0.01]\), without affecting horizontal hyperactivity (Fig. 2). The maximum response was observed at 40 mg/kg when carbamazepine reduced rearing by 50%. However, this effect failed to reach statistical significance \((\ p = 0.07)\) due to high variability (Fig. 2c, d).

Olanzapine (0.1, 0.3, 1.0 mg/kg i.p.) had no effect on spontaneous locomotion, but attenuated amphetamine-stimulated activity, in both horizontal counts \([F(4, 38) = 14.4, \ p < 0.0001]\) and rearing \([F(4, 38) = 7.0, \ p < 0.0001]\) (Fig. 3a–d). Specifically, at 1.0 mg/kg olanzapine amphetamine-stimulated horizontal activity and rearing were reduced by 40% \((\ p < 0.01)\) and 65% \((\ p < 0.001)\), respectively (Fig. 3b, d). Similarly, ziprasidone (0.1, 0.3, 1.0 mg/kg i.p.) had no effect on spontaneous locomotion, but attenuated amphetamine-stimulated horizontal activity \([F(4, 37) = 26.6, \ p < 0.0001]\) and rearing \([F(4, 37) = 12.3, \ p < 0.0001]\) (Fig. 3e–h). Again, 1.0 mg/kg ziprasidone reduced amphetamine-stimulated horizontal activity and rearing by 40% \((\ p < 0.001)\) and 55% \((\ p < 0.01)\), respectively (Fig. 3f, h). A summary of studies on antimanic drugs in this model is presented in Table 1.

The results of co-administration of olanzapine (0.2 mg/kg) and lithium (30, 50 mg/kg) are shown in Fig. 4 and Table 2. Animals treated with either olanzapine or lithium alone, and those that had olanzapine and lithium co-administered, exhibited normal spontaneous locomotion (Fig. 4). There was a significant overall effect of treatment on rearing \([F(5, 41) = 3.5, \ p < 0.01]\), but not on horizontal activity. Olanzapine (0.2 mg/kg) or lithium (30, 50 mg/kg), when administered alone, failed to influence amphetamine-stimulated activity, either horizontal or rearing (Fig. 4). Planned comparisons revealed reduced amphetamine-stimulated horizontal activity in animals which had olanzapine co-administered with lithium (30 or 50 mg/kg) by ~30% \((\ p < 0.05, \text{Fig. 4c})\). However, horizontal activity of animals treated with the combination was not significantly different from those treated with the individual drugs alone (Fig. 4c). Furthermore, co-administration of olanzapine (0.2 mg/kg) and lithium (50 mg/kg) resulted in reduction of amphetamine-stimulated rearing by ~80% \((\ p < 0.001, \text{Fig. 4e, f})\). Animals in the latter group showed reduced rearing compared to olanzapine alone (Fig. 4f).

**Study II**

A selective GSK3 inhibitor, indirubin (10, 20, 40 mg/kg i.p.), had no effect on spontaneous locomotion.
Fig. 3. Horizontal activity (a, b, c, f) and rearing (c, d, g, h) of male Black Swiss mice tested in two separate experiments with pretreatment of either olanzapine (0.1, 0.3, 1.0 mg/kg i.p.) or ziprasidone (0.1, 0.3, 1.0 mg/kg i.p.) and their corresponding vehicles (1% methylcellulose i.p.) during 60 min of spontaneous (Spont) activity and 90 min following challenge (i.p.) with either vehicle (saline) or amphetamine (2.0 mg/kg, Amph hyperactivity). The data are expressed as time-courses (a, c, e, g) or as total counts (b, d, f, h) of spontaneous locomotion and 60 min of hyperactivity (see text). Each point represents the observed mean (± S.E.M.). ** p < 0.01, *** p < 0.001 compared to corresponding reference (ref) groups (n = 7–9 per group).
Table 1. A summary of all antimanic drugs tested in amphetamine-induced hyperactivity in Black Swiss mice. The table includes name of drug, dose (mg/kg), route of administration, pretreatment time (min) as well as minimal effective dose (MED, mg/kg) suppressing spontaneous and amphetamine (Amph)-stimulated horizontal activity and rears

<table>
<thead>
<tr>
<th>Drug tested</th>
<th>Doses (mg/kg)</th>
<th>Route of administration</th>
<th>Pretreatment time (min)</th>
<th>Spontaneous horizontal activity MED (mg/kg)</th>
<th>Amp-stimulated horizontal activity MED (mg/kg)</th>
<th>Spontaneous rears MED (mg/kg)</th>
<th>Amp-stimulated rears MED (mg/kg)</th>
</tr>
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<tbody>
<tr>
<td>Lithium</td>
<td>50, 100 i.p.</td>
<td>30</td>
<td>–</td>
<td>100</td>
<td>–</td>
<td>–</td>
<td>50</td>
</tr>
<tr>
<td>Valproate</td>
<td>50, 100 i.p.</td>
<td>30</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>10, 20, 30 i.p.</td>
<td>40</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>40</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>0.1, 0.3, 1.0 i.p.</td>
<td>60</td>
<td>–</td>
<td>1.0</td>
<td>–</td>
<td>–</td>
<td>1.0</td>
</tr>
<tr>
<td>Ziprasidone</td>
<td>0.1, 0.3, 1.0 i.p.</td>
<td>60</td>
<td>–</td>
<td>1.0</td>
<td>–</td>
<td>–</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Table 2. A summary of the effects of co-administration of lithium and olanzapine on amphetamine (Amph)-induced hyperactivity in Black Swiss mice. The table includes dose (mg/kg), route of administration, pretreatment time (min), and magnitudes of reduction (and p values) in activities compared to the corresponding reference groups (see text)

<table>
<thead>
<tr>
<th>Drug tested</th>
<th>Doses (mg/kg)</th>
<th>Route of administration</th>
<th>Pretreatment time (min)</th>
<th>Spontaneous horizontal activity MED (mg/kg)</th>
<th>Amp-stimulated horizontal activity MED (mg/kg)</th>
<th>Spontaneous rears MED (mg/kg)</th>
<th>Amp-stimulated rears MED (mg/kg)</th>
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<tbody>
<tr>
<td>Lithium</td>
<td>30 i.p.</td>
<td>30</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>–</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>0.2 i.p.</td>
<td>60</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lithium + olanzapine</td>
<td>30 i.p.</td>
<td>30</td>
<td>30% (p &lt; 0.05)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lithium</td>
<td>50 i.p.</td>
<td>30</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>0.2 i.p.</td>
<td>60</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lithium + olanzapine</td>
<td>50 i.p.</td>
<td>30</td>
<td>30% (p &lt; 0.05)</td>
<td>–</td>
<td>80% (p &lt; 0.001)</td>
<td>–</td>
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</table>

Locomotion, but attenuated amphetamine-stimulated activity, both horizontal counts [F(4, 40) = 40.5, p < 0.001] and rearing [F(4, 40) = 11.5, p < 0.001] (Fig. 5a–d). Specifically, 10, 20 and 40 mg/kg indirubin reduced amphetamine-stimulated rearing by 32% (p < 0.05), 37% (p < 0.05), and 60% (p < 0.001), respectively (Fig. 5d). At the highest dose, indirubin also reduced amphetamine-stimulated horizontal activity by 45% (p < 0.01, Fig. 5b).

Alsterpaullone (10, 30, 100 mg/kg i.p.) had no overall effect on spontaneous locomotion but dose-dependently reduced amphetamine-stimulated horizontal activity [F(4, 40) = 218, p < 0.001] and rearing [F(4, 40) = 32.7, p < 0.001] (Fig. 5e–h). In particular, 100 mg/kg alsterpaullone reduced horizontal hyperactivity and rearing by 25% (p < 0.05) and 55% (p < 0.01), respectively (Fig. 5f, h).

TDZD-8 (5, 10, 30 mg/kg i.p.) had no effect on spontaneous horizontal activity, while reducing spontaneous rearing [F(4, 36) = 6.68, p < 0.001] (Fig. 6a–d). Specifically, in comparison to averaged activity of vehicle-treated groups 10 and 30 mg/kg TDZD-8 reduced spontaneous rearing by ~60% (p < 0.01, Fig. 6d). TDZD-8 also resulted in dose-dependent attenuation of amphetamine-stimulated horizontal activity [F(4, 36) = 58.8, p < 0.001] and rearing [F(4, 36) = 39.1, p < 0.001]. Specifically, horizontal hyperactivity was reduced by 75% (p < 0.001) at 30 mg/kg (Fig. 6b), whereas rearing was reduced by 60% (p < 0.001) at 10 mg/kg and virtually eliminated at 30 mg/kg (Fig. 6d).

AR-A014418 (5, 10, 15, 20 mg/kg i.p.) reduced spontaneous horizontal activity [F(5, 42) = 3.01, p < 0.05] and rearing [F(5, 42) = 3.00, p < 0.05] (Fig. 6e–h). Specifically, spontaneous horizontal activity was reduced by 30–40% at 10 and 20 mg/kg, while rearing was reduced by 70% at 20 mg/kg (Fig. 6f, h). AR-A014418 dose-dependently attenuated amphetamine-stimulated horizontal activity [F(5, 42) = 24.4, p < 0.0001] and rearing [F(5, 42) = 9.01, p < 0.0001].
Fig. 4. Horizontal activity (a–c) and rearing (d–f) of male Black Swiss mice administered either vehicle (1% methylcellulose) or olanzapine (0.2 mg/kg) as treatment 1 and with either vehicle (1% methylcellulose) or lithium (30, 50 mg/kg) as treatment 2 during 60 min of spontaneous (Spont) activity and 90 min following challenge (i.p.) with amphetamine (2.0 mg/kg, Amph hyperactivity). The data are expressed as time-courses (a, b, d, e) or as total counts (c, f) of spontaneous locomotion and 60 min of hyperactivity (see text). Each point represents the observed mean (±S.E.M.). * p < 0.05, *** p < 0.001 compared to corresponding reference (ref) groups (n = 7–8 per group).
Fig. 5. Horizontal activity (a, b, e, f) and rearing (c, d, g, h) of male Black Swiss mice tested in two separate experiments with pretreatment of either indirubin (10, 20, 40 mg/kg i.p.) or alsterpaullone (10, 30, 100 mg/kg i.p.) and their corresponding vehicles (saline i.p.) during 60 min of spontaneous (Spont) activity and 90 min following challenge (i.p.) with either vehicle (saline) or amphetamine (2.0 mg/kg, Amph hyperactivity). The data are expressed as time-courses (a, c, e, g) or as total counts (b, d, f, h) of spontaneous locomotion and 60 min of hyperactivity (see text). Each point represents the observed mean (± S.E.M.).

* p < 0.05, ** p < 0.01 *** p < 0.001 compared to corresponding reference (ref) groups (n = 9 per group).
Fig. 6. Horizontal activity (a, b, c, f) and rearing (c, d, g, h) of male Black Swiss mice in two separate experiments with pretreatment of either TDZD-8 (5, 10, 30 mg/kg i.p.) or AR-A014418 (5, 10, 15, 20 mg/kg i.p.) and their corresponding vehicles (1% methylcellulose i.p.) during 30 min of spontaneous (Spont) activity and 90 min following challenge (i.p.) with either vehicle (saline) or amphetamine (2.0 mg/kg, Amph hyperactivity). The data are expressed as time-courses (a, c, e, g) or as total counts (b, d, f, h) of spontaneous locomotion and 60 min of hyperactivity (see text). Each point represents the observed mean (± S.E.M.). * p < 0.05, ** p < 0.001 compared to corresponding reference (ref) groups. ## p < 0.01 compared to the averaged activity of vehicle-treated animals (ref group, n = 6–8 per group).
Specifically, horizontal hyperactivity was reduced by 30% ($p < 0.05$) at 20 mg/kg (Fig. 6f), whereas it stimulated rearing by 45% ($p < 0.05$) and by >80% ($p < 0.001$), at 15 and 20 mg/kg, respectively (Fig. 6h).

SB216763 (10, 30, 100 mg/kg i.p.) had no effect on spontaneous locomotion, but reduced amphetamine-stimulated rearing [$F(4,40)=39.8$, $p < 0.001$], but not horizontal hyperactivity (Fig. 7a–d). Specifically, 100 mg/kg SB216763 attenuated amphetamine-stimulated rearing by 55% ($p < 0.01$) and rearing by 70% ($p < 0.001$, Fig. 7f, h).

A summary of studies on testing GSK3 inhibitors in this model is presented in Table 3.

Discussion

In our study we fully validated and further characterized amphetamine-induced hyperactivity in Black Swiss mice as a putative model of the manic phase of BD. We confirmed activity of drugs of diverse groups used to stabilize acute mania in the clinic, such as the mood stabilizer lithium, anticonvulsants sodium valproate and carbamazepine, and antipsychotics olanzapine and ziprasidone. Furthermore, co-administration of a mood stabilizer and an antipsychotic drug produced efficacy which exceeded those obtained with either mechanism alone. In addition we showed clear efficacy of several, chemically diverse GSK3 inhibitors in this model.

Amphetamine-induced hyperactivity in rodents has received particular attention as a putative model of mania (Berggren et al. 1978; Berggren, 1985; Borison et al. 1978; Gould et al. 2007; Smith, 1981). Black Swiss mice have been shown to be more sensitive to lower doses of amphetamine compared to several other strains (Flaisher-Grinberg & Einat, 2010; Hiscock et al. 2007). We can speculate that the differences in dopaminergic neurotransmission may mediate differences in sensitivity to amphetamine across the strains. Indeed, the augmented effect of amphetamine on locomotor activity of C57BL6/J mice vs. DBA/2J mice have been reported to be paralleled by an increased dopaminergic neurotransmission in the nucleus accumbens and concurrently a reduced dopamine output in the medial prefrontal cortex, measured using in-vivo microdialysis in freely moving animals (Ventura et al. 2004). However, dopaminergic neurotransmission in Black Swiss vs. other strains remains to be investigated. Interestingly, GSK3β has been shown to be involved in the actions of amphetamine on locomotor activity in mice. Administration of 2.0 mg/kg amphetamine resulted in a reduction of Akt, GSK3α and GSK3β phosphorylation in the striatum of wild-type mice, while amphetamine-induced locomotor activity was attenuated in heterozygote GSK3β knockout mice (GSK3β+/−), which have a ~50% reduction in striatal GSK3β expression (Beaulieu et al. 2004). Furthermore, lithium dose-dependently attenuated amphetamine-induced hyperactivity in C57BL6/J mice (Gould et al. 2007).

In response to 2.0 mg/kg amphetamine, used here to assess effects of antimanic drugs and GSK3 inhibitors, Black Swiss mice exhibited patterns of horizontal activity that followed a typical bell-shaped curve: peaking 20–30 min following dosing and gradually declining over the following 60 min. However, amphetamine-stimulated rearing increased more gradually, peaking 60 min following amphetamine administration and was often preceded by a smaller peak immediately after the injection. Interestingly, according to Beaulieu et al. (2004) administration of 2.0 mg/kg amphetamine resulted in a marked reduction in phosphorylated forms of Akt, GSK3α, and GSK3β in the mouse striatum at 90 min but not 30 min following administration. This coincides with the increases in rearing exhibited by Black Swiss mice in response to amphetamine administration. Indeed, amphetamine-stimulated rearing was more sensitive than horizontal hyperactivity to antimanic drugs and to GSK3 inhibitors. Specifically, among the drugs tested in the model, several of them (lithium, indirubin, TDZD-8, AR-A014418), had lower minimal effective doses (MEDs) in stimulated rearing than in horizontal hyperactivity, while others (valproate, carbamazepine, SB-216763) were only active in amphetamine-stimulated rearing. Further, the effect of co-administering subactive doses of olanzapine and lithium was more robust in amphetamine-stimulated rearing than in horizontal hyperactivity. Thus, measurement of rearing appears to be necessary to optimize the sensitivity of the model. It should be noted that previously spontaneous rearing in a novel environment has been linked to exploration and anxiety, the latter being more prominent in certain experimental set-ups such as the staircase test (Ago et al. 2007; Pollard & Howard, 1986; Simiand et al. 1984).
Fig. 7. Horizontal activity (a, b, c, f) and rearing (c, d, g, h) of male Black Swiss mice in two separate experiments with pretreatment of either SB216763 (10, 30, 100 mg/kg i.p.) or SB627772 (10, 30, 100 mg/kg i.p.) and their corresponding vehicles (saline i.p.) during 30 min of spontaneous (Spont) activity and 90 min following challenge (i.p.) with either vehicle (saline) or amphetamine (2.0 mg/kg, Amph hyperactivity). The data are expressed as time-courses (a, c, e, g) or as total counts (b, d, f, h) of spontaneous locomotion and 60 min of hyperactivity (see text). Each point represents the observed mean (± S.E.M.). ** p < 0.01, *** p < 0.001 compared to the reference (ref) groups (n = 9–10 per group).
in reduction in amphetamine-stimulated rearing in the present model. In contrast, maintaining a high level of GSK3 activity as in GSK3α/β knockin mice (McManus et al. 2005) reduced spontaneous rearing and digging behaviours (Mines et al. 2010).

All clinically active antimanic drugs evaluated in this study reduced amphetamine-induced hyperactivity in Black Swiss mice. This activity can not be attributed to non-specific effects because at the doses tested none of the drugs had any effect on spontaneous locomotor behaviour. Lithium (100 mg/kg) reduced amphetamine-stimulated horizontal activity and rearing of Black Swiss mice. This activity can not be attributed to non-specific effects because at the doses tested none of the drugs had any effect on spontaneous locomotor behaviour. Lithium (100 mg/kg) reduced amphetamine-stimulated horizontal activity and rearing of Black Swiss mice confirming early findings by Gould et al. (2007). Similar doses (100–150 mg/kg) reduced hyperactivity induced by amphetamine in NMRI (Berggren et al. 1978) and in C57BL6J (Gould et al. 2007) mice as well as hyperactivity exhibited by dopamine transporter (DAT) knockout mice (Beaulieu et al. 2004). In a different rodent model of acute mania, lithium (85 mg/kg) prevented disruption of amphetamine-induced pre-pulse inhibition (PPI), but not ketamine-induced disruption in both C57BL6/J and 129SvPaslco mice (Ong et al. 2005). Here we demonstrate the activity of antipsychotic drugs in an animal model of mania. Black Swiss mice treated with olanzapine (1.0 mg/kg) or ziprasidone (1.0 mg/kg), exhibited reduction in amphetamine-induced hyperactivity (horizontal activity and rearing), while exhibiting normal spontaneous locomotion. These doses were similar to those (0.32 and 1.0 mg/kg for olanzapine and ziprasidone, respectively) that were active in a putative rodent model of psychosis, i.e. acute MK-801 induced hyperactivity in BALB/C mice (Bradford et al. 2010). Previously, in another rodent model of acute mania, twice-daily treatment with olanzapine (1.0 or 6.0 mg/kg) failed to alter locomotor hyperactivity induced by icv administration of a sodium pump inhibitor ouabain in the rat (El-Mallakh et al. 2006). Combination drug regimens have been increasingly used throughout the world for the

<table>
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<tr>
<th>Drug tested</th>
<th>Doses (mg/kg)</th>
<th>Route of administration</th>
<th>Pretreatment time (min)</th>
<th>Spontaneous horizontal activity MED (mg/kg)</th>
<th>Amph-stimulated horizontal activity MED (mg/kg)</th>
<th>Spontaneous rears MED (mg/kg)</th>
<th>Amph-stimulated rears MED (mg/kg)</th>
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<tr>
<td>Indirubin</td>
<td>10, 20, 40</td>
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<tr>
<td>Alsterpaullone</td>
<td>10, 30, 100</td>
<td>i.p.</td>
<td>60</td>
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<tr>
<td>TDZD-8</td>
<td>5.0, 10, 30</td>
<td>i.p.</td>
<td>30</td>
<td>10</td>
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<tr>
<td>AR-A014418</td>
<td>5.0, 10, 15, 30</td>
<td>i.p.</td>
<td>30</td>
<td>10</td>
<td>20</td>
<td>20</td>
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<tr>
<td>SB216763</td>
<td>10, 30, 100</td>
<td>i.p.</td>
<td>30</td>
<td>–</td>
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<td>–</td>
<td>100</td>
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<tr>
<td>SB627772</td>
<td>10, 30, 100</td>
<td>i.p.</td>
<td>30</td>
<td>100</td>
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Table 3. A summary of all GSK3 inhibitors tested in amphetamine-induced hyperactivity in Black Swiss mice. The table includes name of drug, dose (mg/kg), route of administration, pretreatment time (min), and minimal effective dose (MED, mg/kg) suppressing spontaneous and amphetamine (Amph)-stimulated horizontal activity and rears.
treatment of bipolar mania (Klein et al. 1984; Möller et al. 1989; Tohen et al. 2002). The advantage of such an approach is particularly evident in those patients that do not respond to conventional monotherapy. For example, in a 6-wk long, double-blind trial in previously non-responsive manic patients co-treatment with lithium or valproate with olanzapine produced a significant improvement in therapeutic outcome (Tohen et al. 2002). In the present study effects of combination drug regimen were evaluated in a rodent model of bipolar mania. Olanzapine (0.2 mg/kg) and lithium (30, 50 mg/kg), when administered at subactive doses failed to influence amphetamine-induced hyperactivity. However, co-administration of olanzapine (0.2 mg/kg) and lithium (50 mg/kg) resulted in an 80% reduction in amphetamine-stimulated rearing and a smaller (30%) reduction in horizontal hyperactivity. Thus, the present model has a predictive validity in the context of testing novel compounds for the potential combinational drug regimen in manic patients.

Although haloperidol was not tested in the present model, we can hypothesize that it may attenuate amphetamine-induced hyperactivity in Black Swiss mice in a manner similar to that seen with olanzapine and ziprasidone. There is convincing clinical evidence that haloperidol is efficacious in manic patients, resulting in response rates that are shorter than those seen with lithium treatment (Tohen & Vieta, 2009). In follow-up studies it would be interesting to test selective dopamine and serotonin ligands for pharmacological characterization of the model. It would be also interesting to test the antipsychotic reserpine, based on its mode of action which differs from that of olanzapine and ziprasidone. To our knowledge reserpine has never been tested in a rodent model of acute bipolar mania. There is some evidence that reserpine is efficacious in manic patients when administered either as monotherapy or when co-administered with lithium (Bacher & Lewis, 1979; Watt, 1958).

It needs to be emphasized that in the present study no negative control compounds were used. Further validation of the model can unquestionably benefit from experiments showing no effect of such compounds as the antidepressants, imipramine or fluoxetine. Other investigators have shown that in a putative model of mania, imipramine, used in the study as a negative control, failed to reduce heightened saccharin preference (Flaisher-Grinberg & Einat, 2009) and amphetamine-induced hyperactivity (Flaisher-Grinberg & Einat, 2010) in Black Swiss mice.

Since GSK3 inhibition has been shown to be a potential target for mediating lithium-induced efficacy in mania and it plays a role in mediating the effects of dopaminergic neurotransmission, we have evaluated a range of structurally diverse GSK3 inhibitors in this putative preclinical model of mania. All compounds tested showed activity, dose-dependently reducing hyperactivity in amphetamine-treated animals. The effect of GSK3 inhibitors can be considered specific since, at doses administered in this study, indirubin, alsterpaullone, SB216763, and SB627772 had no effect on spontaneous locomotor activity, whereas TDZD-8 and AR-A014418 were active at doses that were lower than those which suppressed spontaneous locomotion. Previously indirubin, alsterpaullone, SB216763 and TDZD-8 were active in hyperactivity exhibited by mice lacking DAT (Beaulieu et al. 2004). Moreover, according to Gould et al. (2004) AR-A014418 (30 μmol/kg) resulted in reduced immobility in the forced swim test (FST) in the rat suggesting an antidepressant-like action. This increase was specific and was not due to generalized increase in activity, since this dose also reduced spontaneous and amphetamine-stimulated activity (Gould et al. 2004). Another GSK3 inhibitor (L803-mts), administered icv produced antidepressant-like effect in the mouse FST (Kaidanovich-Beilin et al. 2004). Thus inhibition of GSK3 may have a therapeutic effect in treatment of both manic and depression phases of BD.

It is noteworthy that the increased spontaneous locomotion, slower rates of habituation and reduced immobility in the FST described in Black Swiss mice (Flaisher-Grinberg & Einat, 2010; Hiscock et al. 2007; M. Kalinichev, unpublished data) are very similar to those exhibited by transgenic mice over-expressing GSK3β (Prickaerts et al. 2006) and by DAT KO mice (Giros et al. 1996; Ralph et al. 2001; Ralph-Williams et al. 2003; Zhuang et al. 2001). In this regard, both groups of transgenic mice showed elevated activity and reduced habituation rates to a novel environment (Ralph-Williams et al. 2003; Zhuang et al. 2001) and GSK3β over-expressing mice exhibited reduced immobility in the FST (Prickaerts et al. 2006). Interestingly, GSK3 appears to be involved in mediating the locomotor hyperactivity exhibited by DAT mutant mice (Beaulieu et al. 2004, 2005) as it does in animals over-expressing GSK3β (Prickaerts et al. 2006). Thus, based on phenotypic similarities between mutant mice over-expressing GSK3β, DAT mutants and Black Swiss mice, one could speculate that locomotor hyperactivity and reduced rates of habituation of the latter group, are associated with increased dopaminergic neurotransmission paralleled by either GSK3 over-expression or reduced GSK3 phosphorylation. Thus assessment of GSK3 activity and dopaminergic...
neurotransmission in Black Swiss mice may shed light on this speculation.

It should be emphasized that the acute effects of compounds tested in the present model stand in contrast to the therapeutic effects of antimanic medication in the clinic, which require days and sometimes weeks of treatment. However, the acute effects of antimanic drugs and especially those of GSK3 inhibitors can have important impact on development of new medications for bipolar mania. Previously acute mechanisms of action of tricyclic antidepressants were instrumental for the development of SSRIs. Thus, the present model can be useful for the discovery of targets of acute, rather than long-term, action of antimanic drugs as well as those of potentially novel pharmacotherapies, such as GSK3 inhibitors (Gould et al. 2007).

In summary, amphetamine-induced hyperactivity in Black Swiss mice shows some face and predictive validity in modelling acute bipolar mania and thus may have utility as a screening tool for the evaluation of new/novel antimanic agents. Furthermore, these data further suggest that GSK3 inhibitors may have therapeutic utility in the treatment of BD.

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Statement of Interest

None.

References


