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Black Swiss Mice as a New Animal Model for Mania: A Preliminary Study.

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Abstract

The lack of animal models for mania and bipolar disorder hinders research of this devastating disorder. The need for new models in the field is great and one possible approach for model development is to utilize strain differences. In this context, the present study compares the behavior of Black Swiss mice (BS) and C57BL/6 mice (C57). Studied behaviors include performance in the black/white box, sweet solution consumption and responses to low and high doses of amphetamine. This study further tests the response of the two strains to the prototypic mood stabilizer lithium. Results indicate that compared with C57 mice, BS mice are risk takers, show increased reward seeking behavior and have a stronger response to psychostimulants, behaviors strongly associated with mania. Moreover, the BS mice were less responsive to the treatment-like effects of lithium. It is therefore suggested that the combined phenotype of BS mice that emerges from the different tests may model manic-like behavior and that pending further validation, the BS mice strain may be a practical new model for mania.

Key words: Animal model; bipolar disorder; behavior; Mood stabilizers.

Introduction

Animal models for bipolar disorder (BPD) and, especially, for mania are scarce and this shortage hinders research efforts in the field (Nestler et al. 2002; Tecott and Nestler 2004). A number of approaches have recently been proposed toward the development of new and better models for BPD (Gould and Einat 2007) including modeling disease facets (Einat 2006; Einat 2007a), modeling endophenotypes (Gould and Gottesman 2006), modeling based on molecular or genetic manipulations (Einat and Manji 2006; Machado-Vieira et al. 2004) and models based on strain and individual differences (Einat 2006; Einat 2007b; Malatynska and Knapp 2005).

Strain differences are frequently utilized to model psychiatric disorders, however, this is usually after breeding for behavioral or biochemical features. Some well documented specifically bred rat strains used in the context of psychiatry research are the Flinders Sensitive Line (e.g. (Overstreet 1986)) and the Alcohol Preferring P rats (e.g. (Penn et al. 1978)). Many other strains were suggested as tentative models.

Without discounting the importance of using specifically bred strains, there are some advantages in using standard strains. Practically, standard strains are readily available. Conceptually, specifically bred animals can limit a-priori the range of exploration into the underlying basis of a disorder. Such models have explicit biological differences (targeted through breeding or genetic manipulation) that at times may result in changes that resemble a disease but may not reflect the entire scope of a complex disease.

Standard strains have also been used in the context of psychopathology research. For example, the Fischer 344 and the Lewis inbred rat strains are significantly different in a variety of measures including susceptibility to psychoactive drugs and stress (Cohen et al. 2006; Suzuki et al. 1988). These differences have been used to explore the underlying biology of disease (Cohen et al. 2006) as well as possible new treatment approaches (Vit et al. 2006).

Although different strains have been proposed as models for psychiatric disorders, no strain was yet reported to have manic-like properties. The present study reports that the Black Swiss mice demonstrate manic-like behaviors compared with the C57BL/6 mice and that higher doses of the prototypic mood stabilizer lithium are needed to ameliorate their manic-like behavior. It is therefore suggested that pending further validation, this strain may be used as a model for mania.

Materials and Methods

Black Swiss (BS, Taconic, NY) and C57BL/6 (C57, Harlan, IN) mice were tested for manic-like behaviors (Einat 2006) and response to lithium. Both strains were previously demonstrated to behaviorally respond to high dose amphetamine (the most frequently used model for manic-like behavior) as well as to a high dose lithium treatment (Gould et al. 2007). The C57BL/6 strain was chosen as the comparison strain because it is very frequently utilized in modeling affective disorders and because it is the background strain for many related targeted mutation strains. Male mice (10-12 weeks old; 25±5g) were allowed a few days of adaptation after transportation and were held in a colony room with 12/12 light dark cycle and ad-lib food and water. Experiments were conducted during the light phase of the light/dark cycle in an experimental room separate from the colony room. All experimental procedures were conducted according to NIH guidelines and approved by the University of Minnesota IACUC (protocol # 0610A94146). One group of 32 mice was used for the black/white box experiment, another group (40 mice) was used for the saccharine consumption test and 5 days later for the amphetamine hyperactivity experiment, and a third group (34 mice) was used to study the response to lithium. Statistical analysis (Statistica, Statsoft, Tulsa, OK) utilized either Students' t-test or ANOVA according to the specific design of each experiment. Significance level was set at $p<0.05$.

The black/white box was used to evaluate risk taking behavior. A 45x30x30 cm box was divided into a 1/3 covered black section and a 2/3 well lit white section with a connecting door between. Rodents tend to prefer the dark area but explore the white area (Einat et al. 2005). Reduced time in the white area is a standard measure for anxiety (Sanchez and Meier 1997). By the same token, increased time in the white portion of the box can represent risk-taking behavior (Einat 2006). Mice (n=16/group) were placed in the white portion of the box (with the connecting door open) and their behavior digitally recorded for a 5 minutes session. Recording served to manually score the number of entries into and time spent in the white portion.

The sweet solution consumption test evaluates hedonistic-like or reward seeking behavior (Willner et al. 1996). Mice (n=20/group) were housed individually and supplied with a bottle of 1% saccharin solution (SIGMA, St. Lewis, MI) in addition to their supply of water and food. Bottles were present for 4 days during which weights of saccharine solution and water were taken at the beginning of the experiment and every 24 hours thereafter. Daily consumption of saccharine solution as a percentage of total liquid consumption was computed. This computation is needed to overcome differences that may be related to the different weights of individual mice.

Response to low dose amphetamine was tested after administration of d-amphetamine or vehicle (SIGMA, St. Lewis, MI; n=10/group 0.5 mg/kg IP injection, diluted in saline to a 10 ml/kg volume). Immediately after injection, mice were placed in activity monitors (Opto M3, Columbus Instruments, Columbus, OH) for a 30 minute session in which the number of crossings of infra-red beams was collected. The amphetamine dose was chosen because it was previously reported to be inactive in the induction of hyperactivity in C57 mice (Helmeste and Seeman 1982). To evaluate response to lithium, mice (N=5-6/group) received 10 days of IP injections of vehicle (saline) or lithium chloride 100 mg/kg (SIGMA) once or twice daily, and then tested for amphetamine-induced hyperactivity as described above with a higher dose of amphetamine (2 mg/kg) known to induce hyperactivity in both C57 and BS mice.

Results

The number of entries and time spent in the white portion of the black/white box were significantly higher in BS mice than in C57 mice [Table 1; for number of entries: t(30)=5.25, p<0.01; for time: t(30)=3.5, p<0.01], suggesting increased risk-taking behavior. BS mice consumed more saccharine solution compared with C57 mice, suggesting higher reward-seeking behavior or higher sensitivity to hedonic stimuli of this strain [Table 1; ANOVA, repeated measures across days, Strain effects: F(1,38)=77.9, p<0.001; Post hoc Bonferroni, BS different from C57 at each day (p<0.001)].

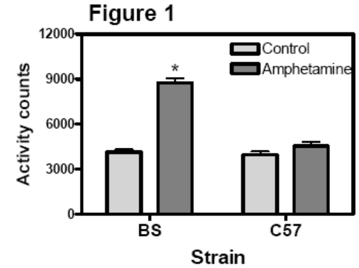
Table 1: Behavioral differences between strains in the black/white box and sweet solution consumption test

TEST	MEASURE	BLACK SWISS (MEAN±SE)	C57BL/6 (MEAN±SE)	STATISTICS
Black/white box	Entries to white	23.5±0.9	16.3±1.0	t(30)=5.25, p<0.01
	Time in white	154.3±3.7	124.8±7.6	t(30)=3.5, p<0.01
Saccharine consumption (% of total liquids)	Day 1	0.83±0.02	0.48±0.03	Repeated measures F(1,38)=77.9, p<0.001; Post hoc Bonferroni, BS different from C57 at each day (p<0.001)
	Day 2	0.77±0.02	0.45±0.04	
	Day 3	0.73±0.03	0.44±0.03	

As expected, C57 mice did not demonstrate hyperactivity after administration of low dose amphetamine. However, BS mice had a significant response with about 100% increase in activity [Figure 1; ANOVA, Strain effect: F(1,36)=69.6, p<0.001; Drug effect: F(1,36)=97.3, p<0.001; Strain x Drug interaction: F(1,36)=59.2, p<0.001].

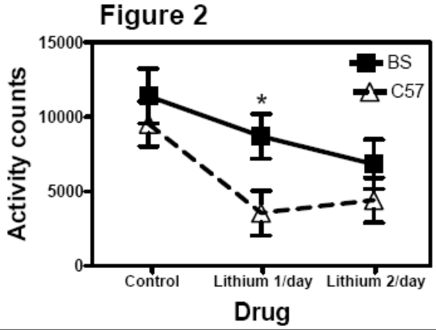
Moreover, the hyperactivity induced by 2 mg/kg amphetamine in C57 mice was ameliorated by one daily injection of 100 mg/kg lithium chloride while two daily injections were needed to reduce the hyperactivity of the BS mice, thus indicating partial resistance to lithium effects (Figure 2; ANOVA, Strain effect F(1,28)=5.86, p<0.05; Lithium effect F(2,28)=5.26, p<0.05; post-hoc Bonferroni test: BS-Lithium 1/day different than C57-Lithium 1/day).

Figure 1



Amphetamine hyperactivity: A low dose (0.5 mg/kg) amphetamine (dark bars) resulted in a large and significant increase in activity in Black Swiss (BS) mice but had no significant effect on C57bl/6 (C57) mice, compared to animals treated with vehicle (light bars). ANOVA, Strain effect: F(1,36)=69.6, p<0.001; Drug effect: F(1,36)=97.3, p<0.001; Strain x Drug interaction: F(1,36)=59.2, p<0.001. Post hoc Bonferroni test: BS amphetamine group different from all other groups (p<0.001).

Figure 2



The hyperactivity induced by 2 mg/kg amphetamine in C57 mice (dotted line) was ameliorated by one daily injection of 100 mg/kg lithium chloride but two daily injections were needed to reduce the hyperactivity of the BS mice (solid line). ANOVA, Strain effect F(1,28)=5.86, p<0.05; Lithium effect F(2,28)=5.26, p<0.05; post-hoc Bonferroni test: BS Lithium 1/day different than C57 Lithium 1/day.

Discussion

The present results demonstrate that BS mice show increased risk-taking behavior, reward-seeking, and response to psychostimulant drugs compared with C57 mice. Such behaviors are all facets of mania (Einat 2006; Einat 2007a; Sadock and Kaplan 2002). Furthermore, BS mice show increased resistance to the effects of the prototypic mood stabilizer lithium. Accordingly, it is suggested that, pending further validation, this strain may serve to model mania. Such a model can be utilized quickly and easily for the screening of new drugs and the differences between the strains can be utilized to further understand possible biological differences that may be involved in the disorder. Such explorations in the area of anxiety research were successfully done using the differences between the Fischer 344 and the Lewis rat strains (Vit et al. 2006) (Cohen et al. 2006).

The present results may be limited for a couple of reasons. (1) BS mice have recessive retinal degeneration (Clapcote et al. 2005) and reduced vision may have altered their behavior in the black/white box. (2) Based on only one saccharine concentration, it is hard to make conclusions regarding response to hedonic stimuli. Yet, the strength of the study comes from the combination of behaviors, each representing a different facet of bipolar disorder. The effects in the black/white box alone can be partially explained by reduced eyesight in BS mice; however, this cannot be a reasonable explanation for the other behavioral differences. Additionally, the battery testing approach used here may have significantly stronger validity compared to any one single test (Einat 2006; Einat 2007a). Furthermore, it is not suggested that the BS mice have behaviors that the C57 mice lack (or vice versa) as both strains explored the white area of the black/white box, consumed saccharine solutions and responded to amphetamine. The differences observed are only in the amount or intensity of response. It is therefore suggested that when presented with a number of stimuli, BS mice respond in a way that may model manic patients. Manic patients show behaviors that are part of the normal behavioral repertoire but in an intensity, rate or susceptibility that go beyond the normal range.

Testing for the response to prototypic drugs is an essential part of model validation (predictive validity) (Willner 1991) and in the present study both strains responded to lithium, albeit at different doses. The increased dose needed to induce a response in the BS strain compared to C57 may seemingly present two problems: (1) People that are not afflicted with bipolar disorder are not affected by lithium treatment, however, C57 mice responded to lithium and therefore may also be considered for mania modeling and (2) since the BS mice do not easily respond to lithium, they may appear to have reduced predictive validity. However, we suggest this may not be the case. Specifically, C57 mice are manipulated by amphetamine to show manic-like behavior and it is the amphetamine effect that responds to lithium while in the other tests their behavior has less manic-like features compared to the BS strain. The data do not preclude the use of C57 mice under specific manipulations to model mania but suggest that the BS strain may be more appropriate. Moreover, the BS mice responded to high-dose lithium administration, modeling the clinical situation where patients with more severe forms of bipolar disorder may need higher drug doses.

Further validation of the model is, however, needed before it can be used. The behavior of BS mice should be compared to additional strains and, possibly, in more tests representing additional facets of mania. Further testing of mood stabilizer treatments are also needed to explore the effects of lithium and other mood stabilizers not only in the amphetamine model but also on other behaviors. Additionally, it would now be interesting to compare the underlying biological differences between BS and C57 mice in the context of genes and molecules that have been implicated in bipolar disorder.

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