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Pharmacological and genetic influences on hole-board behaviors in mice

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Abstract

Head dipping on a hole-board is frequently used as an indicator of exploratory tendencies in rodent studies. Drugs with diverse pharmacological properties alter head dipping suggesting that many neurotransmitter systems are involved in the expression of exploratory behavior. The aim of the current experiments was to determine the effects of several drugs from different classes on head dipping, and to compare the effects of some of these agents in lines of mice that have been selectively bred for divergent expression of head dipping on a hole-board. In the current experiments, the effects on head dipping of three doses each of fluoxetine, desipramine, GBR-12909, methamphetamine, pentylenetetrazol, and diazepam were evaluated in genetically heterogeneous mice. Most drugs altered the number of head dips in a predictable manner, but the effects on locomotion were generally as large as those seen for head dipping. Locomotion could completely account for the effects of fluoxetine and pentylenetetrazol, and to a lesser extent, diazepam. We have also developed replicate lines of mice selectively bred for high (High Exploratory Behavior: HEB) or low (Low Exploratory Behavior: LEB) head dipping on a hole-board and evaluated the effects of diazepam and methamphetamine on hole-board behaviors in these mice. Diazepam increased head dipping and locomotion equivalently in both lines of mice, but methamphetamine stimulated locomotion in HEB mice more than in LEB mice. These results broadly suggest that the effects of most drugs we tested are not specific for head dipping, since almost all drugs tested affected head dipping and locomotion equivalently. However, the results with the genetically heterogeneous mice and HEB and LEB mice suggest that some aspects of the dopaminergic system are involved in head dipping.

Keywords: Hole-board apparatus; Exploratory behavior; Mice; Genetics; Anxiety-like behavior; Drugs

1. Introduction

Head dipping on a hole-board is a commonly used measure in many pharmacological, genetic, and neural lesion studies as an indication of directed exploration (see Crawley, 1985 and Lister, 1990 for reviews). This test is popular because it is short in duration, and the behaviors are discrete and can be quantified with automated apparatus. Head dipping is usually described as being distinct from general locomotor activity, and from the perspective of the experimenter, resembles a directed exploration of the holes in the floor (File and Wardill, 1975). Perhaps because of the different drug classes that have been shown to affect head dipping, hole-board behaviors have variously been described as reflective of some underlying exploratory tendency (e.g. File and Wardill, 1975; Ljungberg and Ungerstedt, 1976) or anxiety-like state (e.g. Takeda et al., 1998).

Drugs with activity at the $GABA_A$ receptor, such as ethanol, barbiturates, and benzodiazepines have well-established effects in apparatus that are thought of as tests of anxiety-like behaviors in rodents. These drugs have also been tested in the hole-board apparatus. For example, Lister (1987b) included the hole-board apparatus in the initial experiments validating the mouse elevated plus maze as a test of anxiety-like behavior, and reported different effects of these drugs on the behaviors measured in the two tasks. Ethanol and chlordiazepoxide increased the amount of time spent on the open arms of the plus maze, which was taken to indicate an anxiolytic-like effect, while neither drug altered head dipping on the hole-board. However, ethanol and chlordiazepoxide have been shown to have effects on head dipping in other studies

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(Bilkei-Gorzo and Gyertyan, 1996; File and Wardill, 1975; Nolan and Parkes, 1973; Takeda et al., 1998), and further, the effects of ethanol on head dipping are known to be strain dependent (Lister, 1987a). As suggested by Lister (1990), distinguishing between the relative influences of anxiety-like and exploratory states in the hole-board solely on the basis of pharmacological experiments is questionable. Rather, it would seem more accurate to say that tasks like the hole-board that are dependent upon the voluntary exploration of an apparatus are influenced by many underlying factors, including exploratory tendencies and anxiety-like states (Crawley, 1985).

Monoamine agonists and antagonists have also been tested in the hole-board, and these studies have generally indicated a role for dopamine, norepinephrine, and serotonin systems in some aspects of hole-board performance. D-amphetamine, methamphetamine (METH), and the dopamine transporter antagonist GBR-12909 have been shown to increase locomotion and decrease or not significantly affect head dipping (File and Wardill, 1975; Lister, 1987b; Morita et al., 2000; Pellow et al., 1985; Takeda et al., 2005), but D-amphetamine has also repeatedly been shown to increase both behavioral measures (Galeotti et al., 2002, 2003, 1997b, 2004; Ghelardini et al., 2002; Ljungberg and Ungerstedt, 1976; Makanjuola et al., 1977). Chronic administration of the norepinephrine transporter (NET) antagonist desipramine has been shown to increase head dipping in some rats as a function of their basal activity (Echandia et al., 1985), while manipulation of the serotonergic system tends to decrease or not affect head dipping behavior (Galeotti et al., 1997a; Takeda et al., 2005; Tsuji et al., 2000).

On the basis of these studies, it would appear difficult to predict the effects of a novel drug or genetic manipulation on hole-board behaviors, as the effects of drugs well-characterized in other apparatus produce inconsistent effects in the hole-board. Further, it is unclear if the changes in hole-board behaviors produced by the different drugs represent effects common to mechanisms underlying exploratory or anxiety-like behavior, or whether the observed behavioral effects are idiosyncratic to a given drug or drug type. Finally, in the studies described above, similar effects of the drug have sometimes been seen on head dipping and locomotor activity measured during the task. This finding is counter to the premise of calling head dipping on the hole-board directed exploratory behavior, since this suggestion was based on the observation that head dipping and locomotion are uncorrelated and therefore measure different underlying tendencies (File and Wardill, 1975).

We have recently artificially selected lines of mice for High or Low Exploratory Behavior (HEB and LEB mice, respectively) as measured by head dipping on a hole-board (Kliethermes and Crabbe, 2006). These mice were bred on the basis of the total numbers of head dips during a 10 min test. After four generations of selective breeding, HEB mice showed approximately 50% more head dips compared to LEB mice, and the difference between the lines was largely unchanged at the fifth generation. Alleles that influence head dipping have segregated within the lines over the course of selection, and these mice therefore provide a valuable tool for examining the biological mechanisms underlying exploratory behavior in the hole-board. In the current experiments, the effects of the monoamine transporter inhibitors desipramine, fluoxetine, and GBR-12909, as well as METH, and the $GABA_A$ modulator diazepam and antagonist pentylenetetrazol (PTZ) were evaluated in the hole-board test. These drugs were first tested in unselected, genetically heterogeneous mice, and were designed to implicate broadly the actions of the various neurotransmitter systems in head dipping behavior using the same apparatus, mouse genotype, and other experimental conditions across the drug types. In order to determine whether head dipping could be dissociated from locomotor activity for the different drugs, we also performed regression analyses on the head dipping and locomotion scores for each drug, and analyzed these residual data along with the raw scores. The effects of diazepam and METH were then evaluated in both replicates of HEB and LEB mice to determine whether selection for divergent head dipping resulted in differential sensitivity to these agents.

2. Materials and methods

2.1. Subjects

2.1.1. WSC mice

Genetically heterogeneous, 55–90 day old WSC-1 and WSC-2 male and female mice were used for the initial characterization of each drug on the hole-board apparatus. These mice were derived from the HS/Ibg outbred stock (McClearn et al., 1970), which was produced from a cross of 8 inbred strains of mice (A, AKR, BALB/c, C3H/2, C57BL, DBA/2, Is/Bi, and RIII) and thus, are potentially polymorphic at any allele at which the progenitor strains differed. The WSC mice have been maintained as two separate breeding populations (WSC-1 and WSC-2) at the Portland Veterans Affairs Medical Center using a rotational breeding strategy that minimizes inbreeding. All mice were housed 3–5 per standard shoebox cage on corncob bedding with food (Purina, 5001) and water available continuously. The mice were maintained on a standard 12 h light/12 h dark (lights on at 0600) at a constant temperature 21 °C.

2.1.2. HEB and LEB selected lines

HEB and LEB mice were selectively bred as independent replicate lines from a common starting population of B6D2F3 mice, and these lines are designated HEB-1 and LEB-1, and HEB-2 and LEB-2. Because the selective breeding experiment was replicated, the finding of a neurochemical or behavioral difference common to both replicates of the HEB compared with LEB lines (i.e. a main effect of selected line in an analysis of the candidate mechanism or behavior) provides strong evidence that common genes mediate the selection trait (head dipping) and the candidate mechanism or other behavior (Crabbe et al., 1990).

Male and female, 50–100 day old HEB and LEB mice from the fourth generation of selection for were used for the experiment with diazepam and the first experiment with METH. Mice from the fifth generation of selective breeding were used for a second experiment with METH. The HEB and LEB mice were locally bred at the Portland Veterans Affairs Medical Center and were maintained under conditions identical to the WSC mice.

2.2. Apparatus

All testing was conducted in four identical 40×40 cm clear acrylic automated activity monitors (Accuscan Systems, Columbus, OH) equipped with 16 infrared beams in the horizontal plane to detect movement, and 8 additional beams placed under the level of an inserted hole-board floor which detected head dips. These two banks of infrared beams provide independent measurements of horizontal activity (beam breaks) and vertical activity. The raw numbers of horizontal beam breaks were converted to a distance traveled using the Accuscan software. Each hole-board was located in an isolated, sound-attenuated box that was equipped with a ventilation fan and fluorescent light that provided approximately 125 lux illumination. Head dips were recorded in any of 4 identical holes (2.9 cm diameter) spaced approximately equidistant from each other in the four corners.

2.3. Procedure

All mice were transported in their home cages from the colony room to the testing room and allowed to acclimate for a minimum of 1.5 h before testing began. Each mouse was weighed, injected intraperitoneally with a dose of the appropriate drug, and placed into a holding cage for 30 min before being placed onto the hole-board for a 10 min test. During the test, the total distance traveled and number of head dips was recorded. For all experiments with WSC mice, 9–14 mice were tested at each dose of all drugs. Approximately equal numbers of WSC-1 and WSC-2 male and female mice comprised each dose group. For the selected line experiments, 9–12 mice of each line and replicate with the exception of LEB-1 were tested at each dose of diazepam and METH. Because of availability problems, 6–8 LEB-1 mice were tested at each of three doses of the two drugs. All mice were experimentally naïve at the time of testing, and were used only once in the current experiments. All procedures used in the current experiments were approved by the Portland Veterans Affairs Medical Center IACUC.

2.4. Drugs

Diazepam, fluoxetine, GBR-12909, METH, desipramine, and PTZ were all obtained from Sigma (St. Louis, MO) and injected at a volume of 0.01 mL/g in saline. For diazepam and GBR-12909, two drops of Tween 20 were added to the solution and the mixtures were sonicated for approximately 15 min to aid in the suspension of the drugs. Control animals received an equivalent injection of saline, to which two drops of Tween 20 were added for the GBR-12909 and diazepam experiments.

2.5. Statistics

Dose effects for each drug were first evaluated separately for head dips and locomotion by one-way analysis of variance (ANOVA). A residual score was also calculated from the regression of head dipping scores on the total distance traveled for each individual, and the effects of dose on the residual score were also analyzed by ANOVA. This was done to determine whether a drug had a significant effect on head dipping after accounting for the effect of locomotion on head dipping, and it allowed us to compare dose–response curves of the residual as compared to the raw head dipping scores. The statistical outcomes of the analyses of residual scores were nearly identical to those we obtained from analyses of covariance. Tukey's post hoc tests were used to determine pair wise differences if a significant effect was found in the ANOVA. Correlations between head dipping and locomotion were evaluated as Pearson's *r*. All results were considered significant at $p<0.05$.

3. Results

3.1. WSC mice

Fluoxetine had a dose-dependent effect on head dipping behavior ($F_{3,\ 39}=10.36$; $p<0.0001$; Fig. 1A). Mice that received 5 or 15-mg/kg fluoxetine did not differ from controls, but a marked reduction in head dipping was observed at the 25-mg/kg dose relative to all other groups ($ps<0.01$). A similar dose–response was obtained for the total distance traveled ($F_{3,\ 39}=8.68$; $p<0.0001$; Fig. 1B). Fluoxetine decreased locomotion relative to all other groups at 25 mg/kg ($p<0.05$), but did not significantly affect locomotion at either of the lower doses ($ps>0.32$). A one-way ANOVA of the effect of fluoxetine dose on the residual scores shown in the inset in Fig. 1A just missed significance ($F_{3,\ 39}=2.61$; $p<0.07$). This effect appears to be due to a higher residual score at the 5 compared with the 25 mg/kg dose.

The NET inhibitor desipramine did not significantly affect head dipping ($F_{3,\ 37}=1.73$; $p>0.17$; Fig. 1C), but dose-dependently reduced the distance traveled ($F_{3,\ 37}=4.56$; $p<0.01$; Fig. 1D). Post hoc analysis revealed that this effect was driven by significantly reduced activity at the 20-mg/kg dose relative to the control group ($p<0.01$); the two lower doses did not significantly reduce locomotion ($ps>0.17$). The effect of desipramine dose on the residual score was also non-significant ($F_{3,\ 37}=0.69$; Fig. 1C inset).

GBR-12909 significantly reduced head dipping and increased locomotion ($Fs_{3,\ 43}>4.12$; $p<0.05$; Fig. 1E and F). For head dipping, this effect that was significant at the 10-mg/kg dose ($p<0.01$), while the 20-mg/kg dose was not significantly different from the controls. The inset in Fig. 1F shows that a significant effect of GBR-12909 dose on head dipping remained after accounting for the effects of locomotion ($F_{3,\ 43}=3.73$; $p<0.05$). This dose effect was largely indistinguishable from that shown for head dipping in Fig. 1E, and the residual score at the 10-mg/kg dose was significantly less than controls ($p<0.05$).

Like GBR-12909, METH also reduced the numbers of head dips ($F_{3,\ 36}=19.82$; $p<0.0001$), an effect that was significant at all doses ($ps<0.001$; see Fig. 2A). METH also tended to increase the total distance traveled, but this effect did not reach significance ($F_{3,\ 36}=2.28$; $p<0.1$; Fig. 2B). An analysis of the residual score shown in the inset in Fig. 2A indicated that a significant effect of dose on head dipping remained after

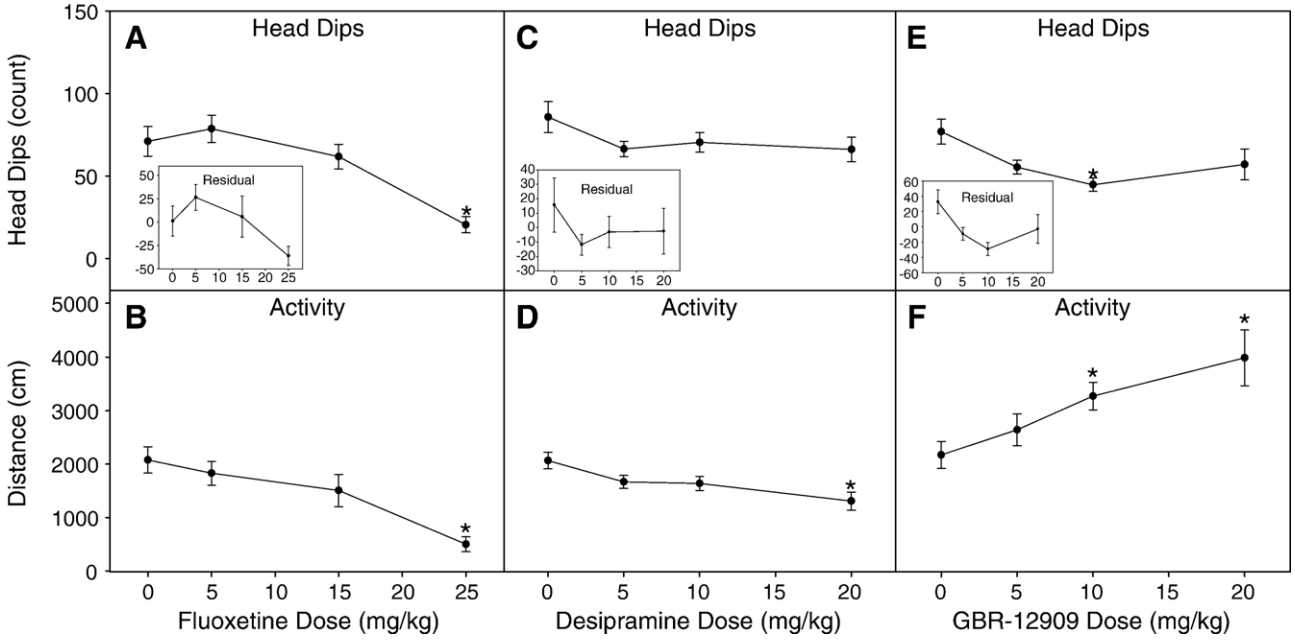

Fig. 1. The effects of fluoxetine (panels A and B), desipramine (panels C and D), or GBR-12909 (panels E and F) on head dipping and distance traveled in a 10-min hole-board test. The insets in panels A, C, and E show the residual score for each drug obtained from the regression of individual head dipping values on the distance traveled during the test. Each point represents the mean±SEM. *$p<0.05$ compared to the vehicle treated mice.

accounting for METH's effects on locomotion ($F_{3,\ 36}=18.82$; $p<0.0001$).

The effects of diazepam on head dipping are shown in Fig. 2C. Diazepam had biphasic effects on head dipping ($F_{3,\ 36}=3.95$; $p<0.05$); a dose of 1 mg/kg tended to increase ($p<0.09$), and 2-mg/kg diazepam significantly increased, head dipping ($p<0.05$), while the 3-mg/kg group was indistinguishable from the controls ($p>0.9$). A similar dose–response was found for locomotion, but this effect did not reach significance ($F_{3,\ 36}=2.12$; $p>0.1$; Fig. 2D). However, the effect of dose on the head dipping residual score was also non-significant ($F_{3,\ 36}=2.14$; $p>0.1$; inset Fig. 2C), indicating that the effects of diazepam on head dipping can largely be accounted for its effects on locomotion.

As indicated by Fig. 2E and F, PTZ decreased both head dipping and the total distance traveled in the hole-board ($Fs_{3,\ 43}>9.32$; $ps<0.001$). For distance traveled, this effect was

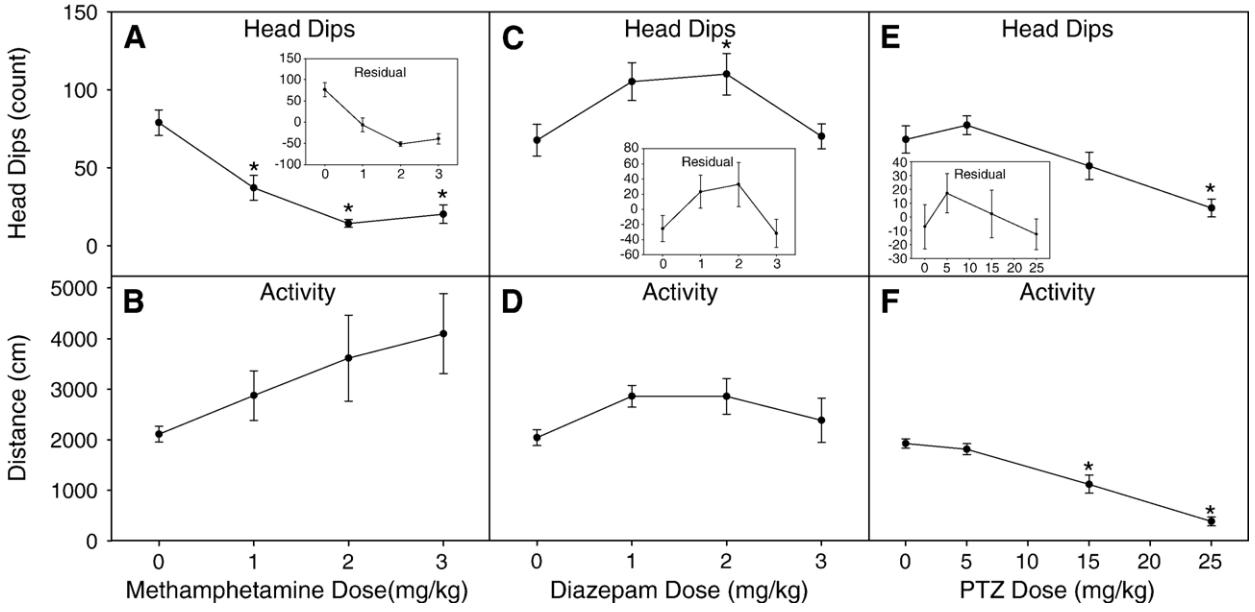

Fig. 2. The effects of methamphetamine (panels A and B), diazepam (panels C and D), or PTZ (panels E and F) on head dipping and distance traveled in a 10-min hole-board test. The insets in panels A, C, and E show the residual score for each drug obtained from the regression of individual head dipping values on the distance traveled during the test. Each point represents the mean±SEM. *$p<0.05$ compared to the vehicle treated mice.

significant at the 15 and 25 mg/kg doses ($p<0.001$), while the effect on head dipping was seen only at a dose of 25 mg/kg. Locomotion accounted completely for the effect of PTZ on head dipping ($F_{3,\ 43}=0.76$; inset Fig. 2E), suggesting that PTZ did not specifically affect head dipping.

3.1.1. Correlational analyses

In order to examine the relationship between head dipping and locomotor activity in the hole-board further, we examined the correlations of these two behaviors across the control groups for the six drugs tested. The values of these correlations ranged from $r=0.12$–0.67, but because of the relatively small sample sizes of the control groups ($n=11$–14), only the two highest correlations ($r=0.61$ and 0.67) were significant at $p<0.05$, although all correlations were positive in direction (data not shown). To increase the power of this analysis, we combined the control groups from all six experiments for a total of 75 mice in the analysis. The correlation obtained was modest, although statistically significant ($r=0.45$; $p<0.01$). This positive correlation is akin to the dose effects seen for diazepam, in that higher activity in the control mice was associated with more head dipping behavior, but unlike those seen for the stimulants METH and GBR-12909, which produced differential effects on head dipping and locomotor activity.

3.2. Selected line experiments

3.2.1. Diazepam

A four-way ANOVA (selected line X replicate X sex X dose) revealed no significant effect of or interactions with sex ($Fs_{1-3,\ 119}<3.23$; $p>0.05$), so further analyses of head dipping were collapsed on sex. A main effect of selected line was observed for the total number of head dips ($F_{1,\ 135}=24.23$; $p<0.0001$; see Fig. 3A and B), an effect that replicated the selection phenotype where HEB mice of both replicates head dip more than LEB mice. However, the selected line difference at the 0-mg/kg dose seen in the second replicate is relatively small (Fig. 3B; see Discussion). A main effect of replicate was also found, where the second replicate head dipped more than the first ($F_{1,\ 135}=5.80$; $p<0.05$). A trend for a line by replicate interaction was also found ($F_{1,\ 135}=3.39$; $p<0.07$). No significant effect of dose ($F_{3,\ 135}=1.20$; $p>0.3$) or interactions of dose with a selected line or replicate were found ($Fs_{3,\ 135}<1.71$; $p>0.16$).

HEB and LEB mice differed in the total distance traveled following diazepam administration ($F_{1,\ 119}=6.77$; $p<0.05$; see Fig. 3C and D), but no main effects of replicate or sex were found ($Fs_{1,\ 119}<1.71$; $p>0.19$). A main effect of diazepam dose was observed ($F_{3,\ 119}=3.9$; $p<0.05$), with the 2-mg/kg dose producing locomotor stimulation relative to vehicle-injected mice ($p<0.05$). A line by sex interaction was also found ($F_{1,\ 119}=6.68$; $p<0.05$) that was driven by significantly higher activity in male HEB mice relative to all other groups ($ps<0.05$; data not shown). There was also a line by replicate interaction ($F_{1,\ 119}=17.46$; $p<0.001$), but no line by dose interaction ($F_{3,\ 119}=0.06$), indicating that overall, HEB and LEB mice did not differ in the response to diazepam.

3.2.2. Methamphetamine

The data from one HEB-2 female receiving 1-mg/kg METH were deleted because of a problem with the hole-board apparatus. HEB mice head dipped more than LEB mice ($F_{1,\ 131}=7.42$; $p<0.01$; see Fig. 4A and B), and METH dose dependently reduced head dipping in both lines ($F_{3,\ 131}=7.22$; $p<0.001$), with doses of 2 and 3 mg/kg reduced significantly below the control levels ($ps<0.01$). Although it appears that HEB mice showed a greater reduction in head dipping with increasing METH dose, no

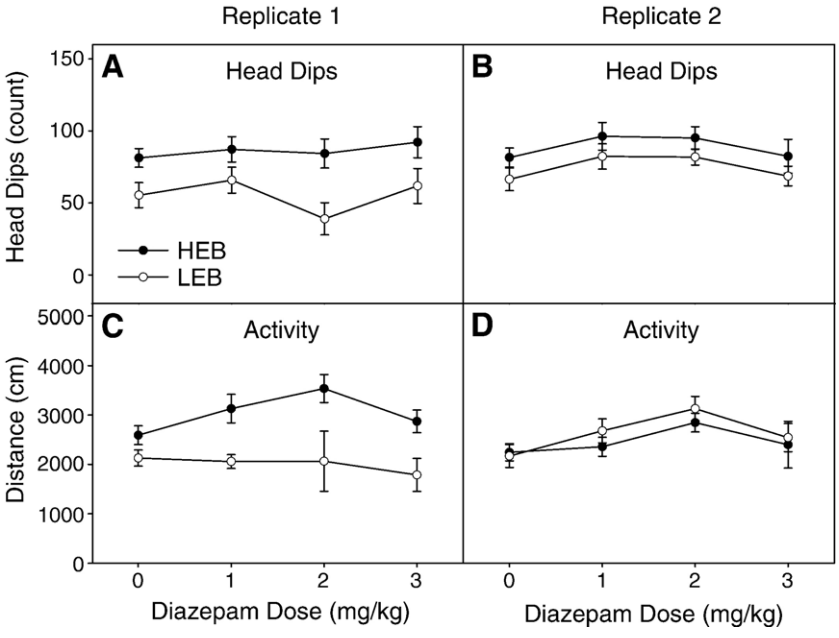

Fig. 3. The effects of diazepam on head dipping (top panels), and distance traveled (bottom panels) in a 10-min hole-board test. Panels A and C depict the effects of diazepam on selected mice of replicate 1, and panels B and D show the effects for replicate 2. Each point represents the mean±SEM.

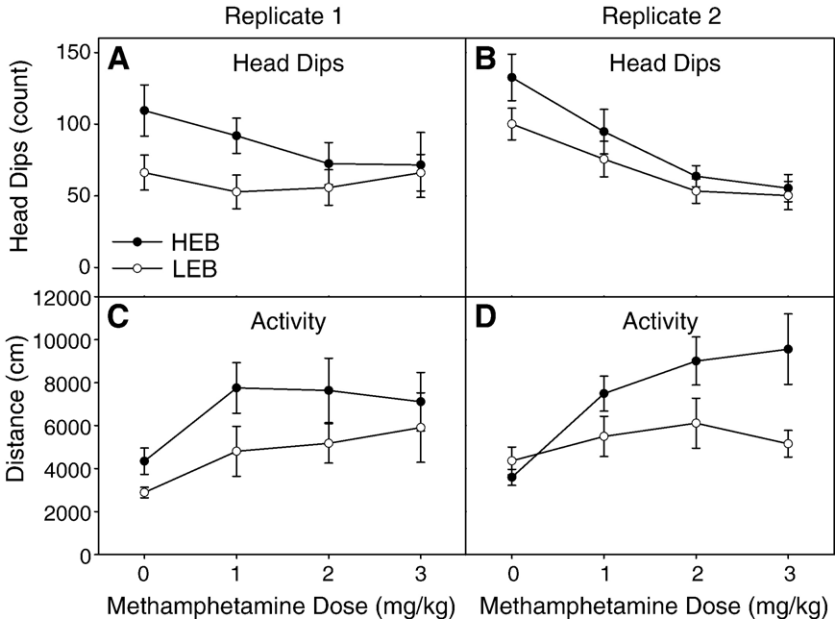

Fig. 4. The effects of methamphetamine on head dipping (top panels), and distance traveled (bottom panels) in a 10-min hole-board test in HEB and LEB mice from the fourth selected generation. Panels A and C depict the effects of methamphetamine on selected mice of replicate 1, and panels B and D show the effects for replicate 2. Each point represents the mean ± SEM. *$p<0.05$ compared to the vehicle treated mice.

line by dose interaction was found ($F_{3,\ 131}=1.13$; $p>0.34$) indicating that HEB and LEB mice are not differentially affected by METH as measured by head dipping.

No sex differences ($F_{1,131}=1.51$; $p>0.22$) or interactions with sex were observed for total distance traveled ($Fs_{1-3,\ 131}<1.98$; $ps>0.16$), so further analyses of activity were collapsed on sex. At all doses, HEB mice moved more than LEB mice (main effect of line: $F_{1,\ 147}=13.91$; $p<0.001$; see Fig. 4C and D), and no effect of replicate was apparent ($F_{1,\ 147}=.34$; $p>.24$). As indicated by a main effect of dose ($F_{3,\ 147}=7.67$; $p<0.001$), METH increased activity overall. This effect was seen at all three doses relative to controls ($ps<0.01$). While there was no significant interaction between line and dose ($F_{3,\ 147}=1.13$; $p>0.33$), it appears from Fig. 4C and D that HEB mice of both replicates showed greater stimulation than LEB mice with increasing doses of METH. Given that the aim of this experiment was to discover any potential differences in the response to METH between HEB and LEB mice, and that a small difference between the lines could be lost in the multiple comparisons in a three-way analysis, we performed a one-way ANOVA of dose within each selected line, collapsed on replicate. This analysis revealed a significant effect of METH dose on the total distance traveled in HEB mice ($F_{3,\ 89}=7.17$; $p<0.001$) but not in LEB mice ($F_{3,\ 66}=1.98$; $p>0.12$), suggesting that there may be a difference in METH sensitivity between HEB and LEB mice.

HEB and LEB mice were still being selectively bred for divergent expression of head dipping while the experiments with diazepam and METH were being conducted. Therefore, at the fifth generation of selection, the potential difference in METH responsiveness seen at the fourth generation was evaluated using a longer, 45-min test session in order to see greater overall locomotor stimulation. This experiment used only saline and 2 mg/kg METH groups, and the mice were placed immediately onto the hole-board after injection.

As with the results from the fourth generation of selection, no selected line differences in the reduction in head dipping caused by METH were apparent (line by dose interaction: $F_{1,\ 79}>0.3$), although METH again significantly reduced the overall number of head dips ($F_{1,\ 79}=7.69$; $p<0.01$; data not shown). No main effect of sex, or interactions of sex with selected line, replicate, or dose were observed for total locomotor activity ($Fs_{1,\ 79}<0.36$), so further analyses of locomotor activity were collapsed on sex. The results presented in Fig. 5A and B show the time course of the total test session in 5-min bins, but all analyses were performed on the session totals only.

Compared to the results from the fourth generation of selection, a greater overall locomotor response to METH was seen in all mice with this longer test session, resulting in a highly significant stimulation relative to saline treated mice in all groups ($F_{1,\ 87}=140.82$; $p<0.0001$; see Fig. 5A and B). Main effects of line and replicate were also observed ($Fs_{1,\ 87}>9.80$; $p<0.01$), with higher activity seen in HEB mice relative to LEB, and greater activity in the first as compared to second replicate. A dose by replicate interaction was also observed ($F_{1,\ 87}=7.25$; $p<0.001$), that was due to significantly greater METH stimulation in the first replicate as compared to the second ($p<0.001$).

In support of the presumed effect seen in the fourth generation, a significant line by dose interaction was observed ($F_{1,\ 87}=11.36$; $p<0.01$), and post hoc tests indicated that HEB mice of both replicates showed more stimulation following 2 mg/kg METH than LEB mice ($p<0.0001$). It appears that this effect was particularly present later in the session. The selected lines did not differ in basal activity in this longer, 45-min test

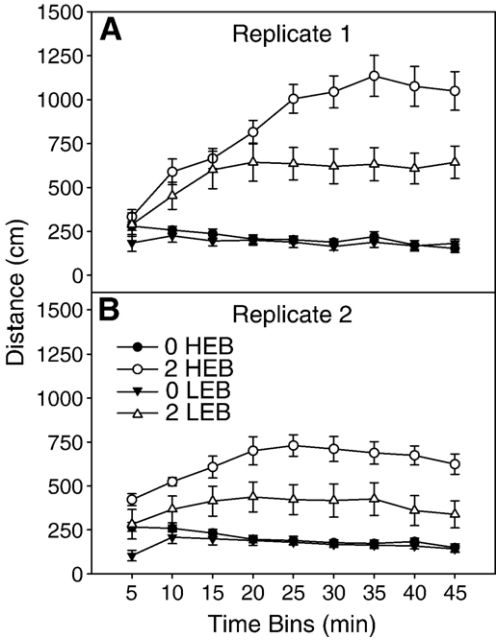

Fig. 5. The effects of methamphetamine on distance traveled in a 45-min hole-board test in HEB and LEB mice from the fifth selected generation. The *X*-axis indicates activity totals for each 5 min time bin. 0 and 2 in the figure legend refer to saline and 2-mg/kg methamphetamine, respectively. Each point represents the mean ± SEM.

session ($p > 0.9$). However, saline treated LEB mice of both replicates appeared to move less than HEBs during the first 5–10 min, but were indistinguishable from HEBs for the remainder of the session, and as mentioned above, did not differ in overall test session activity.

4. Discussion

File and Wardill (1975) demonstrated that, at least for CFW mice, there was no significant correlation between head dipping and locomotion in a 4 or 16 hole hole-board, and that the behavior of these mice changed as a function of repeated exposures or the presentation of objects in some of the holes. These findings were interpreted such that head dipping reflected an underlying tendency to explore novel stimuli that was not the same as more general locomotor activity. Our results with WSC mice partially support this claim of the distinctiveness of head dipping and locomotion, but with the exceptions of METH and GBR-12909, all drugs we tested showed similar dose-related effects on both behaviors. However, our correlational analysis of head dipping and locomotion in untreated mice suggests that at the level of the individual mouse, head dipping and locomotion can vary fairly independently. These results suggest a complicated relationship between head dipping and locomotion measured in the hole-board apparatus.

The literature dealing with the effects of several drug classes on hole-board behaviors is complex, but particularly so for the benzodiazepines (see Lister, 1990). As noted previously (Kamei et al., 2001), depending on the dose and study, benzodiazepines have variously been shown to enhance (de Angelis et al., 1982; Kamei et al., 2001; Nolan and Parkes, 1973; Takeda et al., 1998), reduce (Pellow et al., 1985) or not affect (Lister, 1987b; Sayin et al., 1992) head dipping behavior, in combination with no effect on, enhanced, or reduced locomotor activity. As a potential explanation for some of these differing effects, Bilkei-Gorzo and Gyertyan (1996) showed that the benzodiazepine chlordiazepoxide can increase the number of head dips under relatively normal lighting, decrease head dipping under very bright lights, or not affect head dipping under dark conditions. These authors suggested that head dipping may be a manifestation of exploratory tendencies under conditions of low aversiveness, but may represent escape behavior in more aversive environments: a hypothesis that would appropriately predict increased or decreased head dipping in response to an anxiolytic (or presumably anxiogenic) drug as a function of the lighting intensity of the apparatus. However, it should be noted that the high illumination level, and presumably the most aversive condition used by Bilkei-Gorzo and Gyertyan was 1000 lux, a level 8 times higher than in the current experiments.

Another explanation for these inconsistent effects of benzodiazepines could relate to the choice of rat or mouse as the experimental subject. In general, mice show a modest to relatively robust locomotor stimulation in response to low doses of benzodiazepines (Crabbe et al., 1998; Lister, 1990), while rats show only locomotor depression at any dose (Lister, 1990). Consistent with this, the WSC mice in the current experiments showed biphasic locomotor stimulation in response to diazepam that statistically accounted for the effects of diazepam on head dipping, which showed a similar dose–response function. However, previous results with diazepam in WSC mice in a different apparatus showed no significant increase in locomotion with increasing diazepam dose, but a dose-dependent effect to reduce an anxiety-related behavior (Kliethermes et al., 2003). This suggests that the apparatus in which the behavior is measured, in addition to the use of rats or mice, likely influences findings of locomotor stimulation with low dose diazepam.

The locomotor stimulant and anxiolytic-like response to low dose diazepam also varies across inbred strains of mice (Crabbe et al., 1998; Griebel et al., 2000), indicating that some aspect of diazepam sensitivity is under genetic control. However, no consistent differences between the HEB and LEB selected lines were observed for the effects of diazepam on either the head dipping measure or on locomotor stimulation. While this clearly does not rule out the involvement of the $GABA_A$ system in the selection trait, or especially, in exploratory head dipping in general, it does at least suggest HEB and LEB mice are probably not differentially influenced by this common anxiolytic drug. This could imply that behavioral differences between HEB and LEB mice are not due to some type of anxiety-like state induced by the hole-board apparatus, or to an inborn, genetic difference in an anxiety-like trait between the selected lines. Rather, assuming that the primary factors influencing exploration of a novel apparatus are the contrasting effects of an anxiety-like state and the motivation to explore the apparatus (see Crawley, 1985), a primary difference between HEB and LEB mice could be a differential motivation to explore the hole-board apparatus. However, more experiments using additional

tests traditionally associated with anxiety-like states are obviously needed to address this hypothesis.

As indicated in the introduction, the effects of D-amphetamine and METH on hole-board behaviors are also not consistent across published studies. In the current experiments, the WSC mice tested with METH or GBR-12909, showed differential effects on the two measured behaviors: each drug reduced head dipping and increased locomotion, although not significantly in the case of METH. The finding of this dose-dependent negative relationship between head dipping and locomotor activity makes it unlikely that the positive relationships seen for most other drugs were due to an artifact of the computer system we used to measure hole-board behaviors, although such a contribution cannot be explicitly ruled out.

Our results with the two stimulants are consistent with the notion that head dipping and locomotion are competing behaviors, in which the expression of one behavior interferes with that of another (Lister, 1990). This relationship between head dipping and locomotion for METH contrasts with that seen for diazepam and also seen in undrugged mice, where head dipping was positively correlated with locomotor activity. This might indicate that the head dipping response does not 'mean' the same thing when the animal is under the influence of drugs from different drug classes, since the relationship between the presumably exploratory head dip and locomotor activity changes across drugs, and is also likely different between mice and rats (see above). Further, trying to compare across the effects of different drugs on the basis of hole-board behaviors may not be justified if the goal is to understand the neurobiology underlying a hypothetical exploratory state, but is a reasonable means to examine the effects of the drug on the measurable responses of head dipping and locomotion.

Compared with LEB mice, HEB mice showed a greater locomotor response to METH at the fourth, and especially, fifth generations of selection when a longer testing session was utilized. Since both replicates of HEB differ from LEB mice in this response to METH, this suggests that shared genes may underlie head dipping and the locomotor stimulant response to METH (Crabbe et al., 1990). These shared genes might be involved in monoaminergic, and most likely, dopaminergic function, a suggestion consistent with a large body of literature relating to the dopaminergic system in exploratory and novelty seeking tasks (see Bardo et al., 1996 for review). Because the correlated responses to selection were assessed after relatively few generations of selection, the potential for genetic drift to have contributed to either the response or correlated responses to selection is reduced (Falconer and Mackay, 1996). However, the selected lines did not differ in their sensitivity to METH's effects on head dipping, the trait on which the selection was based. This could be due to a floor effect on the total number of head dips that made it impossible to detect this effect of METH. That is, although a main effect of METH on head dipping was found, the ability to detect a selected line by dose interaction was hampered by the low overall numbers of head dips, even in HEB mice, possibly due to the competing effects of the drug on head dipping and locomotion.

The different procedures used for the first METH and diazepam experiments versus those used for selection could also have contributed to this unexpected effect. In the first METH and diazepam experiments, each mouse was first weighed, injected, and then placed into a holding cage for 30 min prior to being placed onto the hole-board. During selection, the mice were placed immediately onto the hole-board apparatus, so their experience with the hole-board was likely very different from that of the mice in the current experiments. The genes that regulate the naïve hole-board response for which the HEB and LEB mice were selected could be distinct from those regulating the behavioral response seen after the relatively stressful injection procedure. Similarly, any acute stress response to the handling procedure during selection could have contributed to the behavioral differences between the selected lines, although in a previous experiment we found that HEB and LEB mice did not differ in basal or task-induced corticosterone levels (Kliethermes and Crabbe, 2006). Whether HEB and LEB mice differed in the corticosterone response to the injections in the current experiments is unknown. Although the selected line difference in the total number of head dips was present in the diazepam and first METH experiment, the magnitude of the difference between the HEB and LEB lines was reduced, particularly in the second replicate, which might indicate that other factors did affect the behavior of HEB and LEB mice in the current experiments.

The NET and SERT antagonists, desipramine and fluoxetine, dose dependently reduced locomotor activity in WSC mice, but only fluoxetine significantly affected head dipping. This latter effect is consistent with reports of an anxiogenic-like effect by an acute administration of fluoxetine in rodent models of anxiety-like behavior (Belzung et al., 2001; Dawson et al., 1995). However, the reduction of head dipping by fluoxetine in the current experiments could be largely accounted for by a dose-dependent decrease in locomotion, indicating that if fluoxetine does alter hole-board exploration, this effect is confounded by altered locomotor activity.

Desipramine, clonidine, and yohimbine have been shown to affect head dipping behavior of rats and mice on the hole-board (Echandia et al., 1985; although see O'Connor and Leonard, 1988; Pellow et al., 1985; Sara et al., 1995). More generally, the adrenergic system has been associated with responses to novelty, including the recognition of novel stimuli (Mansour et al., 2003). The current results showing no significant effect of desipramine on head dipping behavior were therefore clearly unexpected. As with diazepam, however, this null result could be due to the conditions of the test, the use of rat or mouse, or perhaps even the dose of drug administered. Dose, however, does not seem likely, as equivalent doses of desipramine to those used in the current experiments have been shown to be effective in the forced swim test in multiple inbred strains of mice (Lucki et al., 2001), and significant effects of the drug were found on concurrently measured locomotor activity, indicating that the drug was having an effect under the conditions of the test. Acute administration of desipramine minimally influenced the measured hole-board behaviors, suggesting that at least for WSC mice, the noradrenergic system is probably not substantially involved in head dipping behavior.

The experiments with WSC mice show that GBR-12909 and METH produce relatively independent effects on head dipping

and locomotion, although both drugs affected both behavioral measures. The other drugs tested showed similar effects on head dipping and locomotion, indicating that their effects were not specific for head dipping. Consequently, the effects of a drug or a genetic manipulation on locomotor activity should be included when interpreting the effects of any treatment on hole-board behaviors, and the effects observed on the hole-board should be corroborated by assessing performance in additional exploratory or anxiety-related tasks. Our findings of the relatively specific effects of GBR-12909 and METH on head dipping in WSC mice, and that HEB and LEB mice differ in the locomotor stimulant response to METH, are consistent with a large body of literature implicating dopaminergic function in locomotion, exploration, and novelty seeking. Since both GBR-12909 and METH affect synaptic dopamine levels through direct and indirect effects at the dopamine transporter, respectively, further experiments are needed to assess how and where dopamine acts to affect hole-board behaviors.

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