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Pharmacology, Biochemistry and Behavior 84 (2006) 120–127
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Influence of genetic background on alcohol drinking and behavioral phenotypes of 5-HT3 receptor over-expressing mice

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Received 13 December 2005; received in revised form 13 April 2006; accepted 25 April 2006
Available online 12 june 2006

Abstract

Behavioral effects of genetic manipulations are influenced by the background genetics of mouse strains used for the creation of transgenic mice. One strategy to address whether background genes may compromise interpretation of phenotype is the production of congenics. 5-HT_3 receptor over-expressing mice have been behaviorally characterized on a B6SJL/F2 background (B6SJL/F2-OE mice), and were found to consume less ethanol failed to develop conditioned place preference to moderate doses of cocaine and demonstrate improved hippocampal-dependent learning. To assess the contribution of parental strain genetics to these behaviors, we bred the transgene onto two well-defined backgrounds that differ in ethanol consumption and contextual fear conditioning, C57Bl/6J (B6) and DBA/2J (D2) strains. The behavioral phenotype of B6SJL/F2-OE was recapitulated in C57Bl/6J-OE mice. However, the effect of transgene over-expression on behavior was only apparent for one aspect of the novelty test using DBA/2J-OE mice. Results underscore the need to consider the genetic environment conferred by strain selection on the effects of genetic manipulation in mice.

Keywords: DBA/2; C57Bl/6; Congenic; Ethanol consumption; Fear conditioning; Novelty; Anxiety; Serotonin receptor

1. Introduction

5-HT_3 receptor over-expressing mice were originally produced on a B6SJL/F2 background (B6SJL/F2-OE) to study the role of this receptor in the rewarding properties of drugs of abuse (Engel et al., 1998). More recently, the behavior of B6SJL/F2-OE mice has been characterized in learning and memory paradigms utilizing fear conditioning (Harrell and Allan, 2003). Given that behavior is comprised of complex traits which are impacted by a number of genetic and epigenetic factors, the background genetics of transgenic animals may influence behavior. In order to avoid over-interpreting the impact of a genetic manipulation on behavior, a common strategy is the creation of congenics to carefully control for the influence of background genes (Crawley, 1999).

Over-expressed 5-HT_3 receptors are functional and have been shown to have characteristics indistinguishable from endogenous receptors (Sung et al., 2000). In rat, activation of 5-HT_3 receptors in the nucleus accumbens increases dopamine release by 400% (Campbell and McBride, 1995). In the nucleus accumbens, 5-HT_3 receptors are normally expressed in low densities (Barnes et al., 1990a; Kilpatrick et al., 1987; Waeber et al., 1988). As expected, over-expression has been shown to potentiate dopamine release in the nucleus accumbens (Allan et al., 2001). Whole-cell patch-clamp recordings in dissociated frontal cortex neurons from B6SJL/F2-OE mice revealed that ethanol potentiates the function of 5-HT_3 receptors (Sung et al., 2000), a mechanism that may underlie increased dopamine release.

Additionally, B6SJL/F2-OE mice consume less ethanol in a two-bottle free-choice test and are more sensitive to the effects of ethanol (Engel and Allan, 1999; Engel et al., 1998). Similarly, conditioned place preference for cocaine is attenuated by 5-HT_3 receptor over-expression, especially at high doses that transgene negative mice find rewarding (Allan et al., 2001). This is proposed to be a consequence of increased sensitivity as B6SJL/F2-OE mice display increased open field activity in response to lower doses of cocaine compared to transgene negative littermates. B6SJL/F2-OE mice appear to be more

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doi:10.1016/j.pbb.2006.04.016

sensitive to the effects of drugs of abuse as a consequence of increased dopamine release in response to drug. Furthermore, this sensitivity and the resultant reduction in ethanol consumption may be influenced by the specific genetic environment provided by the B6SJL background.

Over-expression of the 5-HT_3 receptor has also been shown to enhance hippocampal-dependent contextual fear conditioning, reduce anxiety and enhance inspective behavior (Harrell and Allan, 2003). 5-HT_3 receptors are expressed in structures involved in learning and memory, particularly the hippocampus, amygdala and nucleus accumbens (Barnes et al., 1990a; Kilpatrick et al., 1987; Tecott et al., 1993). Antagonism of the 5-HT_3 receptor by ondansetron has been shown to impair performance during a hippocampal-dependent task (Bratt et al., 1994). However, activation of the 5-HT_3 receptor may also impair some forms of learning and memory (Barnes et al., 1990b). By assessing the impact of 5-HT_3 receptor over-expression with respect to background genetics, we may better understand the effect of this receptor in tasks involving learning and memory.

DBA/2J (D2) and C57Bl/6J (B6) mice differ in both ethanol consumption and contextual fear conditioning. B6 consume more ethanol than D2 mice (Belknap et al., 1993; Crabbe et al., 1999; Paylor and Crawley, 1997), and have better hippocampal-dependent memory as evidenced by improved contextual and trace fear conditioning compared to D2 (Holmes et al., 2002; Lu and Wehner, 1997; Nie and Abel, 2001; Paylor et al., 1994; Stiedl et al., 1999). Contextual fear conditioning requires the hippocampal formation, while the amygdala is necessary for both contextual and cued fear conditioning (Kim and Fanselow, 1992; Phillips and LeDoux, 1992). D2 mice have specific deficits in hippocampal function that likely contribute to poor contextual conditioning. Both D2 and B6 mice have early hippocampal long-term potentiation (LTP) but D2 mice are deficient when tested for late LTP in response to theta burst stimulation. This is thought to be due to reduced protein kinase C (PKC) activity in the hippocampus of D2 mice (Nguyen et al., 2000). In addition to ethanol consumption and hippocampal-dependent learning differences, D2 and B6 mice have different responses to novelty. B6 spend significantly longer exploratory time with a new object than D2 mice (Ammassari-Teule et al., 2000; Thinus-Blanc et al., 1996). Finally, both D2 and B6 show high emotional reactivity in an elevated plus-maze (EPM) (Griebel et al., 2000), indicating that anxiety levels are not different between the strains.

More attention has recently been paid to the effects of background strain on behavioral phenotypes of genetically manipulated animals (Contet et al., 2001; Crabbe et al., 1999; Crawley et al., 1997; Dellu et al., 2000; Logue et al., 1997), and rightly so. Even the lethality of a mutation can differ in severity on different genetic backgrounds (Becker et al., 2003). Therefore, phenotype is not only the result of the targeted gene in transgenic animals, but also reflects interactions with background genes and should be as carefully controlled as any other experimental variable (Crawley et al., 1997). By breeding the 5-HT_3 receptor transgene onto two backgrounds, the D2 and B6, that behave differently on tasks previously used to assess the effect of 5-HT_3 receptor over-expression, we can better assess the pleiotropic effects of this receptor on behavior.

2. Materials and methods

2.1. Animals

All of the procedures employed in the current studies were approved by the University of New Mexico Laboratory Animal Care and Use Committee. 5-HT3 over-expressing mice were developed by our laboratory and are described elsewhere (Engel et al., 1998). To produce congenic mice, we used a technique termed speed congenics (Wong, 2002). First, we bred B6SJL-OE males to B6 and D2 females. By selecting pups for back-crossing based on coat color (black for B6 and dilute brown agouti for D2) and gene presence (confirmed by PCR), we sped the creation of our congenics and achieved transgenic animals with >99% of the new strain genotype after 5 back-crosses (Falconer, 1981). To verify that the over-expression did translate into an increase number of 5HT3 receptors in brain, binding of 125I-DAIZAC (5-HT3 antagonist, Mason et al., 1996) to coronal brain slices from mice back-crossed in generations 3 and 5 was performed as described in Engel et al. (1998). Mice were housed individually for ethanol consumption, or 2–4 per cage for all other tests, in a room with a 12-h light/dark cycle (lights on at 0800 h). Standard chow and water were available ad libitum. Adult mice, 60–120 days old, were used in the present experiments. Behavioral testing was conducted between 0800 and 1500 h.

2.2. Ethanol consumption

Mice from the first (N1), third (N3) and fifth (N5) back-crossed generations were tested to follow ethanol consumption over the course of breeding. Mice were housed individually and subjected to a two-bottle choice test in which the mice were offered 10% (w/vol) ethanol in H_2O in one bottle and H_2O in another bottle. Bottles (50 ml) equipped with balled-sipper tubes were used to limit loss of fluid by evaporation and dripping. The position of the tubes was switched daily to avoid a place bias. Fresh ethanol and water were prepared and weighed daily. Data were collected for 10 days and the mean grams per kilogram ethanol consumed daily calculated. Mice were weighed before and at the end of the experiment to check for weight loss.

2.3. Fear conditioning

Mice from the N1 and N5 generations and B6SJL/F2 mice were assessed. Fear conditioning took place in a Coulbourn® Habitest™ Modular Test System with a stainless steel grid floor for administration of the footshock. The apparatus was located within a sound-attenuated chamber. 70% isopropanol was used to clean the walls and floor after the removal of each mouse from the training context. Conditioning protocols were adapted from those described by Paylor et al. (1994). Mice were assigned to two groups, paired tone shock (PTS) or immediate

shock (IS). The tone-conditioned stimulus was an 80 dB, 6 Hz clicker. An unconditioned stimulus of an electric footshock (0.6 mA) was used. Both IS and PTS training took 4.5 min each. Twenty-four hours after training, mice were reintroduced to the conditioning context for the context retention test and scored by an observer blind to genotype. The conditioning context was again cleaned with 70% isopropanol between animals. The tone and the footshock were not delivered during this test session. The animal's behavior was observed and contextual conditioning was assessed using a time-sampling procedure. Every 10 s, the mouse was scored as either moving or freezing. Freezing is the conditioned response measured to reflect the amount of learning and is defined by the absence of movement other than that required for respiration (Paylor et al., 1994). Freezing was scored for 3 min without presentation of the tone to determine levels of freezing in response to the altered context itself. The amount of freezing is represented as the % freezing (# of freezing intervals ÷ total intervals).

2.4. Elevated plus-maze

Mice were tested for behavior using an elevated plus-maze modified from Pellow et al. (1987). Mice from the N5 generations and B6SJL/F2 mice were tested. Animals were placed in the center square (6 × 6 cm) of a Plexiglas maze shaped in a cross elevated 2 ft above the ground. The supports that elevate the maze were made of clear Plexiglas and were positioned in the middle of the arms so the mouse was unable to detect them. The maze had two open arms (30 × 6 cm each) and was located in a moderately lit, sound attenuated room. Open arms consisted of the clear Plexiglas floor and no walls. The closed arms were covered with black contact paper and had walls 6 cm high also covered with black contact paper. Groups were composed of 3 naïve animals × 2 genders × 2 genotypes. An observer blind to genotype monitored behavior for 5 min. The percentage of time spent in the open arms, closed arms and center area as well as the number of entries into the open and closed arms was recorded.

2.5. Novel object exploration

Mice from the N5 generations and B6SJL/F2 mice were tested for response to novelty. The test was adapted from the protocol previously described by Grailhe et al. (1999). An open field apparatus was used measuring 17 in.×17 in. with 8-in. high Plexiglas side-walls. The floor of the apparatus was black and divided up into five areas: four equal quadrants and one center area with a diameter of 14 cm. The experiments were carried out in a dimly lit room with the aid of a video camera to minimize any affects of stress/anxiety. An observer blind to genotype analyzed the tapes. The floor and walls of the open field were wiped with 70% isopropanol before each test session. The test consisted of two 5-min periods. For the first 5 min, mice were placed into the center area and allowed to acclimate. During the second 5 min, latency to approach the object was recorded, the time spent in the center was assessed, the total number of center line crosses and the number of total lines crossed were tallied. The object was a pink and green striped gray cube (1 in.^{3}) with an open side. The cube was placed in the center with the open side facing the mouse.

2.6. Basal open field activity

Ethanol-stimulated activity was assessed using an open field apparatus (102.5 × 102.5 × 47.5 cm) (Opto-varimex, Columbus Instruments) was equipped with a series of photo-beams. Activity was monitored by a computer. Mice were placed in the open field apparatus for 15 min and distance traveled was recorded. On the next day, mice were given an i.p. injection of 1.5 g/kg ethanol prepared in saline. Five minutes later, mice were placed back in the open field arena and distance traveled was recorded for 15 min.

2.7. Analysis

Two-way analysis of variance (ANOVAs) (background × transgene presence) were used in each of the tasks. The number of animals in each group ranged from 7 to 10.

3. Results

3.1. B6SJL/F2-OE and B6-OE mice consume less ethanol, while D2-OE do not

Ethanol consumption was assessed for transgene positive and negative mice from the first (N1), third (N3) and fifth (N5) congenic generations. The effect of 5-HT$_3$ receptor overexpression on ethanol consumption depends on the strain in which it is expressed. In all generations tested, B6-OE mice display significantly reduced ethanol consumption compared to transgene negative mice of the same background (Fig. 1A). However, a reduction in ethanol consumption was never noted for D2-OE mice (Fig. 1B). D2 mice, regardless of transgene presence, consume very low amounts of ethanol. A main effect of background was found for each N1 [$F(1,35)=425.32$, $P<0.0005$], N3 [$F(1,35)=459.41$, $P<0.0005$] and N5 generations [$F(1,35)=349.24$, $P<0.0005$]. A main effect of transgene presence was also found for each N1 [$F(1,35)=56.0$, $P<0.0005$], N3 [$F(1,35)=33.85$, $P<0.0005$] and N5 generations [$F(1,35)=6.33$, $P<0.017$]. Interactions of background and transgene presence were found for N1 [$F(1,35)=54.50$, $P<0.0005$], N3 [$F(1,35)=30.721$, $P<0.0005$] and N5 generations [$F(1,35)=4.35$, $P<0.05$].

3.2. B6SJL/F2-OE and B6-OE mice display improved contextual fear conditioning, whereas D2-OE mice do not

Fear conditioning to the context was determined for transgene positive and negative mice from N1 (Fig. 2A) and N5 (Fig. 2B) generations as well as B6SJL/F2 mice. None of the IS groups differed in freezing behavior and are not reported here. Transgene presence improved conditioning on B6SJL/F2 and B6 backgrounds. A main effect of background was found for N1 [$F(2,51)=38.64$, $P<0.0005$]

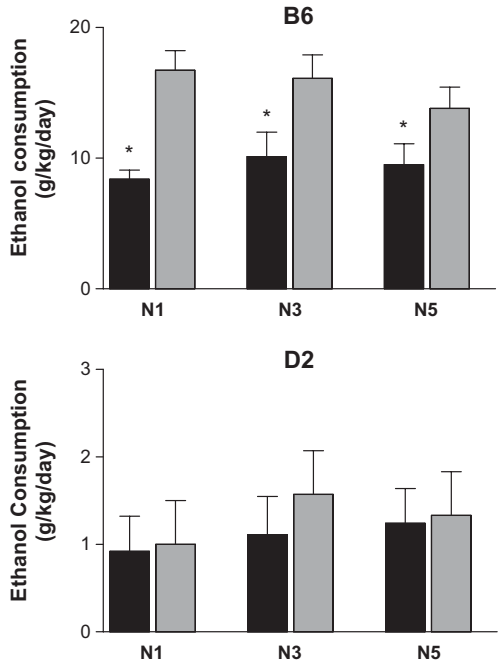

Fig. 1. Ethanol consumption is influenced by 5-HT_3 receptor over-expression and background. Data are AVG±S.E.M., (black bar) transgene positive, (gray bar) transgene negative. * indicates significant effect ($P<0.05$) of transgene presence.

and N5 [$F(2,51)=16.15$, $P<0.0005$]. A main effect of transgene presence was also found for N1 [$F(1,51)=10.9$, $P<0.003$] and N5 [$F(1,51)=11.32$, $P<0.001$]. A background × transgene presence interaction was found for the N5 generation as well [$F(2,51)=4.55$, $P<0.15$].

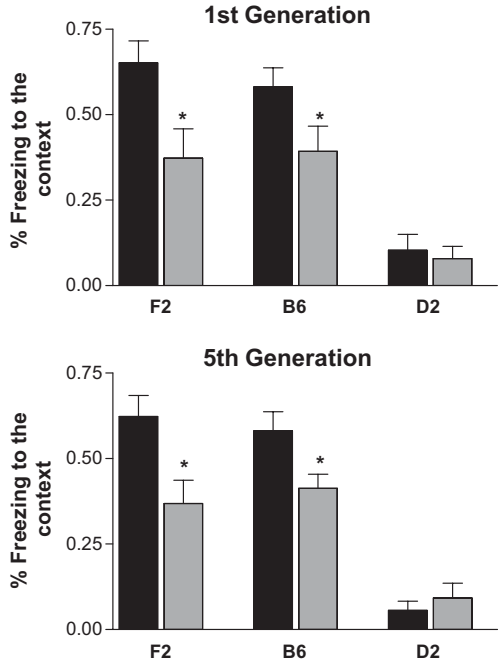

Fig. 2. Contextual conditioning is influenced by 5-HT_3 receptor over-expression and background. Data are AVG±S.E.M., (black bar) transgene positive, (gray bar) transgene negative. * indicates significant effect ($P<0.05$) of transgene presence.

3.3. B6SJL/F2-OE and B6-OE, but not D2-OE mice, display an anxiolytic phenotype in the EPM

Anxiety-related behaviors were assessed for transgene positive and negative mice from the N5 generations as well as B6SJL/F2 mice in the EPM. Number of entries into open arms (Fig. 3A) and time spent in the open arms (Fig. 3B) and time spent in the closed arms (Fig. 3C) are reported here. Again, the transgene effect (anxiolysis) that had been previously observed was recapitulated using the B6SJL/F2 and B6 but not the D2 strain. For the measure of number of entries, main effects of background [$F(2,51)=164.56$, $P<0.0005$] and transgene presence [$F(1,51)=51.66$, $P<0.0005$] were found, as was an interaction between background and transgene presence [$F(2,51)=13.93$, $P<0.0005$]. For the measure of percent time spent in open arms, main effects of background [$F(2,51)=113.64$, $P<0.0005$] and transgene presence [$F(1,51)=103.81$ $P<0.0005$] were found. An interaction of background and transgene presence was detected [$F(2,51)= 31.667$, $P<0.0005$]. There were no significant differences in time spent in the closed arms.

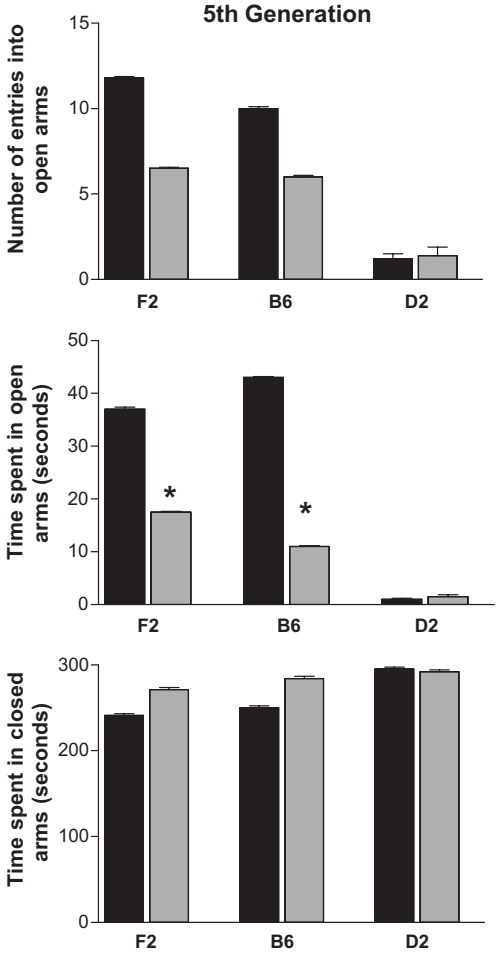

Fig. 3. Anxiety measures in the EPM are affected by 5-HT_3 receptor over-expression and background. Data are AVG±S.E.M., (black bar) transgene positive, (gray bar) transgene negative. * indicates significant effect ($P<0.05$) of transgene presence.

3.4. Both B6-OE and D2-OE spend more time near a novel object

Inquisitive and inspective behaviors were assessed in an open area after the insertion of a novel object for transgene positive and negative mice from the N5 generations, as well as B6SJL/F2 mice. As total number of lines crossed and latency to approach the novel object did not differ between groups, only the total number of entries into the center (Fig. 4A) and time spent near the novel object (Fig. 4B) are reported here. For the number of entries into the center area where the novel object was placed, an effect of transgene presence was found for the B6 but not the D2 background (Fig. 4A). Surprisingly, in the measure of time spent near the novel object, transgene presence had an effect for both B6 and D2 backgrounds (Fig. 4B). For the measure of total entries into the center area, main effects of background [$F(2.51)=35.0$, $P<0.0005$] and genotype [$F(1,51)=20.311$, $P<0005$] as well as an interaction between background and transgene presence [$F(2,51)=3.63$, $P<0.05$] were found. Percent time spent near the novel object was influenced by background [$F(2.51)=17.95$, $P<0.0005$] and transgene presence [$F(1,51)=708.82$, $P<0.0005$].

3.5. Ethanol increases locomotor activity in both B6-OE and D2-OE

Basal activity and ethanol-stimulated activity behaviors were assessed in an automated open arena for transgene positive and negative mice from the N5 generations, as well as B6SJL/F2 mice. Basal activity did not differ significantly between genotypes nor did ethanol-stimulated activity (Fig. 5). There was a main effect of treatment (basal vs. ethanol-stimulated) for distance traveled [$F(5,84)=49.57$, $P<0.0001$] but no genotype effect or interaction present, suggesting that ethanol produced a similar increase in activity among all the genotypes tested.

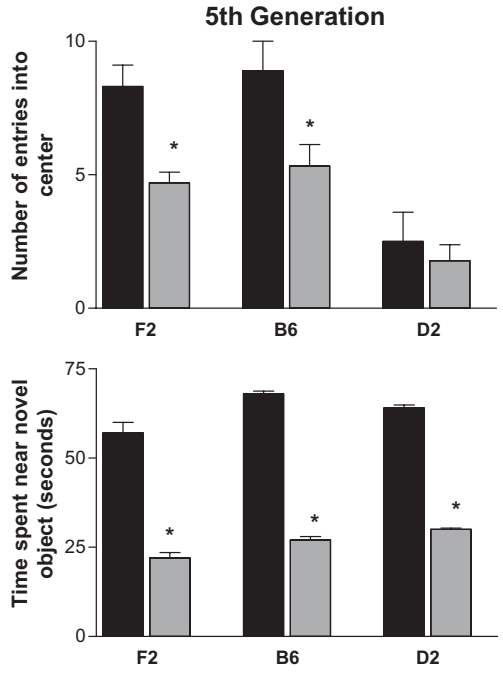

Fig. 4. Inspection of a novel object is enhanced by 5-HT$_3$ receptor over-expression regardless of background. Data are AVG±S.E.M., (black bar) transgene positive, (gray bar) transgene negative. * indicates significant effect ($P<0.05$) of transgene presence.

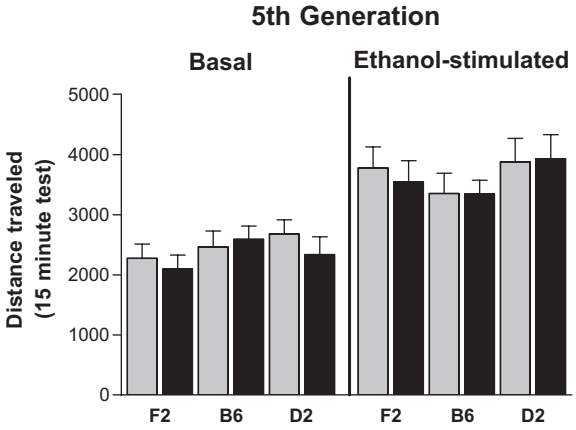

Fig. 5. Ethanol stimulation of open field activity is not altered by 5-HT$_3$ receptor over-expression regardless of background. Data are AVG±S.E.M., (black bar) transgene positive, (gray bar) transgene negative. Right portion of the graph shows basal activity and left portion show ethanol stimulated activity.

4. Discussion

The behavioral phenotype of B6-OE mice supports the original findings in B6SJL/F2-OE mice. Ethanol consumption was reduced, contextual fear conditioning was improved, a reduction in anxiety was indicated using the EPM, and inspective behavior toward the novel object was increased. D2-OE mice did not show any of these pleiotropic effects of transgene expression, save for an increase in the amount of time spent near the novel object, a measure of inspective behavior (Grailhe et al., 1999). The expression of the transgene did not alter ethanol-stimulated open field activity in any of the genotypes. It is also possible that the transgene expression on the D2 background would have influenced ethanol behaviors where this strain shows sensitivity (e.g., ethanol-stimulated activity, fear-potentiated startle and ethanol-conditioned place preference). In our study, we only tested ethanol-stimulated activity and found no effect of the transgene on any of the genotypes tested. The reason for this overall lack of effect of transgene expression on the D2 background may be due to differences in intracellular signaling directly influencing 5-HT3 receptor activity, or alternately due to differences related to dopamine as a consequence of 5-HT3 receptor over-expression.

Ethanol consumption is one of the more stable behaviors in mice (Crabbe et al., 1999), and so was observed in all three N1, N3 and N5 congenic generations to follow the effect of the transgene over-time. The effect of background strain was stable over all generations (Fig. 1). One reason that D2-OE mice do not show reduction in ethanol consumption could be a floor-effect. DBA mice drink very little regardless of transgene presence so it may not be possible for this strain to consume any less alcohol, especially given that the bottle position is changed daily, and any attempt to avoid ethanol consumption, if found

aversive, would still result in low levels of drinking similar to levels observed. We previously reported that ethanol is not rewarding, and indeed may be aversive, at higher doses for B6SJL/F2-OE (Engel and Allan, 1999; Engel et al., 1998). The data presented here support this effect of 5-HT3 receptor over-expression. Furthermore, this experiment demonstrates the critical impact that background choice can have on behavior. While the presence of the transgene reduced ethanol drinking in the B6 and B6SJL strains, it is possible that its expression in the D2 could have resulted in an anomalous result, resulting in an increase in drinking. While this was unlikely, we included the D2-OE mice in this test. The use of B6 mice, and not D2, is advised when a reduction in ethanol consumption is expected as a consequence of genetic manipulation.

Over-expression of the 5-HT3 receptor improved contextual fear conditioning on the B6SJL/F2 and B6 backgrounds but not the D2. As D2 mice have poor contextual fear conditioning (Paylor et al., 1994), it was speculated that improvements in contextual conditioning would be most evident on this background. Reduced PKC activity, previously reported in the hippocampus of D2 mice (Bowers et al., 1995; Wehner et al., 1990) may explain the lack of effect of the transgene on hippocampal-dependent learning. 5-HT3 receptors in Xenopus oocytes are potentiated by PKC activation (Sun et al., 2003; Zhang et al., 1995). Thus, D2 mice may have reduced potentiation of the 5-HT3 receptor, even when over-expressed. As potentiation of the 5-HT3 receptor is proposed to contribute to the reduction in ethanol consumption, and may also underlie enhanced learning and memory in B6SJL/F2-OE mice, the D2 PKC activity deficit may preclude potentiation of the 5-HT3 receptor. While it has been proposed that D2 mice could provide an appropriate genetic background to study enhancement of hippocampal-dependent learning and memory, this strain's usefulness may be limited due to an intrinsic deficit in intracellular signaling. We propose that molecular manipulations aimed at improving hippocampal-dependent learning that may be mediated in part by PKC activity, such as 5-HT3 receptor over-expression, might not be able to overcome the learning deficit of the D2 strain.

Alternately, the lack of improvement in contextual conditioning noted for D2 mice could be due to differences in nucleus accumbens (NAc) function in this task. Inactivation of the NAc selectively reduces contextual fear conditioning (Haralambous and Westbrook, 1999; Riedel et al., 1997; Westbrook et al., 1997). Additionally, NAc function appears to vary in relation to a strains' propensity to develop contextual conditioning (Ammassari-Teule et al., 2000). As the 5-HT_3 receptor modulates dopamine release in the NAc (Allan et al., 2001; Campbell and McBride, 1995; Jiang et al., 1990; Sung et al., 2000; Wozniak et al., 1990), it is possible that over-expression modulates conditioning in a strain-dependent manner. Dopamine release has been implicated in reward processing, but is also affected by aversive conditions, such as fear conditioning, and may influence learning (Horvitz, 2000, 2002; Schultz, 2002). Therefore, differences in NAc activity between strains may influence behaviors altered by 5-HT_3 receptor over-expression.

Anxiety, as measured in the EPM, also remained unchanged by 5-HT3 receptor over-expression on the D2 background, whereas B6SJL/F2-OE and B6-OE displayed reduced anxiety in the EPM. The reason for this is not clear. Both D2 and B6 strains are emotionally reactive in the EPM (Griebel et al., 2000), indicating that 5-HT3 receptor over-expression should affect both similarly. While fearful reactions are generally considered to be controlled by the amygdala (Davis and Whalen, 2001; Rogan and LeDoux, 1996), the hippocampal formation is also important (File et al., 2000; Gonzalez et al., 1998). In fact, the hippocampal formation is thought to be capable of coding critical aspects of anxiety, allowing for the integration of anxiety states and learning cues. It is therefore possible that hippocampal deficits, noted for D2 mice, underlie the inability of 5-HT3 receptor over-expression to modify anxiety-related behaviors in this strain.

The one task where transgene presence produced a change in behavior on all backgrounds was the novelty exploration task. Time spent near the novel object was increased for all three strains. However, total number of entries into the center area near the novel object was not increased in D2-OE mice. This could be influenced by heightened anxiety-related behavior in D2-OE mice relative to B6-OE and B6SJL/F2-OE mice. It is possible that there is some threshold related to anxiety that influences the number of entries into the center. However, once this threshold is crossed and the animal is in the center near the novel object, exploration of the object reflected by time spent near it is enhanced as a consequence of over-expression of the 5-HT_3 receptor.

The exploration of a novel object may depend on dopamine, the release of which is potentiated in 5-HT_3 receptor over-expressing mice (Allan et al., 2001; Sung et al., 2000). In human brain, the ventral striatum (NAc) responds to novelty in the absence of awareness (Berns et al., 1997). Furthermore, heightened exploratory behavior in a novel environment predicts a greater DA response in the NAc (Hooks et al., 1991). This suggests that greater dopamine release in the NAc, mediated by activity at over-expressed 5-HT_3 receptors, may influence the propensity to explore novel objects or environments.

A recent study by Kelley et al. (2003), using a targeted deletion of the 5-HT3A receptor subunit gene, found a reduced level of anxiety associated behaviors in the null mice, indicating that the 5-HT3A subunit may modify anxiety-related behaviors. While the over-expression in our model did not specify the 5HT3A subunit, this subunit was the clone used in our over-expression model and is the upregulated subunit. The work of Kelley et al. (2003) suggests that the reduced anxiety seen in our model may be due to proteins associated with the 5HT 3A subunit, rather than due to a direct effect of the 5HT3A subunit itself. In another series of elegant studies, Hodge et al. (2004) found that the 5-HT(3A)-null mice did not differ from wild-type littermate controls on ethanol intake and preference, and administration of a 5-HT3 antagonist decreased intake of both sweetened and unsweetened ethanol in wild-type but not in the 5HT3A null mutants. These findings indicate that the reduction in alcohol drinking produced by blocking the 5-HT3 receptor is

dependent on the presence of the 5-HT-3A subunit. Curiously, removal of the 5-HT3A subunit by null mutation had no effect on ethanol intake or preference. Suggesting the role that the 5HT3A subunit has over ethanol consumption does not operate in both directions. However, our data would suggest that the 5HT3A subunit plays a role in limiting ethanol drinking. While subunit over-expression is not technically the opposite of subunit deletion, these two findings suggest that more than simple addition and subtraction are taking place in these manipulations.

The 5-HT3 receptor has two known receptor subunits, A and B, which form homomeric 5-HT3A receptors and heteromeric 5-HT3A/B receptors with a stoichiometry of 2A:3B. The B subunit by itself does not form a functioning channel. It is the 3A/B heteromeric form of the receptor, which is most common in mammalian systems and resembles the native receptor (Barrera et al., 2005). Using atomic force microscopy to study the architecture of 5-HT3A and 5-HT3A/B receptors, Barrera et al. (2005) found that, with the heteromer arrangement of A and B subunits, there will be three types of subunit interface (2×A–B, 2×B–A and 1×B–B) and these different interfaces provide nonequivalent agonist-binding sites. In our model, the upregulation of the A subunit only would increase the number of 5-HT3A homomeric channels as well as possibly some heteromeric channels with altered stoichiometries. Over-expression of the single subunit would create channels with different kinetic properties; additionally, it would result in an increase in the Hill coefficient for agonist and antagonist association as well as alter the level of cooperation between the two subunits (Davies et al., 1999; Dubin et al., 1999). Thus, interpretation of these data are cautioned by the understanding that changes in the expression of one subunit within a heteromeric receptor structure can produce complex changes in the receptor architecture producing functional variabilities.

Acknowledgments

This work was supported by an Army Grant, DAMD 17-01-1-0680.

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