Published in final edited form as:

Brain Res. 2008 March 10; 1198: 124–131.

5-HT_{2C} and GABA_B receptors influence handling-induced convulsion severity in chromosome 4 congenic and DBA/2J background strain mice

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Abstract

Progress towards elucidating the underlying genetic variation for susceptibility to complex central nervous system (CNS) hyperexcitability states has just begun. Genetic mapping analyses suggest that a gene(s) on mid-chromosome 4 has pleiotropic effects on multiple CNS hyperexcitability states in mice, including alcohol and barbiturate withdrawal and convulsions elicited by chemical and audiogenic stimuli. We recently identified *Mpdz* within this chromosomal region as a gene that influences alcohol and barbiturate withdrawal convulsions. *Mpdz* encodes the multi-PDZ domain protein (MPDZ). Currently, there is limited information available about the mechanism by which MPDZ influences drug withdrawal and/or other CNS hyperexcitability states, but may involve its interaction with 5-HT_{2C} and/or GABA_B receptors. One of the most useful tools we have developed thus far is a congenic strain that possesses a segment of chromosome 4 from the C57BL/6J (donor) mouse strain superimposed on a genetic background that is >99% from the DBA/2J strain. The introduced segment spans the *Mpdz* gene. Here, we demonstrate that handling-induced convulsions are less severe in congenic vs. background strain mice in response to either a 5-HT_{2C} receptor antagonist (SB242084) or a GABA_B receptor agonist (baclofen), but not a GABA_A receptor channel blocker (pentylenetetrazol). These data suggest that allelic variation in *Mpdz*, or a linked gene, influences SB242084- and baclofen-enhanced convulsions. Our results are consistent with the hypothesis that *Mpdz*'s effects on CNS hyperexcitability, including alcohol and barbiturate withdrawal, involve MPDZ interaction with 5-HT_{2C} and/or GABA_B receptors. However, additional genes reside within the congenic interval and may also influence CNS hyperexcitability.

Keywords

mouse; genetic; serotonin; MPDZ; MUPP1; seizure

1. Introduction

Individual risk for idiopathic epilepsy and other central nervous system (CNS) hyperexcitability phenotypes including predisposition to alcohol and drug withdrawal

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convulsions is regarded as strongly genetic (Annegers, 1996; Sander et al., 1997). Complex generalized seizures characterize at least half of idiopathic epilepsies (Annegers, 1996), and are also the most characteristic and severe type of seizure that occurs in alcohol and drug withdrawn individuals, as well as rodent models of alcohol and drug withdrawal (Rogawski, 2005). Over the past decade, a number of genes have been associated with rare monogenic idiopathic epilepsies that have relatively simple inheritance (Mulley et al., 2005), but progress towards elucidating the genetic variation underlying susceptibility to complex CNS hyperexcitability phenotypes has just begun.

Quantitative trait loci (QTLs) are chromosome sites containing a gene(s) at which allelic variation affects a complex (quantitative) trait. Animal models and QTL analyses provide powerful tools to dissect key sites of neuronal excitability and to identify the underlying genetic factors (Flint, 2003). QTL analyses in common inbred strains of mice have identified seizure susceptibility loci on mid-chromosome 4, including QTLs for convulsions elicited by chemical, electrical and audiogenic stimuli (Neumann and Collins, 1991; Martin et al., 1995; Ferraro et al., 1997) as well as alcohol and drug withdrawal convulsions elicited by handling (Buck et al., 1997, 1999; Fehr et al., 2002; Bergeson et al., 2003; Shirley et al., 2004). Thus, a gene or genes on mid-chromosome 4 may have pleiotropic effects on multiple seizure phenotypes. For drug withdrawal convulsions, based upon positional cloning using robust behavioral models as well as sequence and expression analyses, the most compelling gene in the critical 1.8 Mb chromosomal segment is *Mpdz* (Shirley et al., 2004). This gene and its human homolog encode the multi-PDZ domain protein (MPDZ, also called MUPP1) and show widespread expression in the brain (Ullmer et al., 1998; Simpson et al., 1999; Sitek et al. 2003). The MPDZ protein is thought to affect neuronal excitability by altering the rate or fidelity of signal transduction mediated by a protein(s) with which it interacts, similar to other members of the PDZ domain protein family (Tsunoda et al., 1997; Fanning & Anderson, 1999; Sheng and Sala, 2001). Plausible mechanisms involve MPDZ's interaction with 5-HT_{2C} and/or GABA_B receptors (Ullmer et al., 1998; Becamel et al., 2001; Balasubramanian et al., 2007), both of which influence neuronal excitability and are important therapeutic targets for epilepsy as well as alcohol and drug abuse (Prather et al., 1991; Semenova and Ticku 1992; Lal et al., 1993; Rezazadeh et al., 1993; Bettler et al., 1998; Gatch et al., 2000; Bowery, 2006).

One of the most useful tools we have developed thus far is a congenic strain that possesses a segment of chromosome 4 from the C57BL/6J (B6) donor strain superimposed on a genetic background estimated to be >99% from the DBA/2J (D2) mouse strain (Fehr et al., 2002). Through the elimination of genetic "noise" from additional QTLs on other chromosomes, comparisons of congenic and background strain mice are invaluable to investigate the gene(s) and mechanism(s) underlying a QTL's behavioral effects in a relatively isolated context. Previously, we reported that this congenic strain exhibits significantly less severe alcohol withdrawal CNS hyperexcitability than background strain mice (Fehr et al., 2002). Here, we compare the chromosome 4 congenic and background strains for their susceptibilities to CNS hyperexcitability in response to three chemiconvulsants with well-defined sites of action: SB242084, a selective 5-HT_{2C} receptor antagonist (Kennett et al., 1997); baclofen, a selective GABA_B receptor agonist (Blake et al., 1993); and pentylenetetrazol (PTZ), which exerts its convulsant effects by impairment of GABA_A-mediated neurotransmission (Corda et al., 1991; Kulkarni and George, 1995). As in previous work from our laboratory, we use the handling-induced convulsion (HIC) as a sensitive measure of CNS hyperexcitability because it is a well-established index to assess genetic differences in chemiconvulsant sensitivity and severity of alcohol and drug withdrawal (Goldstein, 1973; Crabbe et al., 1980, 1991a, 1993). Although HICs can be exhibited by naïve mice, and their severity differs among genotypes (Crabbe et al., 1980), they are also increased and decreased in severity by convulsant and anticonvulsant drugs, respectively (Crabbe et al., 1991a). D2 and B6 strain mice do not differ in brain PTZ levels (Kosobud et al., 1992), but the impact of potential strain differences in

pharmacokinetics for many other chemiconvulsants is not known. For this reason, we measured plasma baclofen levels in both chromosome 4 congenic and D2 background strain mice in order to assess whether potential strain differences in drug metabolism may influence the results observed.

The present studies implicate a potential role for allelic variation in *Mpdz*, or a linked gene, on SB242084 and baclofen-enhanced HICs, but not PTZ-enhanced HICs. We discuss potential roles of 5-HT_{2C} and/or GABA_B receptors in mediating *Mpdz*'s influence on alcohol and drug withdrawal and other CNS hyperexcitability phenotypes influenced by a QTL on chromosome 4.

2. Results

2.1. Baseline handling-induced convulsions (HICs)

Individual mice and different strains can vary in baseline (pre-drug) HIC scores. No significant main effect of sex ($F_{[1,190]} = 0.4, p=0.9$) or sex by strain interaction ($F_{[1,190]} = 1.8, p=0.2$) was observed in baseline scores. However, after collapsing the data across both sexes for all of the mice tested, we observed a small but significant difference between chromosome 4 congenic and background strain mice in average baseline HIC scores (mean $\pm$ SEM = 0.18 ± 0.06 and 0.51 ± 0.08 , $n = 97$ per genotype, respectively; $p < 0.05$), with both strains showing extremely low baseline HIC scores. This is consistent with previous data on baseline HIC scores in chromosome 4 congenic and background strain mice (Shirley et al., 2004). Although small, this difference is significant. In order to create an index of chemiconvulsant response that is independent of individual differences in baseline HIC scores and reflects the genotype-dependent difference in convulsion severity, all post-chemiconvulsant HIC scores were corrected for the individual's baseline score.

2.2. Chemiconvulsant-enhanced HICs

We compared chromosome 4 congenic and D2 background strain mice for severity of HICs enhanced by three chemiconvulsants with well-defined sites of action: SB242084, baclofen, and PTZ. No significant effects of sex or sex by strain interactions were apparent (all $p > 0.4$) for SB242084 ($F_{[1,27]} = 0.7$ and $F_{[1,27]} = 0.03$, respectively), baclofen ($F_{[1,49]} = 0.2$, $F_{[1,49]} = 0.1$), or PTZ ($F_{[1,90]} = 0.3$, $F_{[1,90]} = 0.2$), so the data for both sexes were combined for the remainder of the analysis. In all cases, the chemiconvulsant doses used to enhance HIC scores were insufficient (subthreshold) to elicit the convulsions characteristic of higher doses of the drug, such as tonic hindlimb extensor seizures.

For SB242084-enhanced convulsions, our results show a main effect of dose ($F_{[1,31]} = 25.4$, $p < 0.001$), with more severe HICs apparent in congenic and background strain animals treated with 10 mg/kg SB242084 compared to animals treated with 1 mg/kg SB242084. We also observed a main effect of strain, with congenic mice having significantly less severe HICs in response to both 1 and 10 mg/kg SB242084 compared to background strain animals ($F_{[1,31]} = 22.3$, $p < 0.001$, Fig 1). The interaction of strain and dose was not significant ($F_{[1,31]} = 0.4$, $p = 0.5$).

For baclofen, we observed a main effect of strain, with congenic mice having significantly less severe HICs in response to baclofen compared to background strain animals ($F_{[1,53]} = 10.4$, $p < 0.005$, Fig 2). Our results also show a main effect of dose ($F_{[1,53]} = 40.6$, $p < 0.001$), with more severe HICs apparent in congenic and background strain mice treated with 10 mg/kg baclofen as compared to mice treated with 5 mg/kg baclofen. No significant strain $\times$ dose interaction was observed ($F_{[1,53]} = 0.6$, $p = 0.5$).

Because association analyses using a panel of standard inbred mouse strains suggested that *Mpdz* allelic status might be genetically correlated with seizure response to PTZ (Fehr et al., 2004), we also compared congenic and background strain mice for severity of HICs following PTZ administration. Our results show a main effect of dose ($F_{[2,96]} = 48.9, p < 0.001$), with post-hoc analysis showing that animals treated with 5 or 15 mg/kg PTZ had significantly less severe HICs than animals treated with 30 mg/kg PTZ. In contrast to the two other chemiconvulsant drugs tested, no strain difference in PTZ response was apparent ($F_{[1,96]} = 0.01, p = 0.9$; Fig 3), nor was a strain $\times$ dose interaction found ($F_{[2,96]} = 0.3, p = 0.8$).

Taken together, our results demonstrate that baclofen and SB242084 dose-dependently enhanced HICs, and that congenic animals showed significantly less severe convulsions compared to background strain animals in response to both chemiconvulsants. In contrast, PTZ-enhanced HICs were comparably severe in congenic and background strain mice, suggesting that the chromosome 4 QTL gene(s) influences some, but not all, CNS hyperexcitability phenotypes.

2.3. Brain and blood chemiconvulsant levels

Using an established LC-MS assay (Miksa and Poppenga, 2003), individual chromosome 4 congenic and D2 background strain mice were assessed for plasma baclofen levels 1, 2 and 4 hours after baclofen administration (12 mg/kg, i.p.). These time points were chosen based upon data indicating that baclofen produces the majority of its behavioral effects, including proconvulsant activity, within four hours of its administration (Jacobson and Cryan, 2005). The results of this analysis showed no significant main effects of strain ($F_{[1,29]} = 1.4, p = 0.2$), time of sample collection post-baclofen administration ($F_{[2,29]} = 0.7, p = 0.5$), nor a strain $\times$ time interaction ($F_{[2,29]} = 0.3, p = 0.8$). The lack of a significant difference is likely due to the considerable individual variability in plasma baclofen concentrations (Table 2).

3. Discussion

There is currently very little information on the identity of genes involved in complex CNS hyperexcitability states including alcohol and drug withdrawal convulsions and other complex generalized seizures (Annegers, 1996; Rogawski, 2005), although it is commonly accepted that multiple genetic factors are at play (Schmidt and Sander, 2000; Noebels, 2003). Identification of the complex gene and protein interactions that exacerbate these phenotypes is necessary to determine their mechanisms of action and, ultimately, to develop more effective treatments for these disorders in clinical populations.

Several analyses have detected seizure susceptibility QTLs on mid-chromosome 4 (Neumann and Collins, 1991; Martin et al., 1995; Ferraro et al., 1997) in mice, although most are still suggestive associations and mapped to relatively large regions. For convulsions associated with the alcohol and barbiturate withdrawal syndromes, positional cloning, sequence and expression analyses identify the multi-PDZ domain protein gene, *Mpdz*, as the most compelling gene within the critical 1.8 Mb chromosomal segment of chromosome 4 (Shirley et al., 2004). Currently, there is limited information available about the mechanism by which *Mpdz* influences withdrawal convulsions and/or other CNS hyperexcitability phenotypes. Some PDZ domain proteins possess enzymatic activity, but the MPDZ protein (encoded for by *Mpdz*) apparently has no such intrinsic activity, so it is plausible that it affects neuronal excitability by altering the rate or fidelity of signal transduction mediated by one or more of the proteins with which it interacts. MPDZ's interaction with 5-HT_{2C} receptors has been confirmed *in vivo* (Becamel et al., 2001), and is mediated via a PDZ recognition motif (X-S/T-X-V) at the intracellular C-terminus of the 5-HT_{2C} receptor that binds to the tenth PDZ domain in MPDZ (Becamel et al., 2001; Parker et al., 2003). Here, we demonstrate that chromosome 4 congenic mice show significantly less severe convulsions in response to SB242084, a selective 5-

HT_{2C} receptor antagonist, than background strain mice. This is consistent with the hypothesis that allelic variation in *Mpdz*, or a linked gene, affects 5-HT_{2C} receptor function and thereby influences convulsion severity. A plausible mechanism involves MPDZ interaction with 5-HT_{2C} receptors in the basal ganglia, as this circuit is implicated in genotype-dependent differences in ethanol withdrawal convulsions between the D2 and B6 progenitor strains and between the chromosome 4 congenic and background strains (Kozell et al., 2005; Chen et al., submitted), as well as other CNS hyperexcitability states (Deransart and Depaulis, 2002; Dematteis et al., 2003; Biraben et al., 2004; Gernert et al., 2004; Magill et al., 2005). Serotonergic pathways are intimately involved in the modulation of basal ganglia physiology, and anatomical evidence shows that the basal ganglia receives strong serotonergic innervation and expresses high levels of serotonin receptors including 5-HT_{2C} receptors (reviewed in Di Giovanni et al., 2006).

MPDZ also interacts with GABA_B receptors (Balasubramanian et al., 2007), although this interaction has yet to be confirmed *in vivo*. We report here that baclofen increased HIC severity to a lesser degree in chromosome 4 congenic versus background strain mice. This is consistent with the hypothesis that allelic variation in *Mpdz*, or a linked gene, affects GABA_B receptor function. GABA_B receptors have been implicated in a variety of CNS hyperexcitability states (Schuler et al., 2001; Brown et al., 2003; Gassmann et al., 2004; Merlo et al., 2007). Baclofen is proconvulsant for some types of seizures, including models of generalized absence seizures (Marescaux et al., 1992; Snodgrass, 1992), and anticonvulsant in other seizure models (Amabeoku and Chickuni, 1992), suggesting that different brain regions and/or cellular mechanisms influence different seizure types (Carai et al., 2002). Furthermore, in an active neuronal network the net effect of GABA_B receptor activation on neuronal excitability is extremely complex due to the dynamic spatial and temporal complexities of synaptic activity within integrated inhibitory and excitatory circuits. Such a scenario could explain observations that both GABA_B receptor agonists and antagonists can be proconvulsant (Mott et al., 1989) and anticonvulsant (Ault and Nadler, 1983). Interpretation of baclofen effects in whole animals is further complicated by substantial variability in baclofen plasma concentrations following its i.p. administration and by uncertainty of the actual drug concentrations at GABA_B receptor sites in the brain. Although we were successful in measuring baclofen levels in plasma, we were unable to measure baclofen in the brain. This may be a limitation of the LC-MS assay used, which has not previously been used to measure baclofen in the brain. Alternatively, although baclofen's effects in the brain have been confirmed (Carter et al., 2006), baclofen levels may be substantially lower in the brain compared to plasma, perhaps due to its efflux from the brain (Deguchi et al., 1995). Future studies will attempt to circumvent this confound by assessing the behavioral effects of baclofen (and other chemiconvulsants) administered intracerebroventricularly.

These studies contribute significantly to progress in understanding the genetic determination of chemiconvulsant-enhanced HICs and other behaviors, but there are some limitations. First, although *Mpdz* is a known quantitative trait gene (QTG) for drug withdrawal convulsions (Shirley et al., 2004), and its protein product directly interacts with 5HT_{2C} and GABA_B receptors, additional genes reside within the congenic interval and could potentially influence SB242084 and/or baclofen-enhanced convulsions. More definitive confirmation that *Mpdz* status influences SB242084 and/or baclofen-enhanced convulsions, as well as drug withdrawal convulsions, will likely require verification using D2/B6 bacterial artificial chromosome transgenics for *Mpdz* and/or RNA interference methodologies. Second, not all chemiconvulsant levels were assessed following their i.p. administration. We were unable to measure SB242084 levels because appropriate assays do not yet exist. Although PTZ levels were not measured, previous work shows that the B6 and D2 progenitor strains do not differ in brain PTZ levels (Kosobud et al., 1992). Because chromosome 4 congenic and background

strain mice differ across far less of their genomes (~2%), it is highly unlikely that PTZ levels differ between congenic and background strain mice.

Our results were consistent with previous data (Shirley et al., 2004), showing a significant strain difference between chromosome 4 congenic and D2 background strain mice in baseline HICs, which may suggest that a gene(s) within this region influences basal convulsion status. Although these results are significant, baseline HIC scores in both strains were extremely low (<1), and all results were corrected for baseline in order to account for these differences.

In summary, chromosome 4 congenic mice have less severe convulsions in response to 5-HT_{2C} and GABA_B receptor modulation, as well as less severe withdrawal from alcohol (Fehr et al., 2002), but do not differ from background strain mice in PTZ-enhanced HICs. Similarly, PTZ-enhanced HICs do not differ in Withdrawal Seizure-Prone (WSP) and Withdrawal Seizure-Resistant (WSR) mice, which were selectively bred based on the basis of high and low HIC severity, respectively, after chronic alcohol exposure (Crabbe et al., 1991a), and also differ in barbiturate withdrawal severity (Crabbe et al., 1991b). Our results demonstrate that a gene or genes within the congenic interval influences CNS hyperexcitability mediated by 5-HT_{2C} and/or GABA_B receptor modulation. One intriguing possibility is that the same gene identified as a quantitative trait gene for alcohol and barbiturate withdrawal convulsions (Shirley et al., 2004), *Mpdz*, may also influence 5-HT_{2C} and/or GABA_B receptor-enhanced HICs. If true, this would also implicate MPDZ's interaction with 5-HT_{2C} and/or GABA_B receptors in mediating its influence on alcohol and barbiturate physiological dependence and associated withdrawal. Given the growing body of evidence that dysregulation of serotonergic and GABAergic transmission are involved in substance abuse as well as CNS hyperexcitability, increasing our understanding of genetic determinants that regulate 5HT_{2C} and/or GABA_B receptor function has clear translational relevance.

4. Experimental procedures

4.1. Mice

Male and female mice of the chromosome 4 congenic (D2.B6-ISCS1; Fehr et al., 2002) strain were bred at the Portland VA Medical Center in the Portland Alcohol Research Center Animal Production core or in our colony at the Department of Comparative Medicine at Oregon Health & Science University. Male and female D2 strain mice were purchased from the Jackson Laboratory (Bar Harbor, ME) or bred in our colony. To develop the congenic strain, the B6 donor strain was crossed with D2 mice to yield B6D2 F₁ animals, which were backcrossed to D2 mice. Selection of breeders for this and subsequent backcrosses was based on genotyping at four MIT markers within and flanking the chromosome 4 QTL (*D4Mit263* at 3.2 cM; *D4Mit192* at 6.3 cM; *D4Mit347* at 42.5 cM and *D4Mit245* at 42.5 cM). In this manner, 10 generations of backcrossing were carried out. This was followed by an intercross between N10 animals to generate the finished congenic strain (donor interval homozygotes). Thus, excluding the congenic interval, the genetic background for ISCS1 is estimated to be <0.05% B6 and >99.95% D2. Genotyping was performed to identify the boundary regions of the introgressed donor region from the donor (B6) strain, which comprised ~2% of the genome. This region includes *Mpdz*, which is located in the middle of chromosome 4 at 38.6 cM (80.9–81.0 Mb; Ensemble build 37).

Approximately equal numbers of congenic and D2 background strain mice from both genders were tested in all of the behavioral experiments. Food (Purina 5001 lab chow) and water were freely available at all times. Mice were housed in groups of 2–4 in wire-mesh cages with corn-cob bedding (Bedocob). Procedure and colony rooms were kept at a temperature of 21±1°C. Lights were on in the colony from 0600–1800 hr, and testing was initiated between 0700 and 0900 hr. Mice were 60–100 days of age at the time of testing (mean age ± SEM: D2 males,

91.9 ± 2.9; D2 females, 97.1 ± 1.7; congenic males, 85.4 ± 3.6; congenic females, 99.1 ± 3.3), and were age-matched within each sex.

4.2. Drugs

SB242084 (6-chloro-2,3-dihydro-5-methyl-N-[6-[(2-methyl-3-pyridinyl)oxy]-3-pyridinyl]-1H-indole-1-carboxamide dihydrochloride), PTZ (6,7,8,9-tetrahydro-5H-tetrazoloazepine), and racemic baclofen (β -(aminomethyl)-4-chlorobenzenepropanoic acid hydrochloride) were purchased from Sigma Chemical Company (St. Louis, MO). Baclofen and PTZ were dissolved in sterile saline, and SB242084 was dissolved in sterile saline containing 8% β -cyclodextrin and 25 mM citric acid. The drugs were administered by intraperitoneal (i.p.) injection in a volume of 0.01 ml/g body weight, and the doses used were based upon previous work (Kennett et al., 1997; Dalton et al. 2006; Humenick et al., 1994; Crabbe et al., 1991a). Doses administered for each chemiconvulsant are as follows: SB242084 (1 or 10 mg/kg), baclofen (5 or 10 mg/kg), PTZ (5, 15 or 30 mg/kg). In all cases, the chemiconvulsant doses used to enhance HIC scores were insufficient (subthreshold) to elicit the convulsions characteristic of higher doses of the drug, such as tonic hindlimb extensor seizures.

4.3. Handling-induced convulsions

The handling-induced convulsion (HIC) was used to index changes in CNS hyperexcitability following administration of chemiconvulsant drugs as in previous work (Kosobud and Crabbe, 1986). SB242084, baclofen and PTZ-enhanced HICs were indexed and scored on a scale ranging from 0 to 7. Briefly, each mouse is picked up gently by the tail and, if necessary, spun gently through a 180° arc. Details of the HIC scoring system are given in Table 1.

All mice were scored twice, 20 min apart, to establish their individual baseline HIC score. The first and second baseline scores were significantly correlated ($p = 0.001$). Drug or vehicle was then administered. As the three chemiconvulsants tested may differ in their pharmacokinetics, different time courses of testing were chosen based on previous work. PTZ-enhanced HICs were measured 1, 3, 5, 8, 12, 15, 20 and 60 minutes post-drug. This time period was selected based on the time course of PTZ elicited convulsions in a panel of mouse strains (Crabbe et al. 1991). Baclofen-enhanced HICs were assessed every 30 minutes for 6 hours post-drug. This time period was chosen based on data indicating that baclofen produces the majority of its behavioral effects, including proconvulsant activity, within four hours of its administration (Jacobson and Cryan, 2005). SB242084-enhanced HICs were measured hourly beginning 1 hour post-drug through hour 10 post-drug. This time period selected was based on its effects on electroconvulsive seizure threshold (Kennett et al. 1997). To correct for individual differences in baseline (pre-drug) HIC scores, the average baseline HIC score for each mouse was subtracted from its individual post-drug HIC scores to calculate corrected HIC values ($\geq$ zero). Using the time course data for the HIC values, we calculated the area under the curve (AUC) as an overall measure of HIC response to each chemiconvulsant.

4.4. Baclofen levels

Trunk blood samples were collected from individual naïve male chromosome 4 congenic and D2 background strain mice ($n = 3-10$ per strain) 1, 2 and 4 hours post-baclofen (12 mg/kg, i.p.). None of the subjects were scored for HICs. Plasma was extracted by centrifugation ($914 \times g$ for 20 minutes) in a tube containing 7.2 mg ethylenediaminetetraacetic acid (EDTA). All plasma samples were shipped on dry ice to the New Bolton Toxicology Center at the University of Pennsylvania (Philadelphia, PA), where they were analyzed for plasma baclofen concentration using liquid-chromatography/ mass-spectrometry (LC-MS) methodology as previously described (Miksa and Poppenga, 2003).

4.5. Statistical Analysis

Analysis of Variance (ANOVA) was used to determine the effects of strain, sex and convulsant dose on area under the curve (AUC) scores, which is a value obtained for each individual by summing all post-chemiconvulsant HIC scores. All AUC scores were corrected for baseline HIC scores. ANOVAs were also used to determine effects of strain on baclofen concentrations in plasma. Post-hoc Tukey tests were used where appropriate and a two-tailed significance level of $\alpha=0.05$ was used in all cases.

Acknowledgments

National Institute of Health Grants AA011114, DA05228, AA10760, AA07468 and AA13685, and a grant from the Department of Veterans Affairs supported this work.

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Abbreviations

QTL, quantitative trait loci; HIC, handling-induced convulsion.

Figure 1. SB242084, a selective 5-HT_{2C} receptor antagonist, produced less severe handling-induced convulsions (HICs) in chromosome 4 congenic (○) vs. D2 background strain (●) mice

The mice were scored for baseline (pre-drug) handling-induced convulsions immediately before i.p. administration of SB242084 (A) 1 mg/kg or (B) 10 mg/kg (the arrow marks injection of SB242084 at time 0) and hourly for 10 hr after SB242084 administration. Data represent the mean scores $\pm$ SEM scores for baseline and post-SB242084 HICs ($n=17$ congenic and $n=18$ D2 strain mice). In some cases the error bars are smaller than the symbol. *Inset:* SB242084-enhanced HIC severity was calculated as the area under curve (AUC) corrected for individual differences in baseline HIC severity, and was significantly lower in congenic vs. D2 background strain mice after 1 or 10 mg/kg SB242084 (* $p < 0.001$).

Figure 2. Baclofen, a selective GABA_B receptor agonist, produced less severe handling-induced convulsions (HICs) in chromosome 4 congenic (○) vs. D2 background strain (●) mice
 The mice were scored for baseline (pre-drug) handling-induced convulsions immediately before i.p. injection of baclofen (A) 5 mg/kg or (B) 10 mg/kg (the arrow marks injection of baclofen at time 0), and scored every 30 minutes for 6 hours after baclofen administration. Data represent the strain mean $\pm$ SEM for baseline and post-baclofen HICs ($n=26$ congenic and $n=31$ D2 mice). In some cases the error bars are smaller than the symbol. *Inset:* Baclofen-induced HIC severity was calculated as the area under curve (AUC) corrected for individual differences in baseline HIC severity, and was significantly lower in congenic vs. D2

background strain mice after 10 mg/kg baclofen ($*p < 0.05$), and showed a trend in the same direction after 5 mg/kg baclofen ($p < 0.1$).

Figure 3. Pentylentetrazole (PTZ), which blocks GABA_A receptor channels, produced comparably severe handling-induced convulsions (HICs) in chromosome 4 congenic (○) and D2 background (●) strain mice

Mice were scored for baseline (pre-drug) handling-induced convulsions immediately before i.p. injection of PTZ (A) 5 mg/kg, 15 mg/kg (not shown) (B) or 30 mg/kg and then 1, 3, 5, 8, 12, 15, 20 and 60 min after PTZ administration. Arrow marks injection of PTZ at time 0. Data represent the strain mean $\pm$ SEM for baseline and post-PTZ HICs ($n=54$ congenic and $n=48$ D2 mice). In some cases the error bars are smaller than the symbol. PTZ-enhanced HIC severity (AUC) was calculated as the area under curve corrected for individual differences in baseline

HIC severity, and was not significantly different between congenic and D2 background mice after administration of PTZ (5, 15 or 30 mg/kg).

Table 1**Handling-induced convulsion scoring scale**

Symptom	Score
Severe tonic-clonic convulsion, with quick onset and long duration: spontaneous, or elicited by mild stimulus (e.g., lifting cage top).	7
Severe tonic-clonic severe, tonic-clonic convulsion when lifted by the tail: quick onset and long duration, often continuing for several seconds after the mouse is released.	6
Tonic-clonic convulsions when lifted by the tail: onset often delayed by up to 1–2 seconds	5
Tonic convulsion when lifted by the tail.	4
Tonic-clonic convulsion after gentle 180° spin, with onset often delayed by up to 1–2 seconds.	3
No convulsion when lifted by the tail, but tonic convulsion elicited by gentle 180° spin.	2
Facial grimace only after a gentle 180° spin	1
No convulsion after tail lift and spin.	0

Table 2

Baclofen plasma concentrations for CS and D2 background strain mice.

Strain	60 min post-baclofen	120 min post-baclofen	240 min post-baclofen
D2	24850 ± 9926 (7)	17130 ± 11944 (10)	589 ± 464 (3)
CS	8115 ± 5695 (3)	369 ± 136 (9)	109 ± 25 (3)

All data is listed as mean ± SEM. Numbers in parentheses represent number of animals used for each plasma measurement.