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Inactivation of the maternal fragile X gene results in sensitization of GABA_B receptor function in the offspring

Bojana Zupan¹ and Miklos Toth^{1,2}

¹ Weill Cornell Graduate School of Medical Sciences, Cornell University, Neuroscience Program, New York, NY

² Department of Pharmacology, Weill Cornell Medical College, Cornell University, New York, NY

Abstract

Fragile X syndrome is an X linked disorder caused by the inactivation of the *FMR-1* gene with symptoms ranging from impaired cognitive functions to seizures, anxiety, sensory abnormalities and hyperactivity. Although Fragile X syndrome is considered a typical Mendelian disorder, we have recently reported that the environment, specifically the *fmr-1*^{+/-} or *fmr-1*^{-/-} (H or KO) maternal environment, elicits on its own a partial fragile X-like phenotype and can contribute to the overall phenotype of *fmr-1*^{-/0} (KO) male offspring. Indeed, genetically *fmr-1*^{+/0} (WT) males born to H females (H_{maternal}>WT_{offspring}), similarly to KO male offspring born to H and KO mothers (H>KO and KO>KO), exhibit locomotor hyperactivity. These mice also showed reduced D2 autoreceptor function, indicating a possible diminished feedback inhibition of dopamine (DA) release in the nigrostriatal and mesolimbic systems. The GABAergic system also regulates DA release, in part via presynaptic GABA_B receptors (Rs) located on midbrain dopaminergic neurons. Here we show that the locomotor inhibitory effect of the GABA_BR agonist baclofen is enhanced in all progeny of mutant mothers (H>WT, H>KO and KO>KO) as compared to WT>WT mice, irrespective of their own genotype. However, increased sensitivity to baclofen was selective and limited to the locomotor response because the muscle relaxant and sedative effects of the drug were not altered by the maternal environment. These data show that GABA_BR sensitization, traditionally induced pharmacologically, can also be elicited by the *fmr-1* deficient maternal environment.

Fragile X syndrome (FXS) is an X linked neuropsychiatric disorder caused, in most cases, by a triplet expansion in the *FMR-1* gene (Consortium, 1994; Hagerman, 1996). In addition to mental retardation, fragile X individuals display seizures, attention deficit, hyperactivity, anxiety and symptoms associated with autism. Most fragile X individuals inherit a premutated allele (between 55 and 200 repeats) from their mother that undergoes triplet expansion resulting in a full mutation (>200 repeats) and the inactivation of the *FMR-1* gene. In some cases, full mutation is already present in heterozygote carrier mothers who are less affected than males presumably because the mosaic pattern of *FMR-1* inactivation. Premutation in females does not inactivate the *FMR-1* gene but nevertheless seems to increase the frequency of mood and anxiety disorders (Roberts et al., 2008).

The mouse model of FXS (Consortium, 1994) reproduces many human symptoms of the disorder including locomotor hyperactivity, social anxiety/avoidance and sensory hyper-reactivity (D'Hooge et al., 1997; Peier et al., 2000; Chen and Toth, 2001; Spencer et al., 2005; Yun et al., 2006). Although FXS is considered to be a purely Mendelian disorder and it

is assumed that all behavioral abnormalities seen in the mouse model are genetic in nature, we have recently reported that the maternal *fmr-1*^{-/+} (or *fmr-1*^{-/-}) genotype could contribute to the hyperactivity of *fmr-1*⁻⁰ male H>KO (or KO>KO) offspring and it is the sole cause of the increased locomotor activity of genetically normal *fmr-1*⁺⁰ male (H>WT) offspring. Other behaviors such as increased seizure sensitivity and changes in startle reactivity and prepulse inhibition followed Mendelian inheritance (H>WT and WT>WT are similar to each other and different from H>KO and KO>KO mice); thus, the maternal influence affects only specific behaviors in the mouse model of FXS. A maternal genotype effect has also been implicated recently in a human study that analyzed maternal–child interactions in dyads affected by FXS (Wheeler et al., 2007) where maternal behavior can profoundly influence the child's developmental pattern, which, in turn, can influence the affective behavior of the mother.

The striatum is important in the selection, initiation, and execution of voluntary motor responses (Tucker and Williamson, 1984) and lesions to this area in humans can produce hyperactivity (Solanto, 2002). Motor activity is also regulated by mesolimbic DA neurons originating in the ventral tegmental area (VTA) and projecting to the ventral striatum (Koob and Swerdlow, 1988; Szczypka et al., 2001). It has been hypothesized that low tonic DA activity in the striatum via D2 presynaptic autoreceptors leads to a high phasic DA activity and to hyperactivity (Grace, 2001). Consistent with this idea, we found reduced D2 autoreceptor activity in hyperactive offspring born to H and KO mothers (Zupan and Toth, 2008). This indicated that, similarly to locomotor activity, D2 receptor function is regulated in part by the non-genetic factor of H or KO maternal environment.

Activity of mesolimbic DA neurons is also modulated by GABA_BRs at both somatodendritic and presynaptic locations. Somatodendritic GABA_BRs normally inhibit DA release through a K⁺-dependent hyperpolarization mediated by G protein-gated inwardly rectifying potassium (GIRK) channels that decrease the firing rate and the burst firing activity of DA neurons (Engberg et al., 1993; Yoshida et al., 1994; Madden and Johnson, 1998; Labouebe et al., 2007). Presynaptic GABA_BRs in the projection areas of DA neurons normally curtail DA release through the inhibition of Ca⁺⁺ influx into terminals (Zoltay and Cooper, 1994; Smolders et al., 1995). Baclofen, a GABA_BR agonist, has been shown to reduce DA neuron firing pattern and rate in rat midbrain slices (Saitoh et al., 2004; Chen et al., 2005). Reduced firing rates were also observed in the VTA following local microiontophoretic administration of the agonist (Klitenick et al., 1992; Erhardt et al., 2002). Behaviorally, baclofen reduces locomotor activity levels (Cott et al., 1976) and genetic inactivation of the GABA_B1 subunit results in hyperlocomotion (Schuler et al., 2001). In all, the GABA_BR, like the DA D2 autoreceptor, plays a role in regulating DA neuron activity and locomotion. In this study, we aimed to determine if the function of GABA_BRs in modulating locomotor activity is altered in WT or *fmr-1* KO mice reared by mothers with *fmr-1*^{-/+} (H>WT or H>KO) or *fmr-1*^{-/-} (KO>KO) genotypes. We show here an enhanced GABA_BR agonist-dependent behavioral response in male H>WT, H>KO and KO>KO offspring and furthermore, establish that this response is dependent on the maternal *fmr-1* genotype.

Materials and Methods

Animals

fmr1-KO mice (Consortium, 1994) on the FVB (FVB/129P(B6)-Fmr1^{tm1Cgr/J}) background as well as FVB WT mice (FVB/NJ) were purchased from The Jackson Laboratory, Bar Harbor, ME. KO mice were backcrossed at least 5 times at the Jackson Laboratory and once in our animal facility to the FVB background. Experimental subjects were generated through breeding of *fmr1*-KO females and males (KO_{maternal genotype}>KO_{offspring genotype}) or WT females and males (WT>WT) as well as crossing female homozygotes with male WT mice to generate

heterozygote females that were mated with WT males to produce WT and KO littermate offspring (H>WT and H>KO respectively, Fig. 3A)(Zupan and Toth, 2008).

Experiments were conducted using male WT>WT, H>WT, H>KO and KO>KO offspring aged 8–12 weeks. All animals were group housed up to 5 per cage (in most cases 3–4 animals) with 12 hr light/dark cycle with lights on at 6 am. Food and water were available *ad libitum*. Animal experiments were carried out in accordance with the Weill Cornell Medical College IACUC guidelines.

Drugs

(±)-Baclofen was purchased from A.G. Scientific, Inc. (San Diego, CA); CGP46381 was purchased from Tocris Bioscience (Ellisville, MO). All drugs were dissolved in sterile saline and administered intraperitoneally (i.p.) at 0.1ml/10g animal weight.

Behavioral Procedures

Locomotor activity—Locomotor behavior was recorded using infrared beam-equipped activity chambers (27×27cm, Med Associates Inc., St. Albans, VT). WT>WT and KO>KO animals were allowed to habituate to the behavioral testing room for at least 45 minutes prior to the onset of any experiments. Animals were injected with baclofen or vehicle (saline) 30 minutes prior to placement into the activity chamber. Once placed inside the chambers, subjects' locomotor activity was recorded for 30 minutes except in 2h recording sessions, during which activity was measured in 10 min intervals. Cohorts of WT>WT, H>WT, H>KO and KO>KO animals were used to specify the effect of the maternal environment on locomotor activity. Additional groups of WT>WT, H>WT, H>KO and KO>KO animals were pretreated with CGP46381 or saline and then injected again 15 minutes later with baclofen or saline followed by a 30 minute locomotor test 30 min later. Drugs were administered in a crossover design with the sequence of drug administrations randomly assigned and varied between mouse cages. Tests were performed in 7-day intervals for no more than 3 weeks. Habituation to the locomotor chamber does not occur during this type of repeated testing (Zupan and Toth, 2008).

Rotorod—On the first day, subjects were given 4 training trials to learn to walk on a rotating beam (Roto-Rod, Series 8, IITC Life Science, Woodland Hills, CA) at increasing rotation speed up from 2 to 10 rpm and maximal time of 300 seconds. Animals that failed to stay on the rotarod for at least 250 seconds by the 4th trial were excluded from the test. On the second and third days, subjects were injected with vehicle, or 3, 6, or 10 mg/kg baclofen i.p. using a crossover design, 30 minutes prior to being placed onto the accelerating rotating beam (2 to 10 rpm in 300 sec). The order of dosage received was randomly assigned within each group but once the animals received the 10 mg/kg dose they were not reused.

Results

Male *fmr1* KO mice exhibit increased baclofen sensitivity

Administration of baclofen, a selective GABA_BR agonist, has been shown to reduce locomotor activity in mice (Cott et al., 1976). To probe the functionality of the GABA_BR in FXS mice, 3, 6 and 10 mg/kg baclofen (i.p.) was administered 30 minutes prior to the onset of locomotor activity assessment. As Fig. 1A shows, baclofen induced a dose-dependent inhibition of locomotor activity in both *fmr-1*^{+/-0} WT and *fmr-1*^{-/-0} KO animals. However, while 6 and 10 mg/kg were effective in reducing activity in both WT and KO mice equally, only the KO group exhibited a significant locomotor reduction at 3 mg/kg baclofen dose (2-way treatment × genotype ANOVA; treatment: $F_{4,148}=57.68$, $p<0.0000001$; treatment × genotype: $F_{4,148}=7.11$, $p=0.00003$). The injection procedure alone, presumably due to stress, resulted in a reduction

in locomotor activity in the vehicle KO group compared to the no injection KO group (Fig. 1A, $p < 0.01$). However, the loss of hyperactivity in the KO group following injection was not consistently observed (see Fig. 1B and unpublished observations) and may be due to variability to stress in the KO group.

In order to better characterize the baclofen dose that differentiated between the KO and WT mice, we analyzed the locomotor-reducing effect of 3 mg/kg drug over a 120-minute observation period (Fig. 1B). Consistent with data displayed in Fig. 1A, 2-way repeated measures ANOVA showed a significant treatment $\times$ time interaction in the KO ($F_{11, 154} = 2.61$, $p = 0.0045$) but not in the WT group. In the KO group, the greatest reduction in locomotor activity was observed during the first 30 minutes of the trial, a time-course that is consistent with the relatively short half-life of baclofen (Shellenberger et al., 1999; Schuler et al., 2001; Wiersma et al., 2003).

In view of the fact that baclofen is commonly prescribed for its muscle relaxant effect we were interested in assessing whether the increased baclofen sensitivity of KO mice is specific for the locomotor activity or it is more generalized and includes the muscle relaxant/sedative properties of the drug. We tested the effects of 3, 6, and 10 mg/kg baclofen (i.p.) on performance on the rotorod, a well-established test for measuring muscle strength and coordination in rodents (Fig. 2). A 2-way genotype $\times$ treatment ANOVA showed a treatment effect ($F_{3, 73} = 109.35$, $p < 0.00001$) but not genotype effect on rotorod performance. Although the 3 and 6 mg/kg doses resulted in no significant impairment in either KO or WT mice as measured by the time the animals remained on the rotating beam, 10 mg/kg baclofen produced significant and similar impairments in KO and WT mice (Fig. 2). These data show that the observed reduction of locomotor activity at 3 and 6 mg/kg baclofen is unlikely to be caused by sedation and muscle relaxation. Furthermore, these data taken together with the locomotor data described in Fig. 1A indicate that the increased sensitivity of *fmr1* KO mice to the locomotor-reducing effects of baclofen is specific and is not based on systemic GABA_BR supersensitivity.

The increased baclofen sensitivity in the offspring is maternal *fmr-1* genotype dependent

Previous data from our laboratory indicate that the hyperactivity of the *fmr-1* KO mice and the D2 receptor mediated suppression of this phenotype by an agonist is dependent, at least partly, on the *fmr-1* genotype of the mother (Zupan and Toth, 2008). To assess a maternal genotype effect we took advantage of the possibility that with genetically manipulated mice WT offspring can be raised in a wild-type or mutant H maternal environment (WT>WT or H>WT offspring, Fig. 3A and see further details on breeding in Materials and Methods). Previous data showed that H>WT mice have increased locomotor activity as compared to WT>WT mice (Zupan and Toth, 2008). Any behavioral difference between these groups is due to maternal effect and is not the result of genetic factors because H>WT animals were selected for the WT *fmr-1* allele and thus had neither the genetically modified allele nor the associated 129P2/OlaHsd flanking sequences. Also, the at least 6 backcrosses eliminated the majority of randomly dispersed remnants of the original 129 genome guaranteeing that no locus is uniformly and specifically present in the H>WT mice. The breeding scheme shown in Fig. 3A also results in offspring with both offspring and maternal genotype effects (H>KO and KO>KO) and these mice had even higher levels of locomotor activity than H>WT animals (Zupan and Toth, 2008). Given the increased sensitivity of KO male offspring of KO mothers (KO>KO) to baclofen (Fig. 1), which have both offspring and maternal genotype effects, we asked if mice with only the maternal genotype effect (H>WT) would exhibit the increased drug sensitivity as well. We administered 3 mg/kg baclofen i.p. to WT>WT, H>WT, H>KO and KO>KO mice and measured locomotor activity for 30 min, starting 30 min after the administration of the drug. As in previous experiment (Fig. 1A), baclofen suppressed locomotor activity in KO>KO but not in WT>WT mice (Fig. 3B). Importantly, suppression of

the locomotor activity also occurred in H>WT and H>KO mice (2-way group $\times$ treatment ANOVA; treatment: $F_{1,91}=29.4172$, $p<0.00001$ followed by LSD posthoc test) indicating a maternal genotype effect in the locomotor suppressant effect of low dose baclofen (Fig. 3B). The drug induced suppression in H>KO animals was not more pronounced than in H>WT animals suggesting that the offspring KO genotype does not significantly add to the maternal effect under the experimental conditions used here.

The GABA_BR antagonist CGP46381 reveals a role for both maternal and offspring *fmr-1* genotypes in the increased behavioral response to baclofen

The increased sensitivity of H>WT as well as H>KO and KO>KO animals to baclofen raised the question of whether GABA_BRs are tonically activated in these animals to compensate the hyperactivity of these animals. We therefore used the competitive antagonist CGP46381 to block GABA_BRs and assessed if the locomotor activity of H>WT, H>KO and KO>KO animals is increased above the saline baseline. Surprisingly, CGP46381 at 20 and 40 mg/kg had no apparent effect on locomotor activity of these groups of mice (Fig. 4B–D). However, there was a small but significant increase in locomotor activity in the WT>WT group at the 20 mg/kg CGP46381 dose (Fig. 4A; One way ANOVA, CGP treatment: $F_{2,24}=4.80$, $p=0.017$; LSD posthoc saline vs. 20 mg/kg $p=0.007$) indicating that the tonic activity of GABA_BRs may also be under maternal genotype influence.

Next we tested if CGP46381 can antagonize the effect of baclofen given at 6 mg/kg, a dose that elicited a behavioral response in all groups (Fig. 4A–D). Both the 20 and 40 mg/kg CGP46381 doses completely neutralized the baclofen effect in WT>WT mice (One way ANOVA, treatment: $F_{5,50}=11.242$, $p<0.00001$) (Fig. 4A). In contrast, the low 20 mg/kg dose of CGP46381 did not prevent the locomotor suppressant effect of baclofen in H>WT mice and only the larger 40 mg/kg CGP46381 dose was effective in this group (One way ANOVA, treatment: $F_{5,54}=4.641$, $p=0.00135$) (Fig. 1B). This data is indicative of a maternal genotype-dependent increase in baclofen sensitivity in the H>WT animals. In the H>KO (as well as KO>KO) animals, even the higher 40 mg/kg CGP46381 dose was without effect in reverting baclofen-induced suppressed locomotor activity to baseline levels (Fig. 4C and D). Taken together, these data reveal an offspring genotype effect, on top of the *fmr-1* H maternal effect seen in the H>WT mice, on sensitivity to baclofen.

Discussion

Our previous finding showing that WT male mice reared by *fmr-1* H females (H>WT) exhibit locomotor hyperactivity and reduced DA D2 receptor sensitivity to quinpirole indicated that the maternal *Fmrp* deficit elicits, on its own, behavioral and neurochemical changes in brain circuits, principally in the nigrostriatal and mesolimbic DA systems of the offspring. Since these maternally determined phenotypes were measured in adult offspring they are permanent and presumably initiated during the development of the DA system. The DA system is under multiple neurotransmitter control and here we asked if neurotransmitter receptors other than DA are also altered permanently in mice reared by H or KO mothers. GABA_BRs are expressed in both DA and GABAergic neurons in the striatum and VTA and the net effect of receptor activation by the agonist baclofen is a reduction in DA neuronal firing, DA release and locomotor activity (Cott et al., 1976; Cruz et al., 2004; Saitoh et al., 2004; Chen et al., 2005). Interestingly, 3mg/kg i.p. baclofen, a behaviorally subthreshold dose in WT mice, elicited a significant, approximately 2-fold, reduction in locomotor activity in H>WT mice (as well as in H>KO and KO>KO mice) (Fig. 3B). A higher 6 mg/kg dose was required to elicit a comparable suppression of activity in WT>WT mice (Figs. 1A and 4A). The increased baclofen sensitivity is limited to the locomotor behavior because the motor relaxant/sedative effect of the drug was similar in KO>KO and WT>WT mice. These data indicate that the maternal effect

on GABA_BRs or its signaling is not generalized but rather selective for the circuit controlling locomotor activity. It is of note that alterations in the GABA system have previously been shown but it is not known whether they are maternally or genetically determined. Both the mouse and the *Drosophila* model of FXS indicate a GABA/GABA_A receptor deficit (D'Hulst and Kooy, 2007; Chang et al., 2008).

Our data indicate a sensitization-like phenomenon of the GABA_BR in mice elicited by the H or KO maternal environment. Traditionally, behavioral sensitization is the result of repeated drug administration. Sensitization of GABA_BRs has been shown following repeated amphetamine and gamma-hydroxybutyrate (GHB) administration (Zhang et al., 2000; Labouebe et al., 2007). In both of these cases, sensitization was the result of increased coupling. In particular, repeated GHB administration increased the efficiency of GABA_BR-GIRK channel coupling through the downregulation of regulator of G protein signaling 2 (Labouebe et al., 2007). Other possible mechanisms of sensitization in H>WT as well as in H>KO and KO>KO mice may include an increase in the affinity and density of GABA_BRs. Although increased agonist concentration, due to a change in the pharmacodynamics of baclofen, may also be a factor, the similar behavioral response of KO>KO and WT>WT mice in rotarod argues against this possibility. The elucidation of the actual mechanism underlying GABA_BR sensitization requires further studies. Considering that baclofen is an approved drug for spasticity, the increased GABA_BR functionality, if it occurs in FXS, is encouraging because it may allow using suboptimal baclofen doses to control hyperactivity. Since increased neuronal activity likely underlies some symptoms of FXS, activation of the inhibitory GABA_BR by baclofen, similar to the inhibition of the mGluR5 receptor (Bear et al., 2004), may indeed be therapeutically beneficial.

Since activation of the GABA_BR is inhibitory on locomotor activity, it was conceivable that the enhanced drug response of H>WT (as well as H>KO and KO>KO) mice reflects a compensatory upregulation of receptor function. Therefore, we expected a further increase in locomotor activity in H>WT, H>KO and KO>KO mice following the pharmacological blockade of the GABA_BR. This assumption was also consistent with the increased activity of WT>WT mice following 20 mg/kg CGP46381 (Fig. 3A) that reproduced published data (Colombo et al., 2001) and with the hyperactivity of GABA_BR 1 subunit KO mice (Schuler et al., 2001). However, upregulation of GABA_BR function was not compensatory because CGP46381 resulted in no detectable effect in locomotor activity in H>WT, H>KO and KO>KO mice. This suggests that either GABA_BRs are not tonically active or the increased receptor functionality is more difficult to block by the antagonist.

We have previously shown that hyperactivity in *fmr-1* KO male mice reared by *fmr-1* H females (H>KO) was even higher than the activity of H>WT mice suggesting that the offspring genotype, on top of the maternal effect, can also lead or contribute to hyperactivity (Zupan and Toth, 2008). A similar graded response was also observed in GABA_BR sensitivity. While the locomotor suppressant effect of 6 mg/kg baclofen was completely reversed by 20 mg/kg CGP46381 in WT>WT mice, only the 40 mg/kg dose was effective in H>WT mice and even this higher dose was insufficient to neutralize the baclofen effect in H>KO and KO>KO mice. The decreased effectiveness of CGP46381 to block the locomotor-reducing effect of baclofen in mice raised by H or KO mothers is consistent with their increased functional GABA_BR sensitivity and it provides further support for the maternal-genotype mediated modulation of GABA_BR function. Although it is tempting to conclude that the additional increase in locomotor activity and functional GABA_BR sensitivity in H>KO and KO>KO mice is due to an offspring genotype effect (on top of the maternal effect), the maternal and offspring genotype effects can interact in unpredictable ways. Our breeding strategy does not allow the study of the offspring genotype effect in the absence of the maternal H or KO environment; thus, it is possible that the genetic inactivation of the *fmr-1* gene in the offspring enhances the maternal

effect without having its own effect in that offspring. Alternatively, the offspring genotype can have an effect and may even be dominant by suppressing the maternal effect. Further studies will need to be carried out to elucidate the nature of this particular mother-offspring interaction.

The modulation of GABA_BR sensitivity in the H>WT offspring by the maternal effect without the actual transmission of a genetic information indicates that this effect is not mediated by a genetic but rather by an epigenetic mechanism. It has been shown that maternal effects, related to variability in postnatal care, alter the methylation status and chromatin conformation of specific neuronally expressed genes in the offspring and that these epigenetic changes are likely responsible for some of the life-long behavioral alterations seen in the offspring (Kaffman and Meaney, 2007). Since expression of Fmrp is widespread and includes both neuronal and non-neuronal cells, it is possible that the Fmrp deficiency in H mothers alters their physiology and behavior during the gestational and postnatal periods and that these effects impact the epigenome of the offspring leading to the life-long neurochemical abnormalities presented here.

Human studies are consistent with the importance of the mutation status of the mother in FXS. Mothers with FXS have a greater predisposition to anxiety and depression (Mazzocco, 2000; Wheeler et al., 2007) and this could affect the postnatal environment of both healthy and FXS children. Although not demonstrated in FXS directly, studies have shown a negative impact of attention deficit hyperactivity disorder (ADHD) on parenting behaviors (Murray and Johnston, 2006) and improvements in children's behavior when mothers were treated for ADHD (Daly and Fritsch, 1995). Symptoms of ADHD can often be identified in fragile X individuals (Hagerman et al., 1988). It would be important to specify what manifestations or aspects of FXS and neuropsychiatric disorders in general can be transmitted non-genetically because all progeny whether genetically affected or not could be susceptible to these effects.

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Nonstandard abbreviations

FXS

fragile X syndrome

ADHD

attention deficit hyperactivity disorder

GHB

gamma-hydroxybutyrate

GIRK channel

G protein-gated inwardly rectifying potassium channel

DA

dopamine

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A **B** **Figure 1.**

Enhanced sensitivity to baclofen in *fmr-1* KO compared to WT mice. (A) Effect of 3, 6, and 10 mg/kg baclofen on locomotor activity measured for 30 min. Both KO and WT male mice show a dose-dependent reduction in locomotor activity following the i.p. administration of baclofen. However, only the KO mice respond to the lowest 3 mg/kg dose (2-way genotype $\times$ treatment ANOVA followed by Fisher's LSD Post Hoc analysis; NS= not significant, ** $p < 0.01$, *** $p < 0.001$; No injection, $N = 29-32$; vehicle and 3 and 6 mg/kg baclofen, $N = 14-18$; 10 mg/kg baclofen $N = 7-8$ per group). (B) Two-hour time course of the baclofen-induced attenuation of locomotor activity at 3 mg/kg. Baclofen causes no significant behavioral change in WT subjects compared to vehicle controls ($n = 7$ per group) while this dose in KO mice

significantly attenuates locomotor activity as compared to vehicle control (2 way repeated measures ANOVA; Fisher's LSD post hoc, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; $N = 8$ per group).

Figure 2.

The locomotor-reducing effect of 3 and 6 mg/kg baclofen is not mediated by the muscle relaxant or sedative properties of the drug. WT and KO mice respond similarly to baclofen on the rotorod. Post hoc analysis reveals no significant effects until the administration of 10 mg/kg baclofen (2-way genotype $\times$ treatment ANOVA, Fisher's LSD post hoc, *** $p < 0.001$; $N = 10-11$ per group).

A **B** **Figure 3.**

(A) Breeding strategy to generate WT>WT, H>WT, H>KO and KO>KO mice by litter- and non-littermate mating. Breeding involved WT (black), H (grey) and KO (white) mothers. (B) Baclofen at 3 mg/kg i.p. suppresses activity in all groups raised by H or KO mothers (2-way genotype $\times$ treatment ANOVA followed by Fisher's LSD post hoc, NS=not significant, * p <0.05, ** p <0.01, *** p <0.001; N =8–17 per group).

Figure 4.

Reversal of baclofen-induced locomotor suppression by the competitive GABA_BR antagonist CGP46381. (A) Both 20 and 40 mg/kg CGP46381 pretreatments completely reverse the locomotor-reducing effect of 6 mg/kg baclofen in the WT>WT group (One way ANOVA Fisher's LSD post hoc, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; $N = 8-10$ per treatment). In addition, CGP alone increased locomotor activity. (B) Pretreatment with 40, but not 20 mg/kg CGP46381 blocks the locomotor-reducing effect of baclofen in the H>WT group (One way ANOVA, Fisher's LSD post hoc, $N = 10$ per treatment). CGP alone had no effect on activity. (C and D) Neither 20 nor 40 mg/kg CGP46381 pretreatment is effective in fully reversing baclofen-induced reduction of locomotor activity in both the H>KO and KO>KO groups (One way ANOVA Fisher's LSD post hoc, $N = 7-11$ per treatment).