Abnormal Striatal GABA Transmission in the Mouse Model for the Fragile X Syndrome

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Background: Structural and functional neuroimaging studies suggest abnormal activity in the striatum of patients with the fragile X syndrome (FXS), the most common form of inherited mental retardation.

Methods: Neurophysiological and immunofluorescence experiments in striatal brain slices. We studied the synaptic transmission in a mouse model for FXS, as well as the subcellular localization of fragile X mental retardation protein (FMRP) and brain cytoplasmic (BC1) RNA in striatal axons.

Results: Our results show that absence of FMRP is associated with apparently normal striatal glutamate-mediated transmission, but abnormal γ-aminobutyric acid (GABA) transmission. This effect is likely secondary to increased transmitter release from GABAergic nerve terminals. We detected the presence of FMRP in axons of striatal neurons and observed a selective increase in the frequency of spontaneous and miniature inhibitory postsynaptic currents (sIPSCs, mIPSCs) in fmr1-knockout mice. We also observed reduced paired-pulse ratio of evoked IPSCs, a finding that is consistent with the idea that transmitter release probability from striatal GABAergic nerve terminals is higher than normal in these mutants. Finally, we have identified the small noncoding BC1 RNA as a critical coplayer of FMRP in the regulation of striatal synaptic transmission.

Conclusions: Understanding the physiologic action of FMRP and the synaptic defects associated with GABA transmission might be useful to design appropriate pharmacologic interventions for FXS.

Key Words: BC1 RNA, electrophysiology, EPSC, fragile X mental retardation protein, mRNA, synaptic plasticity

Transcriptional silencing of the fmr1 gene on the X chromosome causes fragile X syndrome (FXS), the most common form of inherited mental retardation (1). fmr1 gene encodes the fragile X mental retardation protein (FMRP), an RNA-binding protein involved in mRNA metabolism in neurons (2,3). Patients with FXS show a spine dysmorphogenesis (2). Dendritic spines are a preferential working location of FMRP in neurons (2,4), but evidence is now accumulating that axons also contain this protein (5–7). These findings raise the possibility that FMRP controls synaptic transmission through pre- and postsynaptic actions.

FMRP mediates its complex activity on synaptic transmission by interacting with specific RNA and protein partners. At least 18 putative FMRP-interacting proteins (2,4) and a plethora of FMRP-binding mRNAs have been identified (8–13). In addition, FMRP can also control synaptic protein translation indirectly by associating with either the small dendritic noncoding brain cytoplasmic (BC1) RNA (12,14), or possibly with microRNAs (15), which act as an "adaptor molecules" between FMRP and its target mRNAs.

Abnormal plasticity of excitatory transmission represents a prominent synaptic alteration described in fmr1 knockout (fmr1-KO) mice (4,16–19), a reliable model of FXS showing, as in the human disorder, macroorchidism, learning deficits, hyperactivity, and dendritic spine abnormalities (2). In this model, the possible parallel alteration of inhibitory GABAergic transmission has also been postulated in morphological, neurochemical, and molecular studies (20–26), but it has never been addressed physiologically.

Several brain imaging studies in humans have established an association between reduced expression of FMRP and morphological and functional abnormalities in the basal ganglia of patients with FXS. Accordingly, FXS patients present frontostriatal deficits and larger striatum than normal, abnormalities that tightly correlate with the severity of the disease (27–33). This evidence suggests synaptic deficits in this brain area, but no study thus far has addressed striatal synaptic functioning in models of FXS.

In this study, we investigated both inhibitory and excitatory synaptic transmission in the striatum of fmr1-KO mice. In this model of FXS, we found enhanced γ-aminobutyric acid (GABA)-mediated synaptic inhibition, secondary to loss of presynaptic FMRP-mediated control of transmitter release. By comparing the neurophysiologic effects of fmr1 KO with those of BC1 RNA KO and those of double fmr1 and BC1 KO, we also identified BC1 RNA as a critical coplayer of FMRP in the regulation of striatal synaptic transmission.

Methods and Materials

Mice lacking fmr1 (fmr1-KO) (34), BC1 RNA (BC1-KO) (35), and both fmr1 and BC1 (fmr1-BC1-KO) were employed, along with respective age-matched wildtype (WT) counterparts (2–3 months old) for all the experiments. Animals were maintained in light:dark cycles of 12:12 hours and weaned after 21 days. The WT and KO mice used in these experiments were of different background strains: C57BL/6 for fmr1-KO mice, 129Sv/C57BL/6 for BC1-KO and fmr1-BC1-KO. Appropriate matching strains were used as controls. All efforts were made to minimize animal

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suffering and to reduce the number of mice used, in accordance with the European Communities Council Directive of 24 November, 1986 (86/609/EEC).

Generation of fmr1-BC1-KO Mice

fmr1-KO mice were obtained from Ben Oostra's laboratory (34), and BC1-KO mice were obtained from Jurgen Brosius's laboratory (35). One fmr1-KO female (X^-X^-) was crossed to BC1-KO male (7^-7^-) to generate heterozygous female mice for both the fmr1-BC1-KO loci and fmr1-KO male mice heterozygous for BC1-KO locus. These mice ($X^-X^+/7^-7^+$ x $X^-Y/7^-7^+$) were then crossed to generate the following genotypes: 1) mice lacking ($X^-Y/7^+7^+$) or ($X^-X^-/7^+7^+$) or ($X^-Y/7^-7^+$) or ($X^-X^-/7^-7^+$); 2) mice lacking BC1 RNA ($X^+Y/7^-7^-$) or ($X^+X^+/7^-7^-$); 3) normal male mice ($X^+Y/7^+7^+$); 4) heterozygous mice for BC1 ($X^+Y/7^+7^-$); 5) heterozygous mice for fmr1 ($X^-X^+/7^+7^+$); 6) heterozygous female mice for both fmr1 and BC1 ($X^-X^+/7^-7^+$); 7) mice lacking both fmr1 and BC1 ($X^-Y/7^-7^-$) or ($X^-X^-/7^-7^-$). All mice were genotyped by polymerase chain reaction (PCR) using genomic DNA extracted from the tail. Screening for the presence or absence of the BC1 WT allele was performed using primer BMN176 (5′-GGACAAAGTGGCTCCTCCTG-) and BMN177 5′(-GGAGTATCCGGCATCCTCTG-) to detect the 436 fragment (nt 31094-31530 ID No. AF384675), and screening for the presence or absence of the BC1-KO allele was performed using primer BMN174 5′(-CCCAGCAATGGTGATGAGAC-) and BMN175 5′(-CCTTCTATCGCCTTCTTGACG) to detect the 462 fragment of the mutant allele (nt 28796 ID No. AF384675—NEO cassette). Following an initial cycle at 94°C for 5 min, 20 PCR cycles were performed comprising 1 min denaturation at 94°C, 1 min annealing at 60°C, and 1 min extension at 72°C. The products were electrophoresed on 1.5% agarose gel. The analysis of fmr1 locus was conducted as previously described (34).

Electrophysiology

Corticostriatal coronal slices (200 μm) were prepared from tissue blocks of the mouse brain with the use of a vibratome (36,37). A single slice was then transferred to a recording chamber and submerged in a continuously flowing Krebs solution (32°C, 2–3 mL/min) gassed with 95% O_2-5% CO_2. The composition of the control solution was (in mmol/mL): 126 NaCl, 2.5 KCl, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 2.4 $CaCl_2$, 11 Glucose, and 25 $NaHCO_3$.

The striatum could be readily identified under low-power magnification, whereas individual neurons were visualized in situ using a differential interference contrast (Nomarski) optical system. This employed an Olympus BX50WI (Tokyo, Japan) noninverted microscope with ×40 water immersion objective combined with an infrared filter, a monochrome charge-coupled device camera (COHU 4912), and a PC-compatible system for analysis of images and contrast enhancement (WinVision 2000, Delta Sistemi, Italy).

Whole-cell patch clamp recordings were made with borosilicate glass pipettes (1.8 mm outer diameter; 2–4 MΩ), in voltage-clamp mode, at the holding potential (HP) of −80 mV. Recording pipettes were advanced toward individual striatal cells in the slice under positive pressure, and on contact, tight GΩ seals were made by applying negative pressure. The membrane patch was

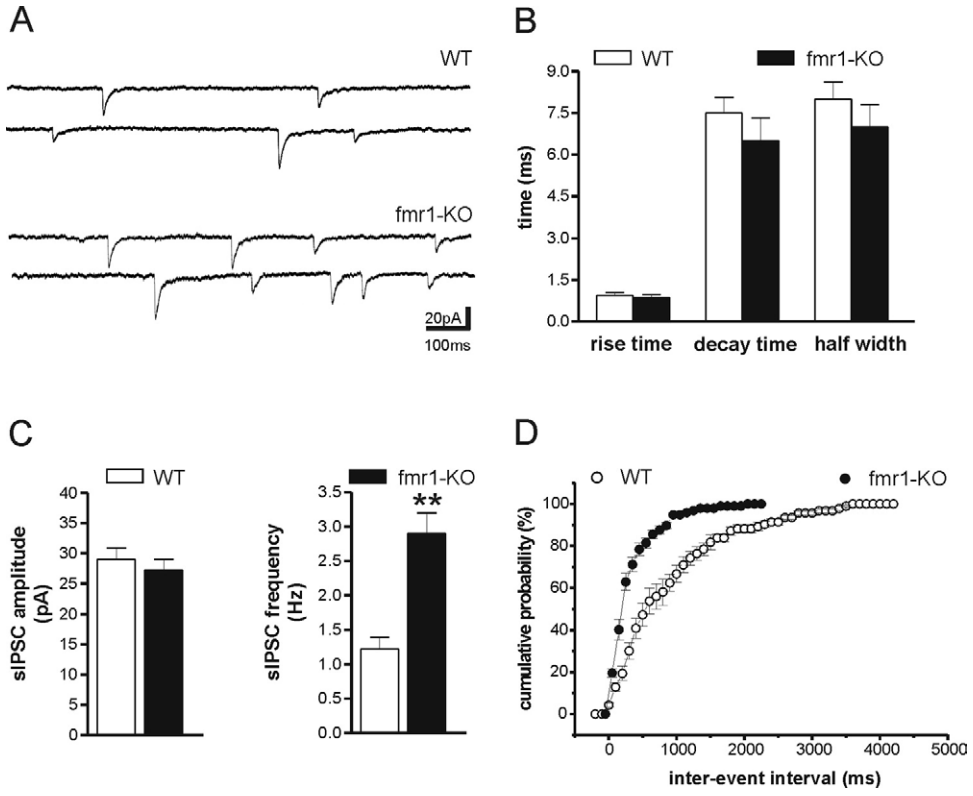

Figure 1. Properties of spontaneous inhibitory postsynaptic currents (sIPSCs) recorded from striatal neurons of wildtype (WT) and fmr1-knockout (KO) mice. **(A)** Examples of voltage-clamp recordings showing striatal sIPSCs (downward deflections) recorded from WT and fmr1-KO mice. **(B)** The graph shows that the kinetic properties (rise time, decay time, and half width) of sIPSCs are similar in the two genotypes. **(C)** The histograms show that the absence of fragile X mental retardation protein remarkably increases the frequency of sIPSCs without altering the mean amplitude of these synaptic events. **(D)** Cumulative distribution of sIPSC interevent interval recorded from WT and fmr1-KO mice.