FAMILIAL HEMIPLEGIC MIGRAINE TYPE-1 MUTATED $\text{Ca}_v2.1$ CALCIUM CHANNELS ALTER
INHIBITORY AND EXCITATORY SYNAPTIC TRANSMISSION IN THE LATERAL SUPERIOR OLIVE
OF MICE.

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Running title: Excitatory and inhibitory synaptic transmission and plasticity in mouse migraine model.

Abstract

$\text{Ca}_v2.1$ Ca^{2+} channels play a key role in triggering neurotransmitter release and mediating synaptic transmission. Familial hemiplegic migraine type-1 (FHM-1) is caused by missense mutations in the *CACNA1A* gene that encodes the α_{1A} pore-forming subunit of $\text{Ca}_v2.1$ Ca^{2+} channels. We used knock-in (KI) transgenic mice harbouring the pathogenic FHM-1 mutation R192Q to study integration of inhibitory and excitatory neurotransmission in the principle neurons of the lateral superior olive (LSO) in the auditory brainstem. We tested if the R192Q FHM-1 mutation differentially effects excitatory and inhibitory synaptic transmission, disturbing the normal balance between excitation and inhibition in the nucleus. Whole cell patch-clamp was used to measure neurotransmitter elicited excitatory (EPSCs) and inhibitory (IPSCs) postsynaptic currents in wild-type (WT) and R192Q KI mice. Our results show that the FHM-1 mutation in $\text{Ca}_v2.1$ channels has diverse effects. Evoked EPSC amplitudes are smaller whereas IPSC and miniature IPSC amplitudes are larger in R192Q KI compared to WT mice. In addition, in R192Q KI mice, the release probability is enhanced compared to WT, at both inhibitory (0.59 ± 0.02 vs 0.48 ± 0.01 , $P = 10^{-4}$, Student's *t*-test) and excitatory synapses (0.72 ± 0.02 vs

0.47 ± 0.01, $P = 4 \times 10^{-5}$, Student's *t*-test). Vesicle pool size is diminished in R192Q KI mice compared to WT mice (71 ± 5 vs 98 ± 6, $P = 0.02$, inhibitory; 28 ± 1 vs 65 ± 2, $P < 0.01$, excitatory, Student's *t*-test). R192Q KI mice present enhanced short-term plasticity. Repetitive stimulation of the afferent axons causes short-term depression (STD) of E/IPSCs that recover significantly faster in R192Q KI mice compared to WT. This supports the hypothesis of a gain-of-function of the Cav2.1 channels in R192Q KI mice, which might trigger alterations in the balance between excitatory and inhibitory inputs thereby altering cortical excitability responsible for FHM.

Key-words: R192Q KI mice; FHM-1; Cav2.1 calcium channels; Excitatory/Inhibitory postsynaptic currents (E/IPSCs); LSO; Short-term synaptic plasticity.

Author contributions

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5. Michel D. Ferrari ⁽³⁾, Arn M.J.M. van den Maagdenberg provide the genetically modified R192Q KI mice.

Introduction

Transmitter release at central synapses is triggered by Ca^{2+} influx through voltage-activated Ca^{2+} channels (VACCs). At many synapses, transmitter release is mediated by multiple Ca^{2+} channel subtypes during early development but increasingly relies on $\text{Ca}_v2.1$ (P/Q-type) Ca^{2+} channels with maturation, as was shown at the neuromuscular junction (NMJ) (Urbano et al 2002, Rosato Siri and Uchitel 1999, Rosato Siri et al 2002) and the calyx of Held (Iwasaki et al. 2000, Fedchyshyn and Wang 2005). It is now accepted that in adult animals, $\text{Ca}_v2.1$ Ca^{2+} channels most efficient to trigger vesicular fusion and transmitter release at a subset of synapses, while other synapses are mediated synergistically by multiple types of Ca^{2+} channels.

Several human channelopathies result from mutations in the α_{1A} pore-forming subunit of $\text{Ca}_v2.1$ Ca^{2+} channels. Familial hemiplegic migraine type 1 (FHM-1) is a mendelian subtype of migraine with aura that is caused by missense mutations in the *CACNA1A* gene that encodes this α_{1A} subunit (Pietrobon 2005, 2010, Ophoff et al. 1996, Vecchia and Pietrobon 2012). Typical migraine attacks in FHM patients are associated with transient hemiparesis and are a useful model to study pathogenic mechanisms of the common forms of migraine (Ferrari *et. al.* 2008). The generation of two knock-in FHM-1 mouse models carrying either the human pathogenic R192Q or S218L missense mutations allowed the first analysis of the functional consequences of FHM-1 mutations on $\text{Ca}_v2.1$ channels and synaptic transmission in neurons expressing the channels at the endogenous physiological level (van den Maagdenberg et al 2010, 2007, 2004, Kaja et al. 2010, Tottene et al 2009, González Inchauspe et al 2010, Adams et al 2010). Cortical spreading depression CSD is believed to be the underlying mechanism of the migraine aura (Lauritzen 1994, Haerter et al. 2005, Pietrobon 2005, Welch 1998, Flippen and Welch 1997) and the knock-in (KI) mice carrying the human FHM-1 R192Q or S218L mutation exhibit a lower threshold for induction of CSD and an increased velocity of CSD propagation (van den Maagdenberg et al. 2004, 2010, Tottene et al. 2009, Eikermann-Haerter et al. 2009). Studies in KI mice revealed multiple gain-of-function effects. The first indication that the gain-of-function of $\text{Ca}_v2.1$ channels produced by FHM-1 mutations could lead to enhanced evoked neurotransmitter release was obtained at the neuromuscular junction (NMJ), where evoked neurotransmission was unaltered at physiological Ca^{2+} ion concentrations but increased at 0.2 mM Ca^{2+} in KI mice (Kaja et al 2005, van den Maagdenberg et al 2004, 2010). In cerebellar granule cells and cortical pyramidal cells of R192Q and S218L KI mice, the $\text{Ca}_v2.1$ Ca^{2+} current density was larger than in WT neurons in a wide range of relatively mild depolarizations, reflecting activation of R192Q $\text{Ca}_v2.1$ channels at 8–9 mV more negative voltages than the corresponding WT channels. $\text{Ca}_v2.1$ current densities were similar in KI and WT neurons at higher voltages (that elicit maximal $\text{Ca}_v2.1$ channel open probability), indicating similar densities of functional $\text{Ca}_v2.1$ channels (van den Maagdenberg et al 2004, 2010, Tottene et al 2009). Using microcultures and brain slices from FHM-1 mice, Tottene et al. (2009) observed an increased probability of glutamate release at the excitatory synapse formed by the cortical layer 2/3 pyramidal cells (PC) and fast-spiking (FS) interneurons, while neurotransmission from the inhibitory gabaergic FS-PC

106 synapses appeared unaltered, despite being mediated by $\text{Ca}_v2.1$ channels (i.e., carrying the FHM-1 mutation).
107 This imbalance of cortical excitation and inhibition was associated with increased susceptibility for CSD in the
108 KI mice, but the underlying mechanism changing synaptic strength in the R192Q mutation is not fully
109 understood. At the calyx of Held, presynaptic KI $\text{Ca}_v2.1$ channels activate at more hyperpolarized membrane
110 potentials than WT channels. This hyperpolarized activation led to a higher inward Ca^{2+} influx when Ca^{2+}
111 currents were evoked by long duration action potentials (like pyramidal cells APs), but not when Ca^{2+} currents
112 were elicited by short duration APs (like the calyx of Held or interneurons APs), suggesting that the shape and
113 duration of the AP triggering transmitter release may be one factor controlling the expression of a synaptic gain-
114 of-function in the FHM-1 KI mice (González Inchauspe et al 2010).

115 Here we analyze the biophysical consequences of R192Q $\text{Ca}_v2.1$ channel mutation at a synapse of the
116 auditory system where these ion channels are normally expressed, together with other type of Ca^{2+} channels. We
117 used brainstem slices containing the lateral superior olive (LSO), which is a nucleus of the superior olivary
118 complex which performs binaural processing (receive inputs from both ears). . The LSO computes interaural
119 intensity differences (IID) for sound localization at high sound frequencies. Ipsilateral excitatory inputs arise
120 from spherical bushy cells of the anterior ventral cochlear nucleus (aVCN) and contralateral inhibitory inputs
121 from the medial nucleus of the trapezoid body (MNTB). So this nucleus allows comparison of both excitatory
122 and inhibitory neurotransmission. We can therefore use this KI animal to explore differential effects of the
123 R192Q mutation on integration of excitatory inhibitory projections in the same neuron. Performing whole cell
124 patch-clamp recordings, we studied glycinergic inhibitory postsynaptic currents (IPSCs) and glutamatergic
125 excitatory postsynaptic currents (EPSCs) onto LSO neurons, in mice carrying the human R192Q FHM-1
126 mutation. Our data show increased release probability, accompanied by a diminished vesicle pool size, enhanced
127 short-term depression (STD) and faster recovery at both excitatory and inhibitory synapses. However, the
128 strength of excitatory and inhibitory synapses is differently affected: EPSC are severely depressed whereas
129 IPSCs are consistently larger in KI mice compared to WT mice. We conclude that a gain-of-function of the
130 $\text{Ca}_v2.1$ channels in R192Q KI mice, differently affects excitatory and inhibitory synaptic transmission, thereby
131 altering the excitation/inhibition balance.

132

133

1. Materials and Methods

134 Generation of the R192Q KI mouse strain was described previously (van den Maagdenberg et al., 2004).
135 In short, codon 192 in exon 4 of the mouse *Cacna1a* gene was modified by gene targeting and now encodes a
136 glutamine instead of an arginine residue. Homozygous R192Q KI and WT mice from the same genetic mixed
137 background of 129 and C57Bl6J were used for our experiments. All experiments were carried out according to
138 National guidelines and approved by local Ethical Committees.

139 *Preparation of brainstem slices.*

140 Mice of P11 to 15 days were killed by decapitation, the brain removed rapidly and placed into an ice-

cold low-sodium artificial cerebrospinal fluid (aCSF). The brainstem was mounted in the Peltier chamber of an Integraslice 7550PSDS (Campden Instruments Limited, UK) vibrating microslicer. Transverse slices of 250 μ m thickness were cut sequentially and transferred to an incubation chamber containing normal aCSF with low calcium (0.1 mM CaCl_2 and 2.9 mM MgCl_2) at 37 °C for 1h. After incubation the chamber was allowed to return to room temperature. Normal aCSF contained (mM): NaCl 125, KCl 2.5, NaHCO_3 26, NaH_2PO_4 1.25, glucose 10, ascorbic acid 0.5, myo-inositol 3, sodium pyruvate 2, MgCl_2 1 and CaCl_2 2. Low sodium aCSF was as above but NaCl was replaced by 250 mM sucrose and MgCl_2 and CaCl_2 concentrations were 2.9 mM and 0.1 mM respectively. The pH was 7.3 when gassed with 95% O_2 -5% CO_2 .

Experimental electrophysiology

Slices were transferred to an experimental chamber perfused with normal aCSF at 25 °C. Neurons were visualized using Nomarski optics on a BX50WI (Olympus, Japan) microscope, and a 40X/0.90 NA water immersion objective lens (LUMPlane FI, Olympus). Whole-cell voltage clamp recordings were made with patch pipettes pulled from thin walled borosilicate glass (Harvard Apparatus, GC150F-15, UK). Electrodes had resistances of 2.9 to 3.2 M Ω when filled with internal solution of the following composition (mM): CsCl 110, Hepes 20, TEA-Cl 10, Na_2 phosphocreatine 12, EGTA 0.5, MgATP 2, LiGTP 0.5 and MgCl_2 1. PH was adjusted to 7.3 with CsOH. To block Na^+ currents and avoid postsynaptic action potentials, 10 mM *N*-(2,6-diethylphenylcarbamoylmethyl)-triethyl-ammonium chloride (QX-314) was added to the pipette solution. Patch-clamp recordings were made using Multiclamp 700B amplifier (Axon CNS, Molecular Devices), a Digidata 1440A (Axon CNS, Molecular Devices) and pClamp 9.0 software. Data were sampled at 50 kHz and filtered at 6 kHz (Low pass Bessel). Whole-cell membrane capacitances (15-25 pF) and series resistances (6-15 M Ω) were registered from the amplifier and compensated by 50-60%. Leak currents were subtracted on line with a P/5 protocol.

To evoke IPSCs a bipolar platinum stimulating electrode was located midway between the LSO and the MNTB, and square pulses of 0.1 ms duration and variable voltage amplitudes were applied through an isolated stimulator (Model DS2A, Digitimer Ltd., UK). To isolate glycinergic IPSCs, CNQX (10 μ M), DLAP5 (50 μ M) and bicuculline (10 μ M) were added to the external solution to block glutamatergic AMPA and NMDA and gabaergic mediated synaptic responses respectively. Glutamatergic EPSCs were evoked by positioning the electrode over the ipsilateral fibers from the cochlear nucleus and isolated using strychnine (1 μ M), AP5 (50 μ M) and bicuculline in the external solution.

Data analysis was done using Clampfit 10.0 (Molecular Devices, USA), Sigma Plot 10.0, SigmaStat 3.5 and Excel 2003 (Microsoft) software. Average data are expressed and plotted as mean $\pm$ sem. Statistical significance was determined using Student's *t*-test or one way repeated measure ANOVA plus Student-Newman-Keuls post-hoc test.

2. Results

2.1 Neurotransmitters mediating E/IPSCs at the LSO

We first confirmed the receptors mediating synaptic transmission at inhibitory MNTB-LSO projections and at excitatory aVCN-LSO projections in P11-P15 WT and R192Q KI mice. Synaptic inputs to the LSO were evoked *in vitro* by electrical stimulation of the presynaptic axons coming from the MNTB and aVCN, respectively. Inhibitory transmission was blocked by strychnine, showing a major contribution by glycine receptors at the MNTB-LSO synapses of both WT and R192Q mice, and in accordance with previous reports (Moore and Caspary 1983, Sterenborg et al. 2010, Giogovaz et al. 2011). Fig. 1A and 1B shows representative IPSCs in LSO neurons from WT and R192Q KI mice in control conditions and after perfusion of strychnine (2 μ M) to block glycine receptors (1A) or (-)-bicuculline methochloride (10 μ M) plus 6-ciano-7-nitroquinoxalina-2,3-dione (CNQX; 10 μ M) and AP5 (50 μ M) to block GABA_A, AMPA and NMDA receptors, respectively (1B). A summary of receptors contributing to inhibitory synaptic transmission onto LSO neurons from WT and R192Q mice (stimulating afferents from MNTB nucleus) is presented in Fig. 1C. Excitatory transmission was mediated almost exclusively by glutamatergic AMPA receptors at the aVCN-LSO synapses of both WT and R192Q KI mice. Fig. 1D and 1E show representative recordings of EPSC in LSO neurons from a WT and R192Q KI mice in control conditions and after adding strychnine (1 μ M) to block glycine receptors (Fig. 1D) or (-)-bicuculline methochloride (10 μ M) plus strychnine (1 μ M) and AP5 (50 μ M) to block GABA_A, glycine and NMDA receptors, respectively (Fig. 1E). Mean contribution of the different types of receptors to excitatory synaptic transmission onto LSO neurons from WT and R192Q KI mice (when stimulating afferents from aVCN nucleus) is presented in Fig. 1F.

We conclude that in mice (P11-15) inhibitory neurotransmission is mainly glycinergic while excitatory synaptic transmission is essentially glutamatergic mediated by AMPA receptors. The subsequent experiments were made isolating glycinergic IPSCs and glutamatergic AMPA EPSCs to examine the role of FHM-1 R192Q mutation on Ca_v2.1 channels in neurotransmitter release at LSO synapses.

2.2 Presynaptic terminals released more glycine but less glutamate in R192Q KI compared to WT mice

Neurons in the LSO are innervated by multiple inhibitory and excitatory projections from the MNTB and aVCN, respectively. The evoked synaptic currents will therefore depend on the intensity of the electrical stimuli applied to the innervating axons. Inputs to LSO neurons were stimulated over a range of intensities. Fig. 2A shows representative IPSCs recorded in LSO neurons from WT and R192Q KI mice (holding potential of -70 mV; in the presence of CNQX, (-)-bicuculline and AP5) during increments in the stimulation voltage. IPSC conductance from R192Q KI mice (n = 15) was significantly larger compared to WT (n = 19) for all stimulating intensities above 1.5 V (Fig. 2B, $P < 0.01$, One-way ANOVA RM, Student-Newman-Keuls post-hoc). IPSC maximal conductance (measured at steady state, 6 V) was 88 ± 8 nS in WT mice (n = 19) and 108 ± 9 nS in the R192Q KI

mice ($n = 15$, $P = 0.001$, Student's t -test). In contrast, EPSC maximal amplitudes (recorded in the presence of strychnine, (-)-bicuculline and AP5) were significantly smaller in R192Q KI compared to WT mice for each stimulus intensity above 8 V (Fig. 2C). Mean EPSC conductance as a function of stimulus are shown in Fig. 2D, * $P < 0.01$, One-way ANOVA, Student-Newman-Keuls post-hoc). EPSC conductance measured at steady state (12 V), were 20 ± 4 nS in WT ($n = 9$) and 9.5 ± 0.9 nS in R192Q KI mice ($n = 8$, $P = 0.02$, Student's t -test). The kinetics of glycinergic IPSCs and glutamatergic EPSCs from R192Q KI were not statistically different than those from WT mice. IPSCs from WT ($n = 19$) and KI ($n = 15$) synapses had respectively a mean rise time of 0.57 ± 0.03 ms and 0.55 ± 0.04 ms, mean half width of 3.7 ± 0.2 ms and 3.6 ± 0.3 ms and mean decay time of 7.3 ± 0.6 ms and 6.7 ± 0.7 ms ($P > 0.05$, Student's t -test). For EPSCs, mean rise time were 0.83 ± 0.09 ms and 0.92 ± 0.09 ms, mean half width are 2.68 ± 0.34 ms and 3.44 ± 0.18 ms and mean decay time were 5.3 ± 0.7 ms and 6.6 ± 0.6 ms for WT ($n = 9$) and R192Q KI ($n = 8$) mice respectively ($P > 0.05$, Student's t -test). We next evaluated the probability of vesicle release by estimating the fraction of the ready releasable pool released by a single action potential. We used an approach based on repetitive stimulation which induced short-term depression (STD) of I/EPSC amplitudes. Assuming that depression is largely caused by a transient decrease in the number of readily releasable quanta, it is possible to estimate the pool size by calculating the cumulative summation of I/EPSC amplitudes for time intervals that are short with respect to the time required for recovery from depression (Schneggenburger et al. 1999). We measured the cumulative sum of I/EPSC amplitudes during a train of 20 stimuli at 20 Hz in WT and R192Q KI mice. Data points in the range between 0.55 s and 1.0 s were fitted by linear regression and back-extrapolated to time 0 (Fig. 3A for IPSCs and Fig. 3C for EPSCs). This estimation effectively takes into account the cumulative I/EPSC amplitudes reached within the first six stimuli, corresponding to a time interval of about 0.25 s, before depression reaches its steady state level. It assumes that recovery from depression is negligible for the time interval of about 0.25 s used for the calculation. The validity of this assumption can be assessed in the analysis of time recovery from STD described in section 2.4 and Fig. 5B and 5D. The zero time intersect gives an estimate of the size of the readily releasable pool of synaptic vesicles (N) multiplied by the mean quantal amplitude (q). The release probability can be estimated by dividing the mean amplitude of the first I/EPSC by the Nq value. Although this calculation may be overestimated because Ca^{2+} influx during the first stimulus is lower than during the last pulses and Ca^{2+} concentration rises during stimulation, the comparison between genotypes indicated that the release probability was significantly higher in R192Q KI than in WT mice at both inhibitory (Fig. 3A inset, $P = 10^{-4}$, Student's t -test) and excitatory (Fig. 3C inset, $P = 4 \cdot 10^{-5}$, Student's t -test) synapses onto LSO neurons. An estimate of the quantal amplitude can be obtained from the amplitudes of spontaneous miniature events (mI/EPSCs). The amplitude of the mIPSCs measured in R192Q KI mice (196 ± 12 pA, $n = 16$) were larger than mIPSC amplitude measured in WT mice (153 ± 7 pA, $n = 32$, $P = 0.002$, Fig. 3B, I); mIPSC frequencies were also higher in synapses from R192Q KI (4.8 ± 0.4 Hz, $n = 16$) compared to synapses from WT mice (2.7 ± 0.2 Hz, $n = 32$, $P = 10^{-5}$, Student's t -test, Fig. 3B, II). Decay times of mIPSCs were not statistically different between

246 R192Q KI (6.7 ± 0.5 ms) and WT mice (5.7 ± 0.5 ms, $P = 0.07$, Student's *t*-test, Fig. 3B, III). The estimated
247 vesicle pool size was obtained dividing the zero time intersect of the cumulative PSC amplitude by the quantal
248 size. This value was slightly smaller in R192Q KI (71 ± 5 , $n = 14$) compared to WT mice (98 ± 6 , $n = 15$), $P =$
249 0.02 , Student's *t*-test, Fig. 3B, IV).

250 Different results were found for the excitatory transmission. Miniature EPSC measured in R192Q KI and WT
251 mice had similar amplitudes (39 ± 3 pA, $n = 13$ in KI and 43 ± 5 pA, $n = 17$ in WT, $P > 0.05$, Student's *t*-test,
252 Fig. 3D, I) and similar frequencies (2.4 ± 0.4 Hz in KI and 2.7 ± 0.4 Hz in WT, $P > 0.05$, Student's *t*-test, Fig.
253 3D, II). Interestingly mEPSC decay times were slower in KI (0.62 ± 0.04 ms) compared to WT (0.49 ± 0.04 ms,
254 $P = 0.02$, Student's *t*-test, Fig. 3D, III).

255 The ready releasable pool size was severely reduced in R192Q KI (28 ± 1 , $n = 8$) compared to WT mice (65 ± 2 ,
256 $n = 8$, $P < 0.01$, Student's *t*-test, Fig. 3D, IV).

257 We conclude that FHM-1 R192Q mutation increased release probability and decreased pool size at both
258 synapses (aiming to a presynaptic effect), even though the IPSC conductance was larger and the EPSC
259 conductance was smaller in R192Q KI compared to WT mice.

260

261 **2.3 Ca^{2+} channels involved in inhibitory and excitatory synaptic transmission in WT and R192Q KI mice**

262 The subtypes of Ca^{2+} channels mediating synaptic transmission were assessed by using specific blockers
263 of the calcium channels. I/EPSCs, evoked at a holding potential of -70 mV, were recorded during sequentially
264 bath application of nitrendipine (10 μ M, L-type antagonist), ω -conotoxin GVIA (2 μ M; N-type blocker), ω -
265 agatoxin IVA (0.2 μ M; P/Q-type blocker) and cadmium (50 μ M; non-selective Ca^{2+} channel antagonist).
266 Representative plots of I/EPSC peak amplitudes as a function of time are shown in Fig. 4A (IPSCs) and Fig. 4E
267 (EPSCs) for both WT and R192Q KI mice.

268 The relative contribution of each type of Ca^{2+} channel to the evoked synaptic currents was determined from the
269 fraction of I/EPSC amplitudes inhibited by the application of the selective Ca^{2+} channel antagonist applied in the
270 sequence shown in Fig. 4A and 4E. While IPSCs were triggered by multiple types of VACC (Fig. 4B), EPSCs
271 were predominantly mediated by $Ca_v2.1$ Ca^{2+} channels (Fig. 4F) in both WT (87 ± 6 %) and R192Q KI mice (75
272 ± 5 %), with no significant differences between the two genotypes.

273 In synapses where multiple VACCs are involved in triggering transmitter release, the degree of cooperativity and
274 efficiency of each type of Ca^{2+} channel depends on their distribution at different release sites and on the distance
275 between the channel and the release sites (Neher and Sakaba 2008, Schneggenburger et al 2002, Wu et al. 1999).
276 The cooperative action of Ca^{2+} on the calcium sensor is reflected in the dependence of the transmitter release on
277 the third or fourth power of the presynaptic calcium influx (Fedchyshyn and Wang 2005; Yang and Wang 2006,
278 Takahashi and Momiyama 1993, Wu and Saggau 1997). Hence, the inhibitory effect of Ca^{2+} channel blockers on
279 IPSCs depends on the order in which drugs are applied, indicating that the effect of each toxin does not cause a
280 linear reduction of release. The effect of ω -agatoxin IVA applied alone (representative traces in Fig. 4C)

inhibited IPSC by $73 \pm 5 \%$ ($n = 5$) and $75 \pm 3 \%$ ($n = 9$) in WT and R192Q KI mice respectively (Fig. 4D) indicating that $\text{Ca}_v2.1$ VACCs play a predominant role at both excitatory and inhibitory LSO synapses.

2.4 Short-term plasticity in synaptic transmission of R192Q KI.

Repetitive stimulation of the LSO synapses causes STD of both excitatory and inhibitory transmission. IPSC and EPSC amplitudes decrease during the train of stimuli until they reach a steady state. EPSCs exhibit stronger STD and a faster decay time course than IPSCs (compare Fig. 5A and 5C for inhibitory and excitatory transmission respectively).

In order to compare WT and R192Q KI synaptic behaviour, we studied the time-course of STD and recovery after 20 Hz stimulation at the LSO inhibitory and excitatory synapses. At both synapses the amplitude of the synaptic currents measured in KI depressed with a faster time course compared to WT. Nevertheless the magnitude of depression at the end of the stimuli was similar between WT and KI mice. Plots of mean IPSC and EPSC amplitudes as a function of time during stimulation, with the corresponding parameters of the exponential decay fitting are shown in Fig. 5A and 5C respectively. Of particular interest is the pair pulse ratio (PPR) calculated as the ratio between the second and the first evoked postsynaptic current during the train. IPSCs had PPRs of 0.86 ± 0.02 (WT, $n = 15$) and 0.81 ± 0.02 (R192Q KI, $n = 14$, $P = 0.015$, Student's t -test) while EPSC had PPRs of 0.67 ± 0.06 and 0.55 ± 0.06 for R192Q KI ($n = 8$) and WT ($n = 8$) mice respectively ($P = 0.003$, Student's t -test).

The time course of recovery from synaptic depression was studied by eliciting a single test I/EPSC at varying time intervals (from 0.1 s to 20 s) following the conditioning train. The fraction of recovery was calculated as follows:

$$\text{Fraction of recovery} = (I_{\text{test}} - I_{\text{ss}})/(I_1 - I_{\text{ss}})$$

where I_1 and I_{ss} are the amplitudes of the first and last I/EPSCs in the train and I_{test} is the amplitude of the test I/EPSC.

The fraction of recovery from STD as a function of time after a 20 Hz stimulus train could be fitted by a single exponential for R192Q KI and WT mice. In line with the stronger STD, excitatory inputs recovered much faster than inhibitory inputs (compare Fig. 5B and 5D). Plots of mean I/EPSC amplitudes as a function of time delay after the train stimuli, with the corresponding parameters of the exponential fitting are shown in Fig. 5B (inhibitory) and Fig. 5D (excitatory). Comparing the two genotypes, we found that the rate of recovery was significantly increased in R192Q KI compared to WT mice, for both inhibitory and excitatory inputs to the LSO neurons. These results support evidence on the importance of Ca^{2+} influx through $\text{Ca}_v2.1$ Ca^{2+} channels in the refilling of the releasable pool of synaptic vesicles: mutations in calcium channels may alter STD and or vesicle replenishment.

3. Discussion and conclusion

316

317 Using knock-in mice carrying the pathogenic FHM-1 mutation R192Q in the *Cacnala* gene that encodes
318 the α_{1A} pore-forming subunit of voltage-gated neuronal $\text{Ca}_v2.1$ (P/Q-type) Ca^{2+} channels, we evaluated the
319 functional consequences of this mutation on inhibitory and excitatory synaptic transmission at the LSO synapse
320 of the auditory system. Excitatory glutamatergic release was almost exclusively dependent on $\text{Ca}_v2.1$ Ca^{2+}
321 channels (Fig. 4E, F). Inhibitory glycinergic release was, on the other hand, mediated by multiple VACCs (Fig.
322 4A, B). The degree of co-operativity and efficiency of each subtype in controlling transmitter release depends on
323 the distribution of the different subtypes of Ca^{2+} channels at different release sites (Mintz et al. 1995; Reid et al.
324 1998). Calcium influx through VACCs increases intracellular Ca^{2+} concentration in a bell-shaped microdomain
325 close to the channel mouth (Llinas and Moreno 1998) which can be modulated by factors that alter calcium
326 buffering and/or diffusion, with a large effect on calcium-triggered cellular events. So, the degree of co-
327 operativity and efficiency of each type of Ca^{2+} channels in controlling transmitter release will also depends on
328 the distance between the channel, the calcium sensor and the release sites (Neher and Sakaba 2008,
329 Schneggenburger et al 2002, Wu et al. 1999). The inhibitory effect of Ca^{2+} channel blockers on IPSCs depends
330 on the order in which each drug is applied. The dependence of the transmitter release on the third or fourth
331 power of the presynaptic calcium influx indicates a cooperative action of Ca^{2+} on the calcium sensor
332 (Fedchyshyn and Wang 2005; Yang and Wang 2006, Takahashi and Momiyama 1993, Wu and Saggau 1997).
333 When the $\text{Ca}_v2.1$ blocker ω -agatoxin IVA was applied alone in WT and R192Q KI mice, IPSCs were inhibited
334 by more than 70 % (Fig. 4C, D). These data reveal that release of vesicle at both excitatory and inhibitory
335 synaptic transmission strongly depends on calcium influx via $\text{Ca}_v2.1$ channels and suggest that $\text{Ca}_v2.1$ Ca^{2+}
336 channels are more suitable for triggering transmitter release and have an efficient and dominant role in
337 controlling neurotransmitter release at central excitatory and inhibitory synapses. It is worth mentioning that the
338 participation of $\text{Ca}_v2.1$ calcium channels in the inhibitory and excitatory neurotransmission onto LSO synapses
339 is opposite to that in cortical synapses. Excitatory glutamatergic neurotransmission at connected pairs of layer
340 2/3 pyramidal cells (PC) and multipolar fast-spiking (FS) interneurons in cortical slices of the somatosensory
341 cortex of WT and KI mouse cortical slices was cooperatively mediated by N-, P/Q- and R- type Ca^{2+} channels,
342 while the FS-PC inhibitory synaptic transmission was exclusively triggered by $\text{Ca}_v2.1$ calcium channels (Tottene
343 et al 2009).

344 FHM-1 R192Q mutation affected inhibitory and excitatory LSO synapses in a different way. As
345 expected from a multiple innervated neuron, IPSC and EPSC amplitudes were dependent on the number of
346 axons recruited during stimulation. At the inhibitory MNTB-LSO synapse, the FHM-1-mutated $\text{Ca}_v2.1$ Ca^{2+}
347 channels resulted in a functional synapse with increased amplitudes of evoked IPSCs in R192Q KI compared to
348 WT mice. In contrast, at the excitatory aVCN-LSO synapse, the mutation gave rise to a reduction in the strength
349 of the EPSC in R192Q KI compared to WT mice. Spontaneous release showed increased amplitudes of mIPSCs

while amplitudes of mEPSCs remained unaltered in R192Q KI compared to WT mice. Frequencies of IPSCs
 (but not of EPSCs) were increased in R192Q KI compared to WT mice.

At both inhibitory and excitatory neurotransmission, FHM-1 R192Q mutation resulted in a higher release
 probability in KI compared to WT. The increment in release probability suggests a presynaptic effect of the
 FHM-1 mutation, which produces an increase in AP-evoked calcium influx. The increase in presynaptic activity
 was previously investigated at the calyx of Held-MNTB synapse, where the big size of the presynaptic terminals
 allowed electrophysiological patch-clamp recordings of presynaptic Ca^{2+} currents (González Inchauspe et al.
 2010). It was reported that R192Q mutated $\text{Ca}_v2.1$ channels activated at more hyperpolarizing potentials and that
 presynaptic Ca^{2+} currents had a faster kinetics of activation. This small shift in voltage dependent activation
 would lead to an increase in Ca^{2+} influx near the release sites during evoked action potentials in the mutant mice.
 On the other hand, a reduced size of the readily releasable pool of vesicles was observed at R192Q KI synapses,
 more prominent in the excitatory synapse (57% of reduction in R192Q KI compared to WT mice) than in the
 inhibitory synapse (27% of reduction in R192Q KI compared to WT mice).

The enhanced inhibitory neurotransmission can be explained by the increase in release probability and in the
 amplitudes of the single quantal events occurring in the MNTB terminals, which compensate in excess the
 slightly reduced vesicle pool size. Moreover, the change in vesicle pool could indicate the occurrence of a
 mechanism that preserves synaptic transmission in face of a dramatic augment of inhibition.

The release probability was also increased at the excitatory glutamatergic nerve terminals. However, the severe
 reduction in vesicle pool size resulted in an overall reduction of the excitatory synaptic currents here reported,
 aiming to recover the balance between excitation and inhibition in R192Q KI synapses. These findings suggest
 the possible occurrence of homeostatic compensatory mechanisms, that could be different at inhibitory or
 excitatory synapses (Turrigiano 2012).

It is interesting to point out that in contrast to our findings at the LSO synapses, at the somatosensory cortical
 synapses studied by Tottene et al. (2009) there was an enhancement in excitatory glutamatergic
 neurotransmission at the PC-FS connection in R192Q KI compared to WT mice while the inhibitory GABAergic
 neurotransmission at FS-PC connections was unaffected by the FHM-1 R192Q mutation. Interestingly it seems
 that at these synapse there were no homeostatic compensatory mechanisms. In summary, in synapse from
 R192Q KI mice, the balance between excitation and inhibition is lost. The differential effect of FHM-1
 mutations on excitatory and inhibitory neurotransmission at different synapses may produce overexcitation in
 certain conditions, but may leave the excitation-inhibition balance within physiological limits in others.

Most relevant was the effect of the FHM-1-mutated $\text{Ca}_v2.1$ Ca^{2+} channels on short-term synaptic
 plasticity. Firstly, it is worth mentioning that excitatory transmission depressed and recovered much faster than
 inhibitory transmission, independently of the genotypes (compare Fig. 5 A with Fig 5 C for depression and Fig.
 5 B with Fig 5 D for recovery). Comparing between genotypes, the time course of STD and recovery during 20
 Hz frequency stimulation was faster in R192Q KI mice at both excitatory and inhibitory LSO synapses. STD is

385 the consequence of depletion of the release-ready vesicle pool (Wang and Kaczmarek 1998, Wu and Borst 1999,
 386 Schneggenburger et al. 1999, von Gersdorff and Borst 2002, Wong et al. 2003, Zucker and Regehr 2002),
 387 although other mechanisms like receptor desensitization (Neher and Sakaba 2001, Taschenberger et al 2002,
 388 Wong et al. 2003, Schneggenburger et al 2002), calcium channel inactivation (Forsythe et al. 1998, Xu and Wu
 389 2005, Muller et al. 2008) and calcium channel inhibition by presynaptic metabotropic glutamate receptor (Von
 390 Gersdorff et al 1997, Takahashi et al. 1996) or AMPA receptors activation (Takago et al. 2005, Scheuss et al
 391 2002, Neher and Sakaba 2001) may be involved. The efficacy of synaptic transmission during repetitive
 392 stimulation is also determined by the rate of recovery from synaptic depression. Postsynaptic currents recover
 393 from STD after many seconds of rest due to recycling of vesicles into the ready releasable pool (Zucker and
 394 Regehr 2002), which is dynamically regulated by Ca^{2+} influx through voltage-gated Ca^{2+} channels in an activity
 395 dependent manner which is highly dependent on the recent history of synaptic activity (Wang and Kaczmarek,
 396 1998). High-frequency stimulation of presynaptic terminals significantly enhances the rate of replenishment.
 397 Wang and Kaczmarek (1998) have shown that Ca^{2+} influx through voltage-gated Ca^{2+} channels is the key signal
 398 that dynamically regulates the refilling of the releasable pool of synaptic vesicles in response to different patterns
 399 of inputs. Therefore, mutations in calcium channels may alter STD and or vesicle replenishment. Considering
 400 that a substantial amount of synaptic depression is due to vesicle depletion, the enhanced short-term depression
 401 (STD) and diminished pair pulse ratio (PPR) in R192Q KI mice (Fig. 5A, C) is consistent with the higher release
 402 probability (Fig. 3A, C, insets) and the smaller vesicle pool size (Fig. 3B, D) that these transgenic mice exhibited
 403 at both excitatory and inhibitory synapses, reinforcing the hypothesis of a presynaptic effect of the FHM-1
 404 mutation.

405 The enhanced rate of recovery from STD observed in R192Q KI mice is an important feature that can modify
 406 synaptic efficacy and excitability. It suggests a faster recycling rate of vesicles into the ready releasable pool
 407 (Wang and Kaczmarek, 1998), and is in agreement with the enhanced preceding synaptic activity (enhanced
 408 STD as shown in Fig. 5A, C). Faster recovery in excitatory and inhibitory transmission might be due to an
 409 increased residual Ca^{2+} in synapses from R192Q KI mice, responsible for accelerating vesicle endocytosis and
 410 refilling process following STD. The rise in residual calcium is a consequence of the mutated $\text{Ca}_v2.1$ channels as
 411 was described at the calyx of Held synapse (González Inchauspe et al. 2010, 2012). It is worth mentioning that
 412 the faster recovery in the R192Q KI model is opposite to the effect observed in knock-out mice with ablated
 413 $\text{Ca}_v2.1$ channels ($\alpha_{1A}^{-/-}$). Synapses from $\alpha_{1A}^{-/-}$ mice show a slower kinetics of recovery after STD compared to
 414 WT mice (Giogovaz et al. 2011, González Inchauspe et al. 2007). Faster recovery of vesicle recycling during
 415 repetitive activity may also contribute to the increased excitability in FHM-1 mutant mice. Although further
 416 studies are needed to determine the exact mechanism involved in the alteration of short-term plasticity observed
 417 in the FHM-1 transgenic migraine mouse model, it is very likely that the increased rate of recovery could result
 418 in important changes in network activity and to dysfunction.

The demonstration that FHM-1 mutations may differently affect synaptic transmission and short-term synaptic plasticity at different cortical synapses suggests that the neuronal circuits that coordinate and dynamically adjust the balance between excitation and inhibition during cortical activity (Shu et al 2003) are very likely altered in FHM-1. The specific dominant and efficient role of Ca_v2.1 channels in controlling fast neurotransmitter release from central excitatory and inhibitory synapses suggests that the human and mouse Ca_v2.1 channelopathies and their episodic neurological symptoms, ranging from migraine to absence epilepsy and ataxia, might primarily be synaptic diseases. The different disorders probably arise from disruption of the finely tuned balance between excitation and inhibition in neuronal circuits of specific brain regions: the cortex in the case of migraine, the thalamus in the case of absence epilepsy and the cerebellum in the case of ataxia. A favored hypothesis considers that the abnormal balance of cortical excitation-inhibition and the resulting persistent state of hyperexcitability of neurons in the cerebral cortex may underlie the increased susceptibility for cortical spreading depression (CSD), which is believed to initiate the attacks of migraine with aura.

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617

618

Figure captions

Fig. 1. Contribution of different types of receptor to inhibitory and excitatory synaptic transmission at the LSO in WT and R192Q KI mice.

A-B. Representative IPSCs recorded from neurons of the LSO in WT and R192Q KI mice, under voltage-clamp conditions at a holding potential of -70 mV. IPSCs were evoked by stimulating the innervating axons coming from the MNTB neurons. The contribution from glycine receptors (A) was determined by adding the specific receptor antagonist strychnine (2 μ M), while the contribution of GABA_A, AMPA and NMDA receptors (B) was assessed by adding (-)-bicuculline methochloride (10 μ M) plus 6-ciano-7-nitroquinoxalina-2,3-diona (CNQX; 10 μ M) and AP5 (50 μ M).

C- Bars plot summarizing the mean inhibition of IPSCs by strychnine (89 ± 6 %, $n = 19$ for WT and 91 ± 7 %, $n = 3$ for KI) and by the addition of (-)-bicuculline methochloride together with CNQX and AP5 (9 ± 5 %, $n = 24$ for WT and 10 ± 6 %, $n = 3$ for R192Q KI mice).

D-E. Representative EPSCs recorded from neurons of the LSO in WT and R192Q KI mice, evoked by stimulating the innervating axons coming from the aVCN. The contribution from AMPA receptors was determined by adding the specific receptor antagonist CNQX 10 μ M (D) while the contribution of GABA_A, glycine and NMDA receptors was assessed by adding (-)-bicuculline methochloride plus strychnine (2 μ M) and AP5 (50 μ M).

F. Bars plot summarizing the mean inhibition of EPSCs by CNQX (89 ± 10 %, $n = 3$ for WT and 95 ± 10 %, $n = 3$ for KI) and by the addition of (-)-bicuculline methochloride together with strychnine and AP5 (14 ± 8 %, $n = 3$ for WT and 12 ± 7 %, $n = 3$ for R192Q KI mice).

Fig. 2. Evoked release is increased at the inhibitory but decreased at the excitatory LSO synapse.

A. Representative glycinergic IPSCs recorded from the soma of an LSO neuron under voltage-clamp conditions at a holding potential of -70 mV for different values of the voltage applied to stimulate the innervating axons coming from the MNTB neurons: 1, 2, 4 and 6 V in increasing order for both WT and R192Q KI mice. Traces are the average of 20 stimuli for each stimulating voltage amplitude.

B. Mean glycinergic IPSC conductance as a function of the voltage amplitude (0.5–8 V) applied to the bipolar platinum stimulating electrode (stimuli of 0.1 ms duration). For each given stimuli intensity above 1.5 V mean IPSC conductance is larger in R192Q KI mice, reaching a steady state value above 6 V (108 ± 9 nS, $n = 15$) significantly higher than in WT mice (88 ± 8 nS, $n = 19$, $P = 0.001$, Student's *t*-test).

C. Representative glutamatergic EPSCs recorded from the soma of an LSO for different voltage amplitudes applied to the bipolar platinum stimulating electrode: 7, 8, 9 and 10 V in increasing order for both WT and R192Q KI mice. Traces are the average of 20 stimuli for each stimulating voltage amplitude.

653 **D.** Mean glutamatergic EPSC conductances as a function of the voltage amplitude (1–12 V) applied to the
654 bipolar platinum stimulating electrode (stimuli of 0.1 ms duration). Mean EPSC conductance is smaller in
655 R192Q KI mice, for stimuli intensities above 6 V reaching a steady state value above 10 V (9.5 ± 0.9 nS, $n = 8$)
656 significantly lower than in WT mice (20 ± 4 nS, $n = 9$, $P = 0.02$, Student's *t*-test).
657 * Significant differences between WT and R192Q KI mice ($P < 0.01$, One-way ANOVA RM, Student-Newman-
658 Keuls post-hoc).

659

660 **Fig. 3. R192Q KI mice present a higher release probability**

661 **A.** Mean cumulative summation of IPSC amplitudes during 20 Hz stimulation in WT and R192Q KI mice as a
662 function of time. A lineal function was fitted to the steady state region for times larger than 0.55 s and back-
663 extrapolated to time 0, to estimate the readily releasable pool size (N) multiplied by the mean quantal amplitude
664 (q). The Nq values obtained for WT and KI are 15.0 ± 0.4 nA and 14.0 ± 0.3 nA respectively. **Inset:** The release
665 probability calculated as the ratio between the mean amplitude of the first IPSC in the train and the Nq value is
666 significantly higher in R192Q KI (0.59 ± 0.02 , $n = 14$) than in WT synapses (0.48 ± 0.01 , $n = 15$; $P = 10^{-4}$,
667 Student's *t*-test).

668 **B.** Spontaneous inhibitory release. Mean amplitudes (I) of mIPSC are increased in R192Q KI mice (196 ± 12
669 pA, $n = 16$) compared to WT (153 ± 7 pA, $n = 32$, $P = 0.002$ Student's *t*-test) and so are the frequencies (II): 4.8
670 ± 0.4 Hz in R192Q KI vs 2.7 ± 0.2 Hz in WT mice ($P = 10^{-5}$, Student's *t*-test). Decay time constants of mIPSCs
671 (III) present no statistical differences between KI (6.7 ± 0.5 ms) and WT mice (5.7 ± 0.5 ms, $P = 0.07$, Student's
672 *t*-test). The estimated vesicle pool (IV) size is diminished at inhibitory synaptic transmission from R192Q KI (71
673 ± 5 , $n = 14$) compared to WT (98 ± 6 , $n = 15$, $P = 0.02$, Student's *t*-test).

674 **C.** Mean cumulative summation of EPSC amplitudes during 20 Hz stimulation in WT and R192Q KI mice as a
675 function of time. The Nq values obtained for WT ($n = 8$) and KI ($n = 8$) are 2.91 ± 0.08 nA and 0.91 ± 0.02 nA,
676 respectively. **Inset:** The release probability is significantly higher in KI (0.72 ± 0.02 , $n = 8$) than in WT synapses
677 (0.47 ± 0.01 , $n = 8$; $P = 4 \cdot 10^{-5}$, Student's *t*-test).

678 **D.** Spontaneous excitatory release. Mean amplitudes (I) of mEPSC are similar in R192Q KI mice (39 ± 3 pA, n
679 $= 13$) compared to WT (43 ± 5 pA, $n = 17$, $P > 0.01$, Student's *t*-test) and so are the frequencies (II): 2.4 ± 0.4 Hz
680 in KI and 2.7 ± 0.4 Hz in WT, $P > 0.05$, Student's *t*-test. Decay time constants of mEPSCs (III) are higher in KI
681 (0.62 ± 0.04 ms) compared to WT mice (0.49 ± 0.04 ms, $P = 0.02$, Student's *t*-test). Mean vesicle pool size is
682 diminished at excitatory synaptic transmission from R192Q KI (28 ± 1 , $n = 8$) compared to WT (65 ± 2 , $n = 8$, P
683 < 0.01 , Student's *t*-test)

684

685 **Fig. 4. Ca^{2+} channel involved in inhibitory and excitatory synaptic transmission at the LSO from WT and**
686 **R192Q KI mice.**

687 **A.** Peak amplitudes of glycinergic IPSCs recorded at a holding potential of -70 mV as a function of time during
688 the sequential application of selective L-, N- and P/Q-type Ca^{2+} channel antagonists: nitrendipine (10 μM), ω -
689 conotoxin-GVIA (2 μM) and ω -agatoxin IVA (200 nM), respectively, in WT (left) and KI mice (right).
690 Horizontal bars indicate the time of drug application.

691 **B.** Average contribution of each channel type to the total glycinergic IPSCs determined by subtracting current
692 amplitudes before and after application of the specific blockers as state in A. The L-type component is $30.8 \pm$
693 0.8% and $31.5 \pm 0.8\%$ in WT ($n = 7$) and KI ($n = 6$) mice respectively ($P = 0.45$, Student's t -test). The P/Q-type
694 component is $36 \pm 1\%$ ($n = 7$) in WT and $40 \pm 2\%$ ($n = 6$) in KI ($P = 0.05$, Student's t -test). The N-type
695 component is $25 \pm 2\%$ in WT ($n = 7$) and $21 \pm 2\%$ in KI ($n = 6$, $P = 0.06$, Student's t -test).

696 **C-** Effect of ω -agatoxin IVA (200 nM) on IPSC amplitudes when this channel blocker is applied alone in WT
697 and KI LSO synapses.

698 **D-** Mean IPSC amplitude inhibition when only P/Q-type Ca^{2+} channels are blocked: $73 \pm 5\%$ in WT ($n = 5$) and
699 $75 \pm 3\%$ in KI ($n = 9$).

700 **E.** Peak amplitudes of glutamatergic EPSCs as a function of time during the sequential application of L-, N- and
701 P/Q-type Ca^{2+} channel antagonists in WT (left) and R192Q KI mice (right). Horizontal bars indicate the time of
702 drug application.

703 **F.** Average contribution of each channel type to the total glutamatergic EPSCs. Excitatory transmission is
704 predominantly mediated by P/Q-type Ca^{2+} channels: $87 \pm 6\%$ ($n = 7$) in WT and $75 \pm 5\%$ ($n = 6$) in KI ($P =$
705 0.05 , Student's t -test). The L-type Ca^{2+} channels contribute with $15 \pm 3\%$ in WT ($n = 7$) and $6 \pm 5\%$ in KI mice
706 ($n = 6$, $P = 0.36$, Student's t -test) while the N-type Ca^{2+} channels contribute with $5 \pm 2\%$ in WT ($n = 7$) and $10 \pm$
707 2% in KI ($n = 6$, $P = 0.4$, Student's t -test).

708

709 **Fig. 5. Enhanced short-term plasticity at inhibitory and excitatory synaptic transmission in R192Q KI**
710 **compared to WT mice.**

711 **A.** Time course of depression of IPSC amplitudes during 1 s stimulation at 20 Hz (conditioning train, recorded at
712 a holding potential of -70 mV). The amplitudes are normalized to the first IPSC amplitude in the train. Data are
713 fitted to a single exponential decay function, with a decay time constant $\tau = 0.19 \pm 0.02$ s in WT ($n = 15$) and $\tau =$
714 0.12 ± 0.01 s in KI R192Q ($n = 14$, $P = 2 \cdot 10^{-5}$, Student's t -test). The depressed IPSC amplitudes at the end of the
715 stimuli reached a steady state value of $52 \pm 2\%$ y $57 \pm 3\%$ of the first IPSC amplitude in the train, in WT and
716 KI R192Q synapses respectively ($P = 0.055$, Student's t -test). **Inset:** pair pulse ratio (PPR), calculated as the
717 ratio between the second and the first IPSC during the train, is significantly smaller in R192Q KI mice ($0.81 \pm$
718 0.02 , $n = 14$) than in WT mice (0.86 ± 0.02 , $n = 15$, $P = 0.015$, Student's t -test).

719 **B.** Time course of recovery from synaptic depression, measured by eliciting a single test IPSC at increasing time
720 intervals following the 20 Hz conditioning train. The fraction of recovery is calculated as $(I_{\text{test}} - I_{\text{ss}})/(I_1 - I_{\text{ss}})$,
721 where I_1 and I_{ss} are the amplitudes of the first and last IPSCs in the train and I_{test} is the amplitude of the test IPSC.

722 Data are fitted to an exponential decay function of first order, with time constants $\tau = 5.9 \pm 0.2$ s (n = 9) and $\tau =$
723 3.4 ± 0.2 s (n = 13) for WT and R192Q KI respectively (P = 0.003, Student's *t*-test).

724 **C.** Time course of depression of EPSC amplitudes, normalized to the first EPSC of the 20 Hz train. The
725 amplitudes of the EPSCs at the end of the stimuli are 30 ± 2 % y 34 ± 2 % of the first pulse for WT (n = 8) and
726 R192Q KI (n = 8, P = 0.075, Student's *t*-test) respectively. Data are fitted with a single exponential decay
727 function, with a time constant $\tau = 0.100 \pm 0.008$ s en WT (n = 9) and $\tau = 0.046 \pm 0.004$ s en KI R192Q (n = 8, P
728 < 0.05 , Student's *t*-test). ***Inset:*** pair pulse ratio (PPR), calculated as the ratio between the second and the first
729 EPSC during the train, is significantly smaller in R192Q KI mice (0.55 ± 0.06 , n = 8) than in WT mice ($0.676 \pm$
730 0.06 , n = 8, P = 0.003, Student's *t*-test).

731 **D.** Recovery of EPSCs after a 1 s train at 20 Hz follows an exponential time course with time constants $\tau = 3.8 \pm$
732 0.2 s (n = 8) and $\tau = 1.54 \pm 0.2$ s (n = 8) for R192Q KI and WT respectively (P = 0.001, Student's *t*-test).

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Fig. 1

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Fig. 2

Figure 3
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Fig. 3

Figure 4

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Fig. 4

Figure 5
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Fig. 5