Impairment of Select Forms of Spatial Memory and Neurotrophin-Dependent Synaptic Plasticity by Deletion of Glial Aquaporin-4

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Abstract

Aquaporin-4 (AQP4) is the major water channel in the central nervous system (CNS) and is primarily expressed in astrocytes. Little is known about the potential for AQP4 to influence synaptic plasticity, although many studies have shown that it regulates the response of the CNS to injury. Therefore, we evaluated long-term potentiation (LTP), and long-term depression (LTD) in AQP4 knockout (KO) and wild type (WT) mice. KO mice exhibited a selective defect in LTP and LTD without a change in basal transmission or short-term plasticity. Interestingly, the impairment in LTP in KO mice was specific for the type of LTP that depends on the neurotrophin BDNF, which is induced by stimulation at theta rhythm (TBS-LTP), but there was no impairment in a form of LTP that is BDNF-independent, induced by high frequency stimulation (HFS-LTP). LTD was also impaired in KO mice, which was rescued by a scavenger of BDNF or blockade of Trk receptors. TrkB receptors, which mediate effects of BDNF on TBS-LTP, were not altered in KO mice, but p75NTR, the receptor that binds all neurotrophins and has been implicated in some types of LTD, was decreased. The KO mice also exhibited a cognitive defect, which suggests a new role for AQP4 and astrocytes in normal cognitive function. This defect was evident using a test for location-specific object memory but not Morris water maze or contextual fear conditioning. The results suggest that AQP4 channels in astrocytes play an unanticipated role in neurotrophin-dependent plasticity and influence behavior.

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INTRODUCTION

AQP4 is one of 14 members of the AQP family, which are membrane proteins that regulate water transport (Verkman, 2008). AQP4 is the primary AQP in the CNS, and is expressed selectively in astrocytes (Nagelhus et al., 2004). Therefore, an AQP4knockout mouse provides an opportunity to investigate the role of fluid regulation by astrocytes.

Using AQP4 KO mice, several studies have demonstrated an important role of AQP4 in the regulation of edema following insults or injury (Papadopoulos and Verkman, 2007). In addition, the extracellular space is altered in AQP4 KO mice (Binder et al., 2004), and AQP4 is colocalized with the potassium channels Kir4.1 and Kir5.1 (Nagelhus et al., 2004). The effects of AQP4 deletion on excitability appear to be complex, however, because AQP4 KO mice had high seizure thresholds relative to WT mice, but increased seizure duration (Hsu et al., 2007). Furthermore, the functional coupling of AQP4 to Kir4.1 or Kir5.1 has not clearly established a role of AQP4 related to Kir4.1 or Kir5.1 (Zhang and Verkman, 2007, Soe et al., 2009) although the response to a K^+ challenge is altered in AQP4 KO mice (Binder et al., 2006). Therefore, one would predict that AQP4 would modulate synaptic transmission, but it is hard to predict exactly how.

It seems likely that AQP4 would influence synaptic plasticity (LTP, LTD), because the increased afferent activity that is required for induction of LTP and LTD would be likely to accentuate any effect of AQP4 on basal transmission. Indeed, AQP4 KO mice have a defect in activity-dependent swelling of astrocytes (Kitaura et al., 2009), and there is growing evidence that glia influence synaptic plasticity (Bains and Oliet, 2007; Haydon et al., 2009). Therefore, we tested the hypothesis that AQP4 influences basal synaptic transmission, LTP, and LTD. The Schaffer collateral synapse in area CA1 was evaluated because LTP and LTD are robust in this pathway normally. We also evaluated hippocampal-dependent behaviors that would potentially be influenced by LTP or LTD in area CA1, including Morris water maze (MWM), contextual fear conditioning (CFC), and object placement (OP; (Tsien et al., 1996, Ji and Maren, 2008, Luiz Assini et al., 2009).

MATERIALS AND METHODS

Animal use met NIH guidelines. Transgenic mice from Dr. Alan Verkman (CD1 strain; Ma et al., 1997), were housed with a 12 hr light:dark cycle and fed *ad libitum*. Chemicals were from Sigma-Aldrich unless otherwise noted. Data are reported as mean ± standard error of the mean(sem) with p<0.05.

Electrophysiology

Extracellular recordings—After isoflurane anesthesia, the brain was placed in ice-cold oxygenated (95%O_2/5%CO_2) sucrose-based artificial cerebrospinal fluid (ACSF, in mM: 126.0sucrose, 5.0KCl, 2.4$CaCl_2$, 1.0$MgSO_4$, 26.0$NaHCO_3$, 1.25NaH_2PO_4, 10.0D-glucose), and 400μm–thick slices were cut in ice-cold ACSF (Vibroslice; Stoelting). Standard recording procedures were used (Supp. Information; Scharfman et al., 2007). All experiments required a stable baseline (fEPSP slope within 95–105% of the mean of the first 3 fEPSPs for 15min). For theta-burst stimulation (TBS), 10 trains of 4 half-maximal stimuli at 100Hz were triggered 200ms apart. For high-frequency stimulation (HFS), 4 trains

(100Hz, 1sec) of half-maximal stimuli were triggered 20sec apart. Low-frequency stimulation (LFS) was a 3Hz, 5min train of half-maximal stimuli. fEPSPs were normalized to the average of the baseline. LTP or LTD was defined from the average of 3 responses evoked 60min after induction. LTP and LTD incidence was defined by greater than 110% change (LTP) or less than 90% (LTD). Analysis used pClamp (Molecular Devices; Supp. Fig. 1).

Whole cell recording—Slices were warmed (32°C) in oxygenated sucrose-ACSF (1hr), placed in a submerged chamber (Model RC-27D; Warner), and perfused with NaCl-ACSF at 2–3ml/min at 32°C (Inline heater, Model TC-324B; Warner). Recording electrodes used 0.85mm inner/1.5mm outer diam. glass (KG-33; Garner Glass) and were filled with 90 potassium gluconate, 40KCl, $2MgCl_2$, 10EGTA, 10HEPES, $0.3Na_2GTP$, $2Na_2ATP$, and $1Na_2$-phosphocreatine (280–300mOsm). Recordings were amplified (Axopatch 200B, Molecular Devices), digitized (Digidata 1322A, Molecular Devices), and analyzed using Minianalysis (Synaptosoft). Spontaneous events were confirmed by manual inspection. Membrane potentials were corrected for liquid junction potentials. Cell that changed series resistance >15% were discarded.

Behavior

The MWM used a 119cm diam. tub with a white platform hidden 1cm below the water surface. The water (20–23°C) was dyed with non-toxic, white, washable paint to conceal the platform. Black cardboard shapes were placed on room walls for external cues. Mice were handled by a blinded experimenter 1×/day for 3–5days and then trained 2×/day (20–30min interval) for 9days with the platform in the same location, and randomly-assigned, alternate starting points. Time was measured for the mouse to find the platform, and stay there for 2sec. The mouse was allowed 60sec to find the platform, and if it did not, it was placed on the platform, and 60sec was designated as its time. One day after the 9-day acquisition phase, mice were subjected to a probe trial to measure retention. The mouse was placed in the water, without the platform, and tracked for 60sec using video and Ethovision software (v. 3.1; Noldus) to determine the time to reach the platform location during the training phase.

For CFC, conditioning and testing took place in 4 PhenoTyper (Noldus) conditioning chambers as previously described (Pham et al., 2008). OP was tested using standard methods with a 1 hr or 24 hr interval between the two trials (Scharfman et al., 2007; Supp. Information).

Immunoprecipitation (IP) and Western blot

fEPSPs were recorded to confirm slices were viable (>5mV amplitude) for 1–3hrs, the time when LTP/LTD was tested, and then frozen in liquid N_2. For p75NTR IP/Western blot, brain slices were lysed (lysis buffer: 1×Tris buffered saline, 1% NP-40, 1%Triton-100, 1mM PMSF, 10% glycerol, and protease inhibitors) for 30min on ice. Lysates were further triturated using a 30g needle, and supernatants were collected following centrifugation (14,000 rpm, 5min). 1.5mg total protein was immunoprecipitated using biotinlyted anti-p75NTR antibodies (#BAF1157; R&D Systems; Supp. Information). Lysates were pre-treated using streptavidin agarose resins (Thermo Scientific), and the supernatant was incubated with biotinylated anti-p75NTR antibodies overnight at 4°C. Streptavidin agarose resins were calibrated with lysis buffer and then added to the supernatant (2hrs, 4°C), rotated, and immunoprecipitates were collected (5min, 5,000rpm). Resins were washed in cold lysis buffer (3×10min), and immune-complexes were resolved by SDS-PAGE. Following transfer, blots were incubated with anti-p75NTR antibodies (1:6,000; Esposito et al., 2001; Supp. Information) for 2hrs at room temperature. Blots were developed using

horse-radish peroxidase-conjugated secondary antibodies and developed with an ECL kit (Amersham). For the TrkB Western blot, 60µg total protein lysates were loaded onto a SDS-PAGE gel, and after transfer, the blot was blocked with 5% skim milk in Tris buffered saline/Tween20 overnight at 4°C. The blot was incubated with anti-TrkB antibodies (1:1,500; #07-225, Millipore; Suppl. Information), anti-β-actin (1:3,000; Sigma) and developed as described above. ImageJ software was used for quantification.

RESULTS

Basal Transmission

Extracellular recordings—WT (n=25 slices, 18 mice) and KO mice (n=19 slices, 15 mice) were age-matched (Suppl. Table 1). For these experiments and others, 1–2 slices/animal were used. There were no significant differences in the input-output curves (fEPSP slope, amplitude, area, and duration; Fig. 1A, Supp. Fig. 2; Two-way repeated measures ANOVA, p>0.05) or the maximum fEPSP slope, amplitude, area, total duration, half-duration, or latency to peak (Table 1). There were no differences in the mean amplitude or latency to peak of the fiber volley (Table 1), or the change in fiber volley amplitude with increasing fEPSP slope or amplitude (Supp. Fig. 2). There were no differences in paired-pulse facilitation of fEPSP slope, amplitude, area, or duration (Fig. 1A; Supp. Table 2).

Whole cell recordings—Whole cell recordings were made from CA1 pyramidal cells using age-matched (Supp. Table 1) mice (n=7/group). Spontaneous postsynaptic currents (sPSCs) were recorded at −90mV for 3min, then 0.6µM tetrodotoxin was added for 15min, and miniature PSCs (mPSCs) were recorded for 3min. As shown in Fig. 1B, sPSC and mPSC frequencies were not significantly different (sPSCs, WT: 7.86±1.39 Hz; KO: 8.18±0.419 Hz; Student's t-test, p=0.6018; mPSCs, WT: 4.41±0.75 Hz; KO: 3.98±1.06 Hz; Student's t-test, p=0.6412), amplitudes were not different (sPSCs, WT: 28.8±2.6 pA; KO: 31.0±2.1 pA; Student's t-test, p=0.5331; mPSCs, WT: 14.1±0.5 pA; KO: 13.28±1.4 pA; Student's t-test, p=0.5757), and there were no differences in cumulative probability (Kolmogorov-Smirnov tests, sPSCs, p=0.1144; mPSCs, p=0.9670; Fig. 1B).

Synaptic plasticity

TBS-LTP—Age-matched WT (14 slices, 11 mice) and KO mice (12 slices, 11 mice; Supp. Table 1) were used. There were no significant differences in post-tetanic potentiation (PTP), evaluated 1, 2, or 3min after TBS (Fig. 1C, Supp. Table 3). LTP was reduced in magnitude in slices from KO mice at 60min (WT: 134.94±6.79%; KO: 105.92±8.78%; Student's t-test, p=0.01582; Fig. 1C). The incidence of LTP was lower in KO mice (WT: 85.7%; KO: 50.0%; χ^2 test, p=0.04918).

Surprisingly, a delayed LTD occurred in KO mice after TBS (Fig. 1C). A possible explanation was that slices from KO mice were unable to maintain an evoked response, because slices are constantly perfused with an aqueous medium, a possible challenge to a slice that might normally require AQP4 to control water balance. Therefore, additional experiments were conducted in slices of KO mice to evaluate whether the fEPSPs decayed slowly. There was no significant decay in the fEPSP slope (Baseline: 100.13±1.09%; After 75min: 98.69±2.37%; n=6 slices; Paired t-test, p=0.2348). Therefore, TBS evoked LTD in KO mice. To examine LTD further, LFS was used to induce LTD.

LTD—Age-matched WT (10 slices, 7 mice) and KO mice (12 slices, 10 mice; Supp. Table 1) were used. Slices that are used long after the dissection exhibit a form of LTD that is distinct (Dudek and Bear, 1993, Bear, 2003), so slices were used at similar times relative to the slice preparation (WT: 2.57±0.35hrs; KO: 3.08±0.29hrs; Student's t-test, p=0.2658).

There were no significant differences in short-term depression (STD; Fig. 1D, Supp. Table 3), but LTD was reduced in KO mice (Fig. 1D; WT: 66.07±8.03%; KO: 107.55±8.87%; Student's t-test, p=0.002816) and LTD incidence was lower (WT: 80.0%; KO: 33.3%; Fisher's exact test, p=0.02861). Interestingly, a delayed LTP followed LFS in KO mice (Fig. 1D).

HFS-LTP—Age-matched WT (11 slices, 10 mice) and KO mice (11 slices, 11 mice; Supp. Table 1) were used. PTP was not different (Fig. 1E; Supp. Table 3). As shown in Fig. 1E, there were no detectable differences in LTP amplitude, 60min after HFS (WT: 155.15±11.78%; KO: 132.07±10.50%; Student's t-test, p=0.1617) or incidence (WT: 90.9%; KO: 54.5%; χ^2 test, p=0.05551).

Behavior

Twenty WT and 19 KO litter-matched mice, 12–19 weeks old, were used for MWM testing and 2–3 weeks later for CFC. WT and KO mice exhibited similar performance (Fig. 2A). Both groups achieved a plateau at approximately day 7, resulting in average time-to-platform values on day 7 that were not different (WT: 26.98±5.78sec; KO: 28.36±4.63sec; Student's t-test, p=0.8520). No differences were observed in the probe trial: the latency to reach the quadrant where the platform was placed during training was 31.38±5.46sec for WT and 27.65±4.50sec for KO mice (Student's t-test, p=0.5990; Fig. 2A, Suppl. Fig. 4). Swimming speed, tested on the day of the probe trial, was not significantly different (WT: 24.12±1.15cm/sec; KO: 26.18±0.76cm/sec; Student's t-test, p=0.1304).

When CFC was tested, both genotypes exhibited similar levels of immobility during conditioning (WT: 11.8±0.02%; KO: 19.0±0.03%; Student's t-test, p=0.3120), and during the retention test (WT: 41.6±0.06%; KO: 50.8±0.07%; Student's t-test, p=0.3170; Fig. 2B).

OP was tested in additional mice (WT: 10–16 weeks-old, n=11; KO: 12–17 weeks-old, n=10; Fig. 2C). WT and KO mice spent the same percent time exploring the objects when they were first presented (Object 1, WT: 48.69±1.45 %; KO: 48.10±2.71 %; Student's t-test, p=0.8470; Object 2, WT: 51.31±1.45%; KO: 51.90±2.71%; Student's t-test, p=0.8470). When mice were retested, WT mice spent more time than KO mice exploring the object that was moved (WT: 64.77±1.81; KO: 52.54±2.21%; Student's t-test, p=0.0003041). KO mice did not prefer the moved object (Object 2; Trial 1: 51.90±2.71% vs. Trial 2: 52.54±2.21%; Paired t-test, p=0.8476) but WT mice did (Trial 1: 51.31±1.45%; Trial 2: 64.77±1.81%; Paired t-test, p=0.00006438). Similar results were obtained in additional mice (WT, n=13; KO, n=14) when a 24 hr interval was used (Fig. 2C; Supp. Information). WT mice spent more time exploring the moved object than the stationary object during the test period (p=0.03559) but the KO mice did not (p=0.13305).

Neurotrophins

The results suggest that there was a defect in synaptic plasticity in KO mice that may be related to BDNF and its TrkB receptor, because maintenance of TBS-LTP requires BDNF and TrkB, whereas HFS-LTP does not (Kang et al., 1997; Chen et al., 1999). The LTD impairment in KO mice also suggested a BDNF-associated mechanism, because LFS induced LTP instead of LTD (Fig. 1D; Supp. Fig. 3) and it has been shown that adding BDNF to slices reduces LTD by inducing a superimposed LTP (Akaneya et al., 1996; Aicardi et al., 2004). Therefore, we asked if scavenging BDNF or interfering with TrkB would rescue LTD. As shown in Fig. 3A1, exposure to TrkB-Fc, in concentrations previously shown to scavenge BDNF (Kang et al., 1997) rescued LTD in KO mice (KO +TrkB-Fc, mean slope 60min after TBS: 71.75±2.36%, n=8 slices, 8 mice; KO+IgG control: 109.01.01±10.76%, n=7 slices, 7 mice; Student's t-test, p=0.005729). K252a (300 nM), a

Trk antagonist, also rescued LTD (KO+K252a, slope 60min after TBS: 55.05±4.560%, n=8 slices, 8 mice; KO+0.05% dimethylsulfoxide (DMSO) control: 95.80±8,003%, n=9 slices, 9 mice; Student's t-test, p=0.001096; Fig. 3A2).

To determine whether the impairments in LTP and LTD in KO mice were related to receptor levels of TrkB, which is critical to TBS-LTP (Chen et al., 2010), Western blots for TrkB (n=4) and IP/Western blots for p75NTR (n=4) were conducted using slices from age-matched WT (38 slices, 11 mice) and KO mice (51 slices, 12 mice; Supp. Table 1). There were no differences in full-length or truncated TrkB between genotypes (Fig. 3B1). However, p75NTR levels were reduced in KO mice (Fig. 3B2; p=0.0001980).

DISCUSSION

Summary

We found that AQP4 KO mice exhibit a defect in TBS-LTP and LTD, without detectable impairments in synaptic transmission and short-term plasticity. Behavior was also impaired in KO mice, but interestingly, only when a test for location-specific object memory was used.

Our data support an unanticipated role for AQP4 in long-term plasticity and spatial memory. The caveat to this conclusion is the possibility that effects were compensatory to changes induced by deletion of AQP4 during development. Therefore, we cannot rule out the possibility that AQP4 deletion had effects during development that contributed to the results. However, the specificity of impairments in KO mice make compensation seem unlikely. The results also provide new insight into the mechanisms underlying glial regulation of synaptic plasticity - an unanticipated role of AQP4. Finding a role of glia in regulating plasticity that is associated with neurotrophins is also important because neurotrophins have many important roles in the CNS.

Regulation of neurotrophin-mediated functions by AQP4

The defect in LTD was rescued by a scavenger of BDNF or an antagonist of Trk receptors, suggesting that the impairment in LTD was caused by excess BDNF released during LFS acting at TrkB to induce LTP, which was superimposed on LTD and therefore reduced LTD, which has been shown in rat cortical slices (Akaneya et al., 1996). However, we cannot rule out other neurotrophin-mediated mechanisms unrelated to BDNF release, such as altered p75NTR, because p75NTR levels were reduced in KO slices. Also, NT-3 could be involved because it binds to TrkB.

It is interesting to consider how AQP4 regulates neurotrophin-dependent plasticity. We suggest that neurotrophins are dysregulated in KO mice. This hypothesis is based on the evidence that neurotrophin levels during induction are critical to LTP (Chen et al., 1999), and the results showing that LTD was rescued by pharmacological antagonism of TrkB activation during induction. One reason could be related to the fact that glia normally release neurotrophins, which has been shown in culture (Parpura and Zorec, 2009). Another reason could be due to the low levels of p75NTR in KO mice. This impairment could be important because p75NTR mediates LTD in response to proBDNF (Woo et al., 2005). However, scavenging neurotrophins that bind to TrkB, or blocking Trk completely rescued LTD, suggesting that reduced p75NTR is not necessary to explain the LTD defect. Nevertheless, reduced p75NTR is significant because of the important functions of p75NTR in the normal developing and adult brain, as well as cell death after injury (Casaccia-Bonnefil et al., 1998; Barker and Kennedy, 2005; Volosin et al., 2007; Angelo et al., 2009). Our data also suggest that AQP4 could be important to the regulation of p75NTR by osmolarity (Ramos et al., 2007).

AQP4 and cognitive function

When interpreting the LTP, LTD and behavioral data, it is important to consider that defects in LTP are not always associated with impairment in hippocampal-dependent tasks, such as the MWM (Jun et al., 1998, Leiva et al., 2009), and vice-versa (von Engelhardt et al., 2008). In the CD1 mouse strain it is important to note that the probe trial is normally not performed well (Adams et al., 2002). Nevertheless, the behavioral data are interesting, because KO mice were impaired in OP, but not MWM or CFS. It is unlikely that OP was selectively impaired in KO mice because performance depended on a relatively short interval between training and testing compared to MWM or CFC, because OP was impaired using a 24 hr interval, similar to CFC. Instead, the reason OP was impaired may be due to the fact that different circuitry is required for this task, or the circuitry required for OP depends more on neurotrophins. Indeed, there is support for the idea that OP is BDNF-dependent, because of studies using transgenic mice that simulate Alzheimer's disease. Early in life, when BDNF levels decline, there are OP defects (Burghardt et al., 2010) as well as impairment in object recognition (Francis et al., 2010).

Implications

The results have potential clinical significance because AQP4 regulates edema after injury (Papadopoulos and Verkman, 2007, Kimura et al., 2010). Our data suggest that AQP4 not only has the potential to regulate edema, but also recovery following injury, because BDNF and p75NTR influence the extent of cell death after injury, and BDNF is important to the plasticity that facilitates recovery (Lykissas et al., 2007).

Acknowledgments

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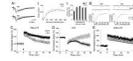
Fig. 1. Selective impairments in synaptic plasticity in AQP4 KO mice
A. 1. Superimposed fEPSPs evoked with increasing stimulus intensities (by increasing the duration of the stimulus, in μsec) show similar responses in WT and KO mice. 2. Quantified fEPSP slopes show similarity of WT and KO mice. 3. Similarity of paired-pulse facilitation of fEPSP slope (half-maximal stimuli) in WT and KO mice.
B. Top: Similar sPSCs in WT and KO pyramidal cells. Bottom: Cumulative frequency plots did not demonstrate differences in genotypes.
C. Comparison of TBS-LTP in WT (black) and KO (white) mice.
D. Comparison of LTD in WT and KO mice.
E. Comparison of HFS-LTP in WT and KO mice.
Arrows denote TBS and HFS; LFS is marked by the bar. Additional data are in the text and Supplemental Information.
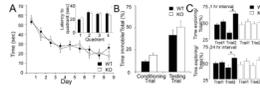
Fig. 2. Selective OP deficit in KO mice
A. In the MWM, there were no significant differences in the average time to locate the hidden platform, plotted for each day animals were tested. Inset: There were no differences between genotypes in the probe trial (1,2,3,4 reflect each quadrant; 4=target quadrant; see also Suppl. Fig. 4). Note that CD1 mice normally do not exhibit a preference for the target quadrant in the probe trial (Adams et al., 2002).
B. In CFC, there were no significant differences between WT and KO mice in the the conditioning or testing trials.
C. KO mice were impaired in OP because they did not spend more time in Trial 2 exploring the moved object, but WT mice did.
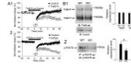
Fig. 3. Neurotrophin signaling in WT and KO mice
A. 1. TrkB-Fc rescued LTD in KO mice. Slices from KO mice were exposed (thick bar) to TrkB-Fc or control IgG for 15min before and after LFS. 2. K252a rescues LTD in KO mice. Experiments analogous to those in part A were conducted except the slices were exposed to K252a (300 nM) or vehicle (0.05% DMSO).
B. 1. Full-length (F) and truncated (T) TrkB levels were not significantly different in WT and KO mice. 2. WT mice had reduced p75NTR receptor levels.

Table 1

Similarity of fEPSPs in slices of WT and KO mice.

	Field EPSP				Duration		Volley	
	Maximum slope	Maximal amplitude	Area under the curve (AUC)	Latency to peak	Half-dur.	Total dur.	Maximal amplitude	Latency to peak
	(V/sec)	(mV)	(mV*msec)	(msec)	(msec)	(msec)	(mV)	(msec)
Wild Type								
mean	0.539	7.49	7.40	3.345	9.52	35.39	3.33	1.721
sem	0.025	0.43	0.815	0.073	0.48	0.77	0.56	0.086
n (slices)	25	25	25	25	25	25	20	20
Knockout								
mean	0.638	8.19	6.89	3.325	9.08	33.17	3.54	1.562
sem	0.058	0.43	0.846	0.086	0.53	1.04	0.46	0.072
n (slices)	19	19	19	19	19	19	23	23
p	0.0991	0.2653	0.6683	0.7661	0.6662	0.2086	0.8099	0.2319

There were no differences in fEPSPs and fiber volleys in slices from WT and KO mice. Half-dur. = half-duration; Total dur. = total duration.