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Aquaporin-4 water channels and synaptic plasticity in the hippocampus

Helen E. Scharfman [a,b], Devin K. Binder [c,*]

[a] Center for Dementia Research, The Nathan Kline Institute for Psychiatric Research, 140 Old Orangeburg Rd., Bldg. 35, Orangeburg, NY 10962, United States
[b] Departments of Child and Adolescent Psychiatry, Physiology and Neuroscience, and Psychiatry, New York University Langone Medical Center, New York, NY 10016, United States
[c] Center for Glial-Neuronal Interactions, Division of Biomedical Sciences, School of Medicine, University of California, Riverside, CA 92521, United States

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ABSTRACT

Aquaporin-4 (AQP4) is the major water channel expressed in the central nervous system (CNS) and is primarily expressed in glial cells. Many studies have shown that AQP4 regulates the response of the CNS to insults or injury, but far less is known about the potential for AQP4 to influence synaptic plasticity or behavior. Recent studies have examined long-term potentiation (LTP), long-term depression (LTD), and behavior in AQP4 knockout (KO) and wild-type mice to gain more insight into its potential role. The results showed a selective effect of AQP4 deletion on LTP of the Schaffer collateral pathway in hippocampus using an LTP induction protocol that simulates pyramidal cell firing during theta oscillations (theta-burst stimulation; TBS). However, LTP produced by a different induction protocol was unaffected. There was also a defect in LTD after low frequency stimulation (LFS) in AQP4 KO mice. Interestingly, some slices from AQP4 KO mice exhibited LTD after TBS instead of LTP, or LTP following LFS instead of LTD. These data suggest that AQP4 and astrocytes influence the polarity of long-term synaptic plasticity (potentiation or depression). These potentially powerful roles expand the influence of AQP4 and astrocytes beyond the original suggestions related to regulation of extracellular potassium and water balance. Remarkably, AQP4 KO mice did not show deficits in basal transmission, suggesting specificity for long-term synaptic plasticity. The mechanism appears to be related to neurotrophins and specifically brain-derived neurotrophic factor (BDNF) because pharmacological blockade of neurotrophin trk receptors or scavenging ligands such as BDNF restored plasticity. The *in vitro* studies predicted effects *in vivo* of AQP4 deletion because AQP4 KO mice performed worse using a task that requires memory for the location of objects (object placement). However, performance on other hippocampal-dependent tasks was spared. The results suggest an unanticipated and selective role of AQP4 in synaptic plasticity and spatial memory, and underscore the growing appreciation of the role of glial cells in functions typically attributed to neurons. Implications for epilepsy are discussed because of the previous evidence that AQP4 influences seizures, and the role of synaptic plasticity in epileptogenesis.

1. Introduction

1.1. Astrocytes and synaptic plasticity

In addition to their long-established roles in structural and metabolic support to neurons, recent studies suggest that astrocytes play a significant role in synaptic plasticity, i.e., the ability of neurons to change the strength of synapses (Barker and Ullian, 2010). First, the structural organization of astrocytes, with numerous perisynaptic processes, indicates that they would be likely to influence many synapses (Ventura and Harris, 1999). Astrocytes are organized into 'domains' and each astrocyte may contact 300–600 dendrites and $\sim 10^5$ synapses (Bushong et al., 2003; Halassa et al., 2007; Ogata and Kosaka, 2002). Second, astrocytes secrete factors that have been shown to directly influence formation of synapses (Ullian et al., 2001), *e.g.* glia-derived cholesterol (Mauch et al., 2001) and thrombospondins (Christopherson et al., 2005). Astrocyte-conditioned media can influence inhibitory synapse formation as well, which appears to be mediated by trkB-dependent mechanisms (Elmariah et al., 2005). Direct neuron-astrocyte contact is also important for excitatory synaptogenesis (Hama et al., 2004). Third, astrocytes influence the trafficking of AMPA receptors (AMPARs) to synapses. In particular, TNF-α, an astrocyte-released cytokine, increases synaptic AMPARs (Beattie et al., 2002). Subsequent studies have shown that TNF-α plays a role in synaptic "scaling" (Stellwagen and Malenka, 2006), and TNF-$\alpha^{-/-}$ mice have impaired activity-dependent plasticity in visual cortex (Kaneko et al., 2008). Fourth, astrocytes directly release factors that affect synaptic transmission. These 'gliotransmitters' include glutamate, ATP, and D-serine (Halassa and Haydon, 2010).

* Corresponding author. Tel.: +1 951 827 2224; fax: +1 951 827 5504.
E-mail address: dbinder@ucr.edu (D.K. Binder).

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Thus, the role of astrocytes in formation and modulation of synapses is well-established. However, the role of astrocyte-specific proteins and transporters in synaptic plasticity is only beginning to be elucidated. In this paper, we review results from experiments using mice with a deletion of the astrocyte-specific channel aquaporin-4 (AQP4) on hippocampal synaptic plasticity and spatial memory function (Skucas et al., 2011).

1.2. Aquaporin-4

AQP4 is one of 14 members of the AQP family, which regulate water transport (Badaut et al., 2002; Verkman et al., 2006). AQP4 is the primary AQP in the CNS, and is expressed selectively in glial cells, primarily in astrocytic endfeet at the blood–brain barrier (Nagelhus et al., 2004; Oshio et al., 2004) although also in the CNS parenchyma in a developmental and laminar-specific manner (Hsu et al., 2011). Because AQP4 is expressed in glia preferentially, an AQP4 knockout (KO) mouse provides an opportunity to investigate the role of fluid regulation by astrocytes.

Using AQP4 KO mice generated in Alan Verkman's laboratory (Ma et al., 1997), a number of studies have shown an important role of AQP4 in the regulation of edema following various types of insults (Manley et al., 2000; Papadopoulos and Verkman, 2007; Verkman et al., 2006). AQP4 also appears to be involved in other types of pathological conditions that are modulated by changes in the extracellular space, such as seizure threshold in epilepsy (Hsu et al., 2007). Normal functions of the CNS are also potentially influenced by AQP4, such as sensory function, because AQP4 KO mice have impaired hearing and smell (Li and Verkman, 2001; Lu et al., 2008; Mhatre et al., 2002).

Less is known about the potential roles of AQP4 in synaptic transmission. There is good reason to suggest a possible contribution of AQP4 to synaptic transmission, because phenomena that are regulated by water transport, such as extracellular space, have robust effects on synaptic transmission (Chebabo et al., 1995; Huang et al., 1997; Simard and Nedergaard, 2004), and AQP4 KO mice have increased extracellular space (Binder et al., 2004b). In addition, metabolic inhibitors of glia decrease synaptic transmission in the hippocampus (Keyser and Pellmar, 1994, 1997). AQP4 is coupled to a subset of potassium channels, Kir4.1 and Kir5.1 (Nagelhus et al., 2004), so it could influence synaptic transmission by effects on $[K^+]_o$ and glial $[K^+]_i$.

Studies that are relevant to this issue have shown that there are prolonged increases in $[K^+]_o$ in response to electrical stimulation in AQP4 KO mice, and an increase in seizure duration (Binder et al., 2006). These studies would suggest that increased excitability would occur if AQP4 channels were deleted. However, the effects of AQP4 deletion appear to be more complex because AQP4 KO mice had higher seizure thresholds than WT mice, not lower thresholds (Binder et al., 2004a, 2006; Hsu et al., 2007). Evaluation of the functional coupling of AQP4 to Kir4.1 or Kir5.1 has not clearly established a functional role related to Kir4.1 or Kir5.1, with evidence against such a role (Zhang and Verkman, 2007) as well as evidence for it (Soe et al., 2009). Therefore, one would predict that AQP4 would modulate synaptic transmission, but it is hard to predict exactly how.

AQP4 could also influence long-term potentiation (LTP), because the increased afferent activity associated with LTP induction would be likely to accentuate any effect of AQP4 on synaptic transmission. For example, during the bursts of neuronal activity that are used to initiate LTP, the expanded extracellular space in AQP4 KO mice might increase further, diluting synaptic concentrations of molecules that are critical to LTP. By effects on $[K^+]_o$, AQP4 could also influence postsynaptic membrane potential, which is important to LTP induction because it regulates the degree of voltage-dependent block of NMDA receptors. Membrane potential can also influence the coordinated pre- and postsynaptic activity that is thought to be important to LTP by depolarizing or hyperpolarizing postsynaptic neurons. Other reasons to suspect a role of AQP4 in synaptic plasticity are based on published data, such as the finding that AQP4 KO mice have a defect in activity-dependent swelling of astrocytes (Kitaura et al., 2009). Furthermore, several studies suggest that astrocytes are particularly prone to swelling (Andrew et al., 2007; Risher et al., 2009; Somjen et al., 1993) and release glutamate when they are stimulated to swell *in vitro* (Abdullaev et al., 2006; Haskew-Layton et al., 2008; Liu et al., 2006). Thus, glial-derived glutamate from astrocytic swelling could influence nearby neurons and their synapses. As discussed above, there is growing evidence that glia play a role in LTP (Filosa et al., 2009; Haydon et al., 2009; Oliet and Bains, 2007; Paerea et al., 2009; Stevens, 2008; Todd et al., 2006), although the proposed roles of astrocytes in LTP do not directly implicate AQP4, and few studies have evaluated long-term depression (LTD).

To address the hypothesis that AQP4 influences synaptic plasticity, and therefore is one of the ways that astrocytes regulate synaptic plasticity, we used hippocampal slices from AQP4 WT and KO mice to evaluate two common forms of LTP, as well as Ltd. The Schaffer collateral synapse in area CA1 is a logical choice because more is known about LTP (Bliss et al., 2004; Kerchner and Nicoll, 2008; Lisman and Raghavachari, 2006; MacDonald et al., 2006) and LTD (Kirkwood et al., 1993; Pöschel and Stanton, 2007) at this synapse than perhaps any other. In addition, tasks that involve the hippocampus have also been studied in AQP4 KO and WT mice, such as the Morris water maze, as well as contextual fear conditioning, and object placement (Luiz Assini et al., 2009; Morris et al., 1982; Parkinson et al., 1988; Phillips and LeDoux, 1992). These tasks not only involve hippocampus, but require area CA1 subfield specifically (Ji and Maren, 2008; Luiz Assini et al., 2009; Tsien et al., 1996), so they complement studies *in vitro* that address synaptic plasticity in area CA1. Here we review the intriguing data collected from these studies and their implications. One of the interesting implications is for epileptogenesis, in light of studies that show an effect of AQP4 on seizures (discussed above), and the suggestions that synaptic plasticity plays a critical role in epileptogenesis (Engel, 2001; Scharfman, 2002; Stefan, 1999).

1.2.1. Synaptic transmission in AQP4 KO and WT mice

In hippocampal slices, Skucas et al. (2011) showed that differences in Schaffer collateral transmission between AQP4 WT and KO mice could not be detected. Slices were made from approximately 1-month-old male WT and KO mice; there were no significant differences in slope, amplitude, the integrated area of the fEPSP (area under the curve), total duration, or half-duration (Skucas et al., 2011). There also were no differences in the latency of fEPSPs, the input–output curve of the fiber volley or latency to peak of the fiber volley (Skucas et al., 2011). When fiber volleys were plotted in relation to fEPSPs, WT and KO mice were similar (Skucas et al., 2011). There were no differences between slices from WT and KO mice in paired-pulse facilitation of fEPSPs either (maximal slope, amplitude, area under the curve, or duration) (Skucas et al., 2011). Tables 1 and 2 present a summary of the data using extracellular recordings in Skucas et al. (2011).

Whole cell recordings were used to ask if higher resolution recordings of single pyramidal cells could identify differences that would be missed by population recordings. The single cell recordings showed that the frequency, amplitude, and cumulative probability of spontaneous postsynaptic currents (sPSCs) and miniature PSCs were not significantly different when pyramidal cells from WT and KO mice were compared (Skucas et al., 2011).

One might expect that a defect in astrocytic AQP4 might alter neurons but there was no detectable difference in the ability to form a gigaseal, or for them to deteriorate after breakthrough

Table 1
Similarity of fEPSPs in slices of WT and KO mice.

	fEPSP						Volley	
	Maximum slope	Maximal amplitude	Area under the curve	Latency to peak	Duration		Maximal amplitude	Latency to peak
	(V/s)	(mV)	(mV∗msec)	(msec)	Half-dur (msec)	Total dur (msec)	(mV)	(msec)
WT								
Mean	0.5394	7.49	7.4	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
KO								
Mean	0.638	8.19	6.89	3.325	9.08	33.17	3.54	1.562
Sem	0.058	0.438	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

Comparisons of fiber volleys and fEPSPs in slices from WT and KO mice. From supplemental material of Skucas et al. (2011).

Table 2
Similarity between slices of WT and KO mice in short-term plasticity.

		Time after stimulation			
		1 min (%)	2 min (%)	3 min (%)	5 min (%)
TBS	WT				
	Mean	159.19	158.32	157.77	160.93
	Sem	6.70	7.10	8.05	6.44
	n (Slices)	14	14	14	14
	KO				
	Mean	153.94	153.47	149.43	148.86
	Sem	6.45	9	4.11	4.14
	n (Slices)	12	12	12	12
	p	0.5811	0.6031	0.3889	0.1423
LFS	WT				
	Mean	72.64	75.38	76.52	84.7
	Sem	2.79	3.21	2.15	3.45
	n (Slices)	10	10	10	10
	KO				
	Mean	80.19	83.61	85.93	90.78
	Sem	3.60	2.94	3.86	4.12
	n (Slices)	12	12	12	12
	p	0.1244	0.0735	0.05751	0.1350
HFS	WT				
	Mean	190.36	200.57	193.31	193.77
	Sem	14.34	9.82	9.39	10.51
	n (Slices)	9	9	9	9
	KO				
	Mean	175.55	161.42	162.1	166.12
	Sem	14.53	11.92	13.16	12.32
	n (Slices)	9	9	9	9
	p	0.4789	0.1935	0.3929	0.2144

Comparisons of PTP and STD for slices from WT and KO mice that were tested using protocols to induce LTP (TBS, HFS) or LTD (LFS). The % potentiation or depression relative to the mean of the baseline period is listed, ±sem. From supplemental material of Skucas et al. (2011).

(manifested by an increase in access resistance >15% in the first 3 min of recording). For WT mice, 8 of the 26 cells (30.7%) that were patch-clamped increased access resistance >15% within the first minutes of recording, and therefore were not used for evaluating PSCs. For KO mice, increased access resistance after the first minutes of recording occurred in 15 of the 35 cells (43.0%), which also were not used for PSC analysis. These percentages were not statistically different (χ^2 test, $p = 0.1632$).

These data suggested that, remarkably, there was no effect of AQP4 deletion on basal or spontaneous miniature synaptic currents. In addition, presynaptic control of synaptic transmission did not appear to be affected based on the lack of differences in paired pulse facilitation. Although no effects of AQP4 deletion were detected, it would be valuable to reassess basal synaptic transmission *in vivo* to determine whether it is affected by deletion of AQP4. There also remains the possibility that AQP4 deletion could modulate some synapses more than others and the Schaffer collateral pathway is relatively unaffected. However, a robust effect of AQP4 deletion was detected on Schaffer collateral LTP, as described below, arguing against this idea.

1.2.2. Synaptic plasticity in vitro: LTP and LTD

1.2.2.1. Theta-burst stimulation (TBS)-LTP. Skucas et al. (2011) showed that LTP was reduced in slices from KO mice compared to slices from WT mice. The mean maximal fEPSP slope, measured 60 min after TBS was 134.9 ± 6.8%, $n = 14$ in WT mice and 105.9 ± 8.8%, $n = 12$ in KO mice (Student's *t*-test, $p = 0.0158$). However, PTP was unaffected, consistent with a lack of effect of AQP4 on presynaptic mechanisms (Table 2). Additional analyses of the data from Skucas et al. (2011), shown in Fig. 1, demonstrated no correlation between the degree of PTP and LTP, using slope of the fEPSP to evaluate the extent of potentiation. This comparison was valuable because the slices from KO mice exhibited reduced LTP, which could have simply been due to a tendency to exhibit weaker PTP.

To probe the differences in TBS-LTP further, the incidence of LTP was analyzed, based on the definition that LTP is an increase in fEPSP slope >110% of the baseline mean, measured 60 min after TBS. In this analysis, an assumption was made that LTP is distinct from LTD and can be considered as "binary" responses to repetitive stimulation (either LTP or no LTP in response to high frequency stimulation; either LTD or no LTD in response to LFS) rather than a "continuum" where repetitive stimulation can lead to potentiation, very little effect, or depression. This assumption was made to examine incidence of LTP for those experiments that used high-frequency trains. Interestingly, the incidence of LTP was statistically different in WT and KO mice: 86.7% of slices from WT mice exhibited LTP, whereas 6/12 (50.0%) of the slices from KO mice demonstrated potentiation (χ^2 test, $p = 0.0492$). Interestingly, when only those slices that exhibited LTP were compared, the potentiation of the slope of the fEPSP was not significantly different (Fig. 1). For those slices that were from WT mice, the slope was 140.7 ± 6.5% of baseline; for KO mice, the slope was 127.3 ± 5.0% of baseline ($p = 0.1563$; data from Skucas et al., 2011). These data suggested that the differences between WT and KO mice were not present in the slices which exhibited LTP, but were in the slices which did *not* have LTP. Therefore, the data from slices without LTP were evaluated for both WT and KO mice. The comparison was intriguing – although few WT mice lacked LTP so statistical comparisons could not be made. There appeared to be LTD in the slices from KO mice that failed to exhibit LTP, rather

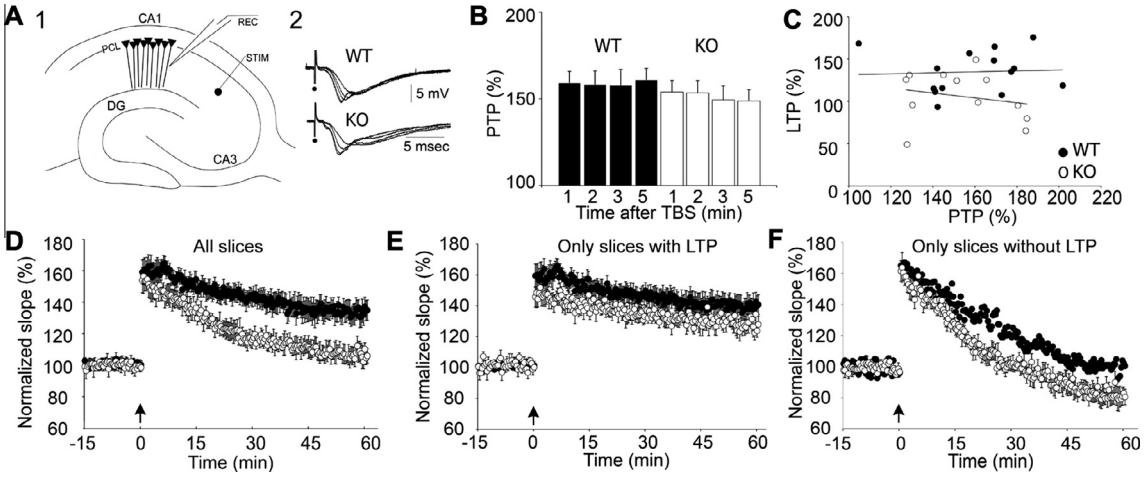

Fig. 1. HFS-LTP in AQP4 WT and KO mice. (A1) A diagram shows the recording and stimulating electrode locations that were used to record fEPSPs. (A2) A representative fEPSP, evoked using a range of stimulus strengths from minimum to maximum. Examples are from a WT mouse (top) and AQP4 KO mouse (bottom). (B) Mean (±sem) percent PTP of fEPSP slopes at 1, 2, 3, and 5 min after TBS were similar for WT (black bars) and KO mice (white bars). There were 14 slices from 11 WT mice and 12 slices from 11 KO mice. (C) PTP (measured 1 min after TBS) is plotted as a function of LTP (measured 60 min after TBS) in the same slice. For WT (black circles) and KO mice (white) the correlations were not significant. (D) Pooled data from all slices showed that there was decreased LTP (measured 60 min after TBS) of the fEPSP slope in KO mice (WT, $n = 14$; KO, $n = 12$). (E) Data are shown only for those slices that exhibited LTP (WT, $n = 12$; KO, $n = 6$). (F) Data are shown only for those slices that did *not* demonstrate LTP (WT, $n = 2$; KO, $n = 6$). A and D are from Skucas et al. (2011). B, C, E, F is analyses from the data in D.

than simply a return to the baseline values (Fig. 1). Thus, for all slices from KO mice that did not exhibit LTP, the fEPSP slope was 80.6 ± 8.2% of baseline. However, in two WT mice that did not exhibit LTP, the mean slope (60 min after LTP induction) was similar to baseline (100.5%).

Taken together, the data suggested that the slices from KO mice might have a greater tendency to exhibit LTD after TBS. This could be explained if the KO animals that were used for TBS simply were a bit older than WT mice because LTP declines with age, but this was not the case; there was no significant difference in ages. It was also possible that the slices from KO mice were used at a different delay after the dissection, which is important because slices that are used many hours after the dissection appear to exhibit a form of LTD that is not present if the delay between dissection and recording is brief (Bear, 2003; Dudek and Bear, 1993). However, the delay (time after the slices were placed in the recording chamber to the time they were used to record) was not different (WT, 2.4 ± 0.3 h; KO mice, 2.2 ± 0.2 h; $p = 0.9349$). Another possible explanation for the difference in TBS-induced LTP in WT and KO mice was that the slices from KO mice were unable to maintain an evoked response for prolonged periods of time. Therefore, additional experiments were conducted in slices of KO mice to evaluate whether the fEPSPs decayed over time, even if the baseline was initially stable for 15 min. In these experiments, responses were evoked for 15 min to ensure the baseline was stable, and then another 60 min, at the same frequency (0.05 Hz) and intensity (half-maximal) used for the experiments that were used to evaluate LTP. TBS was never triggered. The results showed that there was no significant decay in the fEPSP slope. The mean slope after the 75 min, expressed as a percent of the mean of the 15 min-baseline period, was 98.7 ± 2.4% ($n = 6$ slices), suggesting that there was no tendency of KO slices to decay over time.

1.2.2.2. LTD. Because TBS induced LTD instead of LTP in AQP4 KO mice, Skucas and colleagues (2011) addressed whether there was an increased ability to induce LTD in response to LFS in AQP4 KO mice. In contrast, the opposite appeared to be the case. Incidence of LTD was also lower in KO mice (WT, 80%; KO, 33%; χ^2-test, $p = 0.0286$). As shown in Fig. 2, there was less LTD in KO mice (107.6 ± 8.9%) than WT mice (66.1 ± 8.0%; $p = 0.0028$. When only those slices that exhibited LTD were examined, the amplitude of LTD was not significantly different (WT, 57.2 ± 6.8%, $n = 8$; KO, 77.9 ± 4.6%, $n = 4$; Student's t-test, $p = 0.0735$; Fig. 2). However, inspection of Fig. 2D suggests that a late phase of LTD was absent in KO mice, approximately 45–60 min after LFS.

Short-term depression (STD), was not different between WT and KO mice (Fig. 2; Table 2), but there was a weaker relationship between STD and LTD in KO mice, consistent with a long-term deficit (Fig. 2).

The results from slices that did *not* exhibit LTD were interesting because it appeared that some of the slices from KO mice that failed to exhibit LTD may have failed, remarkably, because LTP was induced (Fig. 2). In contrast, fEPSPs in slices from WT mice that failed to exhibit LTD simply returned to the baseline values (Fig. 2). Thus, in slices from KO mice that failed to exhibit LTD, the mean slope of the fEPSP, evaluated 60 min after LFS, showed LTP (121.3 ± 9.2%, $n = 8$). In contrast, the slices that failed to exhibit LTD in WT mice, the mean slope of the fEPSP simply returned to baseline values (101.5%, $n = 2$). Because only 2 WT mice lacked LTD, more experiments will be necessary before concluding differences exist between genotypes.

1.2.2.3. High frequency stimulation (HFS)-LTP. The experiments using TBS showed that LTD was induced in KO mice, surprisingly. To determine whether a different type of high frequency train other than TBS would induce LTD in KO mice, HFS-LTP was tested in the studies of Skucas et al. (2011). There were no significant differences in LTP: WT mice exhibited 160.1 ± 14.0% and KO mice showed 134.8 ± 12.6% LTP ($p = 0.2440$; Skucas et al., 2011; Fig. 3). There also were no differences in PTP (Skucas et al., 2011; Table 2; Fig. 3). However, there was a difference in the relationship between PTP and LTP: there was a significant correlation between PTP and LTP for WT mice, but not KO mice (Fig. 3). A difference in the relationship between PTP and LTP suggests that there could have been differences between genotypes in HFS-LTP even if independent analyses at 1 min and 60 min after HFS did not show it.

The incidence of HFS-LTP was not statistically different: 8/9 slices from WT mice exhibited LTP (89%), vs. 5/9 for KO mice (55%; Fisher's exact test, $p = 0.2914$). When only those slices that exhibited LTP were analyzed, the differences were not significant:

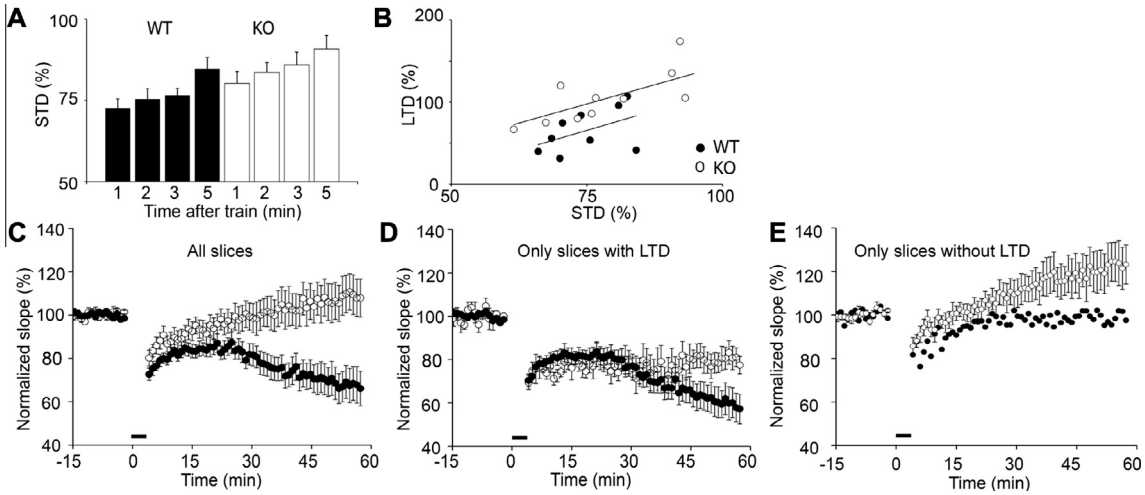

Fig. 2. LFS-LTD in AQP4 WT and KO mice. (A) Mean (±sem) percent STD of fEPSP slopes at 1, 2, 3, and 5 min after LFS were similar for WT (black bars) and KO mice (white bars). There were 10 slices from 7 WT mice and 12 slices from 10 KO mice. (B) STD (measured 1 min after LFS) is plotted as a function of LTP (measured 60 min after LFS) in the same slice (statistical comparisons for (B–E) are presented in the text). (C) Pooled data from all slices showed that there was decreased LTD (measured 60 min after LFS) of the fEPSP slope in KO mice (WT, $n = 10$; KO, $n = 12$). (D) Data are shown only for those slices that exhibited LTD (WT, $n = 8$; KO, $n = 4$). (E) Data are shown for the slices that did *not* exhibit LTD (WT, $n = 2$; KO, $n = 8$). C is from Skucas et al. (2011). A, B, D, E is analyses from the data in C.

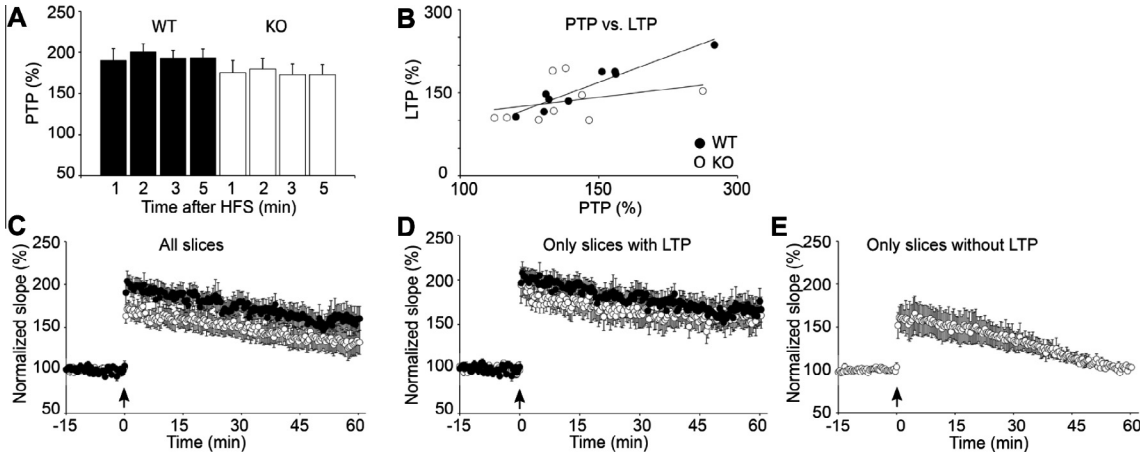

Fig. 3. HFS-induced LTP in AQP4 WT and KO mice. (A) Mean (±sem) percent PTP of fEPSP slopes at 1, 2, 3, and 5 min after HFS were similar for WT (black bars) and KO mice (white bars). There were 9 slices from 8 WT mice and 9 slices from 9 KO mice. (B) The mean percent PTP, 1 min following HFS, is plotted in relation to the mean percent LTP at 60 min. (C) The mean percent potentiation of fEPSP slopes is shown for all slices (WT, $n = 9$; KO, $n = 9$). (D) Data are shown only for those slices that exhibited LTP (WT, $n = 8$; KO, $n = 5$). (E) Data are shown only for those KO slices that did *not* exhibit LTP ($n = 4$). C is from Skucas et al. (2011). A, B, D, E is additional analyses of the data in C.

WT mice exhibited 166.8 ± 14.3% and KO mice showed 160.2 ± 14.4% LTP ($p = 0.7604$; Fig. 3D). Interestingly, in those slices that failed to exhibit LTP, WT mice appeared to be similar to KO mice in that PTP was followed by a return to baseline values. For example, fEPSP slope in KO mice that did not exhibit LTP were 103.0 ± 1.2% of control, 60 min after HFS ($n = 4$). When a slice from a WT mouse failed to exhibit LTP, there also was a return to the baseline values (106.5% of the baseline mean, 60 min after HFS ($n = 1$). Again the fact that only 1 slice from a WT mouse failed to exhibit LTP limits the conclusions, but the data suggest that overall, WT and KO mice were similar in their response to HFS.

1.2.2.4. Mechanisms of impaired long-term synaptic plasticity in AQP4 KO mice. The nature of the defect in TBS-LTP and LTD suggested that the neurotrophin BDNF might be dysregulated in AQP4 KO mice because BDNF and trkB signaling has been implicated in the maintenance of TBS-LTP but not HFS-LTP (Chen et al., 1999; Kang et al., 1997). Moreover, when BDNF levels are increased, LTD can be blocked, and LTP can emerge (Aicardi et al., 2004; Akaneya et al., 1996). Therefore, Skucas et al. (2011) asked if an antagonist of trk receptors, K252a, could rescue the defect in LTD. In addition, the effects of a scavenger of ligands which bind to trkB, trkB-Fc (Binder et al., 1999), were examined.

K252a blocks PKC at a high concentration (Knüsel and Hefti, 1992; Smith et al., 1988) so a lower concentration (300 nM) was used, which is in the range (250–500 nM) that has been used before to specifically block trk receptors. For trkB-Fc, a concentration was used that appeared to be specific based on previous studies (Kang et al., 1997). In addition, we ensured that these drugs, and their vehicles, had no effects on basal transmission. The results showed that both drugs could rescue LTD but there was no effect of the vehicles (Skucas et al., 2011). The data supported the idea that BDNF acting at trkB mediated the effects of AQP4 deletion on LTD, and possibly LTP. However, it was possible that other trk receptors or $p75^{NTR}$, a receptor for all neurotrophins, were involved. Therefore, Western blots were conducted on hippocampal slices that were prepared for recordings, placed into the slice chamber un-

til the times recordings would normally be made, but used for western blots instead. The results showed that trkB receptors were unaffected by AQP4 deletion but there was a reduction in $p75^{NTR}$ (Skucas et al., 2011). Because $p75^{NTR}$ contributes to mechanisms that support LTD (Woo et al., 2005), it was suggested that a reduction in $p75^{NTR}$ could have played a role in the deficit in LTD of AQP4 KO mice (Skucas et al., 2011).

Although the ways that neurotrophin-related mechanisms influenced LTP and LTD in AQP4 KO mice are not completely clear, a link between AQP4 and neurotrophins was established. In itself this link is important; further discussion is provided below (part V).

1.2.3. Hippocampal-dependent behavior

The results from studies of LTP and LTD in Skucas et al. (2011) suggested a preferential defect of AQP4 KO mice in TBS- induced LTP and LTD. To determine if this effect was sufficient to produce a difference in behavior *in vivo*, another group of WT and KO mice were used by Skucas et al. (2011) that were approximately 3 months old. These mice were subjected to three behavioral tasks that have been shown to be dependent on the hippocampus: the Morris water maze, contextual fear conditioning (CFC), and object placement (Ji and Maren, 2008; Luiz Assini et al., 2009; Morris et al., 1982; Parkinson et al., 1988; Phillips and LeDoux, 1992; Tsien et al., 1996). The Morris water maze showed no substantial differences between genotypes, most likely because the background strain (CD1) is known to have a defect in this task (Adams et al., 2002).

In CFC, the means of the groups showed that AQP4 KO mice had different means for the time spent immobile, with the AQP4 KO mice spending more time in immobility during the conditioning phase of the task (WT, 11.8 ± 0.02%, $n = 20$; KO, 19.0 ± 0.03%, $n = 19$; $p = 0.3120$), and the test phase (WT, 41.6 ± 0.1%, $n = 20$; KO, 50.8 ± 0.1%, $n = 19$; $p = 0.3170$) but the differences were not significant. The results suggest that AQP4 KO mice had normal long-term memory for contextual fear.

In object placement, WT mice performed significantly better than KO mice. WT mice and KO mice spent the same percent of the trial period (3 min) exploring the objects when they were first presented (Object 1, WT, 48.7 ± 1.0% of the trial duration was spent exploring; KO, 48.2 ± 1.7%; Student's *t*-test, $p = 0.9098$; Object 2, WT, 51.5 ± 2.1%; KO, 51.9 ± 2.7%; Student's *t*-test, $p = 0.8517$). During Trial 2, WT mice spent more time exploring the object that was moved (Object 2; 64.3 ± 2.1%) compared to the object that was not (Object 1; 35.2 ± 2.5%; $p < 0.001$), suggesting that they could discriminate between objects that were in a familiar location and a new location. KO mice explored Object 1 and 2 for a similar time (49.0 ± 3.0 vs. 51.0 ± 3.1%; $p = 0.1207$), suggesting a defect in object placement memory.

1.2.4. Summary of the effects of AQP4 deletion

1.2.4.1. AQP4 regulates selective aspects of plasticity and spatial memory. The results of Skucas et al. (2011) show that slices of AQP4 KO mice exhibit a reduction in TBS-induced LTP and LFS-induced LTD of the Schaffer collateral pathway. However, a significant deficit in HFS-LTP was not detected. In addition, Skucas et al. (2011) showed that there were no detectable deficits in PTP. Baseline synaptic transmission appeared to be similar in WT and KO mice. There were no detectable differences in performance using common methods to evaluate long-term hippocampal-dependent memory, such as the Morris water maze and CFC. However, Skucas et al. (2011) showed that there was impairment in KO mice in a task that is used to probe short-term hippocampal-dependent memory, object placement (used here with a short delay between test and trial so short-term memory was probed). Taken together, the data from Skucas et al. (2011) suggest that AQP4 is critical to certain types of synaptic plasticity and behavior, and support the growing appreciation that glia are important to plasticity and memory. The results also suggest a novel and specific regulation of learning and memory by water balance, although it is seems likely that the effects of water balance would be indirect rather than direct.

1.2.4.2. AQP4 as an astrocytic regulator of the direction of synaptic plasticity. The results also show a remarkable modifiability in the response to trains of afferent input that appears to be regulated by glia and AQP4. A subset of slices from KO mice exhibited LTD instead of LTP in response to TBS, and a subset of slices from KO mice exhibited LTP instead of LTD in response to LFS. In these atypical slices, the amplitude of LTP and LTD could be substantial (up to 174% of baseline for LTP and 49% of baseline for LTD). The implication is that glia can regulate the directionality of activity-induced synaptic plasticity.

One way to consider this idea is based on the Bienenstock–Cooper–Munro (BCM) model (Bienenstock et al., 1982) which suggests that a given stimulus frequency can evoke either LTP or LTD – *i.e.*, LTP and LTD are a 'continuum' rather than 'binary'. The outcome (potentiation, little change, or depression) would depend on additional factors. One could be related to astrocytic AQP4. The common example for this model, however, is prior history of activity at the synapse. Thus, the ability of a train of afferent input to elicit LTP or LTD depends on the prior history of activity at the synapse, called "metaplasticity" (Bear, 2003). This is an intriguing way to consider the data, in light of the evidence that BDNF (Sajikumar and Korte, 2011) and glia (Ben Achour and Pascual, 2010; Sajikumar and Korte, 2011) exert effects that support metaplasticity. For example, prior synaptic activation can increase the concentration of TrkB at the postsynaptic membrane, where it can subsequently mediate effects of BDNF released during LTP induction (Waterhouse and Xu, 2009).

1.2.5. Potential mechanisms that link AQP4 to select forms of long-term synaptic plasticity and spatial memory

1.2.5.1. AQP4 regulation of TBS-LTP. The results from Skucas et al. (2011) suggest that induction of long-term synaptic plasticity in slices from KO mice was impaired in specific types of plasticity, TBS-induced LTP and LFS-induced LTD. In addition, the data obtained from KO mice indicated a phenomenon that was absent in WT mice: procedures that normally induce TBS-LTP could lead to LTD in KO mice, and the protocol that induced LTD could evoke LTP in KO mice. How can the results be explained by deletion of AQP4?

A potential explanation is suggested by studies that have been published about the role of neurotrophins in LTP and LTD. One of the fundamental differences between TBS-LTP and HFS-LTP is that TBS appears to depend on the neurotrophin BDNF, but HFS-LTP does not (Kang et al., 1997). BDNF and its receptor, trkB, are required for the late phase of LTP (Barco et al., 2005), but BDNF/trkB are also important to the first 60 min after LTP induction ("early" LTP; (Barco et al., 2005; Kang et al., 1997)). BDNF is also potentially relevant because proBDNF, the precursor to mature BDNF, supports LTD by binding to $p75^{NTR}$ receptors (Pang et al., 2004; Woo et al., 2005). This is relevant because hypoosmolarity increases $p75^{NTR}$ (Ramos et al., 2007), and AQP4 KO mice have expanded extracellular space (Binder et al., 2004b), which could act as the functional equivalent of hypoosmolarity by diluting extracellular molecules in a larger space.

The role of proBDNF and mature BDNF in long-term synaptic plasticity may also explain why some slices from KO mice exhibited LTP after LFS. It has been shown that mature BDNF inhibits LTD (Ikegaya et al., 2002; Jiang et al., 2003) and LFS suppresses release of mature BDNF (Aicardi et al., 2004). These data suggest that

release of mature BDNF would block LTD. In addition, release of mature BDNF could lead to LTP, because adding exogenous mature BDNF to hippocampal slices leads to LTP of the Schaffer collateral pathway (Kang and Schuman, 1995). Taken together, if LFS normally suppressed release of mature BDNF in WT mice but this did not occur in KO mice, LTD might result in WT mice, but LTP in KO mice. Importantly, glia release matures BDNF (Parpura and Zorec, 2009). If glial release of mature BDNF was more robust in slices from KO mice that were stimulated with LFS, compared to WT mice, the increase in [mature BDNF]$_o$ could explain the LTP in slices from KO mice.

There are also other explanations for the results that do not depend on neurotrophins. It is possible that TBS led to LTD in some slices because NMDA receptor activation during LTP induction was dysregulated by AQP4 KO. This might lead to a reduced degree of calcium entry, which is significant because relatively low levels of intracellular calcium may be responsible for LTD, whereas higher levels trigger LTP (Johnston et al., 2003; Pöschel and Stanton, 2007; Taniike et al., 2008), although this is not resolved (Lee et al., 2000). One reason that NMDA receptors might be less activated than normal, in response to TBS in slices from KO mice, would be due to atypical pH changes at the synapse during TBS, a consequence of impaired bicarbonate transport, which has been suggested to be regulated by AQP4 (Nagelhus et al., 2004). HFS may still adequately activate NMDA receptors in KO mice, because HFS involves many more stimuli.

Other potential mechanisms may relate to the effects of AQP4 on K^+ regulation. For example, AQP4 KO mice exhibit decreased K^+ reuptake (Binder et al., 2006; Padmawar et al., 2005). The resultant elevation in $[K^+]_o$ would be expected to depolarize neurons and glia. Tonic depolarization of neurons might improve LTP because of greater postsynaptic depolarization during LTP induction. However, it may also impair LTP if the driving force for the fEPSP is reduced by tonic depolarization, or sodium channels become inactivated by tonic depolarization, leading to less postsynaptic firing during induction. Loss of function of Kir4.1 due to deletion of AQP4 would seem unlikely to play a role given that a conditional Kir4.1 KO exhibits deficits in short-term plasticity without affecting LTP (Djukic et al., 2007) and that AQP4 deletion does not affect Kir4.1 function (Zhang and Verkman, 2007).

Pre- and postsynaptic coupling could be important for other reasons. For example, if postsynaptic cells were hyperpolarized during TBS instead of depolarized, LTD would be expected instead of LTP (Stanton and Sejnowski, 1989). TBS could theoretically cause hyperpolarization in the absence of AQP4, due to an increased accumulation of extracellular water, diluting solutes like K^+ and therefore decreasing local $[K^+]_o$. This accumulation of extracellular water might only occur during LTP induction, because glial swelling is defective *in vivo* in AQP4 KO mice in response to high frequency neuronal activity (Kitaura et al., 2009).

AQP4 may also have effects that directly impair glutamate transport. It has been shown that AQP4 KO mice have reduced glutamate uptake because of a reduction in GLT-1, one of the glutamate transporters that is expressed by glia as well as neurons (Zeng et al., 2007). This is interesting in light of the evidence that the late phase of LTP depends on GLT-1a (Pita-Almenar et al., 2006), and that LTP is impaired in a GLT-1 KO mouse (Katagiri et al., 2001). Disruption of GLT-1 also has been shown to inhibit LTP in spinal neurons (Wang et al., 2006). Notably, different types of long-term plasticity seem to be affected differently by GLT-1, based on comparisons between Schaffer collateral stimulation and mossy fiber stimulation (Omrani et al., 2009). Therefore, it is conceivable that effects at GLT-1 may impair TBS-LTP but not HFS-LTP, which would provide an explanation for the results. Importantly, a recent study showed that in AQP4 KO mice, there was an impairment in LTP in the lateral amygdala, and downregulation of GLT-1 was implicated in the defect (Li et al., 2012).

The data support an unanticipated role for AQP4 in synaptic plasticity without impairment of basal synaptic transmission. Given that expression of AQP4 is restricted to glia, the results support previous reports that glia play a role in LTP (Bains and Oliet, 2007; Djukic et al., 2007; Filosa et al., 2009; Ikeda et al., 2007; Todd et al., 2006; Volterra and Steinhauser, 2004), and suggest a new mechanism for the contribution of glia to LTP. Neurotrophins, NMDA receptors, and GLT-1 are likely to play a role. Although it has been well-documented that water regulation and astrocytes influence neurons, and synaptic transmission, our study is the first to demonstrate a direct effect of AQP4 on specific forms of activity-dependent plasticity.

1.2.5.2. AQP4 regulation of object placement performance. In light of the selective effects of AQP4 deletion on TBS-induced LTP, it was important to ask if the effect was large enough to have an influence on hippocampal function. We chose several tasks that are known to be hippocampal-dependent, such as the Morris water maze, contextual fear conditioning, and object placement. Interestingly, there was impairment in KO mice in the object placement task, but no apparent defect in the Morris water maze or contextual fear conditioning. There are studies that have found changes in Schaffer collateral LTP without evidence of behavioral deficits in the Morris water maze (Jun et al., 1998; Leiva et al., 2009), or impairments in Morris water maze without evidence of a deficit in Schaffer collateral LTP (von Engelhardt et al., 2008). Therefore, the lack of difference in Morris water maze and contextual fear conditioning between WT and KO mice should be interpreted cautiously.

Why the KO mice might be impaired using the object placement task and not the Morris water maze or contextual fear conditioning may be related to the fact that the object placement task evaluates a relatively short period of time. Therefore, object placement may be more sensitive to the types of phenomenon that we tested in slices, *i.e.*, "early" LTP (60 min after induction).

1.2.6. Relevance to cognition and disease

1.2.6.1. Cognition. The results provide the first evidence for a role of AQP4 in LTP, LTD, and behavior. Because the type of LTP that was most affected by AQP4 deletion was induced by simulating theta rhythm, and the behavior that was most affected involved short periods of time, the results suggest a physiological role of AQP4 in theta-associated behaviors, such as short periods of spatial exploration. Notably, the idea that aquaporins are involved in cognition has been hypothesized before (Nakada, 2009).

1.2.6.2. Disease. Our results indicating that a single astrocyte molecule, AQP4, influences synaptic plasticity have implications for clinical applications where AQP4 has been considered as a potential regulator of edema (Manley et al., 2000; Papadopoulos and Verkman, 2007). For example, it has been suggested that AQP4 is a possible target for improved pharmacotherapy for traumatic brain injury, spinal cord injury, stroke (Agre and Kozono, 2003; Papadopoulos and Verkman, 2008; Verkman, 2008), and seizure disorders (Hsu et al., 2007). In these various disease conditions, alterations of AQP4 expression, water transport, water balance and ion homeostasis play important roles.

Restoration of water and ion homeostasis represents a novel concept for treatment in addition to standard concepts of neuroprotection. For example, in the context of epilepsy, changes in glial water and potassium homeostasis have been described in multiple animal models and human tissue studies (Binder et al., 2012). In particular, marked downregulation of AQP4 has been observed following status epilepticus in animal models of epilepsy (Lee et al., 2012) as well as in human tissue resected from patients with

epilepsy (Eid et al., 2005). Based on our results, such downregulation of AQP4 may not only lead to increased neural excitability due to abnormalities of water and potassium homeostasis but may also lead directly to abnormalities in synaptic transmission (both LTP and LTD). This provides an potential explanation for the way that astrocytic changes in epilepsy contribute not only to seizures but also to cognitive deficits. Cognitive impairment is very important because patients with temporal lobe epilepsy have many alterations in cognitive function and in particular hippocampal-dependent tasks such as spatial memory (Amlerova et al., 2013; Bell et al., 2011; Brooks-Kayal et al., 2013; Chin and Scharfman, 2013).

Synaptic plasticity is considered to be relevant to epilepsy in many ways. Following insult or injury, the emergence of chronic spontaneous seizures (epileptogenesis) is considered to involve many forms of plasticity, such as potentiation of synapses, reorganization of neuronal circuitry, and alteration in postnatal neurogenesis (Morimoto et al., 2004; Parent, 2002; Scharfman, 2002). The results from Skucas et al. (2011) suggest that AQP4 may be essential to potentiation of glutamatergic pathways in hippocampus, and therefore promote epileptogenesis. In this context, AQP4 deletion would be therapeutic. On the other hand, other experiments that have examined AQP4 KO mice *in vivo* show that there is an increased seizure duration (Binder et al., 2006). In light of this observation, one would predict that AQP4 deletion would exacerbate epilepsy by making seizure less frequent, but more prolonged when they occur. This might depend on the possible increase in convulsive severity by prolongation of seizures. Recent studies have shown marked downregulation of AQP4 expression in the hippocampus during epileptogenesis (Lee et al., 2012), but the effects of AQP4 downregulation on synaptic reorganization and adult neurogenesis in epilepsy are not yet clear. Therefore, more experiments are necessary to role of AQP4 in modulation of these processes underlying epileptogenesis.

In conclusion, the currently available information about AQP4 suggests that strategies to modulate AQP4 may have therapeutic benefit in a variety of CNS diseases although the precise role of AQP4 in some of these diseases, such as epilepsy, is currently unclear. Whether restoration of AQP4-dependent water and potassium homeostasis through novel pharmacological methods will help to prevent or modulate epileptogenesis or 'rescue' cognitive deficits are interesting topics for future investigation. Further understanding of astrocyte-derived factors influencing synapses, such as glial-derived BDNF, will also potentially lead to novel targets for therapeutic approaches.

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