Mammalian target of rapamycin complex 1 activation negatively regulates Polo-like kinase 2-mediated homeostatic compensation following neonatal seizures

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Homeostatic plasticity is characterized by compensatory changes in synaptic strength and intrinsic membrane properties in response to chronic changes in neuronal activity. Neonatal seizures are a naturally occurring source of neuronal overactivation and can lead to long-term epilepsy and cognitive deficits. Using a rodent model of hypoxia-induced neonatal seizures that results in a persistent increase in AMPA receptor (AMPAR) function in hippocampal CA1 pyramidal neurons, we aimed to determine whether there was any evidence of an opposing endogenous homeostatic antiepileptic response. Given that this model results in long-term epilepsy, we also examined mechanisms whereby this homeostasis fails. Whole-cell patch-clamp recordings from neurons in slices removed at intervals following seizure onset revealed an initial up-regulation of AMPAR function that was followed by a transient dynamic attenuation of this enhancement by 48–72 h, although AMPAR function was still increased compared with nonseizure control baseline. This secondary down-regulation of enhanced AMPAR function was coincident with a marked transient increase in expression and function of the Polo-like kinase 2 (PLK2), which has previously been implicated in homeostatic down-regulation of neuronal excitability in cell/slice culture models. The effects were transient and at 1 wk AMPAR function once again became up-regulated, simultaneous with a decrease in PLK2 expression and function. This negative regulation was mediated by subacute postseizure increases in mammalian target of rapamycin (mTOR). Application of the mTOR inhibitor rapamycin prevented post-hypoxic seizure impairment of homeostasis, suggesting that homeostatic plasticity mechanisms may be potentially modifiable therapeutic targets in epileptogenesis.

immature brain | development

Epilepsy is one of the most prevalent neurological disorders, affecting ~1–2% of the United States population (1). The neonatal period is one of the highest incidence periods for seizures across the lifespan, with hypoxic encephalopathy being the most common cause (2, 3). Neonatal seizures are often refractory to conventional antiepileptic drugs and can result in chronic later-life epilepsy and long-term behavioral and cognitive deficits (4, 5).

Using an established neonatal hypoxic seizure (HS) model in rats, we previously showed neonatal HS results in both acute and long-term enhancement in hippocampal and cortical excitability and later-life epilepsy (6–8). We have demonstrated a critical role for seizure-induced early posttranslational modifications of the AMPA receptors (AMPAR) to enhance synaptic excitability (3, 6, 7, 9), and a dependence upon activation of the mammalian target of rapamycin (mTOR) pathway (9).

Neonatal seizure-induced increases in AMPAR function involve mechanisms of receptor trafficking implicated in Hebbian synaptic plasticity (6, 10). Although Hebbian synaptic plasticity is robust in the developing brain at baseline and after seizures, relatively few studies have focused on the status of homeostatic plasticity in the developing brain (11, 12). Homeostatic synaptic plasticity provides neurons with ability to maintain their activity within normal functional boundaries through globally scaling up or down of synaptic strength in response to chronic activity changes (13–15). Homeostatic plasticity (i.e., scaling up) could lower seizure threshold, and can be seen after tetrodotoxin-induced activity silencing in hippocampal slice cultures (16, 17) and by traumatic brain injury-induced decreases in neuron functional activity (18, 19). On the other hand, homeostatic plasticity (i.e., synaptic scaling down) could represent an endogenous antiepileptic response to the naturally occurring neuronal overactivation during seizures.

The molecular mechanisms of homeostatic plasticity are likely to be multifactorial. The Polo-like kinase 2 (PLK2) belongs to the family of serine/threonine kinases involved in cell-cycle progression (20, 21). PLK2 is also critically involved in down-regulation of synaptic strength in response to prolonged increases in neuronal activity (22). Active PLK2 promotes the phosphorylation and degradation of SPAR, a postsynaptic Rap GAP (GTPase-activating protein) and PSD95, and thus mediates compensatory effects through down-regulating AMPAR function in response to elevated activity in vitro (22–24). Here we hypothesized that, in addition to previously documented Hebbian increases in synaptic function (7), PLK2-mediated homeostatic plasticity may provide endogenous protection against HS-induced neural overactivation in immature brain in vivo.

We report here the parallel time course of seizure-induced changes in PLK2 expression with homeostatic compensation of AMPAR function in CA1 pyramidal neurons in slices removed at intervals up to 20 d after in vivo HS in postnatal day (P) 10 rats. Given that we found homeostatic compensation was only transient and incomplete, we further examined potential mechanisms for this failure to correct seizure-induced increases in AMPAR function. Consistent with our prior report of HS-induced activation of the mTORC1 pathway (9), we report here that mTORC1 inhibition can promote PLK2 expression and homeostatic stabilization of neural network excitability. In summary, these results identify a unique and potentially regulatable homeostatic plasticity mechanism that occurs following seizures in the neonatal brain.

Results

Homeostatic Down-Modulation of Seizure-Induced Enhancement in AMPAR Function. To test whether early life HS would induce homeostatic changes in AMPAR function in hippocampal CA1 pyramidal neurons, we performed whole-cell voltage-clamp recordings in post-HS rat pups immediately after HS at P10 and up to 20 d after the initial insult, as well as in littermate controls. In agreement with previous studies (6, 7), slices from post-HS

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rats at 1–3 h time points showed significant increases in both amplitude and frequency of AMPAR-mediated spontaneous excitatory postsynaptic currents (AMPAR sEPSCs) compared with littermate normoxic controls (amplitude: 178.94 ± 7.85% of control, $P < 0.001$, $n = 12$; frequency: 433.19 ± 50.79% of control, $P < 0.01$, $n = 12$) (Fig. 1). At 12 h postseizure, the amplitude and frequency of AMPAR sEPSCs were still significantly elevated compared with littermate normoxic controls (post-HS 12 h: amplitude 162.75 ± 7.03% of control, $P < 0.001$, $n = 9$; frequency: 273.56 ± 39.34% of control, $P < 0.05$, $n = 9$). However, following this acute period of 1–12 h, there was a secondary phase of gradually decreasing up to 72 h after HS (post-HS 24 h: amplitude 137.68 ± 5.63% of control, $n = 9$, $P < 0.01$; frequency 197.23 ± 28.40% of control, $n = 9$ $P < 0.05$; post-HS 48–72 h: amplitude 130.77 ± 6.19% of control, $n = 8$, $P < 0.01$; frequency 195.54 ± 37.33% of control, $n = 8$), although at all times the AMPAR sEPSC amplitude and frequency remained persistently above control baseline. AMPAR sEPSC frequency and amplitude then once again gradually increased at post-HS 12–13 d (post-HS 5–7 d: amplitude 132.52 ± 5.69% of control, $P < 0.01$, $n = 7$; frequency 211.26 ± 35.97% of control, $n = 7$; post-HS 12–20 d: amplitude: 156.85 ± 6.25% of control, $P < 0.01$, $n = 9$; frequency: 186.66 ± 19.36% of control, $n = 9$) (Fig. 1 *B* and *C*). There were no significant changes in rise-time (10–90% of peak) and decay-time (90–10% of peak) of AMPAR sEPSCs at 1 h, 24 h, 48–72 h, 5–7 d, or 12–20 d post-HS compared with littermate controls (Fig. S1). A similar dynamic, multiphasic pattern of change was detected for the miniature AMPAR EPSCs (mEPSCs) with early increases (1 h: amplitude 164.89 ± 7.83% of control, frequency 244.6 ± 35.07% of control, $n = 9$, $P < 0.01$) followed by a secondary decrease (48 h: amplitude 127.12 ± 6.09% of control, $n = 9$, $P < 0.05$, frequency 152.71 ± 23.22% of control, $n = 9$) and a return to enhanced activity 12–20 d after HS (12–20 d: amplitude 148.75 ± 6.91% of control, $n = 9$, $P < 0.01$, frequency 170.83 ± 16.09% of control, $n = 9$, $P < 0.05$). HS did not induce significant changes in the resting membrane potential, input resistance, action potential amplitude, duration, and threshold, or input-output function in CA1 neurons at different time points following HS (all $P > 0.05$, $n = 8$–12) (Figs. S2 and S3). Taken together, these data provide evidence for transient compensatory homeostatic modulation of AMPAR function during the subacute period in response to neural hyperexcitability induced by neonatal HS in CA1 pyramidal neurons. However, this compensation was incomplete and appeared to fail after 7–12 d post-HS, with a subsequent return of progressively increasing AMPA sEPSC/mEPSC amplitude (mEPSC/sEPSC: post-HS 48–72 h: $n = 8$–9, $P < 0.05$; sEPSC: post-HS 5–7 d: $n = 7$, $P < 0.05$) compared with post-HS 12–20 d ($n = 9$) without significant changes in AMPA sEPSC/mEPSC frequency.

PLK2 Expression Is Transiently Increased Following HS. Given the electrophysiological evidence for transient homeostatic regulation following HS, we next evaluated whether this period was associated with alterations in the expression level of mRNA and protein of PLK2, an important mediator of homeostatic plasticity in vitro (22, 23). Real-time PCR quantification of PLK2 mRNA demonstrated significant increases at 1, 6, and 24 h after HS onset in hippocampus compared with littermate controls, peaking at post-HS 6 h (post-HS 1 h: 1.18 ± 0.03-fold increase; post-HS 6 h: 1.70 ± 0.18-fold of increase; and post-HS 24 h: 1.24 ± 0.04-fold increase, $n = 4$, $P < 0.05$) (Fig. 2*A*). Consistently, quantitative analysis of immunohistochemistry in hippocampus showed an overall significant increase in PLK2 protein expression in CA1 pyramidal neurons, prominent in both the cell bodies and the apical and basal dendrites at 48–72 h post-HS (cell body layer: 114.86 ± 3.69% of control, $n = 6$, $P < 0.05$; apical dendritic layer: 111.49 ± 3.22% of control, $n = 6$, $P < 0.05$) (Fig. 2*B*). PLK2 mediates its compensatory functions in part through eliminating dendritic spines and attenuating AMPAR function through phosphorylation and degradation of the SPAR and PSD95 (22–24). We thus examined the expression of SPAR and PSD95 at 48–72 h following HS at P10 using immunocytochemistry and found that SPAR expression was significantly reduced in hippocampal pyramidal cell bodies and apical dendrites of CA1 neurons (88.62 ± 3.06% of control in stratum pyramidale, $n = 6$, $P < 0.05$; 84.93 ± 3.08% of control in stratum radiatum, $n = 6$, $P < 0.05$) (Fig. 2 *C* and *D*). Similarly, PSD95 expression was significantly decreased in the apical dendritic layer (82.83 ± 5.99% of control, $n = 8$, $P < 0.05$) and in the cell body layer (90.45 ± 2.71% of control, $n = 8$, $P < 0.05$) (Fig. 2*B*), consistent with previous studies, immunoblot results showing decreases in PSD95 expression in hippocampus following early-life seizures (25, 26). These results suggest that early-life HS induced functional expression of PLK2 is occurring in parallel with the observed homeostatic compensation of HS-induced hyperexcitability.

PLK2 Activation Mediates Transient Homeostatic Down-Regulation of Seizure-Induced Enhancement of AMPAR Function. To determine whether PLK2 was functionally related to the homeostatic changes in synaptic function, we tested whether PLK2 modulators in vivo could affect the subacute self-downward regulation of AMPAR function observed in slices following HS. The PLK1/2 kinase inhibitor BI-2536 (20 mg/kg, i.p.) at 30 min pre-HS was used to inhibit PLK2 (27, 28) and the proteasome-mediated degradation inhibitor MG132 (5 mg/kg) was chosen to potentiate the effects of PLK2 (23) (*SI Materials and Methods*). Compared with vehicle treatment pre-HS, at 24 and 48 h post-HS, slices from rats treated with BI-2536 showed significantly higher AMPAR sEPSC amplitude (24 h post-HS: 173.94 ± 11.04% of vehicle-treated control, $n = 7$, $P < 0.05$; 48 h post-HS: 169.34 ± 8.77% of vehicle-treated control, $n = 11$, $P < 0.05$) (Fig. 3 *A*–*C*) and

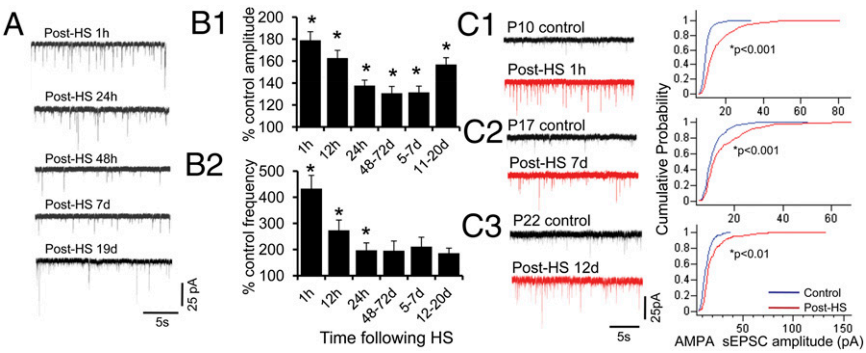

Fig. 1. Dynamic changes of AMPAR function in CA1 pyramidal neurons following neonatal HS at P10. (*A*) Representative traces of AMPAR sEPSCs at a holding potential of −60 mV in hippocampal ex vivo slices from HS rats at different time points (from 1 h to 19 d) following HS. (*B1*) AMPAR sEPSC amplitude shows a dynamic downward regulation pattern following HS at P10, but it is significantly higher in all HS groups relative to that of normoxic controls ($n = 7$–12, $^*P < 0.01$). (*B2*) AMPAR sEPSC frequency shows a dynamic downward regulation pattern following HS at P10 ($n = 7$–12; $^*P < 0.05$). Error bars indicate SEM (*C1*–*C3*) Representative traces of AMPAR sEPSCs in CA1 neurons from post-HS 1h rat and P10 control (*C1*, *Left*), post-HS 7d rat and P17 control (*C2*, *Left*), and post-HS 12d rat and P22 control (*C3*, *Left*). Cumulative probability plots for AMPA sEPSC amplitude in post-HS rats and their littermate controls (*C1*–*C3*, *Right*) show a significant increase in AMPA sEPSC amplitude following HS (Kolmogorov–Smirnov test, $^*P < 0.01$).

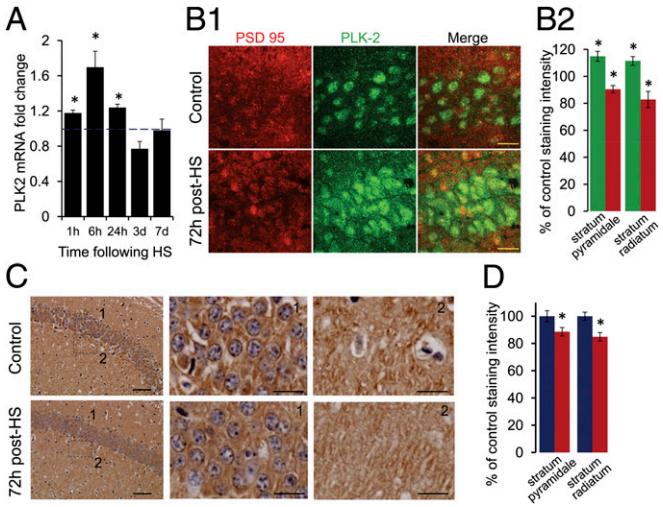

Fig. 2. Neonatal HS increase PLK2 mRNA and protein expression and affect its downstream targets SPAR and PSD95. (*A*) Real-time PCR shows a significant increase in hippocampal PLK2 mRNA at 1, 6, and 24 h post-HS compared with their littermate controls ($n = 4$ per group, $^*P < 0.05$). (*B1*) Double-labeling with PLK2 (green) and PSD95 (red) in a post-HS 72-h rat and an age-matched control. (Scale bars, 25 μm.) (*B2*) Quantification of staining intensity shows significant increases in PLK2 expression (green) and decreases in PSD95 expression (red) in both cell-body layer (*Left*) and apical dendritic layer (*Right*) in CA1 at 72-h post-HS rats compared with age-matched control at P13. $n = 6$–8, $^*P < 0.05$. (*C*) SPAR (DAB) staining shows a decrease in both cell bodies and dendrites of CA1 at 72 h post-HS compared with the age-match control. (Scale bars, 50 μm.) Areas marked with dashed box were shown *Center* and *Right* panels at a higher magnification (Scale bar, 15 μm). (*D*) Quantification of SPAR staining (fluorescent) intensity shows significant decreases in both cell-body layer (*Left*) and apical dendritic layer (*Right*) in CA1 in 72 h post-HS rats compared with age-matched controls at P13. $n = 6$ each, $^*P < 0.05$. Error bars indicate SEM.

frequency (24 h post-HS: 331.26 ± 32.40% of vehicle-treated control, $n = 7$, $P < 0.05$; 48 h post-HS: 311.38 ± 25.87% of vehicle-treated control, $n = 11$, $P < 0.05$) (Fig. 3 *A*, *B*, and *D*). Thus, in vivo administration of BI-2536 prevented the dynamic down-regulation of enhanced AMPAR function seen subacutely after seizures. BI-2536 did not change rise-time (post-HS 24 h: 109.98 ± 14.35% of vehicle-treated control, $n = 7$; post-HS 48–72 h: 106.66 ± 9.19% of control, $n = 11$, all $P > 0.05$) and decay-time (post-HS 24 h: 99.36 ± 4.68% of vehicle-treated control, $n = 7$; post-HS 48–72 h: 103.04 ± 5.14% of control, $n = 11$, all $P > 0.05$).

In contrast, in vivo treatment with PLK2 breakdown inhibitor MG132 resulted in decreasing AMPAR sEPSC amplitude (95.73 ± 6.63% of vehicle-treated control, $n = 5$, $P > 0.05$) (Fig. 3 *E*–*G*) and frequency (150.03 ± 38.74% of vehicle-treated control, $n = 5$, $P > 0.05$) (Fig. 3 *E*, *F*, and *H*) at 48 h post-HS without significant changes in rise-time (105.26 ± 12.69% of vehicle-treated control, $n = 5$, $P > 0.05$) and decay-time (102.26 ± 7.51% of vehicle-treated control, $n = 5$, $P > 0.05$). Importantly, the effects of MG132 could be blocked by coadministration of BI-2536 (amplitude: 150.10 ± 10.76% of vehicle-treated control, $n = 5$, $P < 0.05$; frequency: 260.88 ± 53.71% of vehicle-treated control, $n = 5$, $P < 0.05$) (Fig. 3 *E*–*H*). This modulation of AMPAR sEPSCs was not a result of nonspecific effects of BI-2536/MG132 on AMPAR function during development, as we did not observe any effects in normoxic rat pups (Fig. S4 *A*–*C*). In addition, delayed in vivo administration of BI-2536 after 12 d postseizure (P22) did not affect AMPAR sEPSC amplitude and frequency ($P > 0.05$, $n = 5$) (Fig. S4 *D*–*F*), consistent with the fact that PLK2 expression has already decreased at this time point. Taken together, these results indicate an important role of PLK2 in homeostatic compensation of AMPAR function following neonatal HS. However, unlike in vitro models (22), PLK2 activation is transient in this in vivo model of seizures and did not result in a complete compensation of neuronal excitability to baseline levels.

mTORC1 Pathway Activity Negatively Regulates PLK2-Mediated Homeostatic Plasticity Following Neonatal HS. mTOR is a key regulator of protein synthesis implicated in epileptogenesis in adult models of epilepsy (29–32) as well as in our neonatal HS model (9). As the mTOR pathway component TSC1 interacts with PLK2 (33), we hypothesized that HS-induced mTORC1 pathway activation would affect PLK2-mediated homeostatic regulation of AMPAR function, given that our prior studies have shown that mTORC1 pathway activation occurs in a similar temporal pattern following HS and that treatment with the mTOR inhibitor rapamycin blocks the development of later-life epilepsy (9). Using the same rapamycin treatment paradigm in vivo, we confirmed the previously reported effect of rapamycin treatment on significantly decreasing the subacute enhancement of AMPAR function at 48 h post-HS (amplitude: 96.95 ± 5.03%of vehicle treated control rats, $n = 6$, $P < 0.001$; frequency: 112.31 ± 32.12% of vehicle treated control rats, $n = 6$, $P > 0.05$) (Fig. 4 *D*–*F*). Consistently, immunocytochemical analysis showed significantly increased PLK2 expression in pyramidal neurons in rapamycin-treated HS rats (cell body layer: 122.43 ± 3.97% of vehicle-treated HS group, $n = 6$, $P < 0.05$; apical dendritic layer: 110.04 ± 2.42% of vehicle-treated HS group, $n = 6$, $P < 0.05$). In addition, there was a trend for a decrease in PSD95 expression in rapamycin-treated HS rats compared with vehicle-treated HS rats (cell body layer: 89.32 ± 5.35% of vehicle-treated HS group, $n = 6$, $P = 0.06$; apical dendritic layer: 85.27 ± 4.19% of vehicle-treated HS group, $n = 6$, $P <$

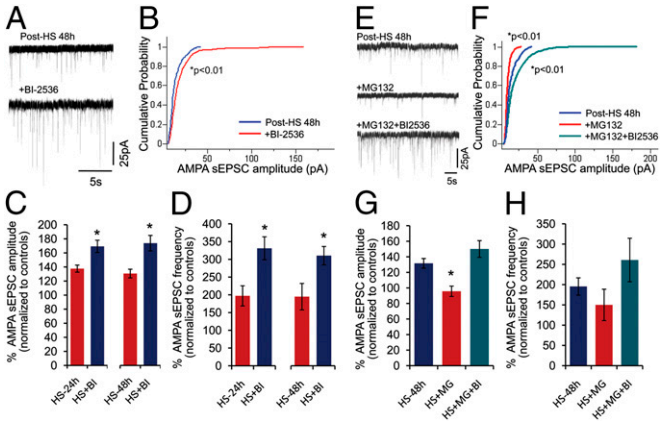

Fig. 3. HS-induced PLK2 activation mediates homeostatic compensation of AMPAR function following HS. (*A*) Typical recordings of AMPAR sEPSCs in hippocampal ex vivo slices from post-HS 48-h rats treated in vivo with vehicle or BI-2536. (*B*) Normalized cumulative distribution of AMPAR sEPSCs shows a significant increase in amplitude in BI-2536–treated HS animals compared with vehicle-treated post-HS 48-h rats ($P < 0.01$, Kolmogorov–Smirnov test). (*C* and *D*) In vivo BI-2536 treatment significantly increased both AMPAR sEPSC amplitude (*C*) and frequency (*D*) at both 24 h ($n = 7$, $^*P < 0.05$) and 48 h ($n = 11$; $P < 0.05$) post-HS. (*E*) Representative traces of AMPAR sEPSCs in hippocampal ex vivo slices from post-HS 48 h rats treated in vivo with either vehicle, MG132, or MG132 plus BI-2536. (*F*) Normalized cumulative distribution of AMPAR sEPSCs shows a significant decrease in amplitude in MG132-treated HS animals compared with vehicle-treated post-HS 48-h rats ($P < 0.01$, Kolmogorov–Smirnov test), which is attenuated by in vivo coadministration of BI-2536 ($P < 0.01$, Kolmogorov–Smirnov test). (*G*) In vivo MG132 treatment significantly decreased AMPAR sEPSC amplitude at 48 h post-HS ($n = 5$, $^*P < 0.05$), which is attenuated by in vivo coadministration of BI-2536 ($n = 5$, $^*P < 0.05$). (*H*) AMPAR sEPSCs frequency trends downward in the MG132-treated HS group at 48 h post-HS ($n = 5$, $P > 0.05$), which is attenuated by in vivo coadministration of BI-2536 ($n = 5$, $P < 0.05$). Error bars indicate SEM.

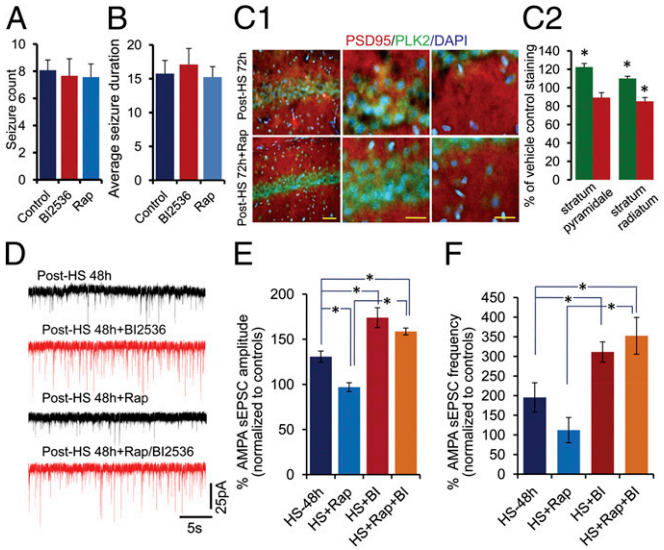

Fig. 4. HS-induced mTORC1 activation negatively regulates PLK2 function. (*A* and *B*) BI-2536 and rapamycin treatments in vivo do not change the number (*A*) and the average duration (*B*) of the initial global hypoxia-induced seizures. (*C1* and *C2*) Triple-labeling with PLK2 (green), PSD95 (red), and DAPI (blue) demonstrate significant increases in PLK2 expression, but PSD95 expression trends downward in both the cell body layer and the apical dendritic layer in CA1 region at post-HS 72 h in rapamycin-treated HS rats compared with vehicle-treated HS rats (n = 6, $^*P < 0.05$). [Scale bar: 35 μm (*C1*, *Left*) and 15 μm (*C1*, *Center* and *Right*).] (*D*) Typical recordings of AMPAR sEPSCs from post-HS 48-h rats treated in vivo with vehicle, BI-2536, rapamycin, or rapamycin plus BI-2536. (*E*) Rapamycin inhibited HS-induced increases in AMPAR sEPSC amplitude at 48 h post-HS ($n = 6$, $^*P < 0.05$), which is attenuated by in vivo coadministration of BI-2536 ($n = 5$, $^*P < 0.05$). (*F*) In vivo coadministration of BI-2536 significantly inhibited rapamycin-mediated attenuation in AMPAR sEPSC frequency ($n = 5$, $^*P < 0.05$). Error bars indicate SEM.

0.05) (Fig. 4*B*), indicating an inhibition of PLK2 expression and function by HS-induced mTORC1 pathway activation.

Given the apparent role of mTOR on seizure-induced PLK2 expression, we next tested whether PLK2 inhibition would occlude the protective effect of rapamycin following HS. Indeed, coadministration of the PLK2 inhibitor BI-2536 completely blocked the rapamycin-mediated attenuation of enhancement in AMPAR sEPSCs at 48 h post-HS (amplitude: 161.31 ± 3.88% of vehicle treated HS rats, $n = 5$, $P < 0.001$; frequency: 352.61 ± 46.65% of vehicle treated HS rats, $n = 5$, $P < 0.01$) (Fig. 4 *C* and *D*). Taken together, these data suggest HS-induced activation of mTORC1 contributed to the failure of PLK2 to mediate a full recovery of neuronal excitability. Importantly, treatment could not be attributed to alteration of the initial seizure stimulus, as neither the PLK2 inhibitor BI-2536 nor rapamycin treatment affected the severity of the initial HS at P10 [number of seizures per rat pup: vehicle: 8.08 ± 1.06, $n = 13$; BI-2536: 7.67 ± 1.37, $n = 9$; rapamycin: 7.57 ± 1.12, $n = 7$; $P > 0.05$) (Fig. 4*A*); average duration of seizures: vehicle: 15.75 ± 1.96 s, $n = 8$; BI-2536: 17.10 ± 2.38 s, $n = 8$; rapamycin: 15.24 ± 1.55s, $n = 7$, $P > 0.05$ (Fig. 4*B*)].

Given the data that PLK2 modulators affected the early alterations in AMPAR function, and the fact early rapamycin treatment in this model can prevent long-term epilepsy, we next tested long-term effects of postseizure manipulations of PLK2 on AMPAR sEPSCs. Slices were prepared from rats at 12–20 d post-HS (P23–P30) treated with rapamycin, MG132, or vehicle 30 min before and 48 h after HS at P10. Compared with slices from vehicle-treated HS rats, rapamycin significantly decreased both the AMPAR sEPSC amplitude and frequency at P23-30 (amplitude: 67.33 ± 2.49% of vehicle-treated HS; frequency: 25.93 ± 2.15% of vehicle-treated HS, $n = 7$, $P < 0.01$) (Fig. 5 *A–D*) to a level comparable to vehicle-treated controls (amplitude: 104.23 ± 2.35% of control, $n = 7$, $P > 0.05$; frequency: 77.21 ± 6.38% of control, $n = 7$, $P > 0.05$). No effects of rapamycin on baseline AMPAR sEPSC amplitude and frequency were detected (amplitude: 108.23 ± 4.85% of vehicle-treated control, frequency: 80.33 ± 12.95% of vehicle-treated control, $n = 5$, $P > 0.05$) (Fig. 5 *E–H*), making it unlikely these were a result of nonspecific effects on AMPAR function during development. Similarly, short-term application of the PLK2 potentiator MG132 further attenuated both the AMPAR sEPSC amplitude and frequency at P23–30 (amplitude: 70.12 ± 6.73% of vehicle-treated HS, $n = 5$, $P < 0.05$; frequency: 36.20 ± 10.04% of vehicle-treated HS, $n = 5$, $P < 0.01$). Taken together, these results support an important role of PLK2 regulated by mTORC1 pathway in early life HS-induced long-term enhancement of AMPAR function.

Discussion

Here we present the important evidence that early-life seizures are associated with a transient endogenous homeostatic down-regulation of enhanced AMPAR function mediated by seizure-induced PLK2 activation. We show that the PLK2-SPAR-PSD95 pathway, previously described in in vitro model systems, appears to be involved in the early endogenous antiepileptic compensation following neonatal seizures. We also reveal that seizure-induced

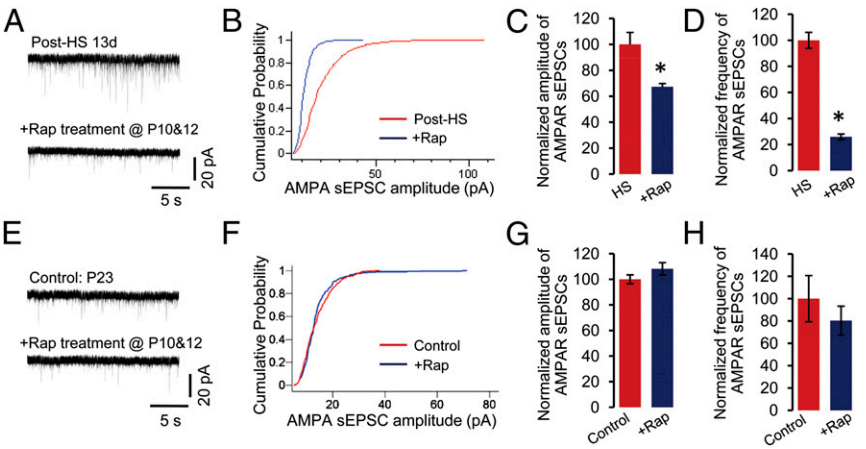

Fig. 5. Short-term rapamycin treatment in vivo prevents HS-induced long-term increase in AMPAR function. (*A*) Typical traces of AMPAR sEPSCs in hippocampal ex vivo slices from post-HS 13-d rats treated in vivo with vehicle or rapamycin at 30 min before and 48 h post-HS. (*B*) Normalized cumulative distribution of AMPAR sEPSCs from short-term rapamycin/vehicle-treated HS rats ($P < 0.01$, Kolmogorov–Smirnov test). (*C* and *D*) AMPAR sEPSC amplitude (*C*) and frequency (*D*) are significantly smaller in the rapamycin-treated HS rats compared with vehicle-treated post-HS rats ($n = 6$–7, $^*P < 0.05$). (*E*) Typical recordings of AMPAR sEPSCs in hippocampal ex vivo slices from P23 control rats treated in vivo with vehicle or rapamycin at P10 and P12. (*F*) Normalized cumulative distribution of AMPAR sEPSCs do not show significant amplitude changes in rapamycin-treated controls compared with vehicle-treated controls ($n = 5$, $P > 0.05$, Kolmogorov–Smirnov test). (*G* and *H*) No significant difference of AMPAR sEPSC amplitude (*G*) and frequency (*H*) between rapamycin-treated P23–P30 controls and vehicle-treated P23–P30 controls ($n = 5$, $P > 0.05$). Error bars indicate SEM.

mTORC1 pathway activation negatively regulates PLK2 function in the immature brain, which may in part underlie the failure of the homeostatic mechanisms to completely reverse the postseizure changes, resulting in epileptogenesis (8). Furthermore, we show that manipulation of PLK2 function through inhibition of mTORC1 pathway with rapamycin rescues homeostatic compensation following HS, and in curtailment of mTOR-mediated progressive network excitability and later-life epilepsy, as shown in our prior work (9). Hence, PLK2 mediated homeostatic compensation following HS may represent a therapeutic target for antiepileptogenesis.

PLK2 Activation Mediates Homeostatic Down-Regulation of Enhanced AMPAR Function Following Neonatal Seizures. Here we report a multiphasic pattern to AMPAR function following seizures at P10. There was an initial increase up to 12 h, then a secondary down-regulation of this enhancement, albeit not back to baseline levels seen in controls, followed by a third phase of a gradual re-emergence of further increases in AMPAR sEPSCs in a seizure model that results in long-term epilepsy monitored using video-EEG recordings after several weeks (8). Although a direct link between the enhancement of AMPAR function and later-life epilepsy is ideal, it would only be possible in future studies where homeostatic plasticity could be ascertained in vivo in the subacute period so that it could permit survival to later adulthood for correlation with the severity of later life epilepsy.

Induction of homeostatic plasticity can be highly dependent upon the preparation used (34–36), and most studies have been carried out using neuronal cell/slice cultures (22, 37). Little is known about physiological homeostatic responses and their molecular mechanisms induced by seizures in vivo, especially in the immature brain, which is characterized by rapid synaptogenesis and enhanced Hebbian plasticity and homeostatic plasticity (3, 7, 36). Although $GABA_A$ receptor-mediated synaptic inhibition is persistently decreased following HS (38), here we show that the previously described initial enhancement of AMPAR function (6) was also transiently and dynamically down-regulated, but notably not to preseizure baseline levels. In contrast, the homeostatic plasticity in vitro, or synaptic scaling, evoked by prolonged disinhibition in cell/slice cultures is manifested by a more complete and long-lasting suppression of mEPSC amplitude (22, 37). Moreover, the post-seizure transient homeostatic compensation is coincident with a seizure-free latent period starting from 2 d after the initial HS at P10 documented by video EEG (8), suggesting that endogenous homeostatic regulation of excitation may account for the appearance of this brief seizure-free latent period.

The factors that regulate synaptic AMPAR function during homeostatic plasticity are still not fully elucidated (13, 14, 39). Among the known mediators, PLK2 is believed to play a pivotal role in homeostatic synaptic downscaling (22–24). In vitro neuronal cell and slice culture studies have shown an important role of PLK2 in homeostatic down-regulation of AMPAR function and intrinsic excitability in responses to chronic activity increases in hippocampal neurons (22). Here, both PLK2 mRNA (over the course of several hours) and protein expression (over the course of a few days) were transiently increased following HS, consistent with reports on chemoconvulsant or electroconvulsive shock seizures in the adult rodent (40, 41). In addition, we report here that treatment with a PLK2 inhibitor, BI-2536, at P10 attenuated the seizure-induced homeostatic drop in AMPAR function. Conversely, potentiating PLK2 function with MG132 led to full recovery of AMPAR function to control levels. Thus, our data suggest that PLK2-mediated dampening of synaptic strength is present as an endogenous compensation toward stabilizing neuronal activity following HS, and antiepileptogenic therapies could be developed to enhance PLK2 function.

In the immature brain, AMPARs are highly Ca^{2+}-permeable and can mediate long-lasting increases in AMPAR function and synaptic strength (3), in part mediated by synaptic receptor trafficking (6). The small GTPases Ras and Rap are molecular signaling switches critically involved in modulating synaptic AMPAR trafficking during long-term potentiation and long-term depression, and many forms of memory formation (24, 42). Importantly, PLK2 has been shown to directly phosphorylate both Ras and Rap at synapses (24, 43). Indeed, HS-induced increases in PLK2 expression led to degradation of SPAR and associated PSD95 in hippocampal CA1 neurons, which may be because of degradation downstream of Ras/Rap signaling (23, 44). Future studies may warrant examining the role of Ras/Rap signaling in PLK2-mediated homeostatic compensation following early life epilepsy.

Neonatal HS-Induced mTORC1 Pathway Activation Negatively Regulates PLK2-Mediated Homeostatic Compensation. The reemergence of enhancement of AMPAR function following 7–12 d post-HS indicates an incomplete homeostatic compensation. At 20 d post-HS, AMPAR sEPSC amplitude is increased by >50% compared with controls, which is in line with increases seen in other models of epilepsy (45, 46). More importantly, at this time point, the appearance of spontaneous seizures (epilepsy) has been demonstrated in our recent study using video-EEG recordings (8). The failure of homeostatic regulation by HS-induced PLK2 activation appears to be at least in part because of mTORC1 pathway activation. Neonatal HS-induced mTORC1 activation (9) inhibits the transient PLK2 activation. In addition, mTORC1 inhibition by rapamycin enhances HS-induced PLK2 expression, which in turn attenuates HS-induced both subacute and long-term enhancement of AMPAR function. These changes are in the context of our previous observation showing that treatment with the mTORC1 inhibitor rapamycin attenuated HS-induced later life spontaneous seizures as well as autism-like behaviors in the identical HS model (9). The mTORC1 signaling pathway has been shown to be critical in regulating protein translation and synaptic plasticity (47, 48). mTORC1 activity was highest in hippocampus and cortex during early development (9). Seizures in both immature and adult brains can induce early increases in mTORC1 expression and function (9, 29, 30). Although overactivation of mTORC1 signaling has been thought to promote the initiation of protein translation (47, 49, 50), there are pathways that are likely compensatory whereby the translational repressor eukaryotic elongation factor 2 is phosphorylated by mTOR activation, and can down-regulate protein translation up to 50% (51, 52). Thus, it is possible that neonatal HS-induced mTORC1 activation alters PLK2 translation, which would be supported by our findings that mTORC1 inhibition by rapamycin increases HS-induced PLK2 protein expression. On the other hand, PLK2 itself can regulate the mTOR pathway, and has been shown to affect mTOR signaling through PLK2–TSC1 interaction during hypoxia in lung tumor cells (33). Blockade of PLK2 function attenuates rapamycin mediated rescue of HS-induced network hyperexcitability (9), suggesting an important interaction and role of PLK2 signaling in mTORC1-mediated epileptogenesis.

In summary, the present study shows that, similar to in vitro (22, 24), PLK2-mediated homeostatic plasticity mediates antiepileptic responses following in vivo neonatal HS. The short-lived compensation appears to be at least in part because of HS-induced mTORC1 activation that negatively regulates PLK2 function and impairs homeostatic regulation of enhanced synaptic strength. Thus, promoting homeostatic compensation with PLK2 potentiators or mTOR inhibitors may represent a unique mechanism for neonatal seizure-induced long-term neuronal network hyperexcitability and provide a unique target to prevent long-term consequences of early life seizures.

Materials and Methods.

Animals. HS were induced in P10 male Long Evans pups (53). Although associated with potential clinical translation limitations, a pretreatment (30

min pre-HS) was used to achieve optimal kinase inhibition at the time of insult or over the first 24 h of a seizure induction (9, 29). All experiments were approved by the Institutional Animal Care and Use Committee at Boston Children's Hospital (Boston, MA). See *SI Materials and Methods* for detailed procedure and treatments.

Immunocytochemistry, Real-Time Quantitative PCR, and Whole-Cell Patch-Clamp Recordings. Immunocytochemistry, real-time quantitative PCR, and whole-cell patch-clamp recordings were performed as previously described (6, 7, 9). ImageJ software (National Institutes of Health) was used to quantify the PLK2, PSD95, and SPAR staining (54). See *SI Materials and Methods* for procedure and analysis details.

Statistics. All data were expressed as mean ± SE and assessed with an α-level of 0.05 using a Student *t* test, a Kolmogorov–Smirnov test, or a one-way ANOVA test followed by post hoc Bonferroni comparison.

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1. Ngugi AK, et al. (2011) Incidence of epilepsy: A systematic review and meta-analysis. *Neurology* 77(10):1005–1012.
2. Jensen FE (2009) Neonatal seizures: An update on mechanisms and management. *Clin Perinatol* 36(4):881–900, vii.
3. Rakhade SN, Jensen FE (2009) Epileptogenesis in the immature brain: Emerging mechanisms. *Nat Rev Neurol* 5(7):380–391.
4. Ronen GM, Buckley D, Penney S, Streiner DL (2007) Long-term prognosis in children with neonatal seizures: A population-based study. *Neurology* 69(19):1816–1822.
5. Berg AT (2011) Epilepsy, cognition, and behavior: The clinical picture. *Epilepsia* 52(Suppl 1):7–12.
6. Rakhade SN, et al. (2008) Early alterations of AMPA receptors mediate synaptic potentiation induced by neonatal seizures. *J Neurosci* 28(32):7979–7990.
7. Zhou C, Lippman JJ, Sun H, Jensen FE (2011) Hypoxia-induced neonatal seizures diminish silent synapses and long-term potentiation in hippocampal CA1 neurons. *J Neurosci* 31(50):18211–18222.
8. Rakhade SN, et al. (2011) Development of later life spontaneous seizures in a rodent model of hypoxia-induced neonatal seizures. *Epilepsia* 52(4):753–765.
9. Talos DM, et al. (2012) The interaction between early life epilepsy and autistic-like behavioral consequences: A role for the mammalian target of rapamycin (mTOR) pathway. *PLoS ONE* 7(5):e35885.
10. Malenka RC (2003) Synaptic plasticity and AMPA receptor trafficking. *Ann N Y Acad Sci* 1003:1–11.
11. Maffei A, Turrigiano G (2008) The age of plasticity: Developmental regulation of synaptic plasticity in neocortical microcircuits. *Prog Brain Res* 169:211–223.
12. Blundon JA, Bayazitov IT, Zakharenko SS (2011) Presynaptic gating of postsynaptically expressed plasticity at mature thalamocortical synapses. *J Neurosci* 31(44): 16012–16025.
13. Turrigiano GG (2008) The self-tuning neuron: Synaptic scaling of excitatory synapses. *Cell* 135(3):422–435.
14. Turrigiano G (2011) Too many cooks? Intrinsic and synaptic homeostatic mechanisms in cortical circuit refinement. *Annu Rev Neurosci* 34:89–103.
15. Pozo K, Goda Y (2010) Unraveling mechanisms of homeostatic synaptic plasticity. *Neuron* 66(3):337–351.
16. Bausch SB, He S, Petrova Y, Wang XM, McNamara JO (2006) Plasticity of both excitatory and inhibitory synapses is associated with seizures induced by removal of chronic blockade of activity in cultured hippocampus. *J Neurophysiol* 96(4): 2151–2167.
17. Trasande CA, Ramirez JM (2007) Activity deprivation leads to seizures in hippocampal slice cultures: Is epilepsy the consequence of homeostatic plasticity? *J Clin Neurophysiol* 24(2):154–164.
18. Avramescu S, Timofeev I (2008) Synaptic strength modulation after cortical trauma: A role in epileptogenesis. *J Neurosci* 28(27):6760–6772.
19. Volman V, Bazhenov M, Sejnowski TJ (2011) Pattern of trauma determines the threshold for epileptic activity in a model of cortical deafferentation. *Proc Natl Acad Sci USA* 108(37):15402–15407.
20. van de Weerdt BC, Medema RH (2006) Polo-like kinases: A team in control of the division. *Cell Cycle* 5(8):853–864.
21. Seeburg DP, Pak D, Sheng M (2005) Polo-like kinases in the nervous system. *Oncogene* 24(2):292–298.
22. Seeburg DP, Sheng M (2008) Activity-induced Polo-like kinase 2 is required for homeostatic plasticity of hippocampal neurons during epileptiform activity. *J Neurosci* 28(26):6583–6591.
23. Pak DT, Sheng M (2003) Targeted protein degradation and synapse remodeling by an inducible protein kinase. *Science* 302(5649):1368–1373.
24. Lee KJ, Hoe HS, Pak DT (2011) Plk2 Raps up Ras to subdue synapses. *Small GTPases* 2(3):162–166.
25. Chen WF, et al. (2006) Alterations in long-term seizure susceptibility and the complex of PSD-95 with NMDA receptor from animals previously exposed to perinatal hypoxia. *Epilepsia* 47(2):288–296.
26. Chen WF, et al. (2007) Impaired expression of postsynaptic density proteins in the hippocampal CA1 region of rats following perinatal hypoxia. *Exp Neurol* 204(1): 400–410.
27. Inglis KJ, et al. (2009) Polo-like kinase 2 (PLK2) phosphorylates alpha-synuclein at serine 129 in central nervous system. *J Biol Chem* 284(5):2598–2602.
28. Nappi TC, et al. (2009) Identification of Polo-like kinase 1 as a potential therapeutic target in anaplastic thyroid carcinoma. *Cancer Res* 69(5):1916–1923.
29. Zeng LH, Xu L, Gutmann DH, Wong M (2008) Rapamycin prevents epilepsy in a mouse model of tuberous sclerosis complex. *Ann Neurol* 63(4):444–453.
30. Buckmaster PS, Ingram EA, Wen X (2009) Inhibition of the mammalian target of rapamycin signaling pathway suppresses dentate granule cell axon sprouting in a rodent model of temporal lobe epilepsy. *J Neurosci* 29(25):8259–8269.
31. Buckmaster PS, Wen X (2011) Rapamycin suppresses axon sprouting by somatostatin interneurons in a mouse model of temporal lobe epilepsy. *Epilepsia* 52(11): 2057–2064.
32. Buckmaster PS, Lew FH (2011) Rapamycin suppresses mossy fiber sprouting but not seizure frequency in a mouse model of temporal lobe epilepsy. *J Neurosci* 31(6): 2337–2347.
33. Matthew EM, et al. (2009) The p53 target Plk2 interacts with TSC proteins impacting mTOR signaling, tumor growth and chemosensitivity under hypoxic conditions. *Cell Cycle* 8(24):4168–4175.
34. Burrone J, O'Byrne M, Murthy VN (2002) Multiple forms of synaptic plasticity triggered by selective suppression of activity in individual neurons. *Nature* 420(6914): 414–418.
35. Karmarkar UR, Buonomano DV (2006) Different forms of homeostatic plasticity are engaged with distinct temporal profiles. *Eur J Neurosci* 23(6):1575–1584.
36. Huupponen J, Molchanova SM, Taira T, Lauri SE (2007) Susceptibility for homeostatic plasticity is down-regulated in parallel with maturation of the rat hippocampal synaptic circuitry. *J Physiol* 581(Pt 2):505–514.
37. Turrigiano GG, Leslie KR, Desai NS, Rutherford LC, Nelson SB (1998) Activity-dependent scaling of quantal amplitude in neocortical neurons. *Nature* 391(6670): 892–896.
38. Sanchez RM, Justice JA, Zhang K (2007) Persistently decreased basal synaptic inhibition of hippocampal CA1 pyramidal neurons after neonatal hypoxia-induced seizures. *Dev Neurosci* 29(1–2):159–167.
39. Sun Q, Turrigiano GG (2011) PSD-95 and PSD-93 play critical but distinct roles in synaptic scaling up and down. *J Neurosci* 31(18):6800–6808.
40. Kauselmann G, et al. (1999) The polo-like protein kinases Fnk and Snk associate with a Ca(2+)- and integrin-binding protein and are regulated dynamically with synaptic plasticity. *EMBO J* 18(20):5528–5539.
41. Newton SS, et al. (2003) Gene profile of electroconvulsive seizures: Induction of neurotrophic and angiogenic factors. *J Neurosci* 23(34):10841–10851.
42. Stornetta RL, Zhu JJ (2011) Ras and Rap signaling in synaptic plasticity and mental disorders. *Neuroscientist* 17(1):54–78.
43. Evers DM, et al. (2010) Plk2 attachment to NSF induces homeostatic removal of GluA2 during chronic overexcitation. *Nat Neurosci* 13(10):1199–1207.
44. Ang XL, Seeburg DP, Sheng M, Harper JW (2008) Regulation of postsynaptic RapGAP SPAR by Polo-like kinase 2 and the SCFbeta-TRCP ubiquitin ligase in hippocampal neurons. *J Biol Chem* 283(43):29424–29432.
45. Jin X, Prince DA, Huguenard JR (2006) Enhanced excitatory synaptic connectivity in layer v pyramidal neurons of chronically injured epileptogenic neocortex in rats. *J Neurosci* 26(18):4891–4900.
46. Isaeva E, Isaev D, Savrasova A, Khazipov R, Holmes GL (2010) Recurrent neonatal seizures result in long-term increases in neuronal network excitability in the rat neocortex. *Eur J Neurosci* 31(8):1446–1455.
47. Kelleher RJ, 3rd, Govindarajan A, Tonegawa S (2004) Translational regulatory mechanisms in persistent forms of synaptic plasticity. *Neuron* 44(1):59–73.
48. Richter JD, Klann E (2009) Making synaptic plasticity and memory last: Mechanisms of translational regulation. *Genes Dev* 23(1):1–11.
49. Banko JL, Hou L, Poulin F, Sonenberg N, Klann E (2006) Regulation of eukaryotic initiation factor 4E by converging signaling pathways during metabotropic glutamate receptor-dependent long-term depression. *J Neurosci* 26(8):2167–2173.
50. Gong R, Park CS, Abbassi NR, Tang SJ (2006) Roles of glutamate receptors and the mammalian target of rapamycin (mTOR) signaling pathway in activity-dependent dendritic protein synthesis in hippocampal neurons. *J Biol Chem* 281(27): 18802–18815.
51. Scheetz AJ, Nairn AC, Constantine-Paton M (2000) NMDA receptor-mediated control of protein synthesis at developing synapses. *Nat Neurosci* 3(3):211–216.
52. Sutton MA, Taylor AM, Ito HT, Pham A, Schuman EM (2007) Postsynaptic decoding of neural activity: eEF2 as a biochemical sensor coupling miniature synaptic transmission to local protein synthesis. *Neuron* 55(4):648–661.
53. Jensen FE, et al. (1998) Acute and chronic increases in excitability in rat hippocampal slices after perinatal hypoxia In vivo. *J Neurophysiol* 79(1):73–81.
54. Manning SM, et al. (2008) NMDA receptor blockade with memantine attenuates white matter injury in a rat model of periventricular leukomalacia. *J Neurosc* 28(26): 6670–6678.