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Sos2 is dispensable for NMDA-induced Erk activation and LTP induction

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Abstract

N-methyl-D-aspartate (NMDA) receptor-induced activation of extracellular signal-related protein kinase (Erk) plays important roles in various neuronal functions including long-term potentiation (LTP). Son of sevenless (Sos) proteins have been implicated in NMDA-induced Erk activation in neurons of young mice. However, contribution of each of the two Sos isoforms, Sos1 and Sos2, has not been clarified. In this study, Sos2 involvement in NMDA-induced Erk activation was examined. We observed no defect in Erk phosphorylation induced by NMDA treatment of cortical neuronal cultures from Sos2-/- newborn mice. Moreover, theta-burst induced LTP induction in the hippocampus of Sos2-/- mice was also normal. Finally, Erk activation by either depolarization or BDNF treatment was also normal in cultured neurons from Sos2 knockout mice. These results imply that Sos1 is the major regulator of these well-known neuronal Sos functions and suggest that a novel function for Sos2 in neurons remains to be determined.

Keywords

NMDA receptor; Erk; Sos; RasGRF; LTP

Introduction

LTP in CA1 hippocampal pyramidal neurons is believed to represent one form of the cellular basis for learning and memory [9]. The synapses between CA3 and CA1 neurons in this region of the hippocampus use glutamate as a neurotransmitter. NMDA-type glutamate receptor (NMDA-R) engagement induces elevation of intracellular calcium, which in turn activates Erk. This event is required for many types of LTP [2-4]. The GTPase Ras is a main activator of Erk, and two types of Ras activators, Ras-guanine nucleotide-releasing factor (RasGRF) and Sos, have been implicated in NMDA-induced Erk activation. RasGRF1 and RasGRF2 both have IQ motifs that allow for activation of the proteins by calcium-calmodulin binding [6]. Sos1 and Sos2 are thought to be activated by calcium-activated Pyk2 and Src tyrosine kinases through adaptor proteins like Grb2 [14]. RasGRF proteins begin to contribute to NMDA-R function at ~ day 20 in the mouse, just when the hippocampus begins to contribute to learning and memory. Before that time Sos, instead of RasGRF, seems to be involved [15]. Consistent

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with this model, expression levels of RasGRFs are very low after birth but increase during adolescence and reach maximum levels at around one month. In contrast, Sos expression levels are high just after birth and decrease over time and are lowest in adulthood. Thus, to activate Ras and Erk, NMDA -Rs appear to switch from using Sos proteins to RasGRF proteins during at a key stage in hippocampus development [15].

Sos proteins also mediate growth factor receptor pathways, such those that respond to neurotrophins, to activate Erk [1]. Sos1 and Sos2 have similar molecular structures, and both proteins mediate EGF-induced Erk activation. However, it has been shown that Sos1 participates in both short- and long-term Erk activation by EGF treatment, while Sos2-dependent signals are predominantly short-term [10]. Sos1 is required for embryo development because Sos1-/- mice are embryonic lethal [10]. On the other hand, Sos2-/- mice are apparently normal and reach fertility [5]. This lead to the hypothesis that Sos2 might be involved in physiological processes other than those that are critical for survival, such as synaptic plasticity.

In this study, we examined Sos2 involvement in NMDA-induced Erk activation and LTP induction in young mice. We found Erk activation by NMDA was normal in Sos2-/- neuronal cultures prepared from newborn mice. We also found no defect in LTP in the hippocampus of young Sos2-/- mice. These results indicate that Sos2 is not a significant mediator of NMDA-induced Erk activation or LTP induction in young mice.

Materials and Methods

Cortical neuronal cultures

Sos2 -/- mice were obtained as described previously [5]. Cortical neurons were prepared from the brains of newborn mice as previously described [16]. Briefly, cortices were dissected from newborn mouse brains, dissociated with papain, then plated onto poly-L-lysine/laminin-coated 6-well plates in Basal Medium Eagle supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 IU/mL penicillin and 100 μg/mL streptomycin. One day later, the medium was changed with Neurobasal medium supplemented with B27 supplement, 100 IU/mL penicillin, 100 μg/mL streptomycin and 2 mM L-glutamine. The cultures were used 7 days after plating. The cultures were incubated with 100 μM NMDA (Calbiochem), 100 ng/mL BDNF (Peprotech, Rocky Hill), or 50 mM KCl for the times indicated in the figures. NMDA was dissolved in water at 100 mM. KCl was dissolved in water at 1 M. BDNF was dissolved in water at 100 μg/mL. These stock solutions were diluted into culture media and added to the cell cultures. Cells were lysed in buffer A containing 10 mM Tris–HCl (pH 7.5), 1 mM EDTA, 150 mM NaCl, 1% NP-40, 0.1% SDS, 1 mM PMSF, 10 μg/ml aprotinin, 10 μg/ml leupeptin and 1 mM $NaVO_3$. The cleared lysates were subjected to SDS–PAGE and immunoblotting with different antibodies as indicated. Primary antibodies used are as follows; anti-Erk polyclonal antibody (Santa Cruz), anti-phospho-Erk monoclonal antibody (Cell Signaling), anti-Sos1 polyclonal antibody (Santa Cruz), anti-Sos2 polyclonal antibody (Santa Cruz). Secondary antibodies used are as follows; horseradish peroxidase (HRP)-conjugated anti-mouse IgG (H+L) and HRP-conjugated anti-rabbit IgG (H+L) (Jackson ImmunoResearch).

Hippocampal Slice Preparation

Mutant and control mice (14-day-old) were anesthetized with CO_2 until shallow breathing occurred and animals were unresponsive. Then, the animals were decapitated and the brain was quickly removed and submerged in ice-cold oxygenated sucrose-enhanced artificial cerebrospinal fluid (ACSF) cutting solution (206 mM sucrose, 2 mM KCl, 2 mM $MgSO_4$, 1.25 mM NaH_2PO_4, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 26 mM $NaHCO_3$, 10 mM D-glucose [pH 7.4], 315 mOsm). Transverse slices (350 μm thickness) from the middle portion of each hippocampus were cut with a vibroslicer (Vibratome). After dissection, slices were incubated

in ACSF that contained the following (in mM): 124 NaCl, 2 KCl, 2 $MgSO_4$, 1.25 NaH_2PO_4, 2 $CaCl_2$, 26 $NaHCO_3$, 10 D-glucose (pH 7.4), 310 mOsm, in which they were allowed to recover for at least 90 min before recording. A single slice was then transferred to the recording chamber and submerged in a continuous perfusion of ACSF that had been saturated with 95% O_2 and 5% CO_2. All of the perfusing solution contained 50 μM picrotoxin (Sigma) to block $GABA_A$ receptor-mediated inhibitory synaptic responses. Slices were incubated in the recording chamber for 20 min before stimulation.

Electrophysiological Recordings

To record field excitatory postsynaptic potentials (fEPSPs) in the CA1 region of the hippocampus, standard procedures were used. A unipolar stimulating electrode (World Precision Instruments, Sarasota, FL) was placed in the Schaffer collaterals to deliver test and conditioning stimuli. A borosilicate glass recording electrode filled with ACSF was positioned in stratum radiatum of CA1, 250–350 μm from the stimulating electrode. Test stimuli were applied at low frequency (0.05 Hz) at a stimulus intensity that elicited a field excitatory postsynaptic potential (fEPSP) amplitude that was ˜50% of maximum, and the test responses were recorded for 20–30 min prior to beginning the experiment to assure stability of the response. To induce LTP, four theta burst stimulations (TBS) separated by 20 s were applied to the CA1 area. The TBS consisted of 15 bursts of 4 pulses at 100 Hz, delivered at an interburst interval of 200 ms were used to induce LTP. The field potentials were amplified 100× using Axon Instruments 200B amplifier and digitized with Digidata 1322A. The data were sampled at 10 kHz and filtered at 2 kHz. Traces were obtained by pClamp 9.2 and analyzed using the Clampfit 9.2. The fEPSP magnitude was measured via the initial fEPSP slope, and three consecutive slopes (1 min) were averaged and normalized to the mean value recorded 20 min before conditioning stimulus. Data were pooled across animals of the same genotype and are presented as mean ± SEM. Values expressed here represent 60 min time points after conditioning stimulus was initiated, unless stated otherwise. The following statistical analysis was carried out: the same time window samples of the control and treatment were compared by paired, two-tailed Student's t test. The difference between the effects of different groups was tested by one-way analysis of variance (ANOVA). An effect was considered significant if $p < .05$. Student-Newman-Keuls post-hoc tests were used to examine the significance of multiple pairwise comparisons.

Results

Cortical neuron cultures were prepared from postnatal day 0 pups and used 7 days after plating. Figure 1a shows neuron cultures from wild type and Sos2-/-mice. There was no apparent difference at the macroscopic level. Absence of Sos2 protein in Sos2-/- neurons was confirmed by immuno-blotting (Figure 1b). Sos1 expression was similar in both wild type and Sos2-/- neurons (Figure 1b).

These neurons were stimulated with NMDA, and Erk activation was examined by immuno-blotting with phospho-specific Erk antibody. In wild-type neuronal cultures, NMDA stimulation activated Erk ~ 4-fold (Figure. 2). In Sos2-/- neurons, NMDA stimulation activated Erk to a similar degree (Figure. 2). Thus, Sos2 is not the predominant mediator of NMDA-induced Erk activation in these cells.

Erk is also known to be activated by KCl-induced depolarization and brain derived neurotrophic factor (BDNF) treatment [11,12]. Both depolarization and BDNF induced Erk phosphorylation in Sos2-/- neuronal cultures to a degree similar to that of cultures from wild-type mice (Figure 2), indicating that Sos2 is not the major mediator of Erk activation by these stimuli.

To assess contribution of Sos2 in LTP induction, we used extracellular recording in CA1 region of hippocampus after theta-burst stimulation, a protocol known to function through Erk activation [8,13]. In hippocampal slices from two week-old Sos2-/- mice, a development stage where RasGRF is not required for LTP induction [7], there was no significant defect in LTP (Figure 3) (wild type, 144.5 ± 5.7%; Sos2-/-, 143.1 ± 7.0%).

Discussion

In this study, we showed that Sos2 is dispensable for both NMDA,-induced Erk activation in cortical neuron cultures from neonatal mice, and for LTP induction at the Schaffer-CA1 synapses in brain slices from young mice that is known to require NMDA receptor signaling.

Two types of Ras activators, Sos and RasGRF, have been implicated in NMDA-activation of Erk, as well as NMDA receptor-mediated LTP induction. However, their influence depends upon the age of the animal. RasGRF1 and RasGRF2 are required for these biological processes only after ~ day 20 in the mouse, and as such RasGRF knockout mice show no defects in NMDAR activation of Erk or LTP before this time [7,15]. In contrast, Sos proteins appear to contribute to these processes in younger mice. This idea is supported by indirect data showing that in neurons and brain slices from young mice, NMDA stimulates complex formation between Grb2 and Shc, adaptor proteins known to promote Sos activation, and NMDA activation of Erk is blocked by an inhibitor of Src, a known intermediary in Sos activation induced by calcium in neurons [15]. This idea is consistent with their opposite expression profiles; RasGRF proteins are expressed at their highest levels in the adult, whereas Sos proteins are expressed at their highest levels in the young [15].

We showed here that Sos2 is not required for NMDA activation of Erk nor LTP induction in CA1 area of hippocampus of young animals before adolescence. Sos1 is expressed in Sos2 knockout neurons at the same level as in wild type neurons, implying that increased signaling through Sos1 is not masking a suppressed Erk signaling through Sos2 in Sos2 knockout mice. Increased RasGRF signaling does not seem to be involved either because we showed previously that GRF proteins do not contribute to NMDA-induced Erk in young neuron cultures [15] and we found here that the level of GRF proteins also did not rise in Sos2 knockout neurons (data not shown). Thus, it is reasonable to speculate that Sos1, not S0s2, mediates NMDA-induced Erk activation. Sos1 homozygous knockout mice are not available because they are embryonic lethal [10], Unfortunately, we have been unable to transiently knockdown Sos1 levels in neuronal cultures using shRNA to test this possibility directly.

In summary, we showed that Sos2 is dispensable for NMDA-induced Erk activation in cortical neuron cultures, and for LTP induction in young mice. We also showed it was not involved in depolarization or NGF induced Erk either. Further study will be needed reveal, what could be novel function for Sos2 in young neurons of the brain.

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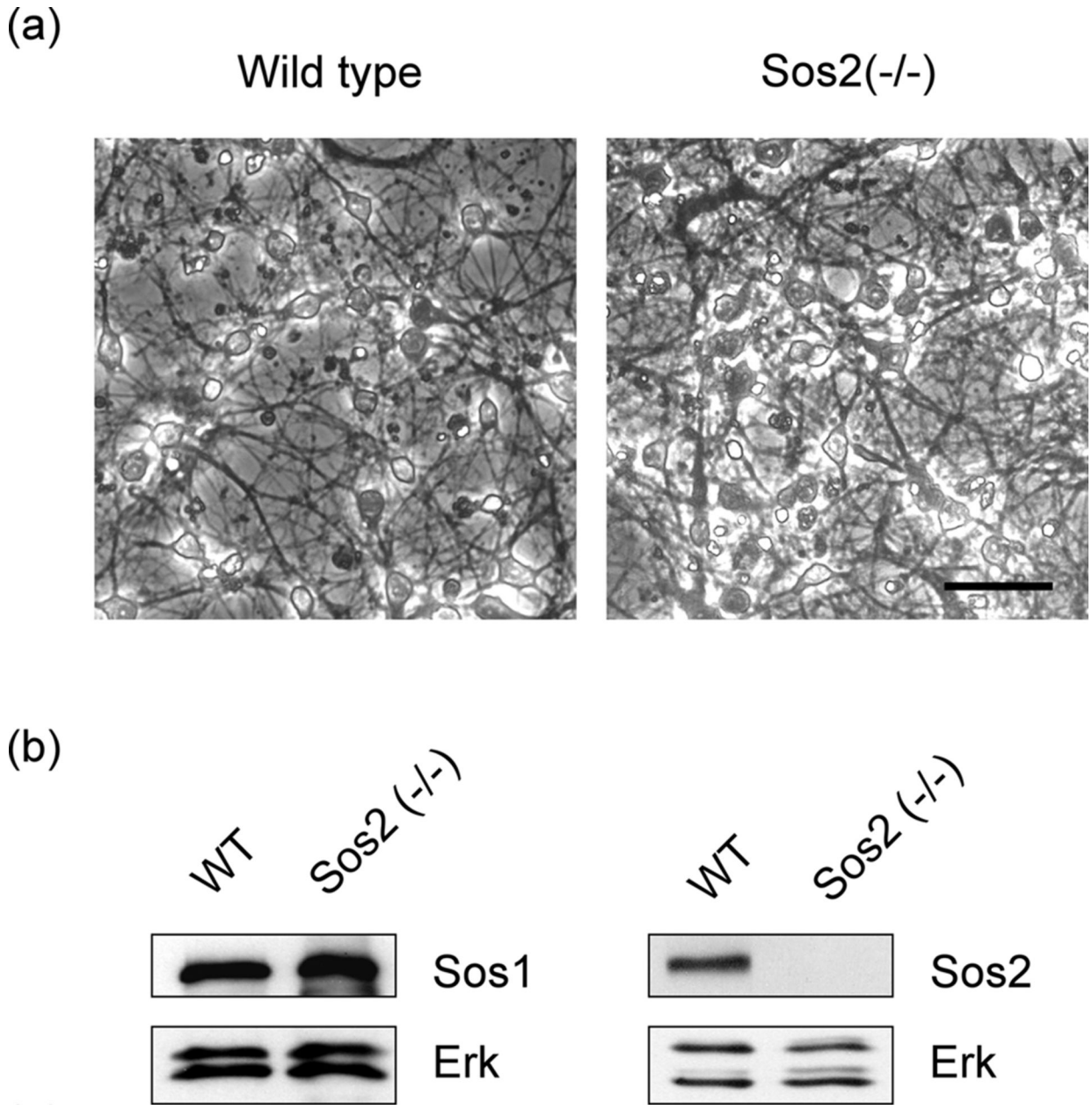

Figure 1.
Cortical neuron culture. Cortical neurons were prepared from P0 mice and cultured for 7 days. (a) Phase contrast images of wild type and Sos2-/- cortical neuron cultures at 7 days in vitro. Bar, 50 μm. (b) Expression of Sos1 and Sos2 in wild type and Sos2-/- neuron cultures.

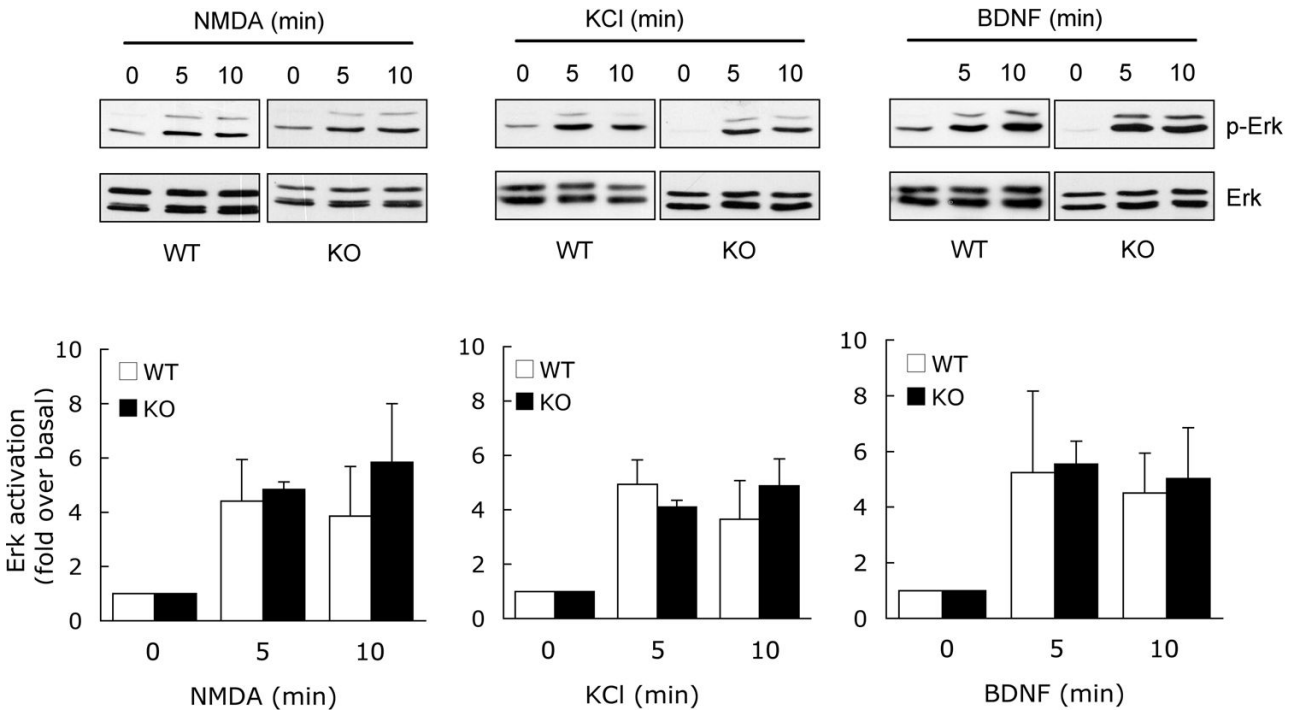

Figure 2.

Erk activation by NMDA, KCl, and BDNF in wild type and Sos2-/- neuron cultures. Neuron cultures were stimulated with 100 μM NMDA, 50 mM KCl, or 100 ng/mL BDNF for indicated times and the lysates were subjected to western blotting for total Erk and phospho-Erk. Bar graphs represent the averaging (±SD) of data from at least three experiments.

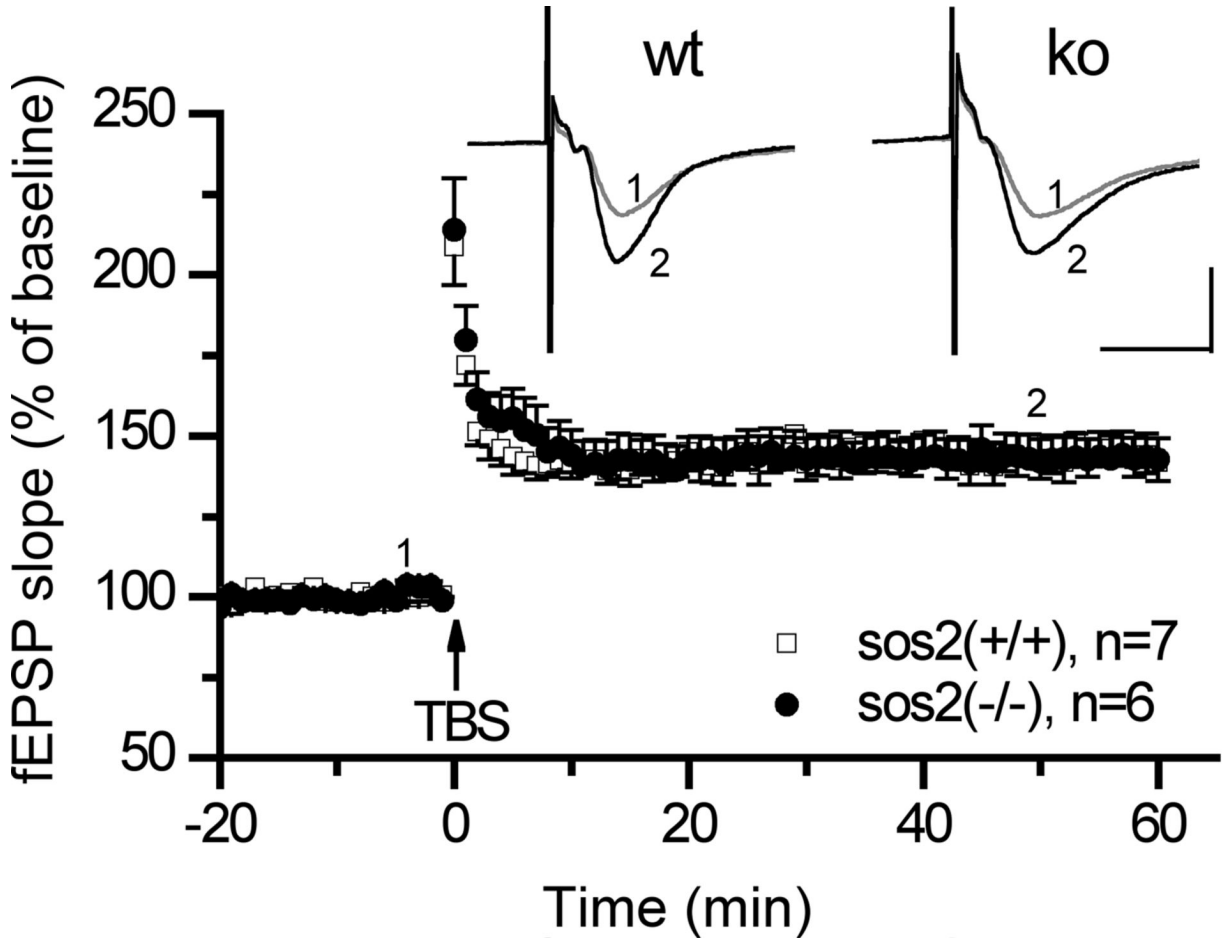

Figure 3.

LTP induction in Schaffer collateral-CA1 synapses in wild type and Sos2-/-. Theta burst stimulation (TBS, 15 bursts of 4 pulses at 100 Hz, interburst interval 200 ms) was used to induce LTP in the CA1 region of the hippocampal slices of P14 wild type and Sos2-/- mice. The recorded fEPSP slope is expressed as percent of baseline ± SEM, and data were pooled from each group.