Input-specific synaptic plasticity in the amygdala is regulated by neuroligin-1 via postsynaptic NMDA receptors

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Despite considerable evidence for a critical role of neuroligin-1 in the specification of excitatory synapses, the cellular mechanisms and physiological roles of neuroligin-1 in mature neural circuits are poorly understood. In mutant mice deficient in neuroligin-1, or adult rats in which neuroligin-1 was depleted, we have found that neuroligin-1 stabilizes the NMDA receptors residing in the postsynaptic membrane of amygdala principal neurons, which allows for a normal range of NMDA receptor-mediated synaptic transmission. We observed marked decreases in NMDA receptor-mediated synaptic currents at afferent inputs to the amygdala of neuroligin-1 knockout mice. However, the knockout mice exhibited a significant impairment in spike-timing-dependent long-term potentiation (STD-LTP) at the thalamic but not the cortical inputs to the amygdala. Subsequent electrophysiological analyses indicated that STD-LTP in the cortical pathway is largely independent of activation of postsynaptic NMDA receptors. These findings suggest that neuroligin-1 can modulate, in a pathway-specific manner, synaptic plasticity in the amygdala circuits of adult animals, likely by regulating the abundance of postsynaptic NMDA receptors.

STD-LTP | thalamic pathway | cortical pathway | autism

A number of studies have indicated that synaptically localized cell adhesion molecules not only trigger de novo synapse formation but also play a critical role in regulating both synaptic transmission and synaptic plasticity (1). The heterophilic cell adhesion molecules—neurexins and neuroligins—have emerged as important regulators of synaptic function in mature neural circuits (2). Among the several isoforms, neuroligin-1 (NLGN1) has been reported to be present in the postsynaptic density of excitatory synapses (3) and interacts with the postsynaptic scaffolding protein PSD-95 via a specific PDZ binding motif (4). We and others have shown that in the adult brain NLGN1 is critically involved in the maintenance of currents mediated by N-methyl-D-aspartic acid type glutamate receptors (NMDARs) but not by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid type glutamate receptors (AMPARs) (5, 6). Given the importance of NMDARs for synaptic plasticity and neuropathology (7, 8), it seems particularly important to understand how NLGN1, a candidate gene in autism, controls NMDAR-mediated synaptic transmission.

Certain forms of synaptic plasticity are regulated by NMDARs (9), and NLGN1 is expressed ubiquitously in various brain areas (2, 3). Thus, it is likely that NLGN1 expression regulates synaptic plasticity as well as the maturation and refinement of neural networks. Indeed, we have previously found that appropriate levels of NLGN1 are required for normal development of pairing-induced LTP at the auditory thalamic inputs to the lateral nucleus of the amygdala (LA) (6). In addition to the thalamic inputs, LA has another auditory input from the auditory cortex (10). Synaptic plasticity induced in the cortical pathway also contributes to the formation and consolidation of fear memories (11–13). It is therefore important to determine whether NLGN1 can modulate synaptic plasticity in each of these two pathways of the amygdala so as to elucidate the physiological consequences of NLGN1 on synaptic plasticity in the circuits of the amygdala that are critically involved in processing sensory information and storage of emotional memories.

To examine how NLGN1 regulates synaptic transmission at the cellular level and whether NLGN1 controls STD-LTP induced at the convergent inputs to the LA, we used both the viral knockdown method and a knockout (KO) mouse model. Electrophysiological analyses revealed that NLGN1 sustains NMDAR-mediated synaptic transmission by regulating the abundance of NMDARs at postsynaptic sites. We also found that NLGN1 expression modulates STD-LTP only at a subset of synapses in the amygdala circuits of adult animals.

Results

Voltage-Independent Regulation of NMDAR-Mediated Synaptic Transmission by NLGN1 Expression. To elucidate the cellular mechanisms whereby NLGN1 controls NMDAR-mediated excitatory postsynaptic currents (NMDAR-EPSCs), we extended our study with mutant mice deficient in *NLGN1* (5). We first analyzed the intrinsic membrane properties and the somatic excitability of the LA principal neurons from *NLGN1* KO and wild-type (WT) littermate control mice. However, we did not detect any difference in all of the measured parameters between *NLGN1* KO and WT littermate mice (Table S1). These results revealed that the expression of NLGN1 was not directly involved in regulating intrinsic membrane characteristics and neuronal excitability of the principal neurons of the LA.

We examined the voltage-dependency of EPSCs. We elicited EPSCs by stimulating the internal capsule (thalamic inputs; Fig. 1*A*), and constructed current-voltage (I–V) curves for each type of EPSC. We found that I–V curves of AMPAR-EPSCs were linear and indistinguishable between *NLGN1* KO and WT littermate mice (Fig. 1*C*). By contrast, amplitudes of NMDAR-EPSCs were reduced in *NLGN1* KO mice not only at positive holding potentials but also at a negative potential (Fig. 1 *B* and *C*).

To corroborate our findings obtained from *NLGN1* KO mice, we also used lentivirus containing a small hairpin RNA sequence targeted to *NLGN1* (shNLGN1) to transiently deplete NLGN1 in the LA. We initially confirmed the efficacy of shNLGN1 by both in vitro expression test and in vivo viral delivery to the amygdala

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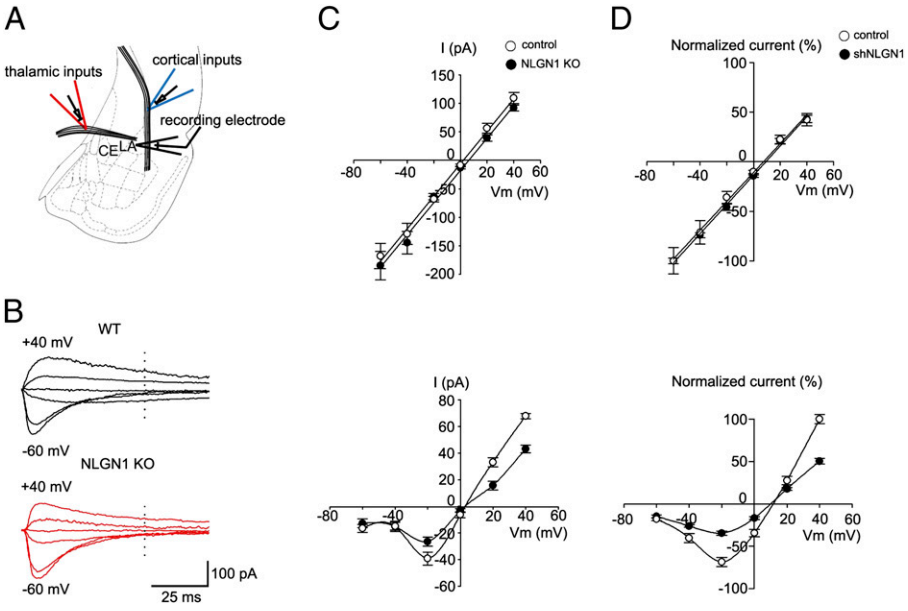

Fig. 1. Voltage-independent decreases in NMDAR-mediated synaptic currents by the deletion or depletion of NLGN1. (*A*) A schematic representation of an amygdala slice shows placement of recording and stimulation electrodes for either cortical or thalamic pathway. LA, lateral amygdala; CE, central amygdala. (*B*) EPSCs were evoked by stimulating the thalamic inputs at holding potentials from −60 mV to +40 mV in WT (*Upper*) and *NLGN1* KO mice (*Lower*). Each trace is an average of three EPSCs at each holding potential. AMPAR-EPSCs were measured at the peaks of the responses, and NMDAR-EPSCs were measured at 50 ms (vertical dotted line) after the onset of EPSCs. (*C*) Current-voltage relationships of AMPAR- (*Upper*) and NMDAR-EPSCs (*Lower*) in WT control (n = 6, open circles) and *NLGN1* KO mice (n = 6, closed circles). (*D*) Current-voltage relationships of AMPAR- (*Upper*) and NMDAR-EPSCs (*Lower*) in uninfected control (n = 16, open circles) and shNLGN1-infected LA neurons of adult rats (n = 14, closed circles). The amplitudes of each type of EPSCs were normalized to mean current amplitudes from uninfected control cells at either −60 mV (for AMPAR-EPSCs) or +40 mV (for NMDAR-EPSCs).

(Fig. S1). In the amygdala slices prepared from rats where shNLGN1 was infused 3–4 days earlier, we constructed I–V curves for either NMDAR-EPSCs or AMPAR-EPSCs. We further isolated AMPAR- and NMDAR-EPSCs by the use of corresponding antagonists as described in *SI Materials and Methods*. Though AMPAR-EPSCs, which we measured in the presence of a NMDAR antagonist, D-2-amino-5-phosphonopentanoic acid (APV), were similar between shNLGN1-infected and control neurons, NMDAR-EPSCs in the presence of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were significantly reduced in shNLGN1-infected neurons (Fig. 1*D*), similar to those in the *NLGN1* KO mice. The deletion or depletion of NLGN1 resulted in a similar level of inhibition (up to 50%) of NMDAR-EPSCs throughout all holding potentials (except intersections), indicating that neither deletion nor depletion of NLGN1 produced any voltage-dependent effects on NMDAR-EPSCs, such as the open probability of NMDARs upon membrane depolarization.

Another plausible explanation for the decreases in NMDAR-EPSCs could be the altered channel properties of individual NMDARs due to a change in the subunit composition (14). It was previously reported that switching from NR2B- to NR2A-containing receptors, which are incorporated into synapses of hippocampal neurons, results in decreases in NMDAR-EPSCs as well as their faster decay (15). To explore this possibility, we compared the decay kinetics of evoked NMDAR-EPSCs, but detected no difference (Fig. S2 *A* and *B*). The unaltered decay kinetics of NMDAR-EPSCs indicated that NLGN1 is unlikely to regulate the subunit composition of NMDARs and subsequent changes in the channel gating properties of NMDARs.

NLGN1-Mediated Stabilization of Synaptic NMDARs. The specific decreases in NMDAR-EPSCs could be attributed to a reduced number of NMDARs in the surface plasma membrane of postsynaptic cells. To test this possibility directly, we stimulated all of the NMDARs in the surface membrane by perfusing 1 mM glutamate (50 ms) at +40 mV in the presence of CNQX. After standardization of experimental conditions, such as the puffing parameters and the relative distance between puffing pipettes and somas (Fig. S3*A*), we measured the agonist-induced responses, which revealed no significant difference in the peak amplitudes of the NMDAR-EPSCs (Fig. S3 *B* and *C*). This result suggested that similar numbers of NMDARs are present in the surface membrane independent of the expression of NLGN1, regardless of their specific spatial loci within the membrane.

The observed differential effects of shNLGN1 on the agonist-induced and synaptically evoked NMDAR-EPSCs prompted us to test if NLGN1 might regulate the abundance of NMDARs confined at synapses. To address this question, we analyzed the NMDAR components of mEPSCs. This allowed us to measure the quantal transmission through NMDARs at single synapses (16). Because quantal NMDAR components of mEPSCs are difficult to isolate due to their small amplitude, we turned to a digital subtraction method to obtain pure NMDAR-mediated mEPSCs (17, 18). Under low Mg^{2+}, we first recorded mEPSCs, which contained both AMPAR and NMDAR components, and then recorded again AMPAR-mediated EPSCs at the same neurons in the presence of APV (Fig. 2*A*). By subtracting the average waveform of mEPSCs from that before APV treatment, we obtained NMDAR-mediated mEPSC waveforms (Fig. 2*B*). The mean area under the curve of NMDAR-mediated mEPSCs was significantly reduced in shNLGN1-infected neurons compared with that in uninfected control neurons, indicating the reduced abundance of functional NMDAR at single synapses (Fig. 2*B*). Collectively, these experimental findings support the idea that NLGN1 acts to stabilize functional NMDARs at individual synapses and, as a result, sustain a normal range of NMDAR-EPSCs.

***NLGN1* Deletion Affects STD-LTP in the Thalamic but Not Cortical Pathway.** LA has another major auditory afferent pathway—the cortical inputs in addition to the thalamic inputs (10). A number of studies suggest that synaptic plasticity occurring at the cortico-amygdala synapses can also support fear conditioning, but that it does so differently from that in the thalamic pathway, by responding preferentially to weak or more complex stimuli (11–13). We analyzed the relative contribution of NMDARs and AMPARs to EPSCs at both afferent inputs by measuring NMDAR/AMPAR ratios while stimulating either internal or external capsules, respectively (Fig. 1*A*). Consistently with the I–V relation (Fig. 1*C*), we found significant decreases in NMDAR/AMPAR ratios at thalamo-amygdala synapses of *NLGN1* KO mice, compared with that of WT control mice (Fig. 3 *A* and *B*). Interestingly, we also observed significant decreases in NMDAR/AMPAR ratios in the cortical pathway of *NLGN1* KO mice compared with that of WT littermate controls (Fig. 3 *A* and *B*).

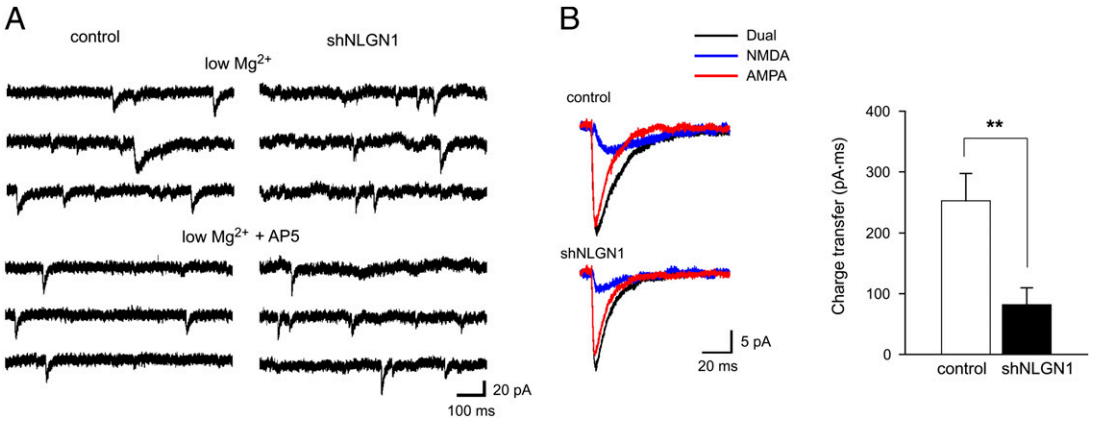

Fig. 2. NMDAR component of mEPSCs maintained by NLGN1. (*A*) Three representative traces recorded at −70 mV from uninfected control- and shNLGN1-infected neurons, respectively, for each condition (low Mg^{2+} vs. low Mg^{2+} + APV) are shown. (*B*) Superimposed averaged traces of mEPSCs obtained in each condition (black, low Mg^{2+}; red, low Mg^{2+} + APV) and the subtracted traces (blue) that yields an average NMDAR-mediated mEPSC recorded from control- (*Left Upper*) and shNLGN1-infected (*Left Lower*) neurons are indicated. A summary histogram for quantal charge transfer through synaptic NMDARs (area under the curve of NMDAR-mediated mEPSCs) is depicted (*Right*); control group, 252.2 ± 44.9 pA·ms (n = 7) vs. shNLGN1 group, 81.3 ± 27.9 pA·ms (n = 5).

Thus, NLGN1 seems to control and sustain NMDAR-mediated synaptic transmission in various neural pathways and brain regions (5, 6).

Activation of NMDARs in the amygdala is believed to be essential for induction of synaptic plasticity and storage of associative fear memory (6, 19–21). Given the marked and similar decreases in NMDAR-EPSCs at both the afferent inputs to the LA of *NLGN1* KO mice, synaptic plasticity might be affected accordingly. To examine this possibility, we used a physiologically relevant protocol to induce STD-LTP. This form of LTP has been widely considered as a physiological model of synaptic modifications during the integration of multiple inputs and thus has emerged as a candidate mechanism for learning-related activity in neural circuits (22, 23). To induce STD-LTP, we delivered 80 presynaptic stimuli at 2 Hz to elicit excitatory postsynaptic potentials (EPSPs) in a current-clamp mode, and each stimulus was paired with an action potential (AP) evoked in a patched postsynaptic neuron following a 5-ms delay. In agreement with previous findings (13, 24), we detected significant LTP in both thalamo- and cortico-amygdala pathways of WT mice (Fig. 3 *C*–*E*). As expected, we observed that STD-LTP was abolished at the thalamo-amygdala synapses of *NLGN1* KO mice (Fig. 3 *C* and *D*). At 30 min after the STD-LTP induction, EPSPs were potentiated to 141.1 ± 6.8% of its baseline in control mice (eight cells from three control mice) but remained at only 104.6 ± 3.2% of its baseline in *NLGN1* KO mice (six cells from three KO mice; Fig. 3 *C* and *D*). However, STD-LTP occurring at the cortico-amygdala synapses remained unaffected in *NLGN1* KO mice (Fig. 3 *E* and *F*). EPSPs at the cortico-amygdala synapses were potentiated to 153.0 ± 7.7% of its baseline in control mice (nine cells from four control mice) and only slightly changed to 140.6 ± 10.1% of its baseline in *NLGN1* KO mice ($P > 0.2$; seven cells from four KO mice; Fig. 3 *E* and *F*). Given the similar decreases in NMDAR-EPSCs in both pathways of *NLGN1* KO mice, the selective impairment in STD-LTP at the thalamo-amygdala synapses was surprising to us. These findings suggest that *NLGN1* deletion might have a differential effect on STD-LTP at the individual afferent inputs to the LA while otherwise producing very similar effects on NMDAR-dependent transmission at both afferent inputs to the LA.

LTP at Thalamo-Amygdala but Not Cortico-Amygdala Synapses Depends on Activation of Postsynaptic NMDARs. Blockade of NMDARs by perfusion of APV blocks STD-LTP in both thalamo- and the cortico-amygdala pathways (25), which indicates that activation of NMDARs exerts an essential role for the induction of STD-LTP. However, expression of heterosynaptic LTP in the cortical but not the thalamic inputs requires the activation of presynaptic NMDARs independent of postsynaptic activity (26). We reasoned that STD-LTP occurring at cortical inputs could be insensitive to a decrease in NMDAR-dependent transmission in *NLGN1* KO mice if STD-LTP in that pathway depends primarily upon presynaptic NMDARs rather than postsynaptic NMDARs. We initially perfused APV (50 μM in perfusion media) and then applied the same STD-LTP induction protocol. Consistent with the previous report (25), LTP in both pathways was almost completely abolished by APV perfusion (Fig. 4). To dissect out the contribution of pre- and postsynaptic NMDARs, we included a noncompetitive NMDAR antagonist MK-801 (0.5 mM) in the patch pipette solution onto the LA principal neurons of WT mice. This intervention abolished LTP in the thalamic pathway but did not affect LTP in the cortical pathway (Fig. 4 *C* and *D*; 30 min after STD-LTP induction, thalamic, 99.2 ± 5.5%, n = five cells/three mice, P = 0.0012, relative to LTP without MK-801 vs. cortical, 136.3 ± 7.5%, n = five cells/three mice, P = 0.18, relative to LTP without MK-801). Taken together, these findings suggest that STD-LTP at the cortical inputs to the LA does not require activation of postsynaptic NMDARs but rather primarily depends upon presynaptic NMDARs.

To confirm the dispensability of postsynaptic NMDARs for STD-LTP induction in *NLGN1* KO mice, we induced STD-LTP at the cortical inputs to the LA of *NLGN1* KO mice after inclusion of MK-801 in the patch pipette. In the range of concentrations of MK-801 that were tested, STD-LTP was reliably initiated and sustained, as was the case in the absence of MK-801 (Fig. S4), arguing that STD-LTP induction in the cortical pathway of *NLGN1* KO mice was also indifferent to activation of postsynaptic NMDARs. Therefore, the intact STD-LTP at the cortical inputs of *NLGN1* KO mice is most likely to result from the intrinsic mechanisms by which STD-LTP was induced in individual pathways of amygdala circuits.

STD-LTP at Thalamo-Amygdala Synapses of *NLGN1* KO Is Restored by Sustained Postsynaptic Depolarization. *NLGN1* deletion did not completely block NMDAR-mediated currents (~50% currents still remained; this study and refs. 5 and 6). We reasoned that postsynaptic [Ca^{2+}] at the thalamo-amygdala synapses would not reach the threshold for LTP induction due to a decrease in the abundance of synaptic NMDARs in *NLGN1* KO mice. LTP

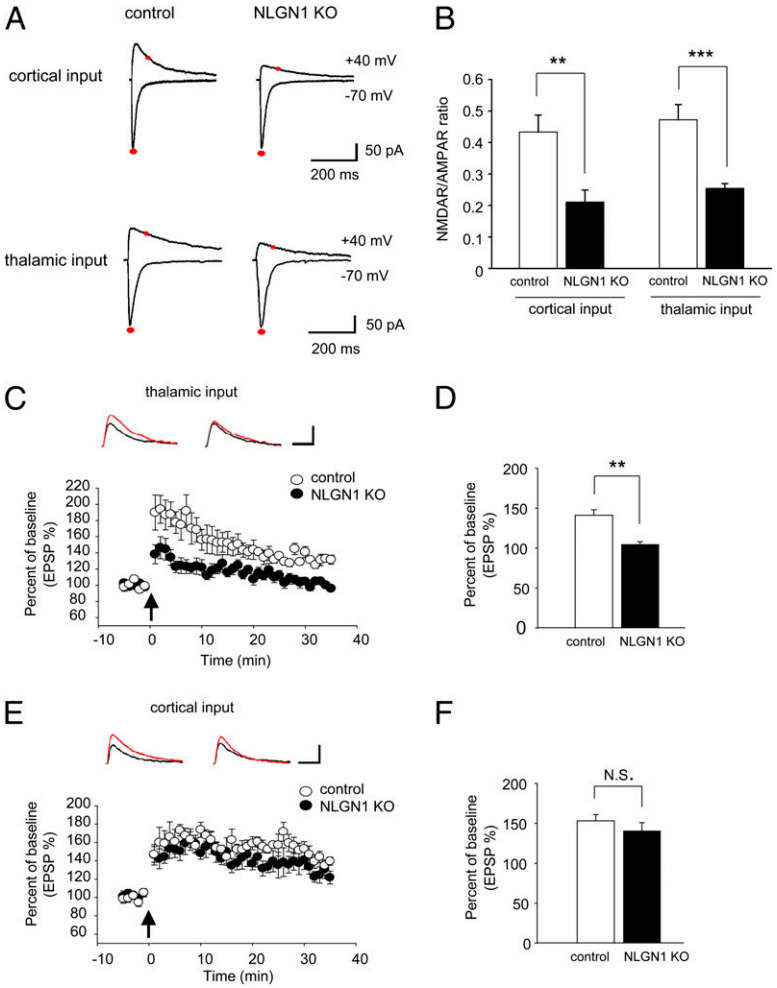

Fig. 3. Similar decreases in ratios of NMDAR/AMPAR but different STD-LTP at cortical and thalamic inputs to the LA of *NLGN1* KO mice. (*A*) Representative traces of EPSCs for obtaining NMDAR/AMPAR ratios are shown. AMPAR-EPSCs (at −70 mV, large red circles) and NMDAR-EPSCs (at +40 mV, small red circles) were measured at either cortical (*Upper*) or thalamic inputs (*Lower*) to the LA of either WT (*Left*) or *NLGN1* KO (*Right*) mice. (*B*) A summary histogram of NMDAR/AMPAR ratios is depicted for the cortical inputs; WT control, 0.43 ± 0.05 (n = 14) vs. *NLGN1* KO, 0.21 ± 0.03 (n = 16) and for the thalamic inputs; WT control, 0.47 ± 0.04 (n = 12) vs. *NLGN1* KO, 0.25 ± 0.01 (n = 12). (*C*) The results of STD-LTP experiments at the thalamo-amygdala synapses are presented. (*Inset*) Average of four EPSPs recorded in individual experiments before (black) and 30 min after (red) the STD-LTP-inducing stimulation in either WT (*Left*) or *NLGN1* KO (*Right*). (Scale bar, 50 ms and 2 mV.) (*D*) A summary histogram of STD-LTP experiments at the thalamo-amygdala synapses 30 min after the STD-LTP stimulation is depicted; WT control, 141.1 ± 6.8% (n = 8) vs. *NLGN1* KO, 104.6 ± 3.2% (n = 6). (*E*) The results of STD-LTP experiments at the cortico-amygdala synapses are presented. (*Inset*) Average of four EPSPs recorded in individual experiments before (black) and 30 min after (red) the STD-LTP-inducing stimulation in WT (*Left*) or *NLGN1* KO (*Right*). (Scale bar, 50 ms and 2 mV.) (*F*) A summary histogram of STD-LTP experiments at the cortico-amygdala synapses 30 min after the LTP-inducing stimulation is depicted ($P > 0.1$); WT control, 153.1 ± 7.7% (n = 9) vs. *NLGN1* KO, 140.6 ± 10.1% (n = 10).

might be restored by normalizing Ca^{2+} influx via NMDARs if the observed blockade of LTP at the thalamic pathway was a direct consequence of a reduced abundance of NMDARs but not because of any subsequent perturbation in biochemical signaling downstream to NLGN1. Toward this end, we further depolarized the postsynaptic LA neurons to −20 mV in between the APs during a stimulation burst (Fig. 5*A Insets*). Indeed, the additional depolarization led to considerable STD-LTP in the thalamic pathway of *NLGN1* KO mice, which had previously been absent without the extradepolarization (Fig. 5*B*; 30 min after STD-LTP induction, *NLGN1* KO neurons with extradepolarization, 127.7 ± 4% of baseline, n = eight cells/four KO mice, relative to STD-LTP in *NLGN1* KO neurons with the standard STD-LTP protocol, 100.9 ± 3% of baseline, n = six cells/three KO mice).

The additional depolarization appeared to rescue STD-LTP at the thalamo-amygdala synapses of *NLGN1* KO mice. However, it has previously been reported that the same extradepolarization can enhance Ca^{2+} influx through voltage-dependent Ca^{2+} channels (VDCCs) at the cortico-amygdala synapses of the LA (27). Accordingly, the sustained depolarization that we applied might rescue STD-LTP by additional activation of VDCCs rather than residual NMDARs at the thalamo-amygdala synapses of *NLGN1* KO mice. We sought to examine whether the restoration of STD-LTP genuinely resulted from the additional activation of NMDARs of *NLGN1* KO mice. Therefore, we applied the same extradepolarization STD-LTP protocol, but now after inclusion of MK-801 in the patch pipette solution (Fig. 5*A*). In this condition, we observed no significant STD-LTP, similar to that obtained when the standard STD-LTP protocol was applied (Fig. 5*B*; 111.6 ± 3% of baseline, n = six cells/three KO mice). These findings suggest that STD-LTP at the thalamo-amygdala synapses was restored by additional activation of residual NMDARs, which presumably leads to the enhancement of Ca^{2+} influx through the remaining NMDARs at synaptic sites.

Discussion

The role of NLGN1 in sustaining NMDAR-EPSCs (5, 6) is likely due to the modification of postsynaptic NMDARs rather than alterations in presynaptic transmitter release, because NLGN1 is localized at the postsynaptic sites (3) and AMPAR-EPSCs were unaffected by either depletion or deletion of NLGN1. NLGN1 might regulate intrinsic channel properties or subunit compositions of NMDARs. However, the I–V curves revealed that the reduction of NMDAR-EPSCs in *NLGN1* KO mice was voltage independent. The voltage-independent reduction of NMDAR-EPSCs was corroborated in shNLGN1-infected principal neurons of the LA of adult rats. Therefore, NLGN1 is not likely to modify intrinsic channel properties such as voltage-dependent Mg^{2+} block, a release of which leads to the depolarization-mediated opening of NMDARs. Furthermore, unaltered decay kinetics of synaptically evoked NMDAR-EPSCs argues against change in the subunit composition of the NMDARs when NLGN1 was deleted. We also detected no decrease in NMDARs-EPSCs, which were elicited by glutamate perfusion, suggesting that the abundance of the total NMDARs in the surface membrane did not change by NLGN1 depletion.

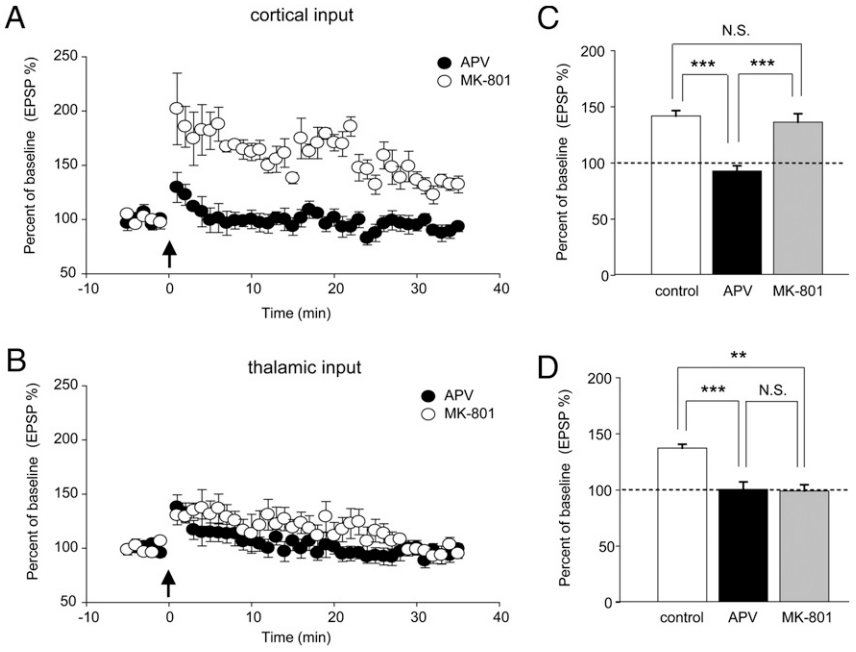

Fig. 4. STD-LTP at the cortico-amygdala synapses dependent upon activation of presynaptic NMDARs. (*A*) STD-LTP in the cortico-amygdala synapses was blocked by 50 μM APV perfusion, but it was normally induced despite inclusion of 0.5 mM MK-801 in the patch pipettes. (*B*) STD-LTP in the thalamo-amygdala synapses was blocked by both perfusion of APV and inclusion of MK-801 in the patch pipettes. (*C*) A summary histogram is depicted for each antagonist intervention at the cortical inputs; control, 141.8 ± 4.7% (n = 7) vs. APV perfusion, 92.9 ± 4.4% (n = 5) vs. MK-801 inclusion, 136.3 ± 7.5% (n = 5), one-way ANOVA followed by Tukey post hoc test. (*D*) A summary histogram is depicted for each antagonist intervention at the thalamic inputs; control, 137.3 ± 3.4% (n = 6) vs. APV perfusion, 100.7 ± 6.4% (n = 6) vs. MK-801 inclusion, 99.2 ± 5.5% (n = 5), one-way ANOVA followed by Tukey post hoc test. These experiments were performed in the LA slices from WT control mice.

Finally, we found that NMDAR-mediated mEPSCs were significantly reduced by the depletion of NLGN1. This quantal analysis provided evidence that the relative abundance of NMDARs at individual synapses could be regulated by NLGN1. However, we cannot exclude the possibility that NLGN1 might regulate the ionic conductance of individual NMDARs in more subtle but voltage-independent ways. The detailed molecular mechanisms underlying the stabilizing role of NLGN1 remain unknown. Two attractive possibilities are (*i*) NLGN1 may facilitate the stable binding of NMDARs to PSD-95 or other scaffolding proteins, or (*ii*) NLGN1 might interact directly with NMDARs and hold them at postsynaptic sites. Further detailed investigations will be required to clarify these issues.

The similar inhibition of NMDAR-EPSCs in both thalamic and cortical pathways, but the specific impairment of STD-LTP in the thalamic pathway of *NLGN1* KO mice was unexpected, especially because STD-LTP at cortico-amygdala synapses has previously been reported to require the activation of NMDARs (25). How does NLGN1 selectively regulate STD-LTP at a subset of synapses on the principal neurons in the LA? Both the induction and expression of LTP at the thalamo-amygdala synapses relies largely upon postsynaptic mechanisms (10, 27, 28). Our findings confirm the requirement of the postsynaptic activities for STD-LTP in the thalamic pathway, which in turn, could account for the impairment in STD-LTP at the thalamic inputs to the LA of *NLGN1* KO mice. However, the induction of LTP at the cortical inputs to the LA recruits more diverse mechanisms involving both pre- and postsynaptic components (10, 13, 19). For example, at least one form of LTP at the cortical inputs can be triggered by activation of presynaptic NMDARs and the subsequent increase in the probability of presynaptic transmitter release (26). Here, we found that STD-LTP in the cortical

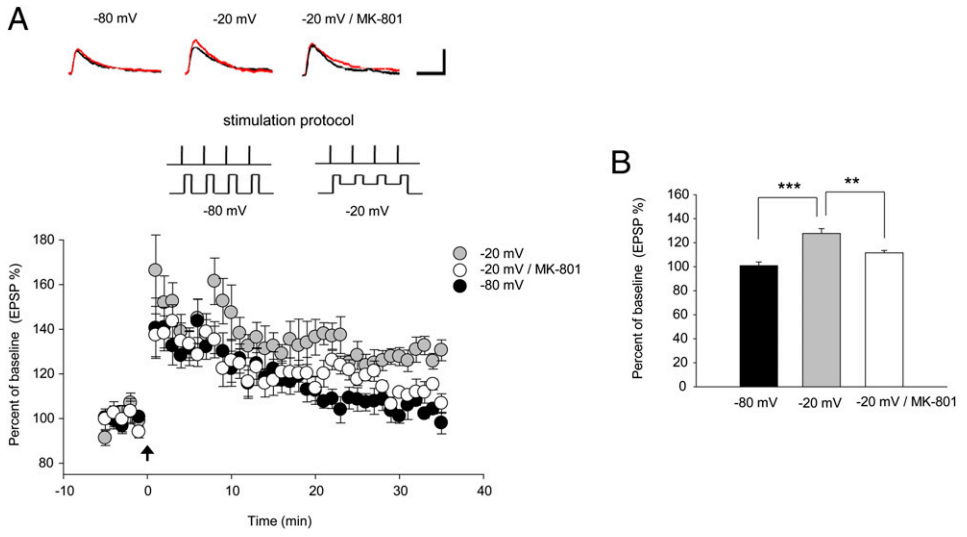

Fig. 5. STD-LTP at the thalamic inputs of *NLGN1* KO mice restored by additional depolarization of postsynaptic neurons. (*A*) Representative traces of EPSPs before (black) and after application (red) of the standard STD-LTP stimulation (−80 mV; *Left*) or additional depolarization (−20 mV; *Center*), or additional depolarization with inclusion of 0.5 mM MK-801 in the patch pipette (−20 mV/MK-801; *Right*) to the postsynaptic LA neurons of *NLGN1* KO mice are shown. (Scale bar, 50 ms and 2 mV.) (*Insets*) Shematic drawings for STD-LTP induction protocols, standard stimulation (−80 mV; *Left*) and stimulation with additional depolarization (−20 mV; *Right*). The results of STD-LTP experiments at the thalamo-amygdala synapses of *NLGN1* KO mice are presented for each stimulation protocol (*Lower*). (*B*) A summary histogram for STD-LTP 30 min after each STD-LTP stimulation protocol is depicted; −80 mV, 100.9 ± 3% (n = 6) vs. −20 mV, 127.7 ± 4% (n = 8) vs. −20 mV/MK-801, 111.5 ± 1.9% (n = 6), one-way ANOVA followed by Tukey post hoc test.

pathway is primarily dependent upon the activation of presynaptic NMDARs. Therefore, the cortico-amygdala synapses can still undergo synaptic modifications to produce STD-LTP despite the reduction of NMDAR-mediated transmission in *NLGN1* KO mice. In the past, there have been inconsistent reports about whether STD-LTP can be induced reliably at the cortico-amygdala synapses of the LA (25, 29, 30; see also ref. 27). Perhaps, this discrepancy is related to the differential contribution of pre- and postsynaptic NMDARs.

STD-LTP has been widely thought to be a more physiologically relevant model for synaptic modifications underlying memory formation (22, 23). Interestingly, *Rap1* KO mice exhibited impairment in STD-LTP in the cortical pathway but not the thalamic pathway, which was attributed to abnormal changes in transmitter release (11). The *Rap1* KO mice also exhibited deficits for fear learning in response to moderately aversive unconditioned stimuli. Taking into account the functional requirement for fear learning substantiated in *Rap1* KO mice, STD-LTP induced at the cortical pathway independently of postsynaptic NMDARs is likely to serve as one of the key mechanisms that can increase the range of flexibility of cortico-amygdala synapses for integration of multiple incoming sensory inputs.

Although the predisposing genetic causes leading to autism is still largely unknown, mutations in the human neuroligin genes have been linked to this disease (31–34). Autism spectrum disorders are characterized by impairments in social and emotional behavior. Social deficits possibly result from the inability to recognize others' emotional expressions or to properly process this information. Altered functions of the amygdala may cause autism-related emotional and social behaviors (35, 36). Therefore, elucidation of molecular mechanism of synaptic plasticity in amygdala circuits would seem to be important for further understanding of impaired social behavior in autistic patients. Although it is not yet clear whether the pathway-specific synaptic plasticity in the amygdala that is regulated by NLGN1 can modulate animal behavior, any possibility of a specific behavioral impact and, in particular, its relevance to autism clearly warrants detailed future investigations.

There is a growing realization of the importance and causal involvement of NLGNs for aberrant synaptic activity at the level of neuronal circuits and also animal behaviors (37). The present data clearly support a regulatory role of NLGN1 for input-specific synaptic plasticity in amygdala circuits by selectively stabilizing postsynaptic NMDARs.

Methods

For detailed experimental procedures, see *SI Methods*.

NLGN1 KO mice were generated by breeding heterozygous mice (2). *NLGN1* KO mice and Sprague-Dawley rats (Orient Co.) were housed on a 12-h light/dark cycle and given ad libitum access to food and water. shNLGN1 lentivirus was produced as described previously (6) and infused to the LA through injection cannulae. Whole-cell recording was made from principal neurons in the dorsolateral division of the LA under an upright microscope with DIC/infrared/fluorescence optics (Olympus). All procedures for animal experiments were performed in accordance with POSTECH guidelines on animal care and use.

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