Postsynaptic *N*-methyl-D-aspartate receptor function requires α-neurexins

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Contributed by Thomas C. Südhof, December 23, 2003

α-Neurexins are neuron-specific cell-surface molecules that are essential for the functional organization of presynaptic Ca^{2+} channels and release sites. We have now examined postsynaptic glutamate receptor function in α-neurexin knockout (KO) mice by using whole-cell recordings in cultured neocortical slices. Unexpectedly, we find that α-neurexins are required for normal activity of *N*-methyl-D-aspartate (NMDA)- but not α-amino-3-hydroxy-5-methyl-4-isoxyzolepropionic acid (AMPA)-type glutamate receptors. In α-neurexin-deficient mice, the ratio of NMDA- to AMPA-receptor currents, recorded as evoked synaptic responses, was diminished ≈50%. Furthermore, the NMDA-receptor-dependent component of spontaneous synaptic miniature responses was reduced ≈50%, whereas the AMPA-receptor-dependent component was unaffected. No alterations in the levels of NMDA- or AMPA-receptor proteins were detected. These results suggest that α-neurexins are required to maintain normal postsynaptic NMDA-receptor function. The decrease in NMDA-receptor activity in α-neurexin-deficient synapses could be due to a transsynaptic effect on the postsynaptic neuron (i.e., α-neurexins on the presynaptic inputs guide postsynaptic NMDA-receptor function) or to a cell-autonomous postsynaptic effect of α-neurexins on NMDA-receptor activity. To distinguish between these two possibilities, we cocultured WT GFP-labeled neurons with neocortical slices from α-neurexin-deficient or control mice. No difference was found between WT neurons innervated by inputs that contained or lacked α-neurexins, indicating that the absence of presynaptic α-neurexins alone does not depress postsynaptic NMDA-receptor function. Our data suggest that, in addition to the previously described presynaptic impairments, loss of α-neurexins induces postsynaptic changes by a cell-autonomous mechanism.

The physiological properties of excitatory synapses of a set of neurons, or even the same neuron, can differ remarkably (1–4). Multiple pre- and postsynaptic processes differentially regulate the strength of synapses. For example, in cortical synapses, the properties of presynaptic release sites formed by a single neuron can be differentially modulated by the postsynaptic target neuron (5). At least in part, this regulation appears to act on the amount of presynaptic Ca^{2+} influx that is induced by an action potential. Conversely, postsynaptic glutamate receptors are modulated by synaptic activity (6, 7). Both of the two principal types of synaptic glutamate receptors, *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxyzolepropionic acid (AMPA) receptors, undergo such use-dependent changes (7–9). The two receptor types are coordinately up- or down-regulated when the total strength of the synaptic inputs into a neuron is held constant during synaptic scaling (9). In contrast, only AMPA receptors are selectively increased during NMDA-receptor-dependent long-term potentiation in the CA1 region of the hippocampus (7). Viewed together, synapses can thus be considered as dynamic units in which discrete adjustments of defined parameters (presynaptic Ca^{2+} influx, the release machinery, and postsynaptic receptor-gated currents) regulate the overall properties of synaptic transmission.

Neurexins are highly polymorphic neuron-specific cell-surface proteins (reviewed in ref. 10). In mammals, three neurexin genes each encode α- and β-neurexins that are transcribed from separate promoters and generate a large molecular diversity by alternative splicing (11–15). Five observations suggest that α- and β-neurexins may function as transsynaptic organizer and/or recognition molecules: (*i*) neurexins resemble a receptor or cell adhesion molecule and are localized to synapses (11, 16); (*ii*) neurexins bind to other synaptic proteins such as the postsynaptic cell-adhesion molecule neuroligin (17–19) and the presynaptic proteins synaptotagmin 1, CASK, and Mint 1 (20–23); (*iii*) neuroligin 1, when expressed in a nonneuronal cell, induces vesicle accumulations in neurons presumably by activating presynaptic β-neurexins (16); (*iv*) neurexins are receptors for α-latrotoxin that stimulates massive neurotransmitter release from presynaptic terminals (11, 24–26); and (*v*) deletion of α-neurexins in mice causes a severe reduction of Ca^{2+}-channel activity and a major decrease in evoked neurotransmitter release (27). Although these studies collectively link neurexins to synaptic transmission, they raise a number of questions. Most notably, α-neurexin knockout (KO) mice exhibit an impairment not only of presynaptic voltage-gated Ca^{2+} currents but also of total Ca^{2+} currents measured in whole-cell recordings (27). At the same time, voltage-gated Na^+ or K^+ channels appear normal. The loss of whole-cell voltage-gated Ca^{2+} currents suggests the possibility that not only presynaptic elements but also postsynaptic channels and receptors are altered in α-neurexin KO mice. However, no morphological pre- or postsynaptic abnormality was noted. Furthermore, spontaneous miniature synaptic responses displayed no change in amplitude for glutamatergic or γ-aminobutyric acid (GABA)ergic synapses, suggesting that postsynaptic responses were not dramatically altered despite a large decrease in mini frequency caused by the presynaptic phenotype (27).

In the present study, we searched for a potential postsynaptic phenotype in triple α-neurexin KO (TKO) mice and examined the relative activities of NMDA and AMPA receptors at glutamatergic synapses. We show that deletion of α-neurexins causes a reduction in NMDA-receptor-mediated postsynaptic currents without affecting AMPA-receptor-mediated currents. This phenotype could not be explained by differences in the protein levels of the major NMDA- and AMPA-receptor subunits. In addition, we analyzed chimeric synapses between presynaptic α-neurexin KO and postsynaptic WT neurons and found that the reduction in NMDA-receptor function is independent of the expression of α-neurexins at presynaptic inputs onto a postsynaptic neuron.

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxyzolepropionic acid; EPSC, excitatory postsynaptic current; NMDA, *N*-methyl-D-aspartate; KO, knockout; SKO, single 2α-neurexin KO; TKO, triple α-neurexin KO.

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Our data suggest that α-neurexins not only act presynaptically, but also have a postsynaptic effect.

Materials and Methods

Animals. Experiments were performed with TKO mice maintained on a mixed C57BL/6 and SV129/SJ genetic background, with littermate single 2α-neurexin KO (SKO) mice or genetically matching WT mice as controls (27). GFP-expressing transgenic mice (28) were kept as heterozygous animals and selected by GFP fluorescence.

Preparation of Cell Cultures. Neocortical slices (0.5-mm thickness) from newborn mice (postnatal day 1) were cultured as described (27, 29) for 6–8 weeks at 37°C in a 5% CO_2 atmosphere on Millicell-CM membranes (Millipore) in 25% MEM/25% Basal Medium Eagle (Invitrogen)/25% horse serum/25% cell culture water/200 mM L-glutamine/20 mM D-glucose, pH 7.35, with media changes every 2 days. For the coculture experiments, neurons from neonatal occipital neocortex from GFP mice were mechanically dissociated after trypsin (0.1%) treatment. A drop of cells (3 μl; ≈1.8–2.4 × 10^3 GFP-expressing cells) was placed on freshly prepared neocortical slice cultures from SKO or TKO mice. After 3 days *in vitro*, cytosine-β-D-arabinofuranoside hydrochloride (10 μM) was added to minimize proliferation of nonneuronal cells.

Electrophysiology. Whole-cell voltage–clamp recordings were established under infrared contrast microscopy in submerged slices superfused (flow rate 2–4 ml/min) with prewarmed artificial cerebrospinal fluid (content in mM: 119 NaCl/2.5 KCl/2.5 $CaCl_2$/1.5 $MgCl_2$/26.2 $NaHCO_3$/1 Na_2HPO_4/10 D-glucose, pH 7.3, equilibrated with 95% CO_2/5% O_2). Analysis of miniature excitatory postsynaptic currents (EPSCs) was performed at a holding potential of −80 mV in the presence of 1 μM tetrodotoxin and 25 μM bicuculline. Recording electrodes (6–8 MΩ) were filled with (in mM): 135 CsCl/20 tetraethylammonium-Cl/2 $MgCl_2$/10 Hepes-CsOH, pH 7.4. For evoked glutamatergic transmission, extracellular field stimulation (0.2-ms pulses repeated at 0.2 Hz in the presence of 25 μM bicuculline) was applied ≈0.1–0.2 mm from the recorded neuron with a bipolar electrode (no. 5755, Science Products, Hofheim, Germany). Stimulation strength was adjusted for monosynaptic responses of high synchrony. Amplitude ratios were calculated by averaging 30 consecutive traces at holding potentials of −80 mV and +40 mV. Recordings were made by using a HEKA EPC-7 patch–clamp amplifier (HEKA Electronics, Lambert/Pfalz, Germany), filtered at 3 kHz and sampled at 10 kHz with a TL-1 interface by using PCLAMP 6 software (Axon Instruments, Foster City, CA). Off-line analysis of postsynaptic currents was performed with CLAMPFIT 8.1 (Axon Instruments) and AUTESP software (H. Zucker, Max Planck Institute for Psychiatry, Martinsried, Germany). Amplitudes of spontaneous and evoked NMDA-receptor-mediated currents were measured 20 ms after the initial rise of the current. Data are expressed as means ± SEM; statistical significance was evaluated with a two-tailed Student *t* test.

Biochemical Procedures. Brains of newborn (postnatal day 1) mice and neocortical cultures grown for 30–40 days were homogenized in 20 mM Hepes, pH 8.1/1 mM EDTA/100 μM PMSF/2 ng/ml aprotinin/2 μM leupeptin, and proteins in the particulate fraction of the homogenate obtained after centrifugation were quantified by immunoblotting by using ^{125}I-labeled secondary antibodies (Amersham Pharmacia–Pharmacia) followed by radioactivity measurements with a Fuji Las3000imager (AIDA 2.02 software, Ray Test, Straubenhart, Germany). Immunoblots of neocortical slice cultures were developed by enhanced chemiluminescence.

Antibodies. To glutamate receptors: NR1 (54.2, Synaptic Systems, Göttingen, Germany), NR1 (CT, Upstate Biotechnology, Lake Placid, NY), phosphorylated NR1 (S896 and S897, Upstate Biotechnology), NR2A and -2B (Chemicon), NR3A (gift of N. Sucher), NR3B (Upstate Biotechnology); and GluR1 and -R2/3 (Chemicon); against miscellaneous proteins: Kv1.4 (Chemicon), Synaptophysin (Sy38, DAKO), SNAP25 (70.1, gift of R. Jahn), HSP70 (Affinity BioReagents, Neshanic Station, NJ).

Results

Analysis of NMDA- and AMPA-Receptor-Dependent Components of Spontaneous EPSCs. In mature synaptic networks, such as those formed in long-term cultures of neocortical slices (29), spontaneous release events occur regularly and can be recorded as miniature postsynaptic currents. We carried out such recordings in cultured neocortical slices from TKO mice to test whether these mice exhibit a postsynaptic phenotype. To monitor only spontaneous EPSCs, we performed whole-cell recordings in the presence of tetrodotoxin to block action potentials and of picrotoxin to block γ-aminobutyric acid (GABA)ergic synaptic transmission (Fig. 1). To separately evaluate AMPA- and NMDA-receptor-mediated currents, we performed these recordings in both Mg^{2+}-containing and -free extracellular solution (Fig. 1*A*). In the presence of Mg^{2+}, spontaneous EPSCs are produced by AMPA-receptor activation, because NMDA receptors are blocked. In Mg^{2+}-free solution, NMDA receptors are unblocked and produce a slowly decaying current that can be quantified and compared to the rapidly decaying AMPA-receptor current (Fig. 1*B*).

As described earlier (27), we found that the frequency of spontaneous events was greatly decreased in neurons from TKO mice that lack all α-neurexins, whereas the overall amplitude of spontaneous EPSCs was similar (see representative recordings in Fig. 1*A*). When we averaged many spontaneous events, we uncovered a major additional difference between WT and α-neurexin-deficient neurons: in the absence of Mg^{2+}, the NMDA-receptor-dependent current was greatly reduced in neurons that lack α-neurexins compared to WT control neurons (Fig. 1*C*). In contrast, the AMPA-receptor-dependent current amplitude was unchanged. We quantified each component in the absence of Mg^{2+} (the AMPA-receptor-dependent component as the peak current, the NMDA-receptor-dependent component as the amplitude of the current 20 ms after the peak). This confirmed that the AMPA-dependent component was indistinguishable between WT and TKO neurons [WT, 12.4 ± 1 pA (n = 13); TKO, 12.7 ± 1 pA (n = 19); P = 0.85], suggesting that the synapses contained similar numbers of functional AMPA receptors. However, the NMDA-receptor-dependent component was decreased by ≈50% (WT, 3.2 ± 0.7 pA; TKO, 1.6 ± 0.3 pA; $P <$ 0.02; Fig. 1*D*), and the ratio of NMDA- to AMPA-receptor-current amplitudes was significantly reduced (WT, 0.25 ± 0.03; TKO, 0.13 ± 0.02; $P <$ 0.01; Fig. 1*E*). These data indicate that deletion of α-neurexins caused a selective loss of NMDA-receptor function.

Contributions of NMDA- and AMPA-Receptor-Dependent Currents to Evoked Synaptic Responses. To measure NMDA-receptor function in α-neurexin KO mice by an independent approach, we examined the contribution of AMPA- and NMDA-receptor-dependent currents to evoked synaptic responses (Fig. 2). In these whole-cell recordings, we compared neocortical slice cultures from TKO mice with control slices from littermate SKO mice, because the SKO has the mildest phenotype of all single α-neurexin KO mice (27). We monitored monosynaptic EPSCs evoked by extracellular field stimulation in the presence of 25 μM bicuculline (to block γ-aminobutyric acid type A receptors) and of 1.5 mM Mg^{2+}. Because of the decrease in evoked synaptic responses in TKO mice (27), we individually adjusted stimulation

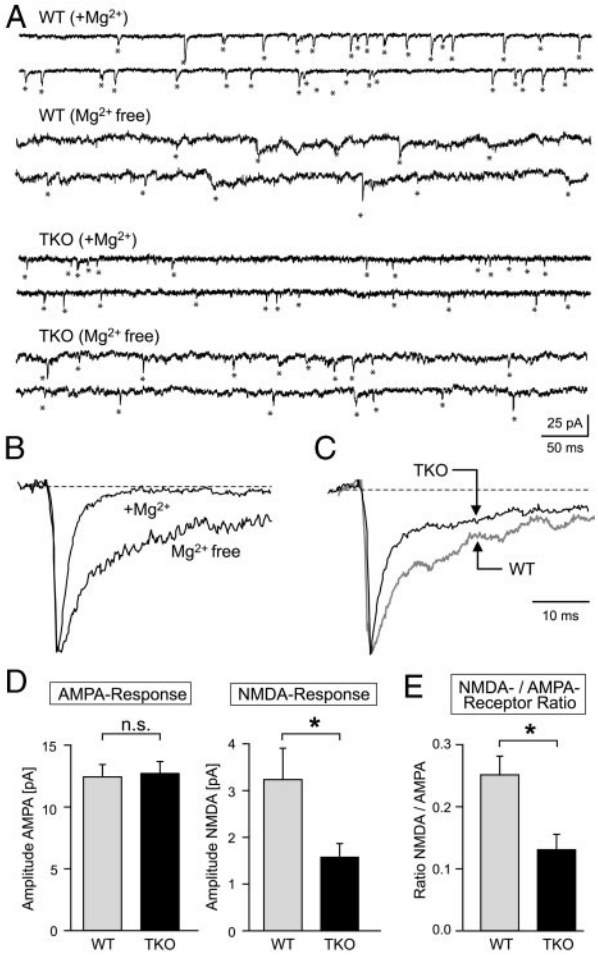

Fig. 1. NMDA- and AMPA-receptor-mediated current components of spontaneous miniature synaptic events in α-neurexin KO mice. (*A*) Representative current traces from whole-cell voltage–clamp recordings of minievents in cultured neocortical slices from WT and TKO mice. Recordings were performed at −80 mV in the presence of 25 μM bicuculline and 1 μM Tetrodotoxin with and without Mg^{2+} in the medium, as indicated. (*B*) Averaged minievents recorded from WT neurons in the presence and absence of extracellular Mg^{2+}. Note that activation of NMDA receptors in Mg^{2+}-free medium leads to a slowly decaying NMDA-receptor current that can be distinguished from the rapidly decaying AMPA-receptor current. (*C*) Averaged current traces of glutamatergic spontaneous synaptic events recorded under Mg^{2+}-free conditions. Arrows indicate the time point of detection of NMDA-receptor-mediated current amplitudes (20 ms after initial rise). (*D*) Quantification of AMPA- and NMDA-receptor-mediated currents in spontaneous synaptic events. (*E*) Quantification of the ratio of NMDA- to AMPA-receptor-mediated spontaneous synaptic currents. In *D* and *E*, data shown are means ± SEM (*, $P <$ 0.01; n.s., not significant).

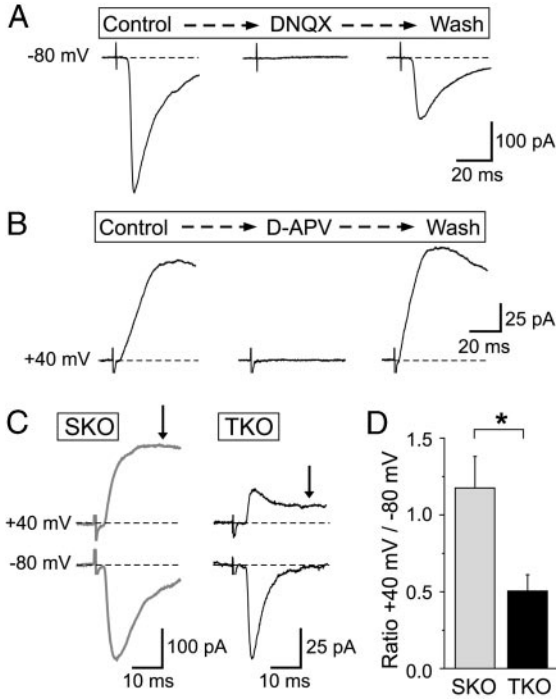

Fig. 2. Deletion of α-neurexins causes a selective decrease in NMDA-receptor-mediated currents affecting evoked synaptic responses. (*A*) Averaged traces ($n = 10$) of AMPA-receptor-dependent evoked synaptic responses from control (SKO) neurons. Currents were recorded at −80 mV in the presence of 25 μM bicuculline and 25 μM 2-amino-5-phosphopentanoic acid (D-AP5). Addition of the AMPA receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX) (10 μM) leads to a complete block of synaptic responses that recovers after washout. (*B*) Averaged traces ($n = 10$) of evoked NMDA-receptor-mediated postsynaptic currents in control (SKO) neurons. Currents were recorded at +40 mV in the presence of 25 μM bicuculline and 10 μM DNQX. Addition of the NMDA-receptor antagonist D-AP5 (25 μM) completely blocks the current; washout leads to full recovery. (*C*) Representative traces of evoked glutamatergic synaptic responses monitored at the indicated holding potentials for SKO and TKO neurons. Note the different scale bars for the two genotypes. (*D*) Quantification of the ratio of synaptic responses recorded in the presence of Mg^{2+} at +40 mV and −80 mV holding potentials that separately monitor NMDA- and AMPA-receptor-dependent currents. AMPA- and NMDA-receptor-dependent currents were measured in the same set of synapses as the peak amplitude of evoked responses at −80 mV and as the current amplitude 20 ms after the initial rise at + 40 mV, respectively (see arrows). Each value was obtained by averaging responses to 30 repetitive stimulations at each holding potential. Data shown are means ± SEM ($n = 14$ for SKO, and 15 for TKO; *, $P < 0.01$).

strengths to produce reliable responses in both genotypes (see different scale bars in Fig. 2*C*). We measured synaptic responses in the same synapses at two holding potentials: at −80 mV in the presence of Mg^{2+} to monitor AMPA-receptor-dependent currents that could be blocked with the AMPA-receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (Fig. 2*A*) and at +40 mV to monitor NMDA-receptor-dependent currents that could be blocked with D-2-amino-5-phosphonovaleric acid (Fig. 2*B*).

Comparison of synaptic responses in TKO neurons lacking all α-neurexins with control responses confirmed a loss of NMDA-receptor-mediated currents in α-neurexin-deficient neurons (Fig. 2*C*). Quantitation of the ratio of evoked NMDA- to AMPA-receptor currents (Fig. 2*D*) indicated a ≈50% reduction in NMDA-receptor function in α-neurexin-deficient neurons compared to control neurons [TKO, 0.5 ± 0.1 ($n = 15$); SKO, 1.2 ± 0.2 ($n = 14$); $P < 0.01$]. This finding confirms that deletion of α-neurexins causes a selective loss of NMDA-receptor-dependent responses at synapses. The observed ratio of NMDA- to AMPA-receptor-mediated currents was significantly higher in evoked (Fig. 2) than in spontaneous synaptic responses (Fig. 1), as described (reviewed in ref. 30).

NMDA- and AMPA-Receptor Subunit Proteins. The reduction in NMDA-receptor-mediated postsynaptic currents could be caused by an overall decrease in NMDA-receptor protein by a change in NMDA-receptor subunit composition or by a decrease of NMDA-receptor function (31). To measure NMDA-receptor expression, we quantified the levels of the NMDA-receptor subunits NR1, -2A, and -2B and of the AMPA-receptor subunit GluR1 in brain homogenates of newborn WT and α-neurexin KO mice. Quantifications, carried out by immunoblotting with ^{125}I-labeled secondary antibodies and PhosphorImager detec-

Table 1. Levels of NMDA-receptor proteins in brains of newborn WT and α-neurexin-deficient KO mice

Protein	Genotype		Statistical significance*
	WT	TKO	
NMDA-R1	100 ± 3	102 ± 3	n.s.
NMDA-R2A and -2B	100 ± 2	97 ± 3	n.s.
NMDA-R3A and -B	ND	ND	n.s.
GluR1	100 ± 1.7	97 ± 2	n.s.
SNAP-25	100 ± 3	96 ± 3	n.s.
HSP70	100 ± 1	98 ± 2	n.s.

Proteins were measured in total brain homogenates by using ^{125}I-labeled secondary antibodies and PhosphorImager detection (see *Materials and Methods*). Data shown are means ± SEMs expressed as percent of normalized WT levels (ND, not detectable). Monoclonal antibodies against heat-shock protein 70 (HSP70) served as reference antibodies.

*Level of significance for null hypothesis (Student's *t* test); n.s., not significantly different.

tion, did not detect changes in NMDA-receptor proteins (Table 1). We obtained no signal for NR3A and -3B in our material, suggesting that their expression may be low. Because the electrophysiological recordings were made in neocortical slices cultured for 4–6 weeks, we also analyzed the expression and composition of glutamate receptors in the actual slice cultures. Again, we detected no significant differences in NMDA- or AMPA-receptor proteins between α-neurexin KO and control slices (Fig. 3). Similarly, we found no variation in the degree of

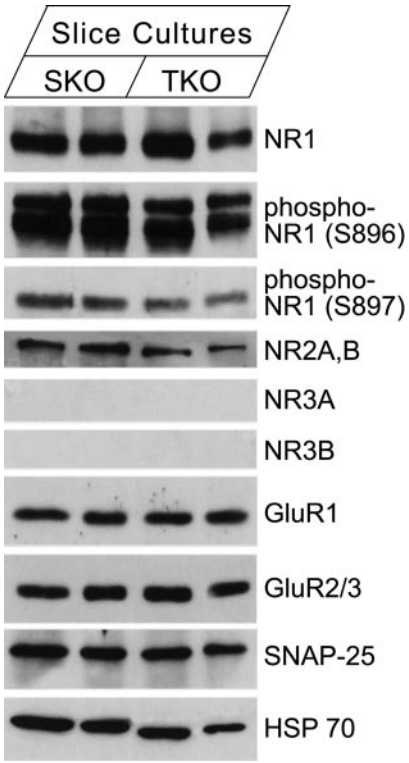

Fig. 3. Receptor protein levels are unchanged in α-neurexin KO mice. Immunoblot analysis of comparable amounts of proteins from control (SKO) and TKO slice cultures. Each lane contains proteins from pooled 10- to 12-slice cultures established from a single mouse. Signals were detected by enhanced chemiluminescence. NR, NMDA-receptor subunit; phospho-NR, phosphorylated NMDA receptor; GluR1 and -R2/3, AMPA-type glutamate receptor subunits 1 and 2/3; HSP, heat-shock protein (loading control). See Table 1 for a quantitation of protein levels in brain homogenates.

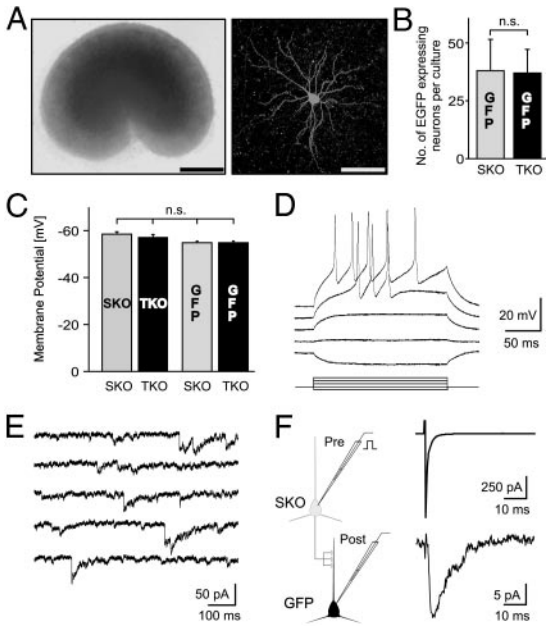

Fig. 4. Coculture and functional integration of GFP-expressing WT neurons with control and α-neurexin-deficient neocortical slices. (*A*) Phase-contrast micrograph of a neocortical slice culture (32 days *in vitro*; *Left*, Bar = 1 mm) and confocal image of a GFP-expressing WT neuron cultured for 28 days on a neocortical slice (*Right*; Bar = 50 μm). (*B*) Quantitation of the number of differentiated GFP-expressing neurons on slice cultures from SKO and TKO mice. (*C*) Membrane potentials of SKO and TKO neurons in neocortical slice cultures and of GFP neurons grown on these slice cultures. The labeling of the bars indicates the type of neuron, and the labeling below the bars indicates the type of slice analyzed. (*D*) Whole-cell measurements of spike trains after depolarizing current injections in GFP neurons cocultured with neocortical slices. (*E*) Spontaneous synaptic responses recorded from GFP neurons cocultured with neocortical slices. (*F*) Paired recordings between a presynaptic α-neurexin control neuron (SKO) in the cultured slice and a postsynaptic GFP-expressing WT neuron (GFP) grown on the slice. (*Left*) The recording configuration. (*Right*) The presynaptic action current (*Upper*) and the postsynaptic evoked response (*Lower*). n.s., not significantly different.

phosphorylated vs. unphosphorylated NMDA-receptor subunit 1 (Fig. 3). These data indicate that the reduction in NMDA-receptor-mediated currents in α-neurexin KO mice was not due to differences in the expression or composition of the receptors.

Culturing Dispersed WT Neurons on Neocortical Slices from α-Neurexin KO Mice. The decrease in postsynaptic NMDA-receptor activity in α-neurexin-deficient synapses could be due to a transsynaptic effect on the postsynaptic neuron (i.e., α-neurexins on the presynaptic inputs guide postsynaptic NMDA-receptor function) or to a cell-autonomous postsynaptic effect of α-neurexins on NMDA-receptor activity. As a first approach to distinguish between these alternatives, we asked whether a WT neuron, when integrated into a synaptic network formed by α-neurexin-deficient neurons, would assume the α-neurexin KO phenotype or retain WT properties of NMDA receptors. We dissociated neocortical neurons from newborn WT mice that express GFP in all neurons (28) and cultured the WT neurons at low density on top of neocortical slices from SKO or TKO mice (Fig. 4*A*).

After 25–40 days of coculture, we analyzed whether the dispersed GFP-expressing WT neurons were morphologically and functionally integrated into the slice cultures. The mean number of GFP-expressing WT neurons was nearly identical on slice cultures from SKO and TKO mice [SKO, 38 ± 14 (n = 303 neurons/8 cultures); TKO, 37 ± 10 (n = 296/8); Fig. 4*B*], as was

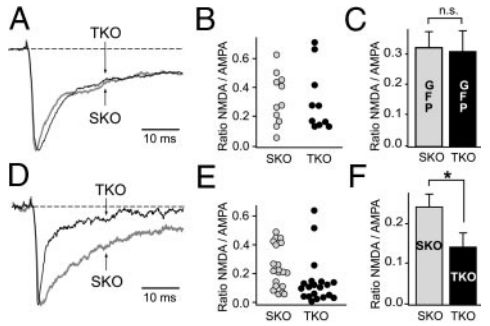

Fig. 5. Reduction of NMDA-receptor-mediated currents is independent of the presence of α-neurexins on presynaptic inputs. The use-dependent induction of the NMDA-receptor impairment was studied in neocortical slice cultures with integrated GFP-expressing WT neurons, as characterized in Fig. 4. (*A*) Superimposed traces of spontaneous miniature synaptic responses recorded in Mg^{2+}-free medium from GFP-positive neurons grown on neocortical slices from control SKO or TKO. Arrows indicate time point at which the amplitude of NMDA-receptor currents was measured (20 ms after rise time). (*B*) Scatter diagram of the ratio of NMDA- to AMPA-receptor-dependent currents in miniresponses recorded from WT GFP-positive neurons grown on SKO and TKO slices. (*C*) Average NMDA- to AMPA-receptor-dependent current ratios obtained from *B*. (*D–F*) Same as *A–C*, except that the recordings were obtained in GFP-negative neurons in slices from SKO and TKO mice to confirm the presence of the NMDA-receptor phenotype in the cocultured slice neurons (compare to Fig. 1*E*). Data shown are means ± SEM (n.s., not significant; *, $P < 0.05$).

the resting membrane potential (Fig. 4*C*). The GFP-positive neurons on slice cultures of both genotypes were similarly capable of generating action potentials (Fig. 4*D*), received action-potential-mediated synaptic inputs (data not shown), and exhibited spontaneous synaptic responses (Fig. 4*E*). Finally, a small number of paired recordings demonstrated functional synaptic transmission between presynaptic slice culture neurons and postsynaptic GFP-positive neurons (Fig. 4*F*). We did not observe autaptic currents in integrated cocultured GFP neurons (data not shown; $n = 14$). Together, these data suggest that GFP-expressing WT neurons integrated into slice cultures of both genotypes and received functional synaptic inputs from the neurons in the slice.

Impaired NMDA-Receptor Function Is Not Caused by the Loss of α-Neurexins from Presynaptic Inputs. The slice cocultures made it possible to ask whether postsynaptic responses in WT GFP-positive neurons assume the α-neurexin-dependent properties of the synaptic network into which they are integrated. To address this, we measured in slice cocultures the NMDA- and AMPA-receptor-dependent amplitudes of spontaneous synaptic miniature responses in the GFP-positive neurons under Mg^{2+}-free conditions (Fig. 5). Strikingly, we detected no significant reduction of the NMDA-mediated component of spontaneous EPSCs when WT neurons were cocultured with slices from TKO mice (Fig. 5 *A–C*). Quantification of the NMDA- to AMPA-receptor-mediated current ratio showed no difference between GFP-positive neurons in SKO slices [0.32 ± 0.05 ($n = 11$)] and in TKO slices [0.31 ± 0.07 ($n = 10$); $P = 0.9$]. As an internal control, we reproduced in the same slice cultures the phenotype of α-neurexin KO neurons as a loss of NMDA-receptor-dependent currents [Fig. 5 *D–F*; NMDA-/AMPA-receptor-dependent postsynaptic current ratios: TKO, 0.14 ± 0.04 pA ($n = 20$); SKO = 0.24 ± 0.03 pA ($n = 20$); $P < 0.05$]. Taken together, these experiments suggest that the impaired NMDA-receptor function in α-neurexin KO neurons is not a consequence of the overall reduction in neurotransmission in the cortical network or of the loss of transsynaptic α-neurexin signaling acting on postsynaptic neurons but represents a cell-autonomous effect of α-neurexins in the postsynaptic cell.

Discussion

In the present study, we have tested whether deletion of α-neurexins, presumptive presynaptic cell-adhesion molecules (10), alters postsynaptic NMDA- or AMPA-receptor-dependent synaptic currents. Our data suggest two major conclusions.

(*i*) We find that α-neurexins are selectively required for maintaining NMDA-receptor function. In α-neurexin KO mice, AMPA-receptor function and AMPA- and NMDA-receptor protein levels were not detectably changed, but the NMDA-receptor-dependent postsynaptic currents, measured by two approaches (Figs. 1 and 2), were decreased ≈2-fold. Thus, in addition to the major changes in presynaptic release (loss of Ca^{2+}-channel function, decrease in spontaneous release, and decline in short-term plasticity; ref. 27), deletion of α-neurexins also causes postsynaptic changes. The loss of NMDA-receptor activity could be due to a decrease in the postsynaptic localizations of NMDA receptors or to an inactivation of properly localized NMDA receptors. Although at present we do not know which explanation is correct, the first alternative is supported by the absence of obvious alterations in NMDA receptor phosphorylation (Fig. 3), a major mechanism of NMDA receptor regulation (32), and agrees with the fact that a similar decrease of NMDA receptor function was observed after stimulation of NMDA-receptor internalization (33).

(*ii*) The loss of NMDA-receptor function in α-neurexin KO neurons is probably caused by the absence of α-neurexin from the postsynaptic neurons (i.e., is cell-autonomous) and is not due to the loss of an α-neurexin signal from presynaptic inputs. The slice coculture assay shows that a GFP-labeled WT neuron, when integrated into a synaptic network composed of α-neurexin-deficient neurons, still has WT NMDA-receptor function (Figs. 4 and 5). Although we observed no autaptic, but at least some heterosynaptic, currents in the slice cocultures (Fig. 4), we cannot at present exclude the possibility that a few synaptic inputs in the seeded WT neurons on top of the slice cultures are autaptic and contribute to the lack of a phenotype.

The postsynaptic decrease in NMDA-receptor function in α-neurexin KO mice is consistent with the decline in whole-cell Ca^{2+} currents in these mice (27). The whole-cell Ca^{2+} currents are presumably mediated by postsynaptic Ca^{2+} channels, although it is unclear whether these channels are located at postsynaptic specializations or are diffusely somatic. The present data confirm that α-neurexins function at synapses, but the requirement of α-neurexins for NMDA-receptor function was unexpected in view of their localization to presynaptic terminals (11, 16). Several possible explanations can be advanced to account for this apparent paradox.

One possibility is that the deletion of α-neurexins disturbs the integrity of synapses, leading to widespread effects on all kinds of receptors, channels, and other neuronal elements. However, brain architecture and synaptic ultrastructure appear to be relatively normal in α-neurexin KO mice, and the functional impairments are selective for voltage-gated Ca^{2+} channels (as opposed to Na^+ or K^+ channels) and to NMDA (as opposed to AMPA) receptors.

A second possibility is that a transsynaptic signaling pathway is responsible, as we suggested to account for the decrease of whole-cell voltage-gated Ca^{2+} currents (27). However, the recordings from postsynaptic WT neurons that were cocultured with α-neurexin-deficient slices argue against this possibility (Fig. 5).

A third hypothesis is that the function of α-neurexins is more general than originally envisioned, and that α-neurexins exert direct pre- and postsynaptic effects. According to this hypothesis, the decrease in voltage-gated whole-cell Ca^{2+} currents observed

earlier (27) and the decrease in NMDA-receptor function shown here could reflect a mechanistically similar change that is directly mediated postsynaptically.

Independent of the mechanism by which α-neurexins alter NMDA-receptor currents, the decrease in NMDA-receptor function in the α-neurexin KO mice suggests that α-neurexins contribute to the regulation of postsynaptic responses. It is striking that postsynaptic Ca^{2+} channels and NMDA receptors both depend at least partly on α-neurexins and both mediate Ca^{2+} influx, consistent with the notion that α-neurexins may be involved in controlling pre- and postsynaptic Ca^{2+} dynamics.

We thank Dr. R. Jahn (Max Planck Institute for Biophysical Chemistry, Göttingen, Germany) and N. Sucher (University of Hong Kong, Hong Kong) for antibodies, H. Hatt and N. Brose for support, and S. Gerke for excellent technical help. This study was supported by grants from the National Institute of Mental Health (R37 MH52804-08, to T.C.S.) and the Deutsche Forschungsgemeinschaft [fellowship award and Grants SFB406 (to M.M.) and SFB509 (to K.G.)].

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