Subunit-specific role for the amino-terminal domain of AMPA receptors in synaptic targeting

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The amino-terminal domain (ATD) of AMPA receptors (AMPARs) accounts for approximately 50% of the protein, yet its functional role, if any, remains a mystery. We have discovered that the translocation of surface GluA1, but not GluA2, AMPAR subunits to the synapse requires the ATD. GluA1A2 heteromers in which the ATD of GluA1 is absent fail to translocate, establishing a critical role of the ATD of GluA1. Inserting GFP into the ATD interferes with the constitutive synaptic trafficking of GluA1, but not GluA2, mimicking the deletion of the ATD. Remarkably, long-term potentiation (LTP) can override the masking effect of the GFP tag. GluA1, but not GluA2, lacking the ATD fails to show LTP. These findings uncover a role for the ATD in subunit-specific synaptic trafficking of AMPARs, both constitutively and during plasticity. How LTP, induced postsynaptically, engages these extracellular trafficking motifs and what specific cleft proteins participate in the process remain to be elucidated.

amino-terminal domain | GluA1 | LTP | AMPA receptor trafficking

Glutamatergic synapses account for the vast majority of excitatory transmission in the brain. At these synapses glutamate typically activates two subtypes of ionotropic glutamate receptors referred to as AMPA receptors (AMPARs) and NMDA receptors (NMDARs). Repetitive activation of these synapses in the hippocampus causes an NMDAR-dependent long-term potentiation (LTP) that is the most compelling cellular model for certain forms of learning and memory. It is well accepted that the strengthening of synapses during LTP is due to the rapid accumulation of AMPARs at synapses (1–3). AMPARs are tetraheteromeric ion channels composed of GluA1 through GluA4 subunits. CA1 hippocampal pyramidal neurons express both GluA1A2 heteromers and GluA2A3 heteromers (4, 5). AMPARs containing the posttranscriptionally edited GluA2 subunit have linear IV relationship and are calcium-impermeable, whereas receptors lacking the edited GluA2 subunit are strongly inwardly rectifying and calcium-permeable. The cytoplasmic C-terminal domains (CTDs) of AMPARs have been proposed to mediate their synaptic trafficking. Specifically, a widely accepted model for the synaptic accumulation of AMPARs during LTP posits that the mode of trafficking depends on the subunit composition of the AMPARs (1, 2, 6–8). It is proposed that GluA1-containing receptors are excluded from the synapse under basal conditions and that activity drives these receptors to the synapse, whereas GluA2A3 heteromers traffic constitutively to synapses. Importantly, the CTDs of GluA1 and GluA2 are proposed to be responsible for this subunit specificity (6, 7). However, recent work has found that both constitutive and activity-dependent trafficking of AMPARs can occur in the absence of the CTDs (9).

In striking contrast to the attention given to the CTDs, the extracellular amino-terminal domain (ATD), which accounts for nearly half of the receptor polypeptide, has received much less attention. Remarkably, although the ATD is proposed to assist in the initial subunit associations involved in the assembly of receptors into functional tetramers (10), truncated subunits lacking the entire ATD can form robust glutamate-activated channels (11). Recent studies have shown that the ATD can modify the gating of AMPARs (12, 13). Given its large size and that it projects midway into the synaptic cleft, one might predict an interaction of the ATD with proteins within the synaptic cleft. Indeed, it has been found that N-cadherin interacts with the ATD of GluA2, but not GluA1, regulating synaptic stability (14), whereas neuronal pentraxins interact with the ATD of GluA4 and regulate receptor trafficking in parvalbumin interneurons (15) as well as in other neuronal subtypes (16).

In recent work we overexpressed GluA1 subunits and found that homomeric GluA1 receptors trafficked to synapses in a constitutive manner (9), in contrast to previous studies (6–8, 17). In the process of explaining these seemingly contradictory results, our attention was drawn to the ATD and we discovered that the presence of a GFP tag on the ATD of GluA1, but not GluA2, masked a critical role for the ATD of GluA1 in the targeting of GluA1-containing receptors to the synapse. Both constitutively active CaMKII and LTP override the GFP masking effect. The presence of the ATD on GluA1, but not GluA2, provides a permissive signal that is essential for synaptic targeting. Thus, we conclude that the ATD imparts a hitherto unrecognized subunit-specific synaptic targeting of AMPARs, both during constitutive and activity-dependent trafficking.

Results

The initial goal of the present study was to understand the basis for the seeming contradiction between our recent work, in which we found that homomeric GluA1 receptors trafficked to synapses in a constitutive manner (9, 18), and previous results primarily from the Malinow laboratory (6–8, 17), in which GluA1 homomers are

Significance

It is generally accepted that trafficking of AMPA receptors underlies synaptic enhancement during long-term potentiation, a cellular model for learning and memory. The role of the cytoplasmic C-terminal domain of AMPA receptors in trafficking has been extensively studied. Here we show that the extracellular amino-terminal domain (ATD) plays a critical role in subunit-specific trafficking. The ATD of GluA1, but not GluA2, is required for the translocation of surface GluA1 homomers to the synapse and this requirement is maintained in GluA1A2 heteromeric receptors. How the GluA1 ATD engages the synaptic cleft proteins remains to be elucidated.

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excluded from the synapse under basal conditions. First, we considered the fact that we used the flip splice variant [GluA1(i)] of GluA1 rather than the flop variant [GluA1(o)], which was used in the previous studies. Even though the flop variant seems to be expressed at higher levels than the flip variant in CA1 pyramidal neurons (19), we used the flip isoform because the desensitization of AMPAR currents in outside-out patches is blocked by cyclothiazide (5), implying the functional dominance of flip isoforms. In this series of experiments we used organotypic hippocampal slice cultures and overexpressed GluA1, which forms homomeric, inwardly rectifying receptors (6) for 2 d (Fig. 1*A*). We confirmed that the expression of GluA1(i) caused an inward rectification of synaptic AMPARs [Fig. 1*A*, GluA1(i)], indicating that GluA1 traffics to the synapse (9). We repeated this experiment but used GluA1 (o) [Fig. 1*A*, GluA1(o)] and found that it caused the same degree of inward rectification, suggesting that the splice variant is not a factor in the synaptic targeting of AMPARs. Another possibility is that the activity in our slice cultures is higher than in previous studies and is responsible for driving the GluA1 receptor into the synapse. We therefore carried out the experiment incubating our slices in the presence of high Mg^{2+} (10 mM), which has previously been used to block constitutive activity (20), but we still observed the same degree of rectification [Fig. 1*A*, GluA1(i) + Mg^{2+}]. Finally, we repeated these experiments but used TTX to fully block any spontaneous activity. Again, the rectification we observed was the same as found in the absence of TTX (Fig. 1*A*, GluA1(i) + TTX], ruling out constitutive activity as the cause for the trafficking of GluA1 to the synapse.

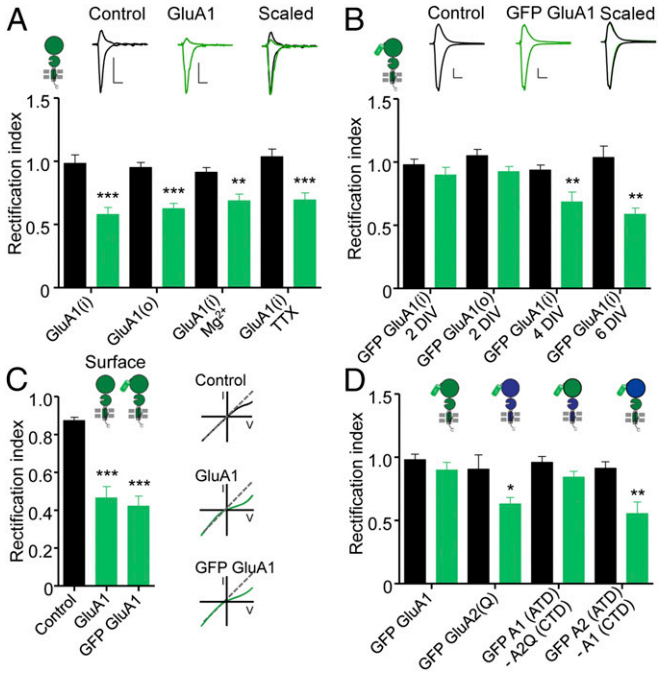

Fig. 1. ATD-tagged GluA1 has normal surface trafficking but impaired synaptic trafficking, unlike ATD-tagged GluA2. (*A*) Representative traces of synaptic rectification experiments in control (black) and WT GluA1-expressing cells (green), scaled and superimposed for comparison in the right-hand panels. Synaptic trafficking of GluA1 is independent of splice variants or basal activity. (*B*) Representative traces of synaptic rectification experiments in control (black) and GFP-tagged GluA1-expressing cells (green). ATD-tagged GFP GluA1(i) and GFP GluA1(o) constructs showed no inward rectification after 2 d of transfection. After 4–6 d, rectification of synaptic currents was observed. (*C*) Both GluA1 and GFP GluA1 showed surface inward rectification. Sample current traces from control (black) and transfected (green) outside-out patches are shown. (*D*) ATD-tagged AMPAR subunits showed different trafficking abilities: GFP GluA1 showed no inward rectification but GFP GluA2 did. Also, GFP A1 (ATD)-A2(Q) (CTD) chimeric receptor showed no inward rectification but GFP A2 (ATD)-A1 (CTD) did. n = 5–23 cells per condition. (Scale bars: 50 pA, 20 ms.) Error bars represent mean ± SEM; *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

We next considered the possible effect of the GFP tag on AMPAR trafficking. In our previous study (9) we expressed the untagged construct in all of our experiments, whereas in prior studies from other groups the amino terminus of GluA1 was tagged with GFP between the third and fourth amino acids after the predicted signal peptide cleavage site, thus presumably in the very distal end of the ATD (21). Expression of the GFP-tagged GluA1 for 2 d did not alter the rectification of the AMPAR excitatory postsynaptic current (EPSC) [Fig. 1*B*, GFP GluA1(i) and GFP GluA1(o) 2 d], in agreement with previous results (6, 7). However, we did find that expression of this construct for 4–6 d caused a rectification similar to that seen for the untagged construct [Fig. 1*B*, GFP GluA1(i) 4 and 6 d].

Why does GFP-tagged GluA1 display impaired synaptic trafficking? There are two major steps in the delivery of AMPARs to synapses. The first step is the assembly of the receptors and their delivery to the cell surface and the second step is the targeting of the surface receptors to the synapse. To determine whether the GFP-tagged GluA1 protein is delivered to the surface, we applied voltage ramps to somatic outside-out patches to measure rectification in response to glutamate application. These currents rectify to the same degree as those seen with the untagged GluA1 construct (Fig. 1*C*), indicating that the GFP-tagged receptor is delivered in normal amounts to the surface. Thus, it seems that the presence of GFP on the ATD of GluA1 interferes with the translocation of the receptor from the extrasynaptic compartment to the synapse.

Previous studies showed that GFP-tagged GluA2(Q) did traffic constitutively to the synapse (7). We also showed that untagged GluA2(Q) could rescue synaptic current in neurons lacking endogenous AMPARs (9). Overexpression of GFP GluA2(Q) receptors, in agreement with previous results (7), caused rectification of the AMPAR EPSC [Fig. 1*D*, GFP GluA2(Q)]. Thus, the GFP tag had no effect on the synaptic trafficking of GluA2(Q).

What accounts for the specific effect of GFP on GluA1 trafficking, but not GluA2? A likely explanation is that there is a functional difference in the ATD between GluA1 and GluA2 and the presence of GFP unmasks this difference. There is precedence for such a proposal, because the ATD of GluA2, but not GluA1, has been reported to promote spinogenesis (14). To test whether the GluA1 and GluA2 ATDs are functionally distinct we placed the GFP-tagged ATD of GluA1 onto GluA2 [GFP A1 (ATD)-A2Q (CTD)] (Fig. 1*D*). The presence of the GFP-tagged ATD of GluA1 on the GluA2 receptor prevented the trafficking of this receptor to the synapse, whereas the presence of the GFP-tagged ATD of GluA2 on the GluA1 receptor [GFP A2 (ATD)-A1 (CTD)] resulted in constitutive synaptic targeting (Fig. 1*D*).

There are two possibilities as to how GFP might be interfering with synaptic targeting of GluA1. First, the addition of the GFP might physically interfere with its entry into the synapse. This seems unlikely given that GFP GluA2 has no difficulty accessing the synapse. Second, the presence of the GFP might be interfering with the ability of the GluA1 ATD to interact with synaptic cleft proteins. To address these possibilities we deleted the ATD of GluA1, referred to as ΔATD GluA1. We first compared the function of this construct to the WT GluA1 in HEK cells (Fig. S1). Consistent with previous results (11), ΔATD GluA1 generated currents at least as large as those observed with the WT receptor. Similar results have been reported for the ATD-lacking GluA2 subunit, referred to as ΔATD GluA2 (22, 23). We also expressed ΔATD GluA1 in pyramidal cells and recorded glutamate-evoked currents in outside-out patches (Fig. 2*A*). We observed pronounced

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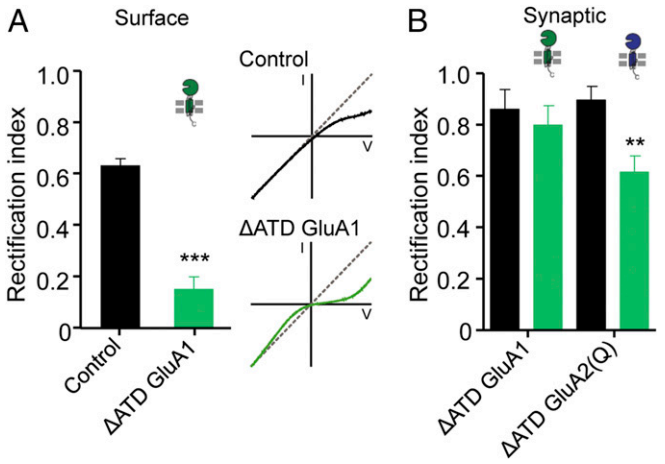

Fig. 2. ATD-lacking GluA1 has normal surface trafficking but, unlike ATD-lacking GluA2, impaired synaptic trafficking. (*A*) Surface rectification experiments in CA1 pyramidal neurons overexpressing ΔATD GluA1 in organotypic slices. Sample current traces in control (black) and ΔATD GluA1-overexpressing (green) outside-out patches are shown. (*B*) Removal of the entire ATD of GluA1 (ΔATD GluA1) impaired synaptic trafficking, whereas removal of the ATD of GluA2(Q) (ΔATD GluA2(Q)) did not affect trafficking. n = 7–12 cells/condition. Error bars represent mean ± SEM; $^{**}P < 0.01$ and $^{***}P < 0.001$.

rectification with this construct, indicating that it is delivered to the surface as well as or better than GluA1 or GFP GluA1 (Fig. 1*C*).

If the GFP were exerting its effect by physically interfering with GluA1 entering the synapse, we would expect that deleting the ATD would allow the receptor to enter the synapse. However, if the GFP is preventing a necessary interaction between the ATD and synaptic cleft proteins, ΔATD GluA1 might be excluded from the synapse. We found that ΔATD GluA1 is excluded from synapses (Fig. 2*B*). Thus, the ATD is required for the translocation of extrasynaptic GluA1 homomers to the synapse. We repeated these experiments with ΔATD GluA2. In striking contrast to ΔATD GluA1, ΔATD GluA2 was able to constitutively traffic to the synapse (Fig. 2*B*). These results have uncovered an unexpected role of the ATD in the subunit-specific trafficking of AMPARs.

Remarkably, although the GFP GluA1 is excluded from the synapse it is reported that a constitutively active form of CaMKII (CA CaMKII) can overcome this exclusion (6). We therefore repeated our experiments but, in addition to expressing GFP GluA1, we also expressed CA CaMKII (T286D/T305A/T306A) (24). It is well established that CA CaMKII mimics and occludes NMDA-dependent LTP (6, 25, 26). As a control in these experiments we repeated the key experiments shown in Fig. 1 but coexpressed an empty vector (GFP) and then compared these results to those in which CA CaMKII is expressed (Fig. S2 A_1 and A_2). As expected, expression of CA CaMKII enhanced AMPAR currents and there was no change in rectification (Fig. S2 A_1, A_2, and B). However, in agreement with previous results (6), when GFP GluA1 is coexpressed with CA CaMKII the currents are now rectifying (Fig. S2 A_1, A_2, and C), indicating that CA CaMKII overrides the masking effects of GFP. We then assessed whether CA CaMKII is capable of driving ΔATD GluA1 to the synapse. As shown in Fig. S2 A_1, A_2, and D, this is not the case. We examined whether CA CaMKII-driven GFP GluA1 synaptic delivery relies on an increase in the surface pool of AMPARs by briefly applying glutamate to cells expressing CA CaMKII alone (Fig. S2*E*), GFP GluA1 (Fig. S2*F*), and GFP GluA1+CA CaMKII (Fig. S2*G*) and examining the surface AMPAR currents. In none of these conditions were the surface currents changed, indicating that the effects on synaptic currents primarily involve a redistribution of surface AMPARs to the synapse.

All of the results presented thus far have relied on the overexpression of GluA subunits on a WT background in organotypic slice cultures. To study the role of the ATD in GluA trafficking we took advantage of *Gria1-3* triple-floxed mice (5), which allow for the complete removal of endogenous AMPARs. We expressed various GluA constructs on this null background. A limitation to this system is that it takes ∼20 d for Cre recombinase transfected cells to lose all their AMPARs and yet the exclusion of GFP GluA1 from the synapse only lasts for a few days. We thus incorporated an inducible Tet-ON system to temporally control the expression of the GluA subunits (Fig. 3 A_1 and A_2) in slice culture. In the absence of doxycycline (DOX), cells expressing Cre and GluA1 generated virtually no synaptic currents (Fig. 3 *B* and *F*), indicating the absence of GluA1 expression in the absence of DOX. However, in the presence of DOX (4 d) there was a partial rescue of synaptic currents (Fig. 3 *C* and *F*). The rescue was less than that in a previous study (9). This is most likely due to the fact that, in the present study, GluA1 was expressed for 4 d after an internal ribosomal entry site (IRES) with an inducible promoter (Fig. 3A_1), whereas the previous study expressed GluA1 after a strong, constitutive promoter for ∼3 wk. In contrast to the expression of WT GluA1, neurons expressing the GFP-tagged GluA1 showed minimal rescue of synaptic currents (Fig. 3 *D*

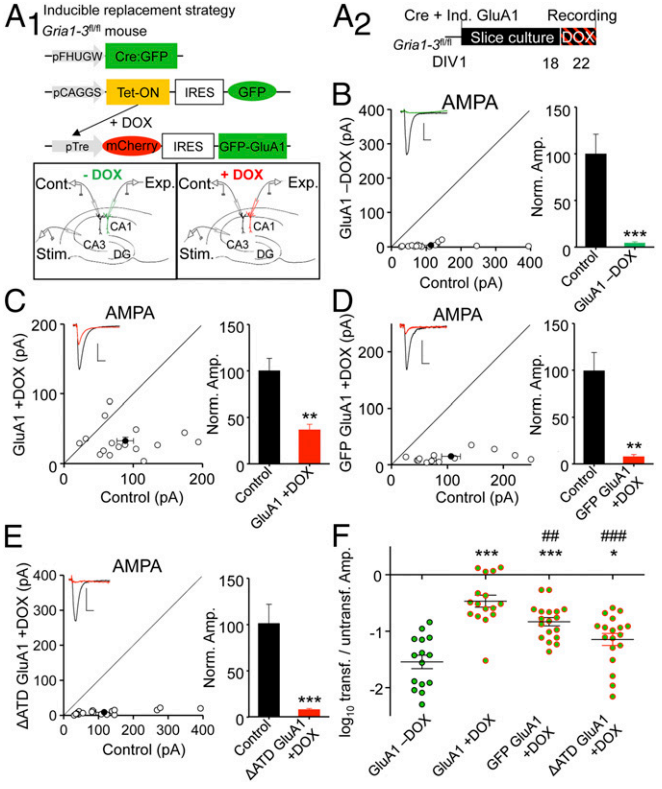

Fig. 3. GluA1, but not GFP GluA1 nor ΔATD GluA1, rescues synaptic AMPAR transmission in AMPAR-null cells in slice culture. (A_1) Scheme of the inducible AMPAR replacement strategy. (A_2) Timeline of the experiment. (*B–E*) Scatterplots showing amplitudes of AMPAR EPSCs for single pairs (open circles) of control and GluA1-replaced cells without DOX (*B*), and +DOX for 4 d (*C*), GFP GluA1 +DOX (*D*), and ΔATD GluA1 +DOX (*E*). Filled circles represent mean ± SEM. Insets show sample current traces from control (black) and transfected (green, –DOX and red, +DOX) neurons. The bar graphs to the right of the scatterplots are normalized to control comparing mean + SEM AMPAR EPSC data. (*F*) Summary of the logarithms of the ratios between transfected and control cells for every pair analyzed in each experiment. n = 15–18 pairs. (Scale bars: 50 pA, 20 ms.) $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ vs. control condition. $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ vs. WT GluA1 condition.

and *F*). Finally, there was little rescue of synaptic currents with the expression of ΔATD GluA1 (Fig. 3 *E* and *F*). In none of these conditions was there a change in the NMDA EPSC (Fig. S3 *A–D*). As expected for the rescue of synaptic currents with GluA1, the synaptic currents were rectifying (Fig. S3*E*).

We repeated these experiments using GluA2(Q). In the absence of DOX there was little remaining synaptic current (Fig. S4 A_1, A_2, and *D*). However, in the presence of DOX there was a substantial rescue of synaptic currents (Fig. S4 *B* and *D*). A similar rescue was obtained with ΔATD GluA2(Q) (Fig. S4 *C* and *D*). In all of these experiments we also recorded the synaptic NMDA current and in none of the experiments did this change (Fig. S5).

Most endogenous AMPARs in CA1 pyramidal cells are heteromers containing GluA1 and GluA2 (4, 5). What effect, if any, does the ATD of GluA1 have on trafficking of heteromeric receptors? On a null background we constitutively expressed the edited GluA2(R), which, in contrast to the unedited GluA2, generates little glutamate activated currents, when expressed on its own, along with inducible ΔATD GluA1 in slice culture (Fig. S6A_1). We then compared cells in the absence of DOX (Fig. S6A_2), which resulted in minimal synaptic currents, to those in its presence (Fig. S6*B*). As addition of DOX did not rescue currents above −DOX levels, we propose that the absence of the ATD in GluA1 prevents the trafficking of heteromeric receptors, indicating the dominance of the ATD of GluA1 in synaptic trafficking. However, this negative result could result from the failure of ΔATD GluA1 and GluA2(R) to form heteromeric receptors. To address this issue we pulled somatic outside-out patches and measure glutamate active currents and, most importantly, rectification. We found that surface currents were of similar magnitude in control and transfected cells (Fig. S6*C*) as well as the rectification index (Fig. S6*D*), indicating that the surface AMPARs are heteromeric.

What role might the ATD play in LTP? Because we are unable to reliably induce LTP in slice culture with a pairing protocol, we turned to in utero electroporation and the Tet-ON system, which we developed in slice culture (discussed above). Starting at postnatal day 15 (P15), we injected the in utero-electroporated pups with DOX, once a day for 6 d, and then made acute slices (Fig. S7A_1). First, we inducibly overexpressed GFP GluA1 by itself in WT mice. Overexpression of GFP GluA1 had no effect on the amplitude of evoked AMPA (Fig. S7A_2) or NMDA (Fig. S7*B*) synaptic currents or on rectification (Fig. S7*D*). We then compared LTP in GFP GluA1 expressing cells to neighboring control cells (Fig. S7*C*). The magnitude of LTP was the same in both groups of cells. However, whereas LTP had no effect on rectification in control cells, GFP GluA1-expressing cells showed clearly rectifying synaptic responses after LTP (Fig. S7*D*). Thus, LTP is able to override the masking action of GFP on the synaptic trafficking of GluA1.

We next examined the effects of in utero overexpression of ΔATD GluA1 on basal synaptic transmission in WT mice to see whether prolonged expression (until P15) of this construct could gain access to the synapse (Fig. S8A_1). ΔATD GluA1 expression had no effect on the amplitude of synaptic currents, indicating that it did not act as a dominant negative (Fig. S8A_2). Unlike GFP GluA1, which gains access to the synapse after 2 d, ΔATD GluA1 remained excluded after 18 d, because there was no change in rectification (Fig. S8*B*). We next expressed ΔATD GluA1 together with Cre in triple-floxed mice (Fig. S8C_1) and observed, on average, a small but significant synaptic current (Fig. S8C_2). Following the induction of LTP, cells expressing ΔATD GluA1 showed a transient potentiation that returned to baseline by 30 min (Fig. S8*D*). Thus, unlike GFP GluA1, LTP is incapable of driving ΔATD GluA1 to the synapse.

We then expressed Cre along with the inducible GluA constructs in triple floxed mice in utero (Fig. 4A_1) to more precisely assess how synaptic trafficking of AMPAR subunits is controlled by LTP. Expression of Cre, together with WT GluA1 in the absence of DOX, resulted in the near abolition of synaptic AMPA currents (Fig. 4A_2), confirming that our inducible construct does not express in the absence of DOX. Applying an LTP pairing protocol resulted in virtually no LTP (Fig. 4*B*), as reported previously (9). What accounts for the small residual currents in the triple-floxed cells? Much of the remaining current is mediated by NMDARs, which can exhibit a small and variable degree of LTP (27). However, the DOX-induced expression of GluA1 partially rescued basal synaptic currents (Fig. 4*C*) and fully rescued LTP (cf. ref. 9) (Fig. 4*D*). By contrast, expressed GFP GluA1 was largely excluded from the synapse under basal conditions (Fig. 4*E*) but fully rescued LTP

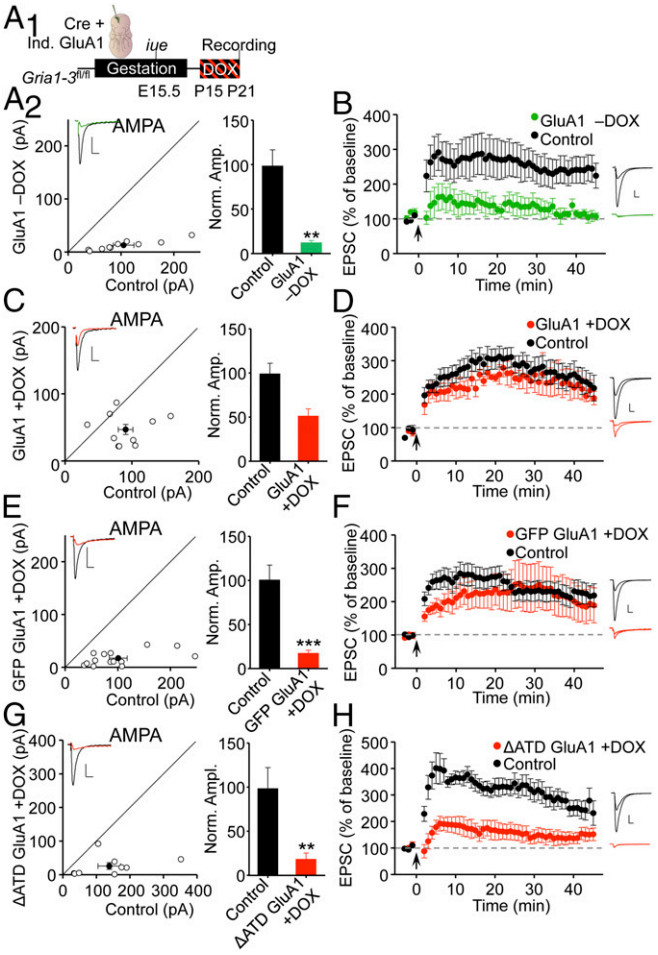

Fig. 4. WT and GFP-tagged GluA1 show normal LTP but ΔATD GluA1 does not. (A_1) Timeline of the experiment. (A_2, *C*, *E*, and *G*) Scatterplots showing amplitudes of AMPAR EPSCs for single pairs (open circles) of control and GluA1-replaced cells by in utero electroporation without DOX (A_2) and with i.p. DOX treatment for 6 d (*C*), GFP GluA1 +DOX (*E*), and ΔATD GluA1 +DOX (*G*). Filled circles represent mean ± SEM. Insets show sample current traces from control (black) and transfected (green, −DOX and red, +DOX) neurons. The bar graphs to the right of the scatterplots are normalized to control comparing mean + SEM AMPAR EPSC data. (*B*, *D*, *F*, and *H*) Plots showing mean ± SEM. AMPAR EPSC amplitude of control (black) and Cre + inducible GluA1-expressing CA1 pyramidal neurons normalized to the mean AMPAR EPSC amplitude before LTP induction (arrow). Experimental cells were transfected with GluA1 −DOX (*B*, $P = 0.04$, min 40) or +DOX (*D*, $P = 0.48$, min 40), GFP GluA1 +DOX (*F*, $P = 0.70$, min 40), or ΔATD GluA1 +DOX (*H*, $P = 0.01$, min 40). Sample AMPAR EPSC current traces from control (black) and electroporated (green, −DOX and red, +DOX) neurons before and after LTP are shown to the right of each graph. $n = 9–14$ pairs in baseline experiments and 6–13 cells per condition in LTP experiments. (Scale bars: 50 pA, 20 ms.) $^{**}P < 0.01$ and $^{***}P < 0.001$.

(Fig. 4*F*). Finally, ΔATD GluA1 was largely excluded from the synapse under basal conditions (Fig. 4*G*) and LTP was severely impaired (Fig. 4*H*). Together, these results demonstrate the critical role of the ATD of GluA1 in both the basal and activity-dependent trafficking of AMPARs. They further show that LTP can override the masking effect that GFP has on basal trafficking of GluA1 but is unable to drive ΔATD GluA1 to the synapse.

In a final series of experiments we examined the effects of LTP on GluA2(Q) trafficking in an AMPAR-null background (Fig. 5A_1). As in the case with inducible GluA1, there was virtually no expression of GluA2(Q) in the absence of DOX (Fig. 5A_2). Furthermore, LTP was absent in these cells (Fig. 5*B*). In the presence of DOX there was a full rescue of synaptic currents (Fig. 5*C*) and LTP (cf. ref. 9) (Fig. 5*D*). Interestingly, unlike full-length GluA2(Q), ΔATD GluA2(Q) only partially rescued synaptic currents (Fig. 5*E*), suggesting a possible role of the ATD of GluA2 in basal trafficking. Curiously, this defect was not seen with either the overexpression experiments (Fig. 2*B*), which may be a less-sensitive assay, or with inducible expression in slice cultures, in which both full-length and ΔATD GluA2(Q) only partially rescued synaptic currents. Despite the partial rescue of basal currents, LTP was fully rescued (Fig. 5*F*). Importantly, in all GluA1 and GluA2(Q) experiments the currents were rectifying (Fig. S9), indicating the responses were mediated by overexpressed AMPARs.

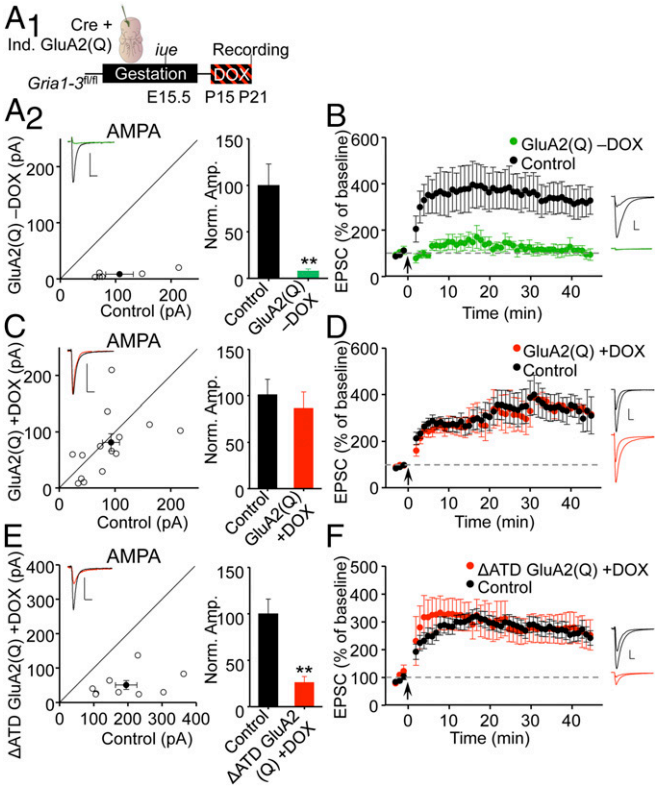

Fig. 5. WT and ΔATD GluA2 show normal LTP. (A_1) Timeline of the experiment. (A_2, *C*, and *E*) Scatterplots showing amplitudes of AMPAR EPSCs for single pairs (open circles) of control and GluA2(Q)-replaced cells by in utero electroporation without DOX (A_2), and with DOX treatment for 6 d (*C*) and ΔATD GluA2(Q) +DOX (*E*). Filled circles represent mean ± SEM. Insets show sample current traces from control (black) and transfected (green, −DOX and red, +DOX) neurons. The bar graphs to the right of the scatterplots are normalized to control comparing mean + SEM AMPAR EPSC data. (*B*, *D*, and *F*) Plots showing mean ± SEM. AMPAR EPSC amplitude of control (black) and Cre + inducible GluA2(Q)-expressing CA1 pyramidal neurons normalized to the mean AMPAR EPSC amplitude before LTP induction (arrow). Experimental cells were transfected with GluA2(Q) −DOX (*B*, $P = 0.02$, min 40) or +DOX (*D*, $P = 0.84$, min 40) or ΔATD GluA2(Q) +DOX (*F*, $P = 0.72$, min 40). Sample AMPAR EPSC current traces from control (black) and electroporated (green, −DOX and red, +DOX) neurons before and after LTP are shown to the right of each graph. $n = 6$–14 pairs in baseline experiments and 4–11 cells per condition in LTP experiments. (Scale bars: 50 pA, 20 ms.) $^{**}P < 0.01$.

Discussion

The literature on the synaptic trafficking of overexpressed AMPARs is confusing. In dissociated neuronal cultures most studies show that epitope-tagged GluA1 receptors (28, 29), including GFP-tagged GluA1 (21), colocalize with synaptic markers. By contrast, imaging experiments (21, 30) and electrophysiological experiments (6, 7) in slice culture report that the GFP-tagged GluA1 is excluded from synapses. This discrepancy between dissociated neurons and slice cultures suggests differences in the way synapses in these two preparations handle expressed AMPARs and raises caution about interpreting AMPAR synaptic trafficking in dissociated neuronal cultures. In the present study we examined a number of possibilities that might account for the apparent discrepancy between our results in slice cultures, in which GluA1 is constitutively trafficked to the synapse (9), and previous results (6–8, 17), where GluA1 synaptic trafficking required activity. In the process we discovered an important role for the ATD in the subunit-specific trafficking of AMPARs to the synapse, both constitutively and during activity.

The primary differences between our previous experiments and those of others include splice variants, the presence or absence of a GFP tag on the ATD of the receptor, and perhaps the level of basal activity in the slice culture. To our surprise we did find that the presence of a GFP tag on GluA1 prevented its appearance at the synapse. However, extending the number of days of expression from 2 d to 4–6 d did result in the appearance of these receptors at the synapse. Remarkably, tagging the GluA2 subunit with GFP did not prevent its trafficking to the synapse. This differential effect of GFP seems to explain the finding that GluA2, but not GluA1, traffics to the synapse constitutively (6, 7), although a recent paper concluded that untagged GluA1 subunits are also excluded from the synapse (17). We are at a loss to explain this difference. It is interesting to note that in a previous study (8) a non-GFP-tagged GluA1 receptor was excluded from the synapse in slice culture. This receptor contained an HA tag inserted between the 28th and 29th amino acid codons after the signal peptide, which might mimic the effect of GFP.

Why does the presence of a GFP tag on GluA1 affect trafficking? Because this receptor expresses in normal amounts on the surface of the neuron, it cannot be due to a problem in the synthesis or delivery of the receptor to the surface of the cell. Rather, the GFP tag disrupts the translocation of the receptor from the extrasynaptic to the synaptic location. There seem to be two possibilities. First, the presence of the large GFP tag may provide a physical constraint, in which case one might expect that the GFP-tagged GluA2 receptor would behave similarly to the GFP GluA1 receptor, which it does not. Second, the GFP tag might mask an interaction of the ATD of GluA1, but not GluA2, with proteins in the synaptic cleft. We favor the latter explanation because deleting the ATD from GluA1, but not GluA2, prevents its targeting to the synapse.

It is of interest to compare the present results with previous results on the ATD of GluA2. The ATD of GluA2 binds N-cadherin and plays an instructive role in spinogenesis, whereas the ATD of GluA1 was devoid of this activity (14). By contrast, our results indicate a specific role of the ATD of GluA1 in synaptic targeting, whereas the ATD of GluA2 lacks this activity. Thus, the ATDs of GluA1 and GluA2 have specific and separable functions in the regulation of spine formation and subunit-specific synaptic trafficking of AMPARs. We argue that, because ΔATD GluA2(Q) shows normal constitutive synaptic trafficking and LTP, whereas both ΔATD GluA1 homomers and ΔATD GluA1A2 heteromers

have impaired synaptic targeting, the presence of the ATD of GluA1 provides the necessary permissive signal required for constitutive and activity-dependent synaptic trafficking of GluA1A2 heteromeric AMPARs, which account for nearly 80% of all AMPARs at CA1 synapses (4, 5). Remarkably, the masking effect that GFP has on the constitutive trafficking of GluA1 can be overridden by the expression of active CaMKII and by LTP. This, in turn, implies that the intracellular signaling pathway initiated by CaMKII can control the functional interactions of the extracellular ATD.

A number of recent studies have drawn attention to the importance of the interactions between the ATD of ionotropic glutamate receptors and a variety of cleft proteins in synaptic trafficking and plasticity. The ATD of the GluD2 receptor, which is selectively expressed at cerebellar parallel fiber–Purkinje cell synapses, binds to the soluble glycoprotein Cbln1, which, in turns, binds to a presynaptic neurexin. This tripartite bridging complex plays a critical role in synapse formation and maintenance (31, 32). Recent evidence has shown that the ATD of kainate receptors is critical for their synaptic localization (33–35). Furthermore, the neuronal pentraxins interact with the ATD of GluA4 (15) as well as other GluA subunits (16). Finally, recent reports have found a critical role for synaptic adhesion molecules in LTP (36–38). These studies highlight the rich interplay between the extracellular domains of ionotropic glutamate receptors and synaptic cleft proteins. Of particular interest is how LTP, which is induced postsynaptically, engages these newly discovered extracellular trafficking motifs, and which specific cleft proteins participate in this process.

While this manuscript was in preparation a paper appeared that presents findings very similar to ours (39).

Materials and Methods

Mouse Genetics. Animals were housed according to the University of California, San Francisco (UCSF)'s Institutional Animal Care and Use Committee (IACUC) guidelines. *Gria1–3*$^{fl/fl}$ mice were genotyped as previously described (5). All experimental protocols involving animals were reviewed and approved by the UCSF's IACUC.

Electrophysiology. All experiments were performed in accordance with established protocols approved by the UCSF's IACUC. Whole-cell recordings were performed as described previously (5). Simultaneous dual whole-cell recordings were made between GFP- and/or mCherry-positive experimental cells as identified by epifluorescence and neighboring nontransfected control cells. Slice cultures were prepared from P6–P8 rat or *Gria1–3*$^{fl/fl}$ mouse pups as described previously (40) and biolistically transfected (*SI Materials and Methods*). Acute slices were prepared from E15.5 in utero-electroporated (*SI Materials and Methods*) P21–P28 mice.

Experimental procedures are described in *SI Materials and Methods*.

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