damage (*3*, *16*–*18*, *21*). Understanding the balance between forebrain and midbrain responses to threat might illuminate the pathophysiology of neuropsychiatric disturbances, including chronic anxiety and panic disorder, where brainstem involvement has long been suspected.

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Supporting Online Material

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Astrocytes Potentiate Transmitter Release at Single Hippocampal Synapses

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Astrocytes play active roles in brain physiology. They respond to neurotransmitters and modulate neuronal excitability and synaptic function. However, the influence of astrocytes on synaptic transmission and plasticity at the single synapse level is unknown. Ca^{2+} elevation in astrocytes transiently increased the probability of transmitter release at hippocampal area CA3-CA1 synapses, without affecting the amplitude of synaptic events. This form of short-term plasticity was due to the release of glutamate from astrocytes, a process that depended on Ca^{2+} and soluble *N*-ethylmaleimide–sensitive factor attachment protein receptor (SNARE) protein and that activated metabotropic glutamate receptors (mGluRs). The transient potentiation of transmitter release became persistent when the astrocytic signal was temporally coincident with postsynaptic depolarization. This persistent plasticity was mGluR-mediated but *N*-methyl-D-aspartate receptor–independent. These results indicate that astrocytes are actively involved in the transfer and storage of synaptic information.

Recent data have demonstrated the existence of bidirectional communication between astrocytes and neurons (*1*). In addition to responding to synaptic activity, astrocytes release gliotransmitters (*2*), which modulate neuronal excitability and neurotransmission (*3*). To investigate the consequences of astrocyte Ca^{2+} elevations on evoked synaptic transmission at single hippocampal synapses, we performed paired recordings from CA1 pyramidal neurons and single astrocytes (*4*). Astrocytes were loaded with the Ca^{2+}-cage *o*-nitrophenyl-EGTA (NP-EGTA) to be selectively stimulated by ultraviolet (UV)–flash photolysis, while we stimulated Schaffer collaterals using the minimal stimulation method that activates single, or very few synapses (*5*, *6*).

First, we established that single synapses were stimulated in our experimental model by quantifying the synaptic transmission properties of the excitatory postsynaptic currents (EPSCs) (Fig. 1). Synaptic responses showed failures and successes in neurotransmitter release [probability of release (Pr) was 0.34 ± 0.02; range, 0.13 to 0.54; $n = 34$]; regular amplitude of successful responses (termed "synaptic potency"; 20.9 ± 1.3 pA; range, 8.5 to 37.5 pA; $n = 34$); and relatively low synaptic efficacy {i.e., the mean amplitude of all responses including failures: 6.9 ± 0.5 pA [range, 2.8 to 10.2 pA; $n = 34$ (fig. S1)]}. Paired-pulse stimulation facilitated the second EPSC relative to the first EPSC [paired-pulse facilitation (PPF) index was 0.48 ± 0.05; $n = 20$ (fig. S1)]. To stimulate astrocytes, we patch-clamped single passive astrocytes located in the stratum radiatum near (<50 μm from) the stimulating pipette. We included NP-EGTA and fluo-4 in the recording pipette to selectively activate single astrocytes and to monitor their Ca^{2+} levels, respectively (Fig. 1A). UV-flash trains evoked astrocyte Ca^{2+} elevations that were reliably repeated by successive stimuli [15 out of 15 astrocytes (fig. S2)].

After the control recording of EPSCs, NP-EGTA–loaded astrocytes were photo-stimulated. In 18 out of 38 neuron-astrocyte pairs [47% (Fig. 1D)] astrocytic Ca^{2+} elevations transiently (~2 min) increased the synaptic efficacy (from 4.8 ± 0.6 pA to 6.2 ± 1.0 pA; $n = 18$; $P < 0.05$). This was due to a transient enhancement of Pr rather than a postsynaptic modulation (Fig. 1, F and G). Indeed, although Pr increased after astrocyte stimulation (from 0.24 ± 0.03 to 0.33 ± 0.04; $n = 18$; $P < 0.001$), the synaptic potency was unchanged (from 15.2 ± 1.3 pA to 15.7 ± 1.9 pA; $n = 18$; $P = 0.96$). Moreover, the PPF index changed from 0.64 ± 0.06 to 0.33 ± 0.10 after astrocyte stimulation [(fig. S3) $n = 18$; $P < 0.01$], which is consistent with a presynaptic mechanism of action. Furthermore, the kinetic properties of EPSCs were unaffected (respective rise and decay time constants before and after astrocyte stimulation were τ_{on} = 1.48 ± 0.22 ms and 1.45 ± 0.23 ms; $P = 0.34$; τ_{off} = 9.80 ± 0.94 ms and 10.31 ± 1.77 ms; $P = 0.43$; $n = 6$). These effects were reliably evoked by successive astrocyte stimulation (Fig. 2A).

In the absence of NP-EGTA or with the NP-EGTA–filled pipette placed outside the cell, UV flashes did not modify synaptic transmission (fig. S4), which indicated that the effects were not due to photo-stimulation of synaptic terminals and that Ca^{2+} elevation in astrocytes is necessary and sufficient to potentiate the synaptic transmission.

We further analyzed whether the astrocyte-induced neuromodulation could also be evoked by stimuli that elevate astrocyte Ca^{2+} through transmitter receptor activation. We used adeno-

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sine 5′-triphosphate (ATP) because, although it may act on neuronal and astrocytic receptors, it is highly effective in elevating astrocytic Ca^{2+} (*3*, *7*). While recording synaptic currents, we locally applied ATP by microionophoresis (from pipette placed in the stratum radiatum >150 μm away from the synapse-stimulating pipette), which elevated Ca^{2+} in a large number of astrocytes (Fig. 2B). Because ATP is converted to adenosine by extracellular adenosine triphosphatases (ATPases), these experiments were performed in the presence of the A1 purinergic receptor antagonist 8-cyclopentyltheophylline (CPT; 2 μM), which increases synaptic transmission (fig. S5), but prevents adenosine-dependent

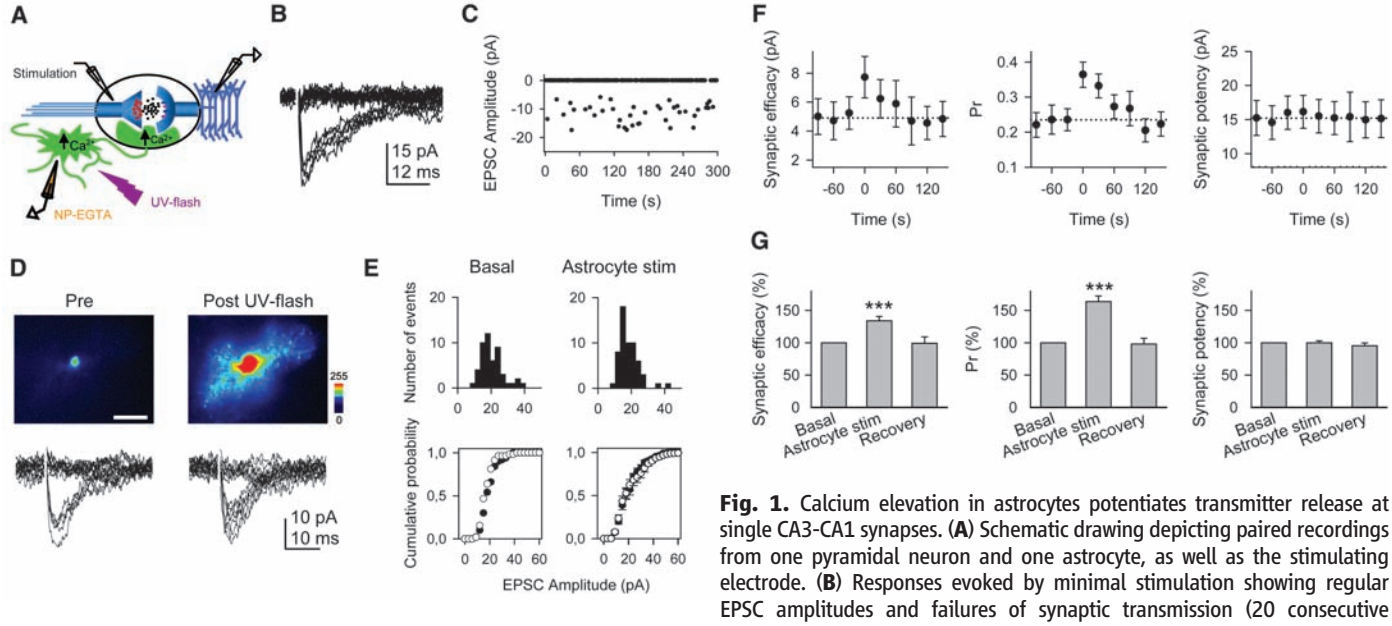

Fig. 1. Calcium elevation in astrocytes potentiates transmitter release at single CA3-CA1 synapses. (**A**) Schematic drawing depicting paired recordings from one pyramidal neuron and one astrocyte, as well as the stimulating electrode. (**B**) Responses evoked by minimal stimulation showing regular EPSC amplitudes and failures of synaptic transmission (20 consecutive stimuli). (**C**) EPSC amplitudes from a representative synapse. (**D**) Pseudocolor images representing fluorescence intensity of a NP-EGTA– and fluo-4–filled astrocyte (top) and synaptic responses [(bottom) 15 consecutive stimuli] obtained from paired whole-cell recordings before and after UV-flash astrocyte stimulation. Scale bar, 20 μm. (**E**) EPSC amplitude histograms (bin width, 3 pA) (top); corresponding cumulative probability plot (bottom, left); and average (n = 7 astrocyte-neuron pairs) cumulative probability plots (bottom, right) of EPSC amplitudes before and after astrocyte stimulation (filled and open circles, respectively). (**F**) Synaptic efficacy (i.e., mean amplitude of responses including successes and failures of neurotransmission); probability of neurotransmitter release (Pr); and synaptic potency (i.e., mean EPSC amplitude excluding failures) (bin width, 33 s; n = 18 astrocyte-neuron pairs). Zero time corresponds to the time of astrocyte stimulation. (**G**) Relative changes of synaptic parameters in the control state, after astrocyte stimulation, and 2 min later (recovery) (n = 18). ***P < 0.001. Error bars indicate SEM.

a

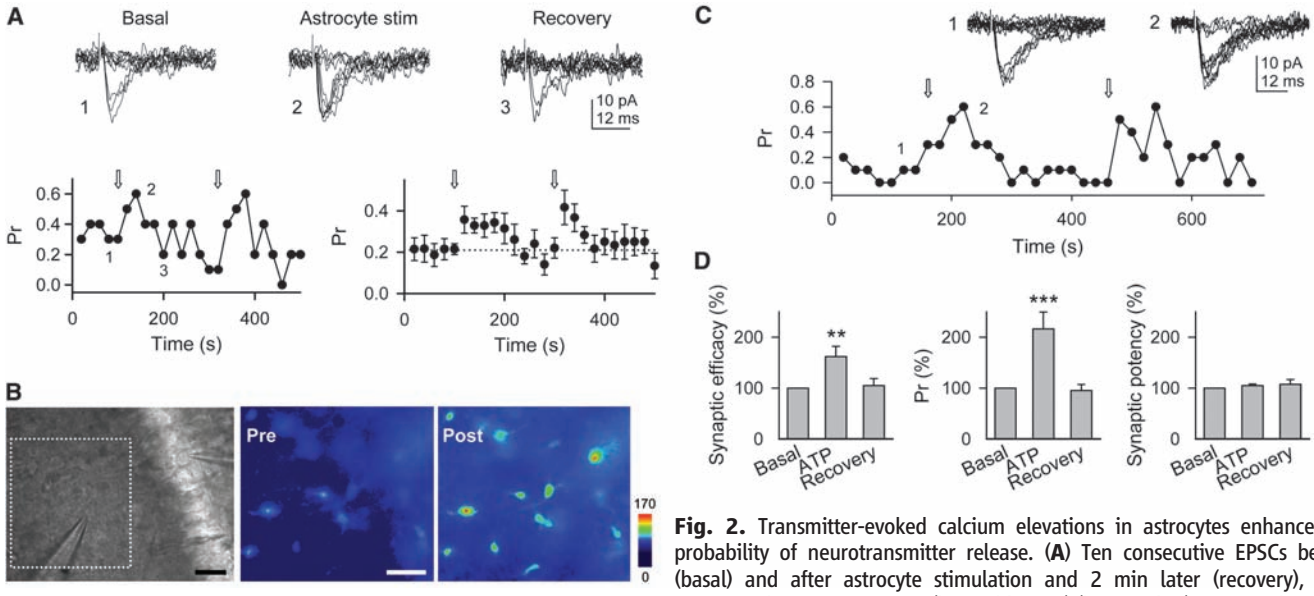

Fig. 2. Transmitter-evoked calcium elevations in astrocytes enhance the probability of neurotransmitter release. (**A**) Ten consecutive EPSCs before (basal) and after astrocyte stimulation and 2 min later (recovery), with corresponding Pr versus time (bin width, 20 s) (bottom, left). Averaged Pr from seven neuron-astrocyte pairs (bottom, right). Arrows indicate astrocyte stimulation. (**B**) Infrared differential interference contrast image showing the recorded pyramidal neuron and the stimulation electrode (left). Pseudocolor images representing fluorescence intensity of fluo-4–filled astrocytes before (pre) and after (post) ATP application through a pipette placed out of the image. Scale bar, 30 μm. (**C**) Ten consecutive EPSCs before (1) and after (2) ATP-evoked astrocyte Ca^{2+} elevations, with corresponding Pr versus time. (**D**) Relative changes of synaptic parameters in the control state, induced by ATP-evoked astrocyte stimulation, and after recovery (2 min later) (n = 16). **P < 0.01, ***P < 0.001. Error bars indicate SEM.

neuromodulation (*8*–*11*). Under these conditions, ATP-evoked astrocyte Ca^{2+} elevations transiently potentiated synaptic transmission in 43% of the synapses (16 out of 37) (Fig. 2C). The analysis of synaptic responses showed an increase of Pr [(Fig. 2, C and D) from 0.30 ± 0.03 to 0.41 ± 0.03 after astrocyte stimulation; n = 16; $P < 0.001$], without changes of synaptic potency (from 17.3 ± 1.1 pA to 17.8 ± 1.1 pA; n = 16; P = 0.47). Consequently, the synaptic efficacy was increased [(Fig. 3D) from 6.0 ± 0.6 pA to 8.0 ± 0.7 pA; n = 16; $P < 0.01$]. Because ATP is not a specific agonist for astrocytes, a direct effect on neurons cannot be totally excluded. However, the comparable effects of astrocyte Ca^{2+} elevations evoked by photo-stimulation or ATP, as well as their equal sensitivity to metabotropic glutamate receptor (mGluR) antagonists (see below), indicate that these effects were not likely due to nonspecific actions of the stimuli.

We next investigated the mechanisms responsible for the astrocyte-induced neuromodulation. Passive astrocytes release gliotransmitters that influence neurotransmission (*7*–*18*). Because some gliotransmitters are released through soluble *N*-ethylmaleimide–sensitive factor attachment protein receptor (SNARE) protein–dependent mechanisms (*19*–*21*), we asked if the cleavage of synaptobrevin 2 by tetanus toxin (TeNT) affected the astrocyte-induced neuromodulation. When the light chain of TeNT was included in the recording astrocyte, photo-stimulation of the NP-EGTA–loaded astrocyte increased its intracellular Ca^{2+} but did not affect the synaptic properties [(Fig. 3A) n = 13]. Therefore, the neuromodulation requires the SNARE protein–dependent gliotransmitter release from astrocytes.

Because glutamate is released by astrocytes through Ca^{2+}- and SNARE protein–dependent mechanisms (*19*–*21*) and can facilitate neurotransmitter release by activation of presynaptic group I mGluRs (*13*, *22*, *23*), we investigated their participation in the synaptic transmission potentiation. After assessing that UV-flash astrocyte stimulation transiently increased Pr, we perfused the group I mGluR antagonists 2-methyl-6-(phenylethynyl)-pyridine (MPEP, 50 μM) and (+)-2-methyl-4-carboxyphenylglycine (LY367385, 100 μM), which are mGluR subtype 5 and mGluR subtype 1 antagonists, respectively. These antagonists blocked the transient potentiation of transmitter release without affecting the astrocyte Ca^{2+} signal [(Fig. 3, B and C) Pr = 0.41 ± 0.11 and 0.37 ± 0.12 before and after astrocyte stimulation; n = 8; P = 0.28). Likewise, the synaptic potentiation induced by ATP-evoked astrocyte Ca^{2+} elevations was inhibited by those antagonists [n = 7 (fig. S6)].

One of the most-studied forms of synaptic plasticity is long-term potentiation (LTP), characterized by a long-lasting increase of synaptic strength caused by the coincidence of pre- and postsynaptic activity (*24*). The results above indicate that astrocytes evoke a short-term potentiation of neurotransmitter release. We then investigated whether the temporal coincidence of the astrocytic signal and the postsynaptic activity could induce a long-lasting modulation of synaptic transmission. We analyzed the long-term effects of pairing the astrocyte Ca^{2+} uncaging with a transient mild depolarization (to –30 mV) of the postsynaptic neuron. We first established that in the absence of astrocyte stimulation, the postsynaptic depolarization per se did not modify the synaptic parameters [n = 6 (Fig. 4, D and E)]. However, when astrocytes were photo-stimulated during the postsynaptic depolarization, both the synaptic efficacy and the probability of transmitter release were persistently (≥60 min) increased in 41% of the synapses (n = 7 out of 17) [(Fig. 4, A, B, and E); synaptic efficacy: 5.1 ± 0.5 pA and 15.5 ± 2.1 pA ($P < 0.01$) and Pr = 0.28 ± 0.04 and 0.71 ± 0.08 ($P < 0.001$), before and 60 min after astrocyte stimulation, respectively; n = 7]. Although two out of seven synapses showed a potentiation of the synaptic potency, on average the synaptic efficacy increase was not accompanied by synaptic potency changes (18.1 ± 1.3 pA and 21.0 ± 2.7 pA, before and 60 min after astrocyte stimulation, respectively; n = 7; P = 0.17). The astrocyte-induced LTP was also evoked by receptor-mediated astrocyte Ca^{2+} signaling because pairing the neuronal depolarization with the ATP-evoked astrocyte stimulation (in the presence of CPT) caused a long-lasting potentiation of synaptic transmission in 46% of the synapses (6 out of 13) (Fig. 4C). This astrocyte-induced LTP was independent of *N*-methyl-D-aspartate receptor (NMDAR) activation because it was unaffected by D-2-amino-5 phosphonopentanoic acid (D-AP5; 50 μM) [(Fig. 4, C and E); 42% of synapses showed persistent potentiation; n = 5 out of 12].

This form of LTP was associated with the astrocyte-induced transient increase of Pr be-

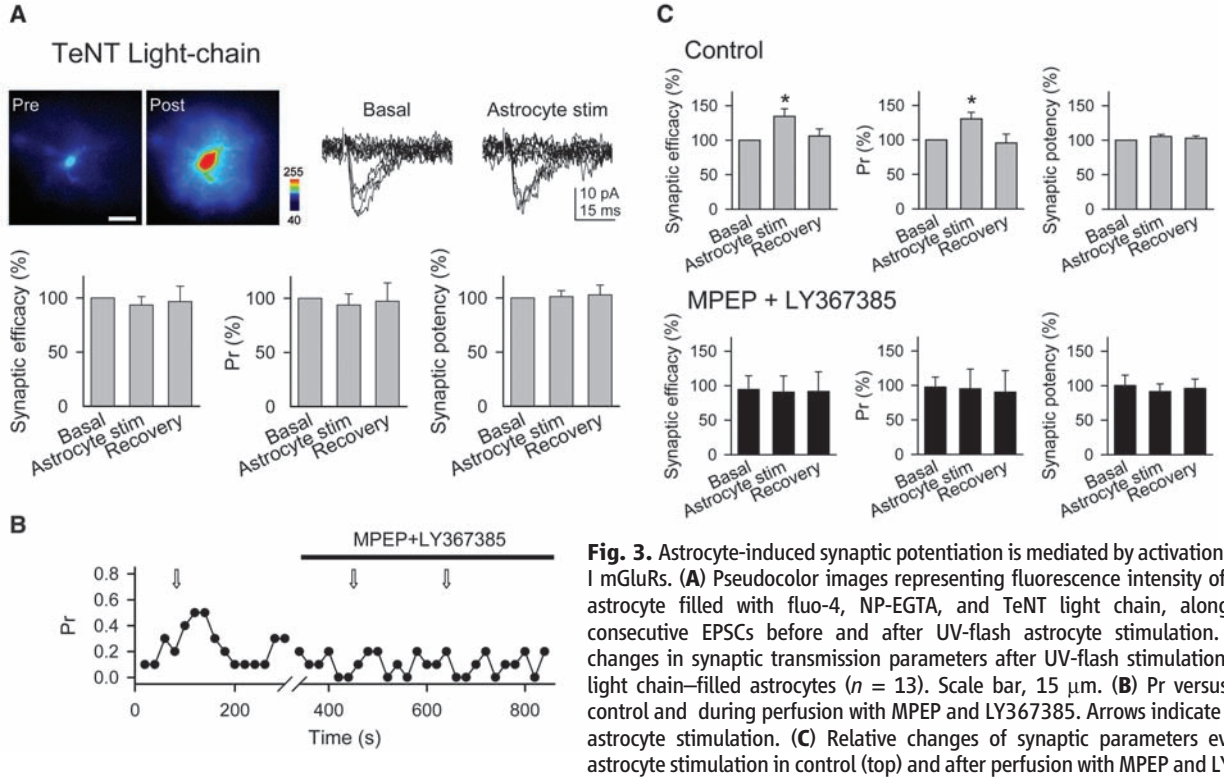

Fig. 3. Astrocyte-induced synaptic potentiation is mediated by activation of group I mGluRs. (**A**) Pseudocolor images representing fluorescence intensity of a single astrocyte filled with fluo-4, NP-EGTA, and TeNT light chain, alongside 12 consecutive EPSCs before and after UV-flash astrocyte stimulation. Relative changes in synaptic transmission parameters after UV-flash stimulation of TeNT light chain–filled astrocytes (n = 13). Scale bar, 15 μm. (**B**) Pr versus time in control and during perfusion with MPEP and LY367385. Arrows indicate UV-flash astrocyte stimulation. (**C**) Relative changes of synaptic parameters evoked by astrocyte stimulation in control (top) and after perfusion with MPEP and LY367385 (bottom) (n = 8). *$P < 0.05$. Error bars indicate SEM.

cause 7 out of 17 synapses showed both transient and long-lasting increase in Pr and no synapses underwent persistent potentiation without transient modulation ($n = 10$). Therefore, we studied whether astrocyte-induced LTP depended on activation of group I mGluRs. In the presence of the antagonists MPEP and LY367385, astrocyte stimulation did not modify the synaptic parameters [$n = 6$ (Fig. 4, D and E)].

Our results show that Ca^{2+} elevation in astrocytes modulates transmitter release probability and evokes long-term synaptic plasticity at single hippocampal synapses without affecting the postsynaptic sensitivity. Although the astrocyte-induced transient synaptic modulation is in agreement with reports of mGluR-dependent neuromodulation [compare (*13*, *22*, *23*)], the NMDAR-independent long-lasting effects are more challenging to our vision of the classical LTP in the CA1 region, where postsynaptic changes underlie its expression [(*25*), but see (*26*)].

These data extend the classical Hebbian model for LTP, which results from concurrent pre- and postneuronal activity, to include astrocytes activity, so that the temporal coincidence the temporal coincidence of astrocyte and postsynaptic activities evokes long-term changes in synaptic efficacy. Therefore, astrocytes are directly involved in the synaptic information transfer and storage by the nervous system.

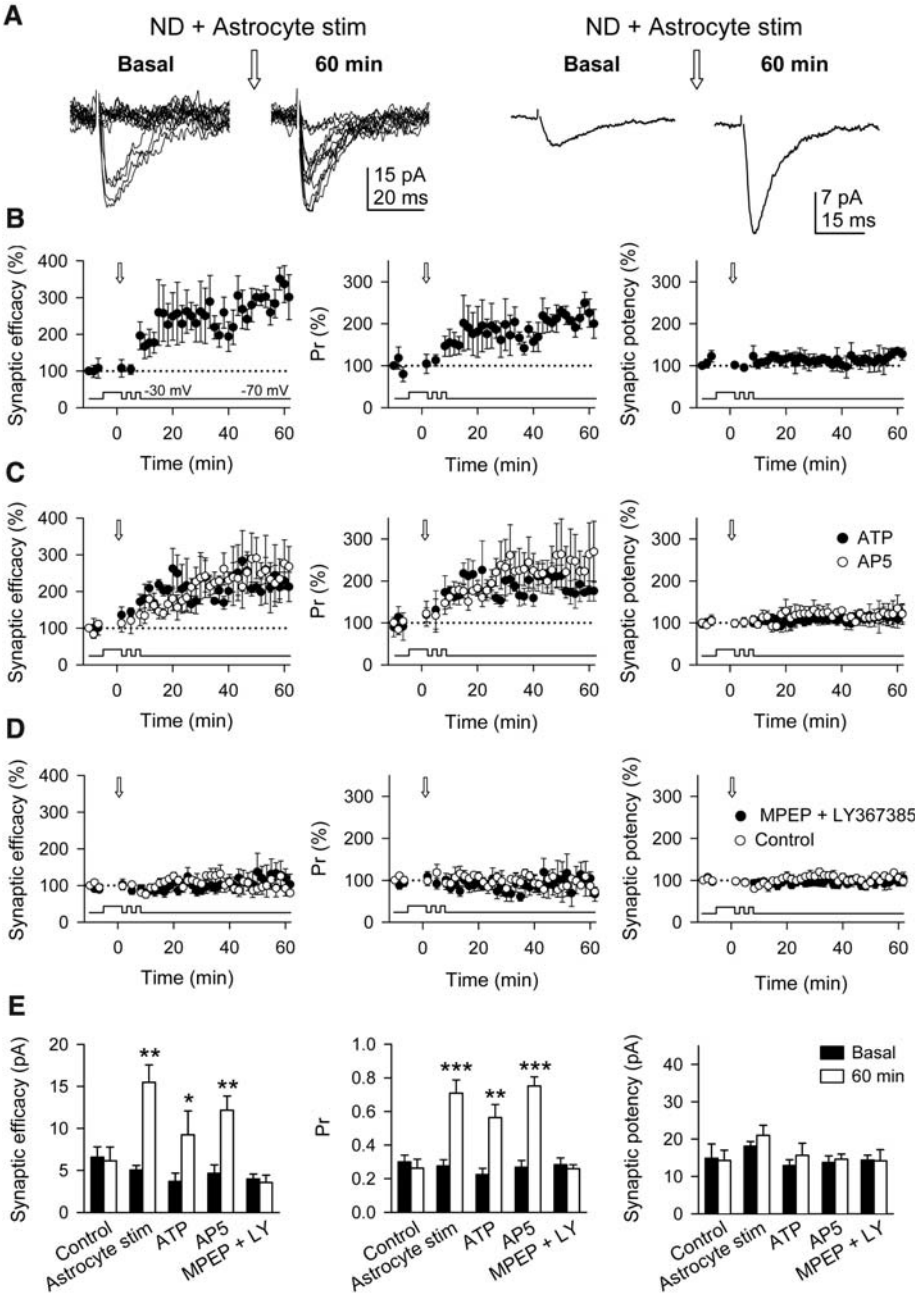

Fig. 4. Temporal coincidence of astrocyte and neuronal signals evoke LTP of synaptic transmission. (**A**) Fifteen consecutive EPSCs (left) and average EPSCs ($n = 50$ trials including failures) (right) before (basal) and 60 min after pairing neuronal depolarization (ND) and UV-flash astrocyte stimulation. (**B** to **D**) Synaptic parameters normalized to baseline and plotted over time. Arrows indicate astrocyte stimulation. (B) Pairing of UV-flash astrocyte stimulation and ND ($n = 7$ astrocyte-neuron pairs). (C) Pairing of ATP-evoked astrocyte stimulation and ND in control (black circles, $n = 6$) and after administering 50 μM D-AP5 (white circles, $n = 5$). (D) Pairing of UV-flash astrocyte stimulation and ND after administering MPEP and LY367385 (black circles); and ND with no astrocyte stimulation (control; white circles); ($n = 6$ for each case). (**E**) Synaptic parameters before and after 60 min under different conditions. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Error bars indicate SEM.

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