

Astrocyte β -Adrenergic Receptor Activity Regulates NMDA Receptor Signaling of Medial Prefrontal Cortex Pyramidal Neurons

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Glutamate spillover from the synapse is tightly regulated by astrocytes, limiting the activation of extrasynaptically located NMDA receptors (NMDAR). The processes of astrocytes are dynamic and can modulate synaptic physiology. Though norepinephrine (NE) and β -adrenergic receptor (β -AR) activity can modify astrocyte volume, this has yet to be confirmed outside of sensory cortical areas, nor has the effect of noradrenergic signaling on glutamate spillover and neuronal NMDAR activity been explored. We monitored changes to astrocyte process volume in response to noradrenergic agonists in the medial prefrontal cortex of male and female mice. Both NE and the β -AR agonist isoproterenol (ISO) increased process volume by $\sim 20\%$, significantly higher than changes seen when astrocytes had G-protein signaling blocked by GDP β S. We measured the effect of β -AR signaling on evoked NMDAR currents. While ISO did not affect single stimulus excitatory currents of Layer 5 pyramidal neurons, ISO reduced NMDAR currents evoked by 10 stimuli at 50 Hz, which elicits glutamate spillover, by 18%. After isolating extrasynaptic NMDARs by blocking synaptic NMDARs with the activity-dependent NMDAR blocker MK-801, ISO similarly reduced extrasynaptic NMDAR currents in response to 10 stimuli by 18%. Finally, blocking β -AR signaling in the astrocyte network by loading them with GDP β S reversed the ISO effect on 10 stimuli-evoked NMDAR currents. These results demonstrate that astrocyte β -AR activity reduces extrasynaptic NMDAR recruitment, suggesting that glutamate spillover is reduced.

Key words: astrocyte; β -adrenergic receptors; extrasynaptic NMDA receptors; glia modulation of synapses; norepinephrine; synaptic modulation

Significance Statement

Astrocyte processes closely associate with glutamatergic synapses, limiting glutamate access to extrasynaptically located NMDA receptors (NMDARs). Norepinephrine activation of β -adrenergic receptors (β -ARs) can change astrocyte morphology and process volume, which can modulate glutamate spillover. β -AR-driven changes to astrocyte process volume may influence NMDA receptor activity, but this has not been explored. Here we show that β -AR activity increases astrocyte process volume in the medial prefrontal cortex (mPFC), an emotion processing region of the brain, and reduces NMDAR activity associated with glutamate spillover in an astrocyte-dependent manner. Our study provides a novel mechanism by which impaired norepinephrine signaling and astrocyte β -AR activity in the mPFC may contribute to the excessive extrasynaptic NMDAR activity observed in certain psychiatric and neurodegenerative disorders.

Received May 29, 2023; revised Oct. 23, 2023; accepted Nov. 13, 2023.

Author contributions: A.P.D.F. designed research; A.P.D.F. performed research; A.P.D.F. analyzed data; A.P.D.F. wrote the first draft of the paper; A.P.D.F. and E.A.N. wrote the paper.

This research was supported by National Institutes of Health Grants R01-EY-026514, R01-EY-026882, R01 NS126166, and P30-EY-011374 to E.N. and University of Minnesota Doctoral Dissertation Fellow Grant to A.D. We thank Stanley Thayer and Paulo Kofuji for lending equipment crucial to the completion of this research.

The authors declare no competing financial interests.

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<https://doi.org/10.1523/JNEUROSCI.0990-23.2023>

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Introduction

Proper functioning of the nervous system relies on tight regulation of glutamate neurotransmission. Astrocytes serve a critical role in this process and carry out various functions vital to synaptic physiology through their complex interactions with glutamatergic synapses (Araque et al., 1999; Oliet et al., 2001; Rusakov, 2001; Halassa et al., 2007; Theodosis et al., 2008). Peripheral astrocyte processes (PAPs) closely associate

with synapses, acting as the primary means of glutamate uptake and as barriers to diffusion from the synaptic cleft (Rusakov, 2001; Huang and Bordey, 2004; Takayasu et al., 2006; Nie and Weng, 2009). As such, astrocytes play a critical role in regulating the extent to which nonsynaptic receptors are activated following glutamate release. In addition to being expressed in the postsynaptic density, NMDA receptors (NMDARs) are often found in extrasynaptic sites (Lozovaya et al., 1999, 2004; Scimemi et al., 2004; Harris and Pettit, 2008; Papouin et al., 2012). Glutamate spillover and recruitment of these extrasynaptically located NMDARs play an important role under various physiological and pathological conditions (Hardingham et al., 2002; Zhang and Sulzer, 2003; Oliet et al., 2004; Delgado et al., 2018).

Astrocyte morphology is dynamic and can drive changes in the extracellular space (ECS), affecting neurotransmitter diffusion from the synapse. PAP morphology is modulated by norepinephrine (NE; Sherpa et al., 2016), serotonin (Müller et al., 2021), oxytocin (Theodosis and Poulain, 2001), long-term plasticity (LTP) induction (Perez-Alvarez et al., 2014; Henneberger et al., 2020), fear memory induction (Badia-Soteras et al., 2022), and caloric restriction (Popov et al., 2020). Studies that observed PAP retraction and reduced astrocyte process volume noted enhanced glutamate spillover and NMDAR activation. Conversely, astrocyte process volume increases led to decreased extrasynaptic NMDAR recruitment (Popov et al., 2020). NE was found to expand PAPs and increase process volume (Vardjan et al., 2014; Sherpa et al., 2016), though its effects on glutamate spillover remain unexplored.

NE modulates the activity of neural circuits, coordinating state changes and adaptive responses to stimuli, wakefulness, arousal, mood, and stress (Benarroch, 2009; Schwarz and Luo, 2015). Various adrenergic receptor (AR) subtypes are expressed on astrocytes, though most prior work has focused on Ca^{2+} signaling elicited by G_q G-protein-coupled receptor (GPCR) α_1 -AR activity (Bekar et al., 2008; Paukert et al., 2014; Tran et al., 2018). Astrocytes also express G_s GPCR β -ARs (Hertz et al., 2010). β -AR activity enhances metabolic support, K^+ uptake, gap junction connectivity, as well as changes in morphology (Giaume et al., 1991; Rosenberg et al., 1994; Laureys et al., 2010; Sherpa et al., 2016; Wotton et al., 2020). Despite evidence that NE and β -AR activity affect astrocyte volume in sensory cortices, the effect of NE has not been tested in emotion processing brain regions where noradrenergic and NMDAR signaling dysfunction result in multiple psychiatric disorders (Breier, 1990; Ruhé et al., 2007; Li et al., 2018; Uno and Coyle, 2019; Tang et al., 2020), nor has the link between noradrenergic signaling and glutamate spillover been explored.

We have now investigated the role of β -AR activity on astrocyte volume in an emotion processing region of the brain with a high degree of noradrenergic innervation, the medial prefrontal cortex (mPFC; Santana and Artigas, 2017), characterizing its effects on pyramidal neuron NMDAR signaling. We show that NE and the β -AR agonist isoproterenol (ISO) increase astrocyte volume in the mPFC and demonstrate that these changes can be blocked by eliminating astrocyte GPCR signaling. We also find that ISO reduces NMDAR postsynaptic currents (EPSCs) associated with glutamate spillover. Blocking astrocyte responsiveness to ISO reversed this reduction of NMDAR EPSCs. Finally, by pharmacologically blocking synaptic NMDARs, we confirmed that ISO reduced extrasynaptic NMDAR EPSCs.

Materials and Methods

Ethics statement

All experimental procedures were approved by and adhered to the guidelines of the Institutional Animal Care and Use Committee of the University of Minnesota.

Brain slice preparation

All experiments used tissue harvested from 5- to 12-week-old male and female C57BL/6 mice. Mice were anesthetized with isoflurane and decapitated for rapid removal of the brain. Three hundred and fifty μm coronal brain slices of the mPFC were taken in oxygenated (95% O_2 , 5% CO_2) and chilled sucrose artificial CSF (aCSF). Brain slices were initially incubated in 32°C aCSF with 0.5 μM sulforhodamine 101 (SR 101; S7635, Sigma-Aldrich) for 10 min to label astrocytes. Slices then recovered in 32°C aCSF for another 20 min and another 30 min in room temperature aCSF until experiments were conducted. During experiments, a single brain slice was transferred to an immersion recording chamber and held stationary by a harp. Slices were perfused with oxygenated, room temperature aCSF solutions at 2.5 ml/min.

Measurement of changes in astrocyte volume

Astrocytes were whole-cell patch-clamped and filled with a fluorescent dye to measure changes in process volume. Layer 2/3 astrocytes were identified based on their labeling by SR 101, small soma size (5–10 μm), hyperpolarized V_{rest} (approximately -80 mV), and distinct morphology (Bushong et al., 2002; Henneberger and Rusakov, 2012; Minge et al., 2021). Astrocytes were filled for 25 min with the gap junction-impermeant dye Alexa Fluor 488 (3,000 MW) dextran (40 μM ; D34682, Invitrogen) prior to the start of recordings. The dye equilibrated through the finer astrocyte processes during this time (Fig. 1A,B). Using a gap junction-impermeant dye eliminated the possibility that observed fluorescent intensity changes were due to diffusion of dye into other astrocytes through gap junctions (Giaume et al., 1991). The fine processes of astrocytes fall below the imaging resolution of confocal microscopy, and dye-filling provides a method of measuring process volume, where fluorescence is proportional to the space occupied by the processes (Henneberger et al., 2020). By comparing the relative brightness of dye-filled processes to baseline fluorescence established prior to an experimental manipulation, observed changes in fluorescence will be proportional to changes to astrocyte process volume.

We captured single focal plane images of a dye-filled astrocyte at 4 min intervals. The z -axis was manually adjusted to adjust for drift over the recording period. Dye-filled astrocytes were imaged using confocal microscopy (Olympus, FV1000) through a 40 $\times$, 1.00 numerical aperture water objective to acquire 512 $\times$ 512 pixel images (0.155 $\times$ 0.155 μm pixels). Three images were acquired and averaged together every 4 min for 20 min in control aCSF to establish a baseline and then for an additional 40 min with either NE (20 μM ; A0937, Sigma-Aldrich), ISO (5 μM ; I5627, Sigma-Aldrich), or control aCSF. Five μM ISO elicits astrocyte morphological changes (Vardjan et al., 2014; Sherpa et al., 2016), recruiting both β_1 - and β_2 -ARs to increase cAMP in astrocytes (Du et al., 2010). Such β -AR activation is observed during sustained NE elevation during vigilance tasks (Wahis and Holt, 2021) and is crucial for astrocyte participation in memory consolidation (Gao et al., 2016). In some experiments, astrocytes were patched with an internal solution containing GDP β S (100 μM ; ALX-480-056, Enzo Life Sciences) to block noradrenergic receptor G-protein activity (Navarrete et al., 2012; Cavaccini et al., 2020).

Sequences of images were registered using a custom MATLAB (2016b) program and then analyzed with Fiji ImageJ. A manual ROI was drawn to encompass filled astrocyte processes (Fig. 1C–F), and the averaged ROI fluorescence was calculated for each time point (Fig. 1G,H). Fluorescence values are reported relative to baseline fluorescence:

Change in Fluorescence from Baseline (%)

$$= 100 * (F_t - F_{\text{Baseline}}) / F_{\text{Baseline}}$$

The fluorescence measurements of agonist-induced changes in astrocyte volume could possibly be skewed if ISO or NE changed the optical properties of the tissue. We tested for this by measuring changes in the

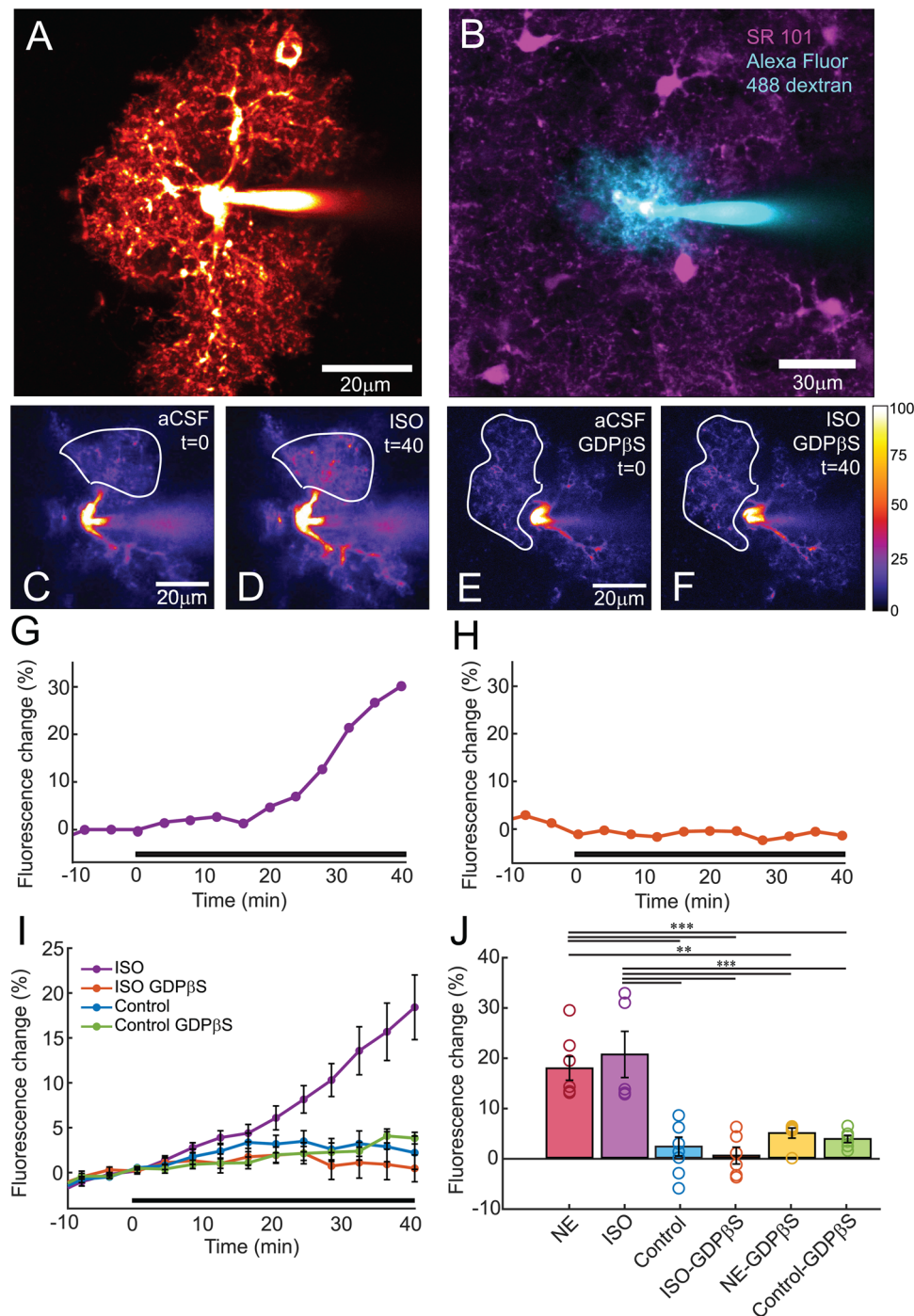


Figure 1. Noradrenergic agonists increase astrocyte process volume in the mPFC. **A**, Confocal image of an astrocyte filled with Alexa Fluor 488 dextran (3,000 MW). **B**, Confocal image of multiple astrocytes labeled with SR 101 (magenta) and a single astrocyte filled with Alexa Fluor 488 dextran (cyan). The Alexa Fluor dye does not spread beyond the patched cell. **C–F**, Example fluorescence images of two dye-filled astrocytes. **C**, Last time point in aCSF. **D**, 40 min in ISO. **E**, Fluorescence intensity remains unchanged in an astrocyte loaded with GDP β S. **F**, Last time point in aCSF. **G**, 40 min in ISO. Pseudocolor scale indicates fluorescence intensity in arbitrary units. **G**, Astrocyte process brightness versus time from the experiment illustrated in **C** and **D**. **H**, Process brightness versus time for an astrocyte loaded with GDP β S, illustrated in **E** and **F**. In **G** and **H**, process brightness is measured in the ROIs indicated in **C–F**; the black bars indicate the time course of ISO incubation. **I**, Mean $\pm$ SEM astrocyte process fluorescence versus time for astrocytes initially in aCSF and exposed to experimental solutions for 40 min (black bar). Experimental solutions include ISO (purple), ISO with GDP β S-filled astrocyte (orange), aCSF control (blue), and aCSF control with GDP β S (green). **J**, Summary of astrocyte process fluorescence changes measured after 40 min incubation in noradrenergic agonists or control solution, with or without GDP β S loading of astrocytes. Mean $\pm$ SEM and data from individual mice are shown. NE ($17.7 \pm 2.4\%$ increase; $n = 7$ mice), ISO ($20.5 \pm 4.6\%$ increase; $n = 5$ mice), control aCSF ($2.21 \pm 1.86\%$ increase; $n = 8$ mice), ISO-GDP β S ($0.40 \pm 1.67\%$ increase; $n = 6$ mice), NE-GDP β S ($4.88 \pm 1.00\%$ increase; $n = 6$ mice), aCSF GDP β S ($3.71 \pm 0.72\%$ increase; $n = 6$ mice). Two-way ANOVA with Tukey–Kramer post hoc multiple comparisons, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

fluorescence of the Alexa Fluor 488-labeled intracellular solution at the tip of the pipette patched onto an astrocyte during the course of ISO or NE application. The imaged fluorescence changed little with agonist

addition, increasing $0.8 \pm 2.0\%$ (mean $\pm$ SEM; $n = 13$ cells) after 40 min in the agonist, demonstrating that the test solutions did not change the optical properties of the tissue.

Measurement of neuronal NMDA and AMPA receptor currents

Layer 5 mPFC pyramidal neurons were patched to record evoked EPSCs. Large soma cells, 300–500 μm from the midline, were whole-cell patch-clamped and filled with Alexa Fluor 594 hydrazide dye (40 μM ; A10438, Invitrogen). Neurons were imaged with confocal microscopy to confirm the identity of the patched cell, based on a prominent apical dendrite (Fig. 2A). Cells with series resistance (R_s) >20 M Ω were excluded from analysis. To evoke EPSCs, a theta-glass pipette (BT-150-10, Sutter Instrument) delivered electric stimuli to Layer 2/3, $\sim$ 150 μm from the patched neuron's apical dendrite (Fig. 2A). Single 100 μs current pulses were delivered through a stimulator (A-M Systems Isolated Pulse Stimulator 2100) and the current amplitude adjusted to elicit 40–260 pA EPSCs. EPSC amplitudes depended on the position of the stimulating electrode and the stimulus strength used to evoke the synaptic potentials. Control NMDAR single stimulus-evoked currents equaled 72.7 ± 37.1 pA (mean $\pm$ SD). Control AMPAR single stimulus-evoked currents equaled -111.8 ± 57.8 pA (mean $\pm$ SD). Ten stimuli bursts were delivered at 50 Hz to elicit glutamate spillover and recruit

extrasynaptic NMDARs (Harris and Pettit, 2008). EPSC traces were zeroed to baseline current and were smoothed for illustrations by a 3 ms moving average filter using MATLAB. Patch clamp responses and stimuli were recorded and controlled using a MultiClamp 700b amplifier (Molecular Devices) and LabChart (ADInstruments). Currents were sampled at 10 kHz and filtered at 3 kHz and R_s compensated to $\sim$ 70%. EPSCs were analyzed using custom MATLAB programs.

Measurement of NMDAR EPSCs. NMDAR currents were isolated by blocking AMPARs and GABA_ARs with CNQX (10 μM ; Cat. No. 1045, Tocris) and gabazine (5 μM ; 5059860001, Sigma-Aldrich), respectively. Patched neurons were voltage-clamped and held at +40 mV to free NMDAR Mg^{2+} block. Five to 10 single EPSCs were averaged together and 2–4 trials of 10 stimuli EPSCs were averaged together. EPSC peak amplitude from 10 stimuli bursts was measured after the 10th stimulus. EPSC charge transfer was calculated as the area under the trace beginning from the current peak to the end of single EPSC recordings and for 1.6 s after the evoked current peak of the 10th stimulus for 10 stimuli

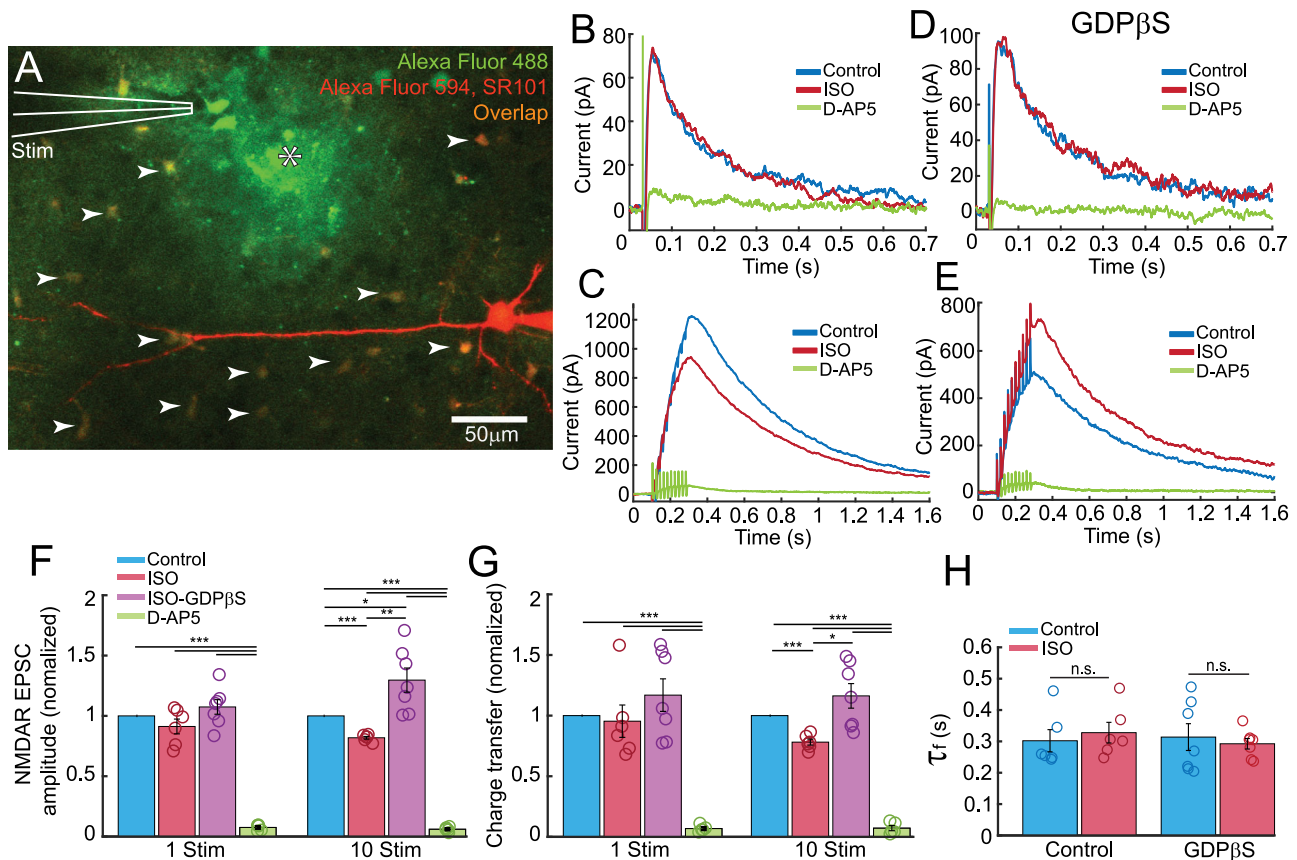


Figure 2. Ten stimuli-evoked NMDAR EPSCs are modulated by astrocyte β -AR activity. **A**, Confocal image of experimental arrangement for EPSC recording. A patched Layer 5 pyramidal neuron is filled with Alexa Fluor 594 (red) while astrocytes are initially labeled with SR 101 (also red). In some experiments, an astrocyte was patched (asterisk) and filled with GDP β S and Alexa Fluor 488 hydrazide (green), which diffused widely through the astrocyte network. SR 101-labeled astrocytes that are filled with GDP β S and Alexa Fluor 488 are orange (arrowheads) and surround the neuron. A theta tube pipette in Layer 2/3 (white lines, labeled Stim) evokes EPSCs in the labeled neuron. **B–E**, NMDAR EPSCs recorded from neurons in control aCSF (blue), after 40 min in ISO (red), and after an additional 5 min in D-AP5 (green). **B**, EPSCs evoked by single stimuli. **C**, EPSCs evoked by 10 stimuli bursts. **D**, EPSCs evoked by single stimuli with the astrocyte network loaded with GDP β S. **E**, EPSCs evoked by 10 stimuli bursts with the astrocyte network loaded with GDP β S. **F**, Summary of NMDAR EPSC amplitudes (normalized to paired control aCSF EPSCs) elicited by single and 10 stimuli. Single stimulus groups include control, all conditions, ISO (0.91 ± 0.06 ; $n = 6$ mice), ISO with GDP β S (1.07 ± 0.06 ; $n = 7$ mice), and D-AP5 (0.08 ± 0.01 ; $n = 5$ mice). Ten stimuli groups include control, all conditions, ISO (0.82 ± 0.01 ; $n = 6$ mice), ISO with GDP β S (1.30 ± 0.10 ; $n = 7$ mice), and D-AP5 (0.06 ± 0.01 ; $n = 5$ mice). **G**, Summary of NMDAR EPSC charge transfer (normalized to paired control aCSF EPSCs) elicited by single and 10 stimuli. Single stimulus groups include control, all conditions, ISO (0.95 ± 0.13 ; $n = 6$ mice), ISO with GDP β S (1.17 ± 0.13 ; $n = 7$ mice), and D-AP5 (0.07 ± 0.01 ; $n = 5$ mice). Ten stimuli groups include control, all conditions, ISO (0.78 ± 0.02 ; $n = 6$ mice), ISO with GDP β S (1.16 ± 0.10 ; $n = 7$ mice), and D-AP5 (0.07 ± 0.01 ; $n = 5$ mice). In **F** and **G**, mean $\pm$ SEM and data from individual mice are shown; one-sample t tests compare normalized experimental groups with null control condition, and paired t tests compare D-AP5 with paired ISO trials. Comparisons between ISO with and without GDP β S use unpaired t tests. All t tests are Bonferroni corrected for multiple comparisons. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. **H**, Summary of the fast decay time constant (τ_f) from 10 stimuli-evoked NMDAR EPSCs. EPSC decay was fit by the sum of two exponentials ($R^2 = 0.9965 \pm 0.0005$; $n = 26$ traces from 13 mice). Groups include no GDP β S control (0.302 ± 0.035 s; $n = 6$ mice), no GDP β S ISO (0.328 ± 0.033 s; $n = 6$ mice), GDP β S control (0.314 ± 0.043 s; $n = 7$ mice), GDP β S ISO (0.292 ± 0.017 s; $n = 7$ mice). Mean $\pm$ SEM and data from individual mice are shown; two-way ANOVA with Tukey–Kramer post hoc multiple comparisons, n.s. between all groups $p > 0.88$.

EPSCs. At the end of an experiment, currents were blocked with D-AP5 (50 μ M; Cat. No. 0106, Tocris) to verify that they were NMDAR EPSCs.

Measurement of 10 stimuli NMDAR EPSC decay constant. The decay constants of NMDAR EPSCs evoked by 10 stimuli bursts were calculated by fitting the EPSC decay after the 10th evoked EPSC with a double exponential using the MATLAB curve fitting tool. The fast decay constant component (τ_f) was compared between control aCSF and ISO recordings.

Measurement of AMPAR EPSCs. We assessed whether ISO affected glutamate release and synaptic activity by measuring AMPAR EPSCs (Ji et al., 2008; Grzelka et al., 2017). AMPAR currents were isolated by blocking NMDARs with D-AP5 (50 μ M). Neurons were voltage-clamped and held at the Cl^- reversal potential, approximately -52 mV (after correcting for the liquid junction potential), to eliminate the GABA_A R contribution to the evoked currents. AMPAR current isolation was confirmed by using CNQX (10 μ M) to eliminate AMPAR EPSCs. For AMPAR EPSCs evoked by 10 stimuli, the peak EPSCs evoked by each stimulus were summed together to reflect the overall synaptic glutamatergic activity, Σ AMPAR EPSCs.

Evaluation of the role of astrocyte β -adrenergic receptor activity on EPSCs. We eliminated astrocyte β -AR-mediated changes to EPSCs by loading the astrocyte network near the recorded neuron with the gap junction-permeable, membrane-impermeant G-protein blocker GDP β S (Navarrete et al., 2012; Cavaccini et al., 2020). An astrocyte in Layer 2/3, ~ 50 μ m from the stimulus electrode, was patched and loaded with an internal solution containing 100 μ M GDP β S and the gap junction-permeable dye Alexa Fluor 488 hydrazide (40 μ M; A10436, Invitrogen) to verify spread of GDP β S. Astrocytes were filled for 30 min before removing the patch pipette. Immediately afterward, a Layer 5 pyramidal neuron was patched and imaged to confirm that its dendrites fell within the region of the GDP β S-filled astrocytes (Fig. 2A).

Measurement of extrasynaptic NMDA receptor currents

We investigated the extent to which ISO reduces extrasynaptic NMDAR currents. Single and 10 stimuli NMDAR EPSCs were initially recorded in aCSF at $+40$ mV. The preparation was then perfused with the activity-dependent NMDAR blocker MK-801 (25 μ M; M107, Sigma-Aldrich; Huettner and Bean, 1988; Harris and Pettit, 2008; Papouin et al., 2012; Yang et al., 2017). Single stimuli were delivered at 1 Hz for 10 min, resulting in the block of synaptic NMDARs (Harris and Pettit, 2008; Papouin et al., 2012). MK-801 was then washed out for 15 min while the neuron was held at -60 mV to prevent further NMDAR activation. Recordings were not included if the post MK-801 single stimulus EPSCs were greater than 15% of the pre MK-801 EPSCs or if the post MK-801 EPSCs in ISO increased by more than 50% after switching from the post MK-801 aCSF.

Solutions and drugs

Brains were sliced in a sucrose solution that contained the following (in mM): 228 sucrose, 1 NaH_2PO_4 , 2.5 KCl, 7 MgCl_2 , 11 glucose, 0.5 CaCl_2 , 0.4 sodium ascorbate, 26 NaHCO_3 , bubbled with 95% O_2 /5% CO_2 , pH 7.35 (300–310 mOsm). aCSF used for recovery and recordings contained the following (in mM): 125 NaCl, 1.25 NaH_2PO_4 , 2.5 KCl, 1.5 MgCl_2 , 20 glucose, 1.2 CaCl_2 , 0.5 L-glutamine, 0.4 sodium ascorbate, 26 NaHCO_3 , bubbled with 95% O_2 /5% CO_2 , pH 7.35 (300–310 mOsm). Solutions were shielded from light to avoid NE and ISO oxidation. The patch pipette solution for patching astrocytes contained the following (in mM): 120 K-gluconate, 4.5 MgCl_2 , 9 HEPES, 0.1 EGTA, 14 tris $_2$ -phosphocreatine, 4 Na_2 -ATP, 0.3 tris-GTP, sucrose to bring the solution to 280–290 mOsm, pH 7.25. Astrocyte internal included either 40 μ M Alexa Fluor 488 Dextran (3,000 MW) for astrocyte volume recordings or 40 μ M Alexa Fluor 488 hydrazide to track GDP β S diffusion for neuronal recordings. For experiments blocking astrocyte GPCR activity, tris-GTP was replaced with 100 μ M GDP β S. The patch pipette solution for patching pyramidal neurons contained the following (in mM): 105 Cs-methanesulfonate, 10 TEA-Cl, 20 HEPES, 10 EGTA, 2 QX-314

Cl^- , 5 Mg-ATP, 0.5 tris-GTP, sucrose to bring the solution to 280–290 mOsm, pH 7.25. The neuronal internal solution included 40 μ M Alexa Fluor 594 hydrazide.

Statistics and analysis

One-sample t tests, two-sample paired and unpaired t tests, and two-way ANOVAs were used, as appropriate. Data are expressed as mean $\pm$ SEM in the text and figures, unless stated otherwise. The sample size (n) of the number of animals tested for each group is reported throughout the text. The sample size for each group was sufficiently powered (0.8) when estimating a moderate effect size of 0.15 and a null hypothesis rejection of $\alpha = 0.05$. Effect size was based on estimates from comparable studies investigating astrocyte volume, changes to glutamate spillover, and extrasynaptic NMDAR activity (Henneberger et al., 2020; Popov et al., 2020). A two-way ANOVA with Tukey–Kramer multiple-comparisons post hoc test was conducted on astrocyte volume changes measured after 40 min perfusion with experimental or control solutions and intracellular GDP β S. Student's one-sample t tests were run between normalized control and ISO EPSC values as well as between control and antagonist EPSC values. Unpaired t tests were used to compare the effects of normalized ISO responses between neuron EPSCs with or without GDP β S-loaded astrocytes. Paired t tests were used to compare antagonist responses with ISO responses in the same experiment. All t tests had Bonferroni correction applied for multiple testing. Two-way ANOVA with Tukey–Kramer post hoc was used to compare fast decay time constants between NMDAR currents in control and ISO and the presence of GDP β S-loaded astrocytes. Data analysis and statistics were carried out using custom MATLAB scripts. The data, images, and MATLAB scripts for analysis and statistics are available upon request.

Results

Norepinephrine and β -adrenergic agonists increase astrocyte volume in the medial prefrontal cortex

Noradrenergic activity alters astrocyte morphology and process volume in certain brain regions (Vardjan et al., 2014; Sherpa et al., 2016). We wanted to determine whether similar morphological changes occur in the mPFC, which receives heavy noradrenergic input. We used a previously established technique to measure process volume, filling astrocytes with a gap junction-impermeant fluorescent dye and imaging dye-filled cells using confocal microscopy (Henneberger et al., 2020; Minge et al., 2021). Fine astrocyte processes can be loaded with dye (Fig. 1A) while being restricted to the patched astrocyte (Fig. 1B). Compared with a stable baseline fluorescence, any change in process fluorescence represents a change in volume. Single images of process fluorescence prior to β -AR agonist ISO exposure (Fig. 1C) and after 40 min in 5 μ M ISO (Fig. 1D) highlight how astrocyte process fluorescence increases following ISO incubation (Fig. 1C,D,G). ISO increased process fluorescence by $\sim 20\%$ after 40 min exposure ($20.5 \pm 4.6\%$ increase; $n = 5$ mice; Fig. 1J). Incubating brain slices with 20 μ M NE for 40 min also increased the fluorescence in processes by $\sim 18\%$ ($17.7 \pm 2.4\%$ increase; $n = 7$ mice; Fig. 1J). Process volume increased significantly with both NE and ISO compared with control recordings measured after 40 min in aCSF ($2.21 \pm 1.86\%$ increase; $n = 8$ mice; ISO vs aCSF, $p = 2.69 \times 10^{-5}$; Tukey–Kramer post hoc; two-way ANOVA; NE vs aCSF, $p = 7.89 \times 10^{-5}$; Tukey–Kramer post hoc; two-way ANOVA; Fig. 1J).

We sought to determine whether the noradrenergic agonist effect on astrocyte process volume could be attributed to the agonists acting directly on astrocytes. To selectively eliminate noradrenergic signaling in astrocytes, we blocked GPCR signaling by loading dye-filled astrocytes with membrane-impermeant GDP β S, a G-protein inhibitor (Navarrete et al., 2012; Cavaccini et al., 2020). Single images of process fluorescence from an

astrocyte loaded with GDP β S prior to ISO exposure (Fig. 1E) and after 40 min in ISO (Fig. 1F) demonstrate that ISO exposure does not increase process fluorescence of GDP β S-filled astrocytes (Fig. 1E,F,H). Instead, while process fluorescence increases with ISO over time, the fluorescence increase is severely blunted by GDP β S, becoming no different from astrocyte fluorescence changes recorded in aCSF (Fig. 1I).

Relative changes in astrocyte process fluorescence are summarized in Figure 1J. Process fluorescence changes were significantly smaller after 40 min in NE for GDP β S-loaded astrocytes ($4.88 \pm 1.00\%$ increase; $n=6$ mice) or 40 min in ISO ($0.40 \pm 1.67\%$ increase; $n=6$ mice) compared with astrocytes lacking GDP β S loading (NE vs NE GDP β S; $p=2.68 \times 10^{-3}$; Tukey–Kramer post hoc; two-way ANOVA; ISO vs ISO GDP β S, $p=1.53 \times 10^{-5}$; Tukey–Kramer post hoc; two-way ANOVA; Fig. 1J). Indeed, GDP β S-loaded astrocyte process fluorescence in either noradrenergic agonist was similar to the fluorescence changes recorded in control aCSF (aCSF vs NE GDP β S, n.s., $p=0.944$; Tukey–Kramer post hoc; two-way ANOVA; aCSF vs ISO GDP β S, n.s., $p=0.990$; Tukey–Kramer post hoc; two-way ANOVA; Fig. 1J). We also verified that GDP β S by itself does not alter astrocyte process volume (aCSF GDP β S, $3.71 \pm 0.72\%$ increase; $n=6$ mice; aCSF vs aCSF GDP β S, n.s., $p=0.996$; Tukey–Kramer post hoc; two-way ANOVA; Fig. 1J). These results indicate that activation of β -ARs expressed on mPFC astrocytes elicit increases in process volume.

Astrocyte β -adrenergic receptor signaling reduces NMDA receptor activation

Since astrocyte volume changes influence glutamate signaling and NMDAR activity, we next sought to understand how β -AR signaling affected NMDAR activity (Oliet et al., 2004; Müller et al., 2021; Badia-Soteras et al., 2022). We used electrical stimulation of afferents in Layer 2/3 to evoke Layer 5 pyramidal neuron NMDAR currents (Fig. 2A). Single stimulus-evoked NMDAR EPSCs remained unchanged by ISO (Fig. 2B,F,G). NMDAR EPSC peak amplitude in ISO was unchanged compared with control aCSF (0.91 ± 0.06 ; $n=6$ mice; one-sample t test with Bonferroni correction; n.s., $p=0.42$) as was NMDAR EPSC charge transfer in ISO (0.95 ± 0.13 ; $n=6$ mice; one-sample t test with Bonferroni correction; n.s., $p=1.00$; Fig. 2F,G).

We then elicited NMDAR EPSCs using a 10 stimulus, 50 Hz burst, a stimulus paradigm associated with glutamate spillover and extrasynaptic receptor recruitment (Harris and Pettit, 2008; Fig. 2C). Although there was no difference observed with single stimulus EPSCs in ISO, ISO significantly reduced high-frequency, repetitive stimulus-evoked NMDAR currents (Fig. 2C). ISO reduced the peak amplitude of these EPSCs by 18% (0.82 ± 0.01 ; $n=6$ mice; one-sample t test with Bonferroni correction; $p=3.20 \times 10^{-5}$) and reduced the NMDAR EPSC tail charge transfer by 22% (0.78 ± 0.02 ; $n=6$ mice; one-sample t test with Bonferroni correction; $p=5.71 \times 10^{-4}$). For both single and repetitive stimuli, the NMDAR blocker D-AP5 reduced evoked currents by over 90% compared with control, confirming that we were measuring NMDAR currents in these recordings (Fig. 2B,C,F,G; 1 stimulus, 0.08 ± 0.01 ; $n=5$ mice; one-sample t test with Bonferroni correction; $p=2.14 \times 10^{-7}$; 10 stimuli, 0.06 ± 0.01 ; one-sample t test with Bonferroni correction; $p=8.64 \times 10^{-8}$).

While ISO-induced astrocyte volume increases (Fig. 1J) may influence neuronal signaling, changes to neuronal NMDAR currents may also be due to direct action of ISO on neurons, which also express β -ARs (Ji et al., 2008; Grzelka et al., 2017). We evaluated the role of astrocytes in the ISO effect on NMDAR EPSCs

by blocking astrocytic β -AR signaling. Prior to recording neuronal NMDAR currents, we patched and loaded the astrocyte network with membrane-impermeant, gap junction-permeable GDP β S (Fig. 2A) to selectively block astrocyte network β -AR signaling and eliminate process volume increases. The spread of GDP β S from the patched astrocyte (Fig. 2A, asterisk) was tracked with a gap junction-permeable Alexa Fluor 488 dye to ensure astrocyte-specific loading (Fig. 2A, arrowheads) near the recorded neuron.

The effect of ISO in reducing NMDAR currents was blocked when astrocytes were loaded with GDP β S, demonstrating that ISO modulation of NMDAR synaptic transmission was mediated, at least in part, by astrocytes. Consistent with the lack of effect of ISO on single stimulus-evoked NMDAR currents, single stimulus-evoked NMDAR EPSCs were unchanged between control aCSF and ISO recordings with astrocyte GDP β S loading, both when measuring peak EPSC amplitude (1.07 ± 0.06 ; $n=7$ mice; one-sample t test with Bonferroni correction; n.s., $p=0.531$) and for charge transfer (1.17 ± 0.13 ; $n=7$ mice; one-sample t test with Bonferroni correction; n.s., $p=0.509$; Fig. 2D,F,G). The impact of ISO on single stimulus-evoked NMDAR EPSC peak amplitude was not different when comparing the absence to the presence of GDP β S-loaded astrocytes in ISO (no GDP β S ISO vs GDP β S ISO; unpaired t test with Bonferroni correction; n.s., $p=0.176$; Fig. 2E,F). GDP β S also had no impact on the effect of ISO on NMDAR EPSC charge transfer (no GDP β S ISO vs GDP β S ISO; unpaired t test with Bonferroni correction; $p=0.51$; Fig. 2G).

Blocking astrocyte β -AR activity with GDP β S reversed the effect of ISO on repetitive stimulus-evoked NMDAR EPSCs from a reduction to an enhancement. When astrocytes were loaded with GDP β S, ISO increased the peak NMDAR EPSC evoked by 10 stimuli by 30% compared with responses in control aCSF (1.30 ± 0.10 ; $n=7$ mice; one-sample t test with Bonferroni correction; $p=0.05$; Fig. 2F), though charge transfer remained unchanged (1.16 ± 0.10 ; $n=7$ mice; one-sample t test with Bonferroni correction; n.s., $p=0.193$; Fig. 2G). Compared with ISO recordings done without GDP β S loading of astrocytes, where ISO reduced repetitive stimulus-evoked NMDAR EPSCs, when loaded with GDP β S, ISO significantly enhanced repetitive stimulus-evoked NMDAR EPSC peak amplitude (no GDP β S ISO vs GDP β S ISO; unpaired t test with Bonferroni correction; $p=5.99 \times 10^{-3}$; Fig. 2F) and charge transfer (no GDP β S ISO vs GDP β S ISO; unpaired t test with Bonferroni correction; $p=1.75 \times 10^{-2}$; Fig. 2G). Results for all normalized NMDAR responses are summarized in Figure 2F,G. While ISO previously reduced 10 stimuli-evoked peak EPSC and charge transfer, the addition of GDP β S to astrocytes led to ISO greatly enhancing peak EPSC and charge transfer. Meanwhile, single stimulus EPSC measures remained unchanged.

As a control for loading of the astrocyte network with GDP β S, three of the six animals from the no GDP β S recordings were conducted with astrocytes loaded with a standard astrocyte internal solution. There were no differences between ISO responses of single or 10 stimuli-evoked NMDAR EPSCs compared with control, whether astrocytes were patched with a control internal or not patched at all (unpaired t test; n.s., $p>0.33$ for all measures).

The decay time constant (τ) of repetitive stimulus-evoked NMDAR EPSCs has been shown to change with altered glutamate uptake and other properties affecting dwell time of glutamate near receptors (Diamond, 2005; Nie and Weng, 2009; Anderson et al., 2015). Since ARs can alter glutamate transport in astrocytes (Hansson and Rönnbäck, 1991, 1992), we compared ISO and control aCSF decay time constants for 10

stimulus-evoked NMDAR EPSCs. Traces were well fit with two exponentials, comprising fast and slow phases of decay and achieved an average R^2 fit of 0.9965 ± 0.0005 ($n = 26$ traces from 13 mice). We found no difference between the fast time constant (τ_f) of decay of control aCSF and ISO recordings, whether astrocytes were loaded with GDP β S or not (no GDP β S control aCSF 0.302 ± 0.035 s vs no GDP β S ISO 0.328 ± 0.033 s vs with GDP β S control aCSF 0.314 ± 0.043 s vs with GDP β S ISO 0.292 ± 0.017 s; Tukey–Kramer post hoc; two-way ANOVA; n.s. between all groups $p > 0.88$; Fig. 2H).

AMPA receptor currents are not affected by β -adrenergic receptor activation

Neurons express β -ARs and ISO could potentially act on presynaptic terminals as well as astrocytes, changing glutamate release (Ji et al., 2008). We measured AMPAR EPSCs with and without ISO and then with GDP β S-loaded astrocytes to determine whether changes in glutamate release could account for the changes we observed in NMDAR activity. Single stimulus

AMPA EPSCs recorded in ISO were not significantly different compared with control EPSCs, although they did trend larger (1.25 ± 0.14 ; $n = 5$ mice; one-sample t test with Bonferroni correction; n.s., $p = 0.292$; Fig. 3A). Similarly, single stimulus AMPAR EPSCs in ISO with GDP β S-loaded astrocytes were not different from control EPSCs, although they trended larger as well (1.24 ± 0.16 ; $n = 6$ mice; one-sample t test with Bonferroni correction; n.s., $p = 0.362$; Fig. 3B). GDP β S did not impact the relative effect ISO had on AMPAR EPSCs (no GDP β S ISO vs GDP β S ISO; unpaired t test; n.s., $p = 1.00$).

We also measured AMPAR EPSCs with the 10 stimulus paradigm to evaluate changes to overall synaptic facilitation or depression in the presence of ISO and GDP β S. The peak AMPAR EPSCs evoked by each of the 10 stimulus pulses were added together to reflect the overall synaptic glutamatergic activity: Σ AMPAR EPSCs (Fig. 3C,D). We observed that Σ AMPAR EPSCs trended larger with ISO compared with control EPSCs (1.28 ± 0.12 ; $n = 5$ mice; one-sample t test with Bonferroni correction; n.s., $p = 0.166$; Fig. 3C,E). Σ AMPAR EPSCs with

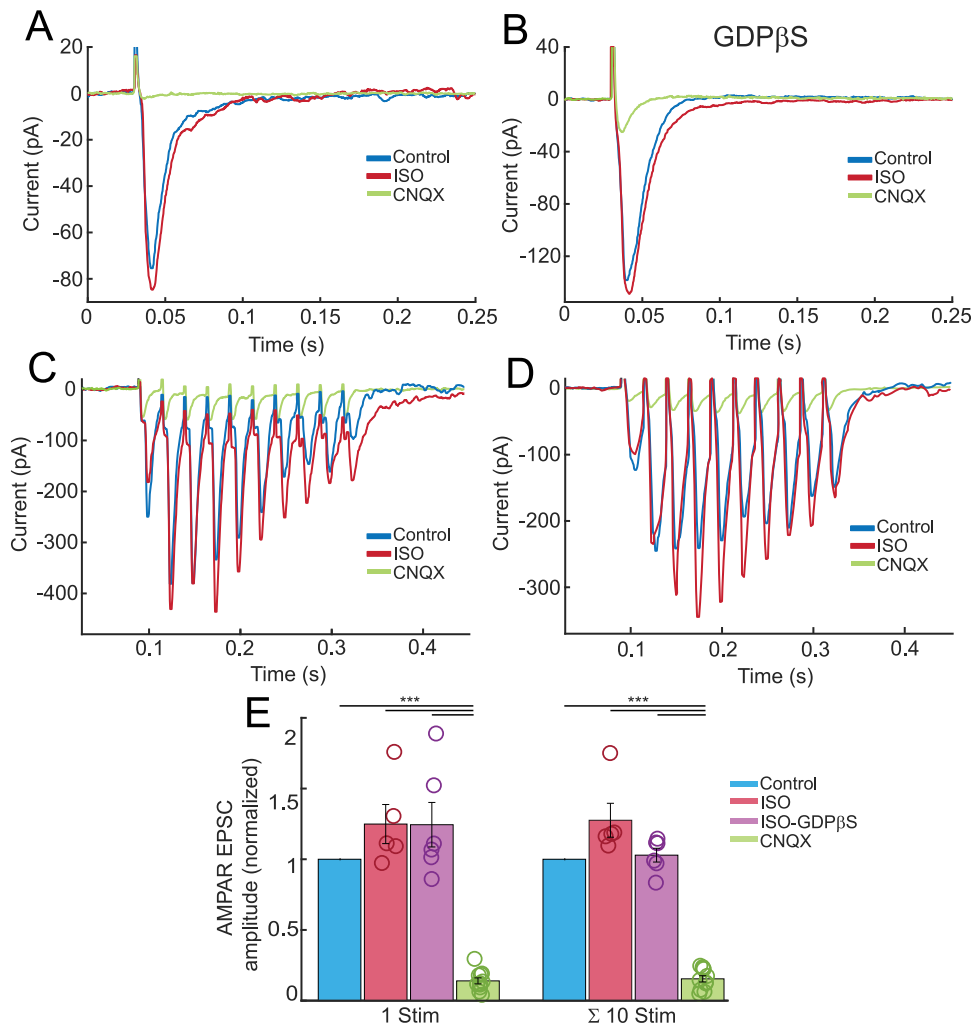


Figure 3. Single and 10 stimuli-evoked AMPAR EPSCs are not modulated by astrocyte β -AR activity. **A–D**, AMPAR EPSCs recorded from single neurons in control aCSF (blue), after 40 min in ISO (red), and after an additional 5 min in CNQX (green). **A**, EPSCs evoked by single stimuli. **B**, EPSCs evoked by single stimuli and the astrocyte network loaded with GDP β S. **C**, EPSCs evoked by 10 stimuli bursts. **D**, EPSCs evoked by 10 stimuli bursts and the astrocyte network loaded with GDP β S. **E**, Summary of single and 10 stimuli-evoked AMPAR EPSCs (normalized to paired controls). Mean $\pm$ SEM and data from individual mice are shown. Single stimulus groups include control, all conditions, ISO (1.25 ± 0.14 ; $n = 5$ mice), ISO with GDP β S (1.24 ± 0.16 ; $n = 6$ mice), and CNQX (0.14 ± 0.02 ; $n = 11$ mice). Ten stimuli-evoked Σ AMPAR EPSC groups include control, all conditions, ISO (1.28 ± 0.12 ; $n = 5$ mice), ISO with GDP β S (1.03 ± 0.05 ; $n = 6$ mice), and CNQX (0.15 ± 0.02 ; $n = 11$ mice). One-sample t tests compare normalized experimental groups with null control conditions, and paired t test compares ISO groups with paired CNQX trials. ISO with and without GDP β S use unpaired t tests. All t tests are Bonferroni corrected for multiple comparisons. *** $p < 0.001$.

GDP β S-loaded astrocytes showed no difference between control and ISO recordings (1.03 ± 0.05 ; $n = 6$ mice; one-sample t test with Bonferroni correction; n.s., $p = 1.00$; Fig. 3D,E). Further, the presence or absence of GDP β S did not affect the relative impact ISO had on Σ AMPAR EPSCs (no GDP β S ISO vs GDP β S ISO; unpaired t test; n.s., $p = 0.223$). Figure 3E summarizes the results, showing that ISO and the presence of GDP β S had no significant effect on single or repetitive stimulus-evoked AMPAR EPSCs. These results suggest that changes in glutamate release cannot account for the ISO-induced reduction in NMDAR currents in response to repetitive stimuli and are instead consistent with a role for β -AR-mediated astrocyte volume changes.

β -Adrenergic receptor signaling reduces extrasynaptic NMDAR currents

Delivering 10 stimuli at 50 Hz is sufficient to elicit glutamate spillover and activation of extrasynaptic NMDARs (Lozovaya et al., 1999, 2004; Scimemi et al., 2004; Harris and Pettit, 2008). We sought to determine whether extrasynaptic NMDARs contributed to the currents reduced by ISO. MK-801 is an activity-dependent NMDAR blocker used to eliminate synaptic NMDARs activated by single stimuli, leaving the unblocked extrasynaptic NMDARs to respond to a high-frequency repetitive stimulus (Lozovaya et al., 1999, 2004; Scimemi et al., 2004; Harris and Pettit, 2008; Papouin et al., 2012; Yang et al., 2017). The process of isolating extrasynaptic NMDARs is illustrated in Figure 4A. After initial

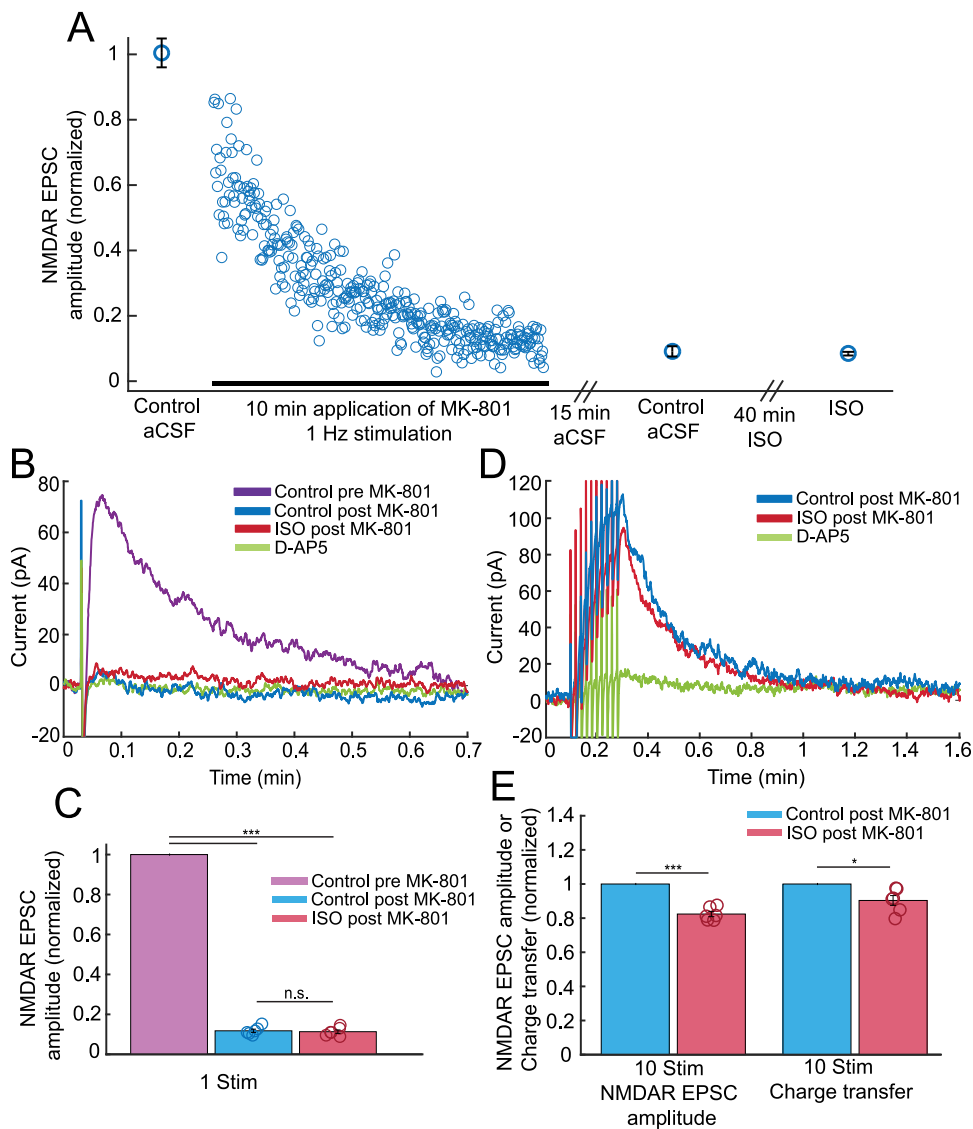


Figure 4. β -AR activity modulates extrasynaptic NMDAR EPSCs. **A**, Single stimulus-evoked NMDAR EPSCs tracking synaptic NMDAR blockade by MK-801, recorded from a single neuron (normalized to pre MK-801). Data points with error bars represent mean $\pm$ SEM. Data points during MK-801 treatment indicate individual EPSCs. Time course of 10 min MK-801 treatment is indicated by black bar. **B**, NMDAR EPSCs evoked by single stimuli, recorded from a single neuron. Pre MK-801 aCSF control (purple), post MK-801 aCSF (blue), post MK-801 after 40 min in ISO (red), and after an additional 5 min in D-AP5 (green). **C**, Summary of single stimulus NMDAR EPSCs (normalized to pre MK-801 control aCSF). Pre MK-801 control aCSF (purple), post MK-801 control aCSF (blue; 0.12 ± 0.01 ; $n = 6$ mice), ISO (red; 0.11 ± 0.01 ; $n = 6$ mice). One-sample t tests compare normalized post MK-801 groups with the pre MK-801 null control condition. Paired t test compares post MK-801 aCSF with post MK-801 ISO. All t tests are Bonferroni corrected for multiple comparisons. n.s., $p > 0.05$, *** $p < 0.001$. **D**, NMDAR EPSCs evoked by 10 stimuli, recorded from a single neuron. Post MK-801 aCSF (blue), post MK-801 after 40 min in ISO (red), and after an additional 5 min in D-AP5 (green). **E**, Summary of 10 stimuli-evoked NMDAR EPSCs and charge transfer (normalized to post MK-801 control aCSF, blue). Post MK-801 ISO NMDAR EPSC amplitude (red, 0.82 ± 0.02 ; $n = 6$ mice) and post MK-801 ISO NMDAR EPSC charge transfer (red, 0.90 ± 0.03 ; $n = 6$ mice). One-sample t tests compare normalized post MK-801 ISO with null post MK-801 control aCSF for NMDAR EPSC amplitude and charge transfer measures. All t tests are Bonferroni corrected for multiple comparisons. * $p < 0.05$, *** $p < 0.001$. Mean $\pm$ SEM and data from individual mice are shown in **C** and **E**.

aCSF NMDAR EPSC recordings with single stimuli, neurons were incubated in MK-801 while delivering stimuli at 1 Hz. After 10 min, NMDAR EPSCs were reduced to near zero and the MK-801 was washed out. Stimulus delivery in MK-801 resulted in blocking single stimulus-evoked synaptic NMDAR currents, which was sustained throughout the rest of the recording session (Fig. 4B). The sustained blockade of single stimulus NMDAR EPSCs is summarized in Figure 4C. After a 15 min washout, post MK-801 control NMDAR EPSCs in aCSF were reduced by 88% (0.12 ± 0.01 ; $n = 6$ mice; one-sample t test with Bonferroni correction; $p = 1.92 \times 10^{-12}$). Synaptic NMDAR blockade was maintained through the 40 min ISO incubation as well, and single stimulus NMDAR EPSCs in ISO were similar to post MK-801 control EPSCs (0.98 ± 0.08 , $n = 6$ mice; pairwise t test with Bonferroni correction; n.s., $p = 1.00$; Fig. 4B,C).

Maintained synaptic blockade allowed us to measure isolated extrasynaptic NMDAR currents in post MK-801 control aCSF and ISO conditions. Isolated extrasynaptic, repetitive stimulus-evoked NMDAR EPSCs were reduced in ISO to a similar degree as the NMDAR currents that were recorded when synaptic NMDARs were unblocked (Figs. 2E, 4D). As summarized by Figure 4E, 10 stimuli-evoked extrasynaptic NMDAR EPSC amplitude was reduced by 18% in ISO compared with control aCSF (0.82 ± 0.02 ; $n = 6$ mice; one-sample t test with Bonferroni correction; $p = 1.80 \times 10^{-4}$). NMDAR charge transfer was reduced by 10% (0.90 ± 0.03 ; $n = 6$ mice; one-sample t test with Bonferroni correction; $p = 0.038$). These results suggest that β -AR activity reduces glutamate spillover from high-frequency repetitive stimulation and that limiting extrasynaptic NMDAR recruitment accounts, at least in part, for the reduced NMDAR EPSCs driven by astrocyte β -AR activation.

Discussion

We have shown that astrocyte β -AR activity increases astrocyte process volume in the mPFC, limiting glutamate spillover and extrasynaptic NMDAR recruitment. We first measured changes to astrocyte process volume utilizing an established method of dye-filling astrocytes (Henneberger et al., 2020) and found that noradrenergic agonists increase astrocyte process volume by ~20%. This is in accord with prior studies which showed that astrocyte β -AR activity results in process expansion in vitro (Vardjan et al., 2014) as well as in the visual cortex (Sherpa et al., 2016), where treatment with ISO results in a 30% reduction in ECS volume fraction. Our work confirms that NE and β -ARs increase astrocyte volume in the mPFC, an unexplored area which is densely innervated by noradrenergic projections (Hoover and Vertes, 2007; Santana and Artigas, 2017). We also effectively blocked noradrenergic agonist-evoked increases in process volume by selectively loading astrocytes with GDP β S. This agrees with findings from multiple studies which demonstrate that GDP β S loading of astrocytes blocks G-protein-mediated signaling from monoaminergic receptors (Navarrete et al., 2012; Corkrum et al., 2020).

We next demonstrated that β -AR activation reduces pyramidal neuron NMDAR currents in an astrocyte-dependent manner. In agreement with this finding, the reverse phenomenon has been reported in other systems: when decreases in astrocyte process volume are observed, enhanced glutamate spillover is seen (Oliet et al., 2004; Henneberger et al., 2020; Müller et al., 2021; Badia-Soteras et al., 2022). Our study found that NMDAR currents evoked by 10 stimulus bursts were reduced by ISO,

consistent with the finding that increasing astrocyte process volume limits glutamate spillover (Popov et al., 2020).

We then demonstrated that β -AR activity reduces extrasynaptic NMDAR recruitment. Several methods have previously been used to approximate or isolate extrasynaptic NMDAR activity. Prior work has shown that a 50 Hz repetitive stimulus overpowers glutamate uptake mechanisms, leading to glutamate spillover and the recruitment of extrasynaptic NMDARs (Lozovaya et al., 1999, 2004; Scimemi et al., 2004; Harris and Pettit, 2008). Others have used the NMDAR activity-dependent blocker MK-801 to eliminate synaptic NMDAR currents through low-frequency stimulation (Milnerwood et al., 2010; Liu et al., 2013; Yang et al., 2017; Pallas-Bazarra et al., 2019). In this work, we used both methods to study extrasynaptic NMDAR currents. Following MK-801 block of synaptic NMDAR currents, the residual extrasynaptic NMDAR current elicited by a 50 Hz repetitive stimulus was reduced by ISO to 82% of control. This decrease is similar to the ISO-induced reduction of the total NMDAR current evoked by 50 Hz repetitive stimulation (82%), suggesting that the ISO-mediated increase in astrocyte volume primarily reduces extrasynaptic NMDAR activation. These findings are consistent with prior work showing that increased astrocyte volume caused by caloric restriction results in decreased extrasynaptic NMDAR activity (Popov et al., 2020).

Although we showed that NE and ISO evoke large increases in the volume of astrocyte processes, we did not establish that the swelling occurs at the distal ends of these processes, which surround synapses in the cortex (Kikuchi et al., 2020). However, previous work has demonstrated that β -AR agonists do evoke astrocyte swelling at synapses. Sherpa et al. (2016) showed in the visual cortex that ISO significantly increased the cytoplasmic volume of astrocytic distal endings. Their EM pictures demonstrate that astrocyte process swelling occurs near synaptic terminals. In addition, Aoki (1992) showed that β -ARs are expressed on astrocyte processes close to synapses in the cortex, suggesting that the action of β -AR agonists is on these distal processes.

The ISO effect could, in theory, be mediated by direct ISO modulation of pyramidal neurons rather than astrocytes, as these neurons also express β -ARs, which can enhance neuronal EPSCs (Ji et al., 2008; Grzelka et al., 2017). Although we found that ISO did not significantly affect single stimulus AMPAR or NMDAR currents, we did find that ISO trended toward increasing AMPAR currents for both single and high-frequency repetitive stimulation. ISO reduced high-frequency repetitive stimulus-evoked NMDAR EPSCs. However, when astrocyte β -AR activity was blocked with GDP β S, ISO also enhanced repetitive stimulus-evoked NMDAR currents, likely because blocking astrocyte β -AR activity unmasked an underlying ISO-induced enhancement of neuronal excitability.

β -AR signaling acts on several astrocyte pathways that could affect NMDAR activity in addition to astrocyte volume changes. Previous work has shown that ISO modestly inhibits glutamate uptake in primary astrocyte cultures (Hansson and Rönnbäck, 1991, 1992). However, reduction of glutamate uptake would enhance glutamate spillover and would increase NMDAR signaling, the opposite of what we observed. Astrocyte β -AR activity also enhances lactate production, which has been shown to support LTP in the hippocampus and enhance EPSCs (Gao et al., 2016). This again runs counter to our observation that ISO reduces NMDAR activation. Astrocytes release gliotransmitters, including ATP and D-serine, that may influence glutamate release and NMDAR activity, respectively (Panatier et al., 2006; Henneberger et al., 2010; Chen et al., 2013). Norepinephrine

elicits astrocyte ATP release and can increase AMPAR signaling (Gordon et al., 2005). Despite this possibility, ISO had no significant effect on synaptic AMPAR or NMDAR single stimulus currents in our experiments. Since D-serine is a co-agonist for NMDARs, changes to D-serine secretion would impact NMDAR activity, although this has not been explored with ISO. Astrocytes can also release glutamate directly (Basarsky et al., 1999; Angulo et al., 2004; Woo et al., 2012). This would, however, result in an increase, not a decrease, in extrasynaptic NMDAR activation.

In our experiments, we preloaded astrocyte networks with GDP β S to eliminate glial responses to neuromodulators, as has been done previously (Navarrete et al., 2012; Cavaccini et al., 2020; Corkrum et al., 2020). This technique may, however, block multiple astrocyte GPCR signaling pathways that could contribute to modulating neuronal EPSCs. Since the astrocyte network was loaded prior to neuronal recordings, changes in baseline neuronal responsiveness must be considered. Despite this caveat, GDP β S loading effectively blocked ISO-induced astrocyte process volume increases and changes in NMDAR and AMPAR responses.

ISO-induced swelling of astrocyte processes could influence extracellular K⁺ levels as well as glutamate spillover. Astrocyte swelling and the resulting decrease in the extracellular space volume would lead to increases in K⁺ concentration, which would raise neuronal excitability. This increase could be offset by increased K⁺ removal by astrocyte K⁺ spatial buffering (Kofuji and Newman, 2009), however. β -AR activation also enhances Na⁺/K⁺-ATPase activity, leading to increased K⁺ uptake into astrocytes (Walch et al., 2020; Wotton et al., 2020) and reduced extracellular K⁺ levels. Thus, the effect of changing K⁺ levels on synaptic currents is hard to predict.

Major depression in humans and depressive-like symptoms in animals are associated with impaired monoamine signaling, including NE (Ruhé et al., 2007; Moret and Briley, 2011). The effects of reduced NE signaling could be mediated by changes in astrocyte morphology. Indeed, retraction and a reduction of astrocyte process volume has been observed in suicide victims diagnosed with depression and mouse chronic stress models that produce depressive-like behavior (Torres-Platas et al., 2016; Codeluppi et al., 2021). Altered astrocyte morphology and process retraction have also been shown to impact neuronal circuits in emotion processing regions of the brain, enhancing fear memory (Badia-Soteras et al., 2022). Reduced astrocyte volume may impair the termination of synaptic glutamate signaling and result in spillover, enhancing extracellular glutamate and extrasynaptic NMDAR activation, which is seen in chronic stress models displaying depressive-like behavior (Li et al., 2018). Enhanced extrasynaptic NMDAR recruitment can also have harmful effects on neurons and on behavior (Hardingham et al., 2002; Milnerwood et al., 2010; Zhou et al., 2013; Hanson et al., 2015; Li et al., 2018), and the blockade of extrasynaptic NMDARs by new fast-acting antidepressants may contribute to depressive symptom relief (Miller et al., 2014; Li et al., 2018; Tang et al., 2020).

Our results are consistent with and support this mechanism of depression. Our observation that noradrenergic-mediated astrocyte volume increase leads to a reduction in extrasynaptic NMDAR activity implies that the opposite is also true. That is, a reduction in noradrenergic activity would lead to astrocyte shrinkage and to an enhancement of extrasynaptic NMDAR activity.

Schizophrenia has been canonically linked to monoamine overactivation, including NE elevation (Breier, 1990; Nagamine, 2020). More recently, a NMDAR hypofunction hypothesis of schizophrenia has been proposed (Lahti et al., 1995; Uno and Coyle, 2019). The hypothesis holds that reduced levels of NMDAR activity contribute to schizophrenic symptoms. Overstimulation of astrocyte β -AR could result in excessive volume increases in these glial cells, limiting NMDAR activation.

Overall, our findings support previous research showing that astrocytes provide a crucial role in modulating neuronal activity and, specifically, glutamatergic signaling. Changes to astrocyte volume during various pathological conditions may well modulate extrasynaptic NMDAR activation. Uncovering novel mechanisms to modulate astrocyte volume changes will further elucidate how astrocyte morphology affects synaptic activity and animal behavior, possibly leading to new strategies for targeting neuropsychiatric disorders.

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