

## Supplementary Materials for

### Astrocytes Modulate Neural Network Activity by $\text{Ca}^{2+}$ -Dependent Uptake of Extracellular $\text{K}^+$

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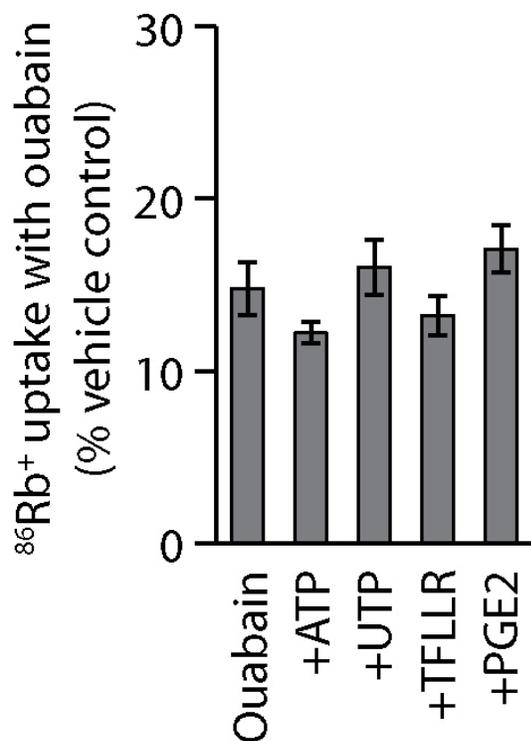
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#### The PDF file includes:

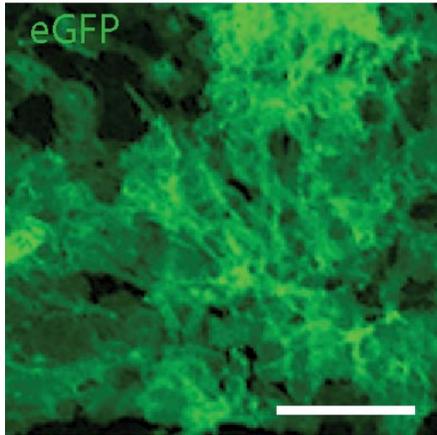
- Fig. S1. Ouabain-insensitive  $^{86}\text{Rb}^+$  uptake.
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## SUPPLEMENTARY FIGURES

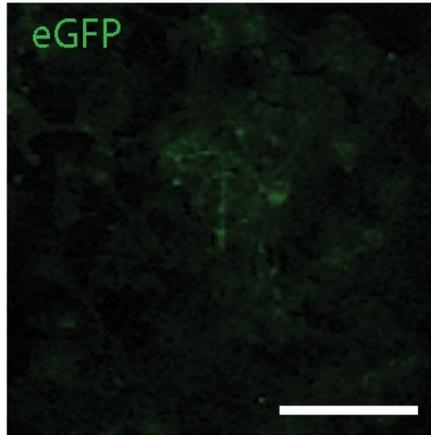


**Fig. S1. Ouabain-insensitive  $^{86}\text{Rb}^+$  uptake.** Ouabain (1 mM) was added to all cultures in addition to the GPCR agonists ATP (100  $\mu\text{M}$ ), UTP (100  $\mu\text{M}$ ), TFLLR (30  $\mu\text{M}$ ), or PGE<sub>2</sub> (20  $\mu\text{M}$ ) (n = 12-16 wells, p=0.075, Kruskal-Willis test).

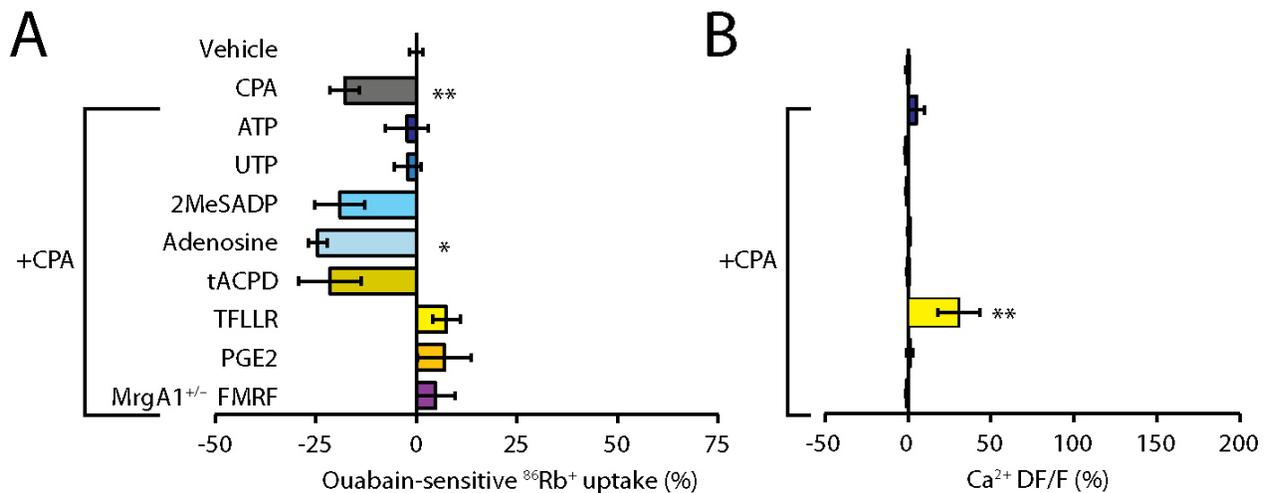
MrgA1<sup>+/-</sup> (-doxycycline)



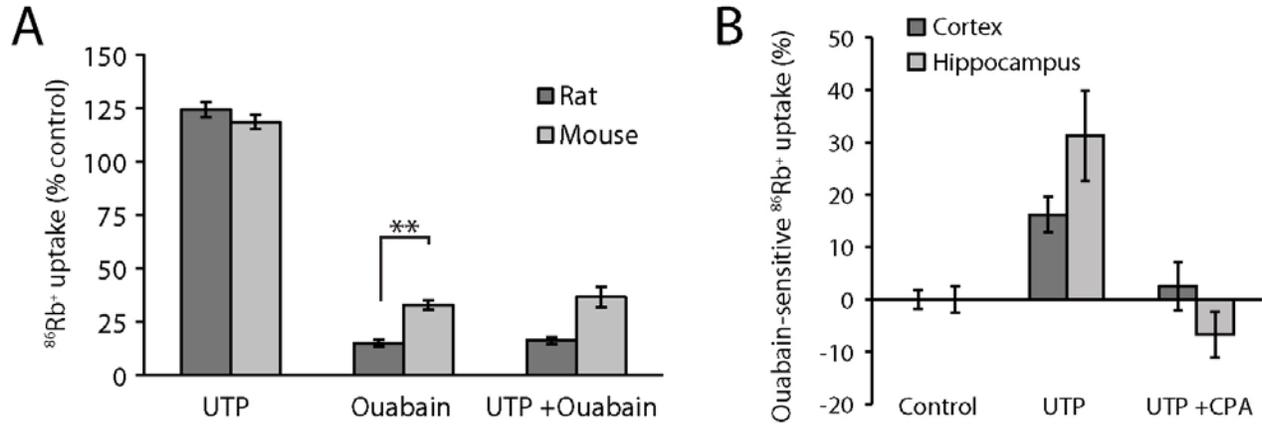
MrgA1<sup>+/-</sup> (+doxycycline)



**Fig. S2. Doxycycline suppresses eGFP expression in cultured astrocytes prepared from MrgA1<sup>+/-</sup> mice.** Cultured astrocytes prepared from MrgA1<sup>+/-</sup> pups treated without or with doxycycline (1  $\mu\text{g ml}^{-1}$ ). Scale bars, 100  $\mu\text{m}$ .

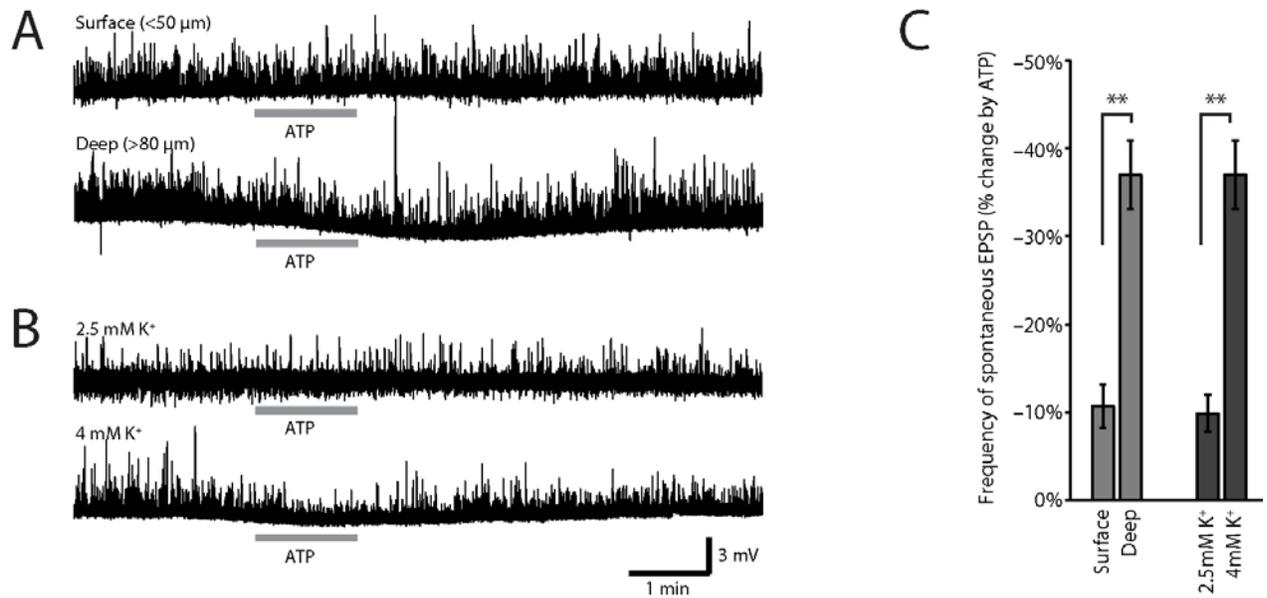


**Fig. S3. GPCR-activated increases in <sup>86</sup>Rb<sup>+</sup> uptake require increases in cytosolic Ca<sup>2+</sup> in cultured astrocytes.** (A) The effect of Gq receptor agonists, ATP (100  $\mu\text{M}$ ,  $n = 11$  wells), UTP (100  $\mu\text{M}$ ,  $n = 14$  wells), 2MeSADP (100  $\mu\text{M}$ ,  $n = 4$  wells), adenosine (100  $\mu\text{M}$ ,  $n = 4$  wells), *t*-ACPD (100  $\mu\text{M}$ ,  $n = 4$  wells), TFLLR-NH<sub>3</sub> (30  $\mu\text{M}$ ,  $n = 6$  wells), PGE<sub>2</sub> (50  $\mu\text{M}$ ,  $n = 6$  wells), and FMRF (15  $\mu\text{M}$  in MrgA1<sup>+/-</sup> mice,  $n = 8$  wells) on <sup>86</sup>Rb<sup>+</sup> uptake in the presence of the ER Ca<sup>2+</sup>-ATPase inhibitor CPA (20  $\mu\text{M}$ ) (\*;  $p < 0.05$  compared to vehicle, \*\*;  $p < 0.01$  compared to vehicle, Bonferroni-Dunn test). (B) Cytosolic Ca<sup>2+</sup> increases in response to the same agonists ( $n = 3$  wells, \*\*;  $p < 0.01$  compared to vehicle, Bonferroni-Dunn test).



**Fig. S4. Comparison of  $^{86}\text{Rb}^+$  uptake in rat versus mouse and rat cortical versus hippocampal astrocytes**

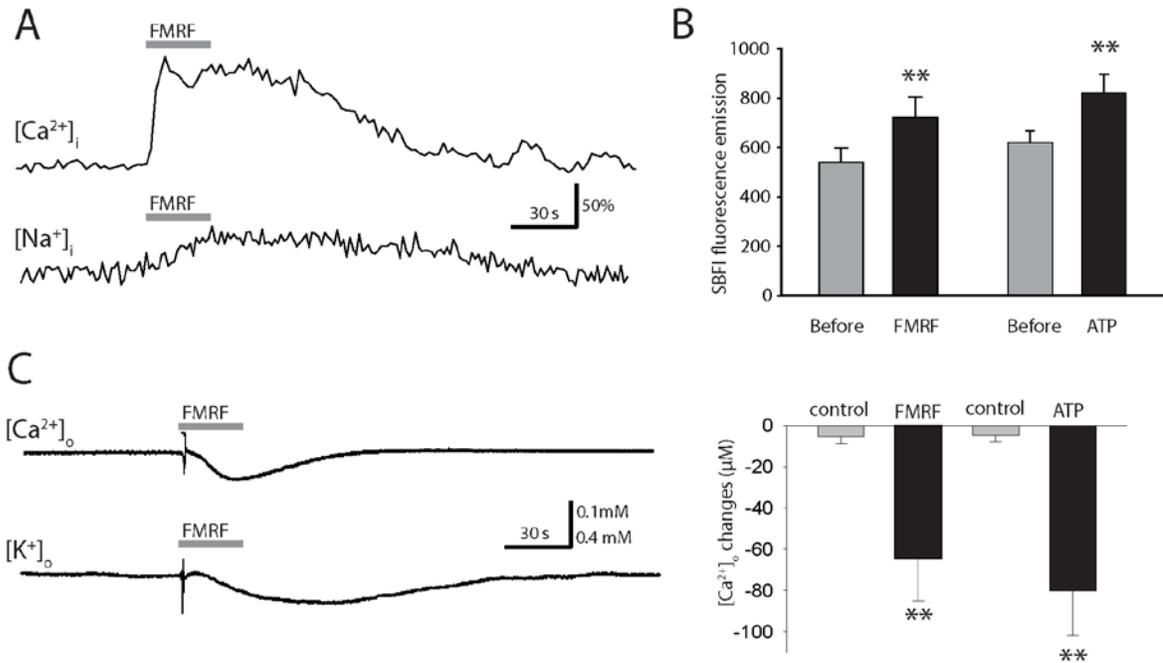
The effect of UTP (100  $\mu\text{M}$ ) or ouabain (1 mM) or both on  $^{86}\text{Rb}^+$  uptake in cultured cortical rat or mouse astrocytes ( $n = 4\text{-}39$  wells, \*\*,  $p = 0.0018$ , Mann-Whitney U-test). **(B)** The effect of CPA pretreatment (20  $\mu\text{M}$ ) on UTP (100  $\mu\text{M}$ )-induced  $^{86}\text{Rb}^+$  uptake in rat cortical or hippocampal astrocyte cultures ( $n = 7\text{-}14$  wells,  $p > 0.05$ , Bonferroni-Dunn test).



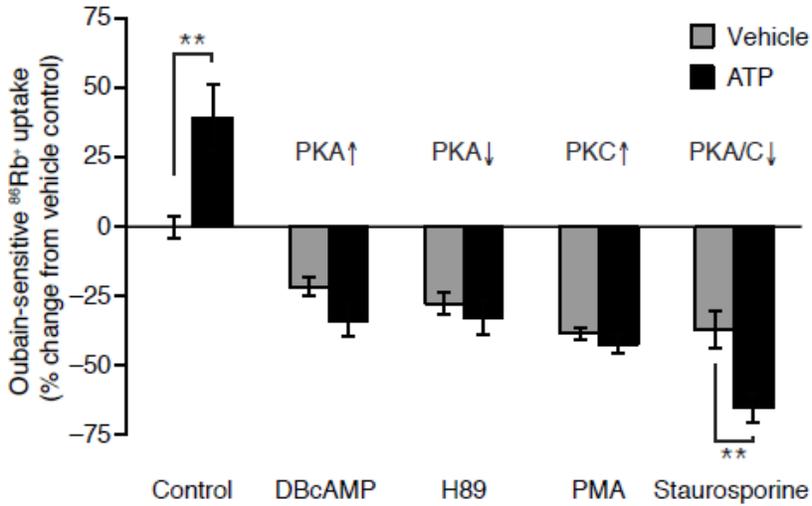
**Fig. S5. Detection of GPCR-activated changes in EPSPs is depth- and  $[\text{K}^+]_{\text{Bath}}$ -dependent in hippocampal slices.**

(A) Effects of agonist-exposure (ATP, 100  $\mu\text{M}$ ) on sEPSPs in superficial (<math><30 \mu\text{m}</math>) and deeper layers (>math>>80 \mu\text{m}</math>). (B) Effects of agonist-exposure (ATP, 100  $\mu\text{M}$ ) on sEPSPs when  $\text{K}^+$  concentration in the perfusate is either 2.5 or 4 mM. (C) Bar graph comparing changes in the frequency of sEPSPs in response to ATP during the 4 experimental conditions shown in panels A and B (n = 5-7 slices, \*\*, p < 0.01, t-test).

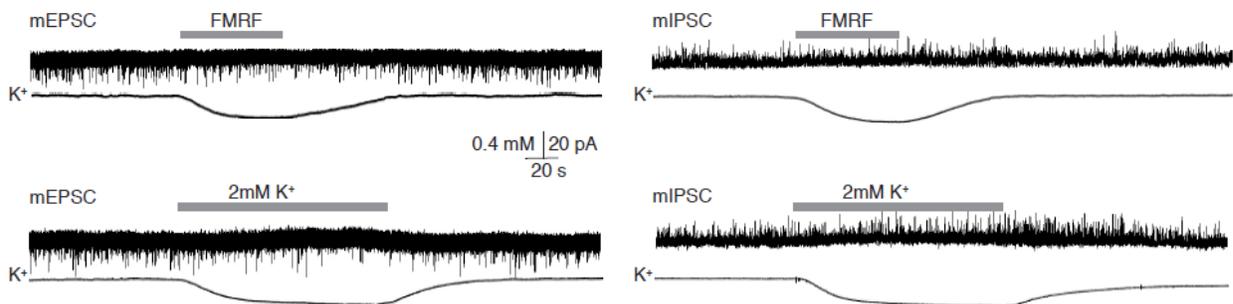
Fig. S6



**Fig. S6. GPCR activation triggers a transient minor increase in intra-astrocytic Na<sup>+</sup> concentration and a minor decrease in extracellular Ca<sup>2+</sup> concentration in hippocampal slices.** (A). Rhod2 (Ca<sup>2+</sup> indicator) and SBFi (Na<sup>+</sup> indicator) both show a transient increase in fluorescence in response to FMRF (15 μM) in slices prepared from MrgA1<sup>+/-</sup> mice. Rhod2 (10 μM) or SBFi (20 μM) were added to the patch pipette solution. (B) Bar graph shows agonist-induced increases in SBFi fluorescence emission (n=5 slices, \*\*, p < 0.01 compared to before, t-test, n = 5 slices). (C) Representative traces of extracellular Ca<sup>2+</sup> (upper trace) and extracellular K<sup>+</sup> (lower trace) recorded simultaneously in a hippocampal slice from a MrgA1<sup>+/-</sup> mouse by ion selective microelectrodes. FMRF (15 μM) exposure induced a small decrease in extracellular Ca<sup>2+</sup> concentration (~ 0.1 mM) concurrent with the decrease in extracellular K<sup>+</sup> concentration (~0.3 mM) (left panel). Comparing [Ca<sup>2+</sup>]<sub>o</sub> changes induced by FMRF- and ATP with control (right panel) (\*\*, P < 0.01, t-test, n = 6 slices).

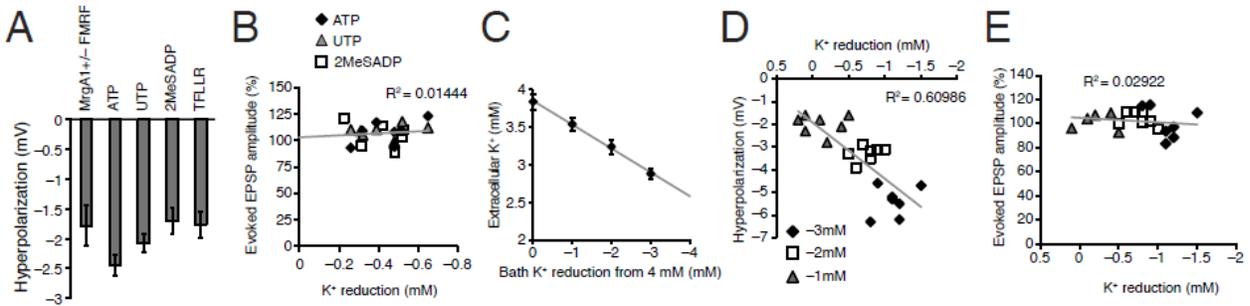


**Fig. S7. Activation of GPCR-induced changes in PKA and PKC activity does not mediate ATP-induced increases in  $^{86}\text{Rb}^+$  uptake in cultured astrocytes.** Effect of N6,2'-O-Dibutyryl adenosine 3',5'-cyclic monophosphate (DBcAMP), (1 mM, PKA activator) and H89 (10  $\mu\text{M}$ , PKA inhibitor), PMA (1  $\mu\text{M}$ , PKC activator), staurosporine (2  $\mu\text{M}$ , PKA and PKC inhibitor) on  $^{86}\text{Rb}^+$  uptake in cultured astrocytes under control conditions and in response to ATP (100  $\mu\text{M}$ ) exposure (n = 4–12 wells, \*\*, p<0.01, t-test).



**Fig. S8. Combined recordings of extracellular  $\text{K}^+$  with mEPSCs or mIPSCs in hippocampal slices.**

Representative traces are shown with experimental manipulation as indicated. Traces displayed here are representative traces and are identical to those in Fig. 5 with the addition of  $\text{K}^+$  measurements using a  $\text{K}^+$  ion selective electrode inserted  $\sim 100 \mu\text{m}$  from the surface of the slice a distance of  $< 50 \mu\text{m}$  from the CA1 cell used for whole cell recording.



**Fig. S9. Effects of GPCR activation or reduction of bath K<sup>+</sup> concentration on extracellular K<sup>+</sup>, neuronal membrane potential, and evoked EPSP in hippocampal slices. (A)** Effect of agonists on the membrane potential changes of CA1 hippocampal neurons. FMRF (15  $\mu$ M) in MrgA1<sup>+/-</sup> mice (n = 6 slices) and ATP (100  $\mu$ M), UTP (100  $\mu$ M), 2MeSADP (100  $\mu$ M), and TFLLR (30  $\mu$ M) in wild type mice (n = 6-8 slices, p > 0.05, ANOVA). **(B)** The amplitude of the evoked EPSP was not changed after application of ATP, UTP, or 2MeSADP (n = 18 slices). **(C)** Changes in extracellular K<sup>+</sup> in response to transient reduction of K<sup>+</sup> from 4 mM to either 3, 2, or 1 mM in the bath solution. A K<sup>+</sup> sensitive microelectrode was inserted ~100  $\mu$ m below the surface of the hippocampal slice and the extracellular K<sup>+</sup> concentration after 2 min is plotted (n = 5 slices). **(D)** The effects of changing extracellular K<sup>+</sup> on the membrane potential changes of CA1 neurons. **(E)** Changes in extracellular K<sup>+</sup> by switching bathing K<sup>+</sup> concentration from 4 mM to 2 mM did not change amplitude of evoked EPSPs ( $R^2=0.03$ ).