

The Impact Of Astrocytic Ca²⁺ On Synaptic Plasticity During Motor Learning

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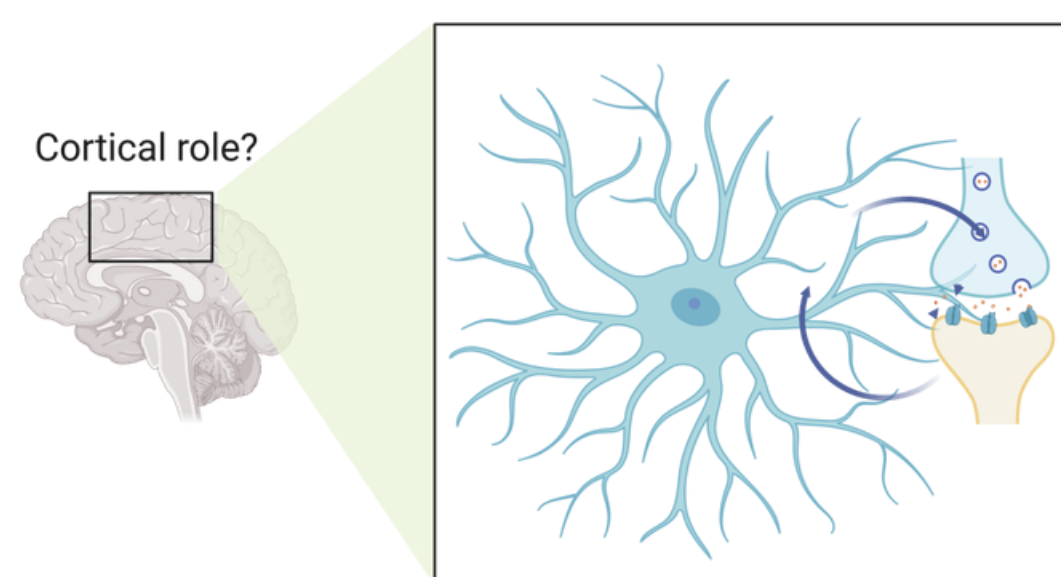
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ABSTRACT

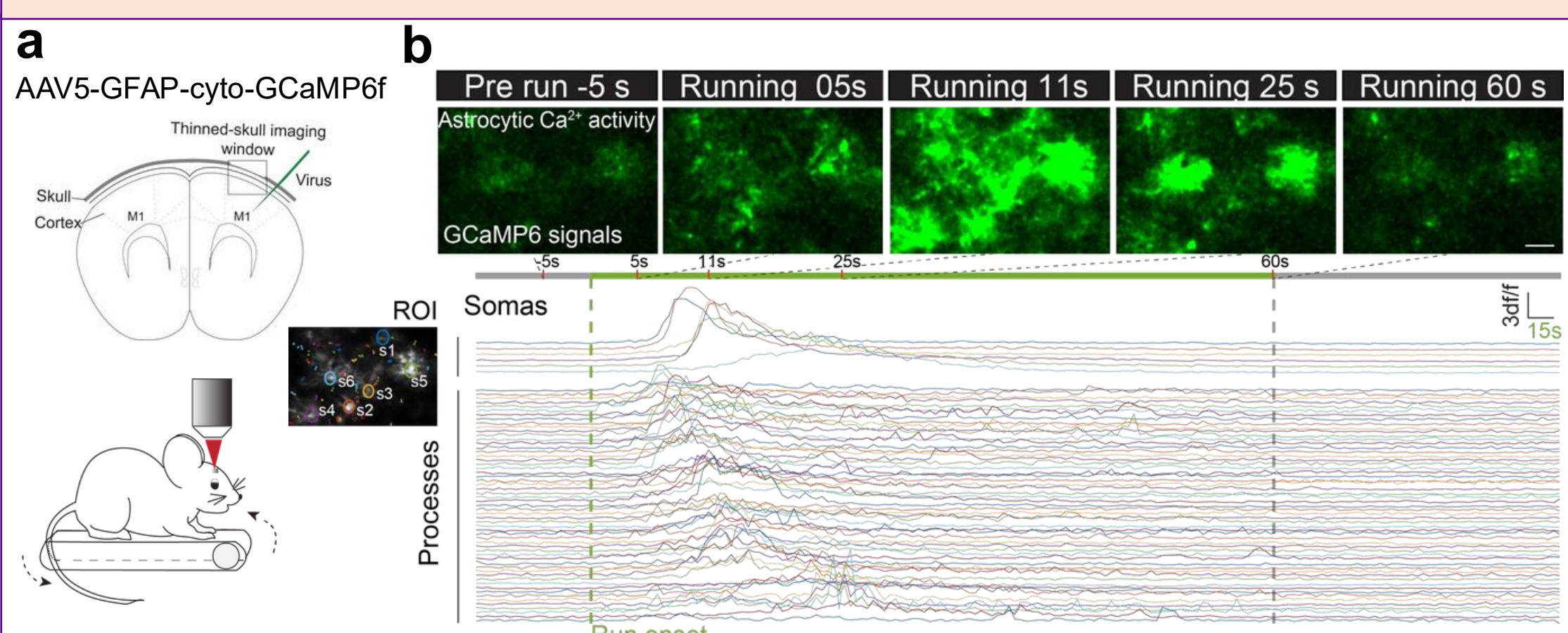
Many lines of evidence indicate that astrocytic Ca²⁺ activity regulates activity-dependent synaptic plasticity, but its role in learning-related synaptic changes in the living brain remains unclear. We found that motor training induced synaptic potentiation on apical dendrites of layer 5 pyramidal neurons, as well as a substantial rise in Ca²⁺ within the processes and somas of astrocytes in the superficial layer of the mouse motor cortex. When motor training-triggered astrocytic Ca²⁺ was reduced either by suppressing $\alpha 1$ adrenergic receptor-IP R2 signaling or activating Gq-DREADD receptors in astrocytes, it led to synaptic depotentiation during motor training and subsequent impairment in performance improvement. Notably, synaptic depotentiation occurred on a fraction of dendrites with repetitive dendritic Ca²⁺ activity. On those dendrites, dendritic spines that were active prior to dendritic Ca²⁺ activity underwent CaMKII-dependent size reduction during motor learning. In addition, the activation of adenosine receptors prevented repetitive dendritic Ca²⁺ activity and synaptic depotentiation caused by the reduction of astrocytic Ca²⁺, suggesting the involvement of adenosine signaling in the processes. Together, these findings reveal a novel function of astrocytic Ca²⁺ in preventing synaptic depotentiation by limiting repetitive dendritic activity during learning.

INTRODUCTION

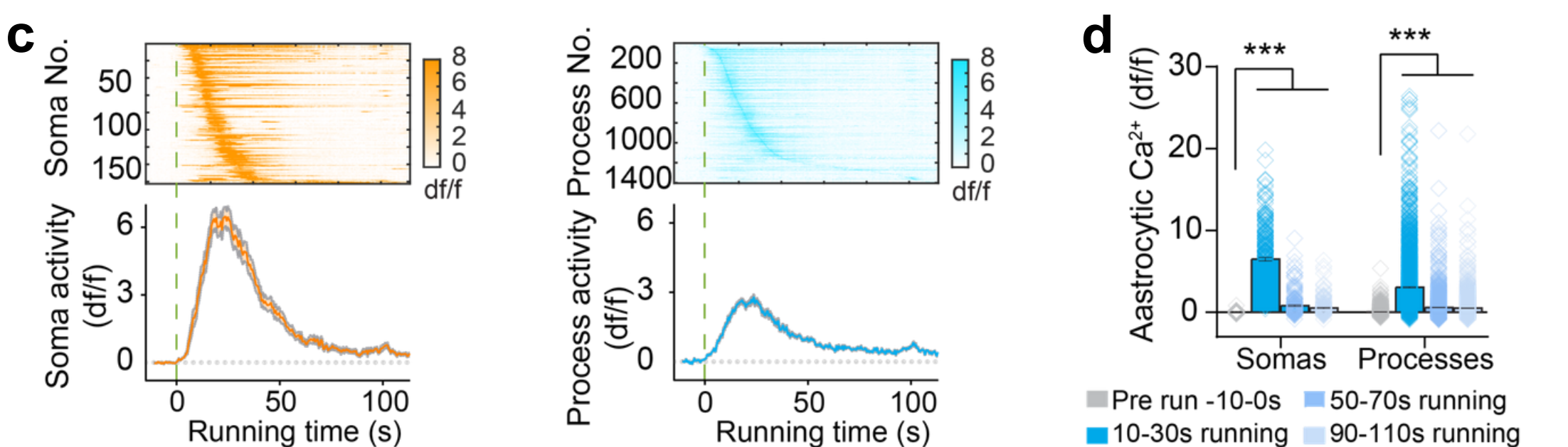
- It has been suggested that intracellular Ca²⁺ signals in astrocytes are important for the LTP through the release of D-serine to enhance NMDA receptor activity or through the release of glutamate to act on metabotropic glutamate receptors.
- Astrocytic Ca²⁺ during the LTP induction causes heterosynaptic depression at unstimulated synapses that involves ATP released from astrocytes and the conversion of ATP to adenosine.
- The malfunction of astrocytes have been linked to depression, epilepsy, pain and several neurodegeneration diseases. Patients with neurological diseases often show altered learning ability and cognitive deficits.



Motor training induces transient Ca²⁺ activity in astrocytes across multiple brain regions



a. Schematic of two-photon imaging of cytosolic GCaMP6f-expressing astrocytes during running; b. Images and fluorescence traces of running-induced astrocytic Ca²⁺ activity.

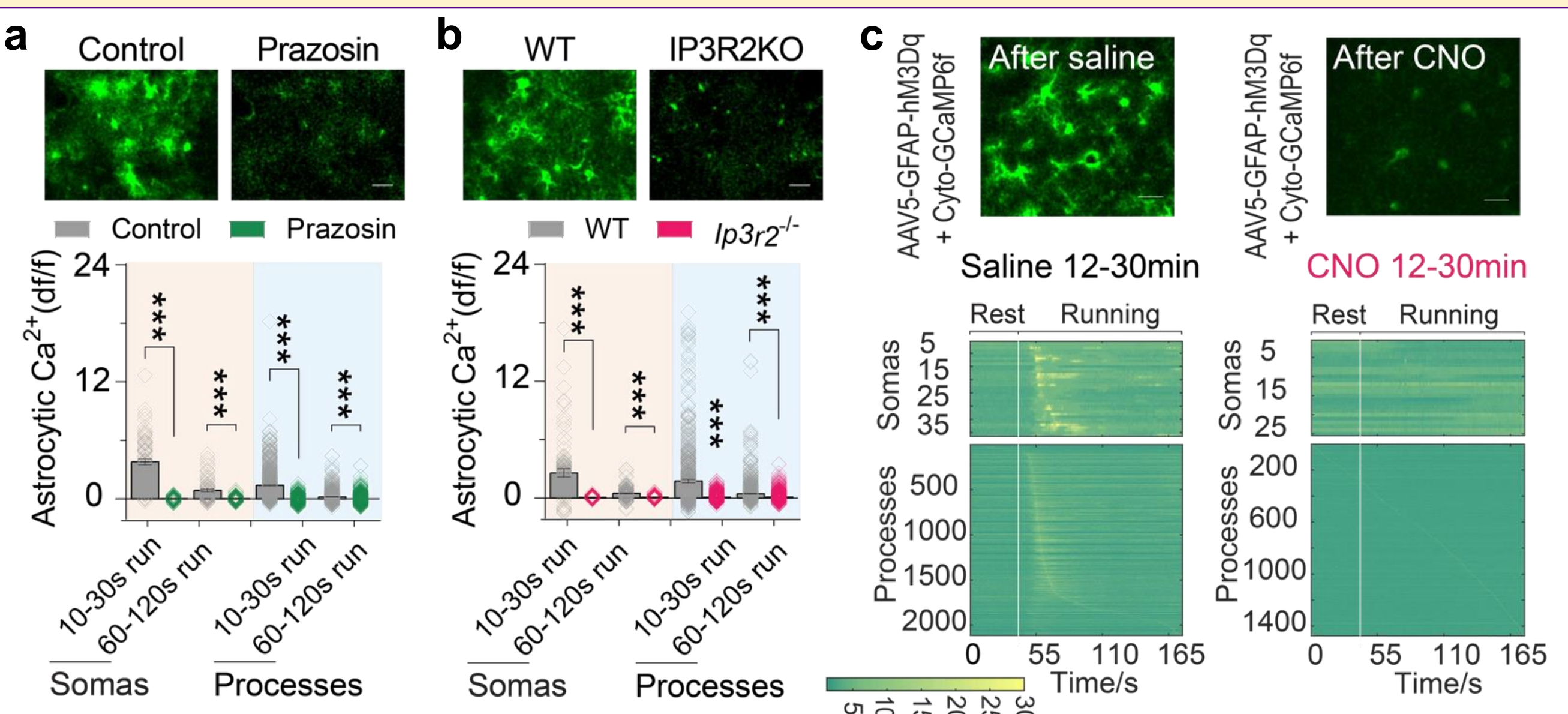


c. Heatmaps (up) and average amplitude of somatic (left) and process (right) Ca²⁺ transients in astrocytes during pre-run and different stages of treadmill running (n=5 mice). Scale bar, 20µm.

d. The average amplitude of astrocytic Ca²⁺ transients significantly increased in both somas (n=162) and processes (n=1405) over different periods of running compared to pre-run levels. Data are mean ± s.e.m. ***P<0.001, Mann-Whitney U test.

e. Heatmaps of astrocytic Ca²⁺ activities during the periods of pre-run and treadmill running in the prefrontal cortex, motor cortex and visual cortex and hippocampus. df/f ranged from 0-0.5 (blue, relatively low activity) to 0.5-1 (red).

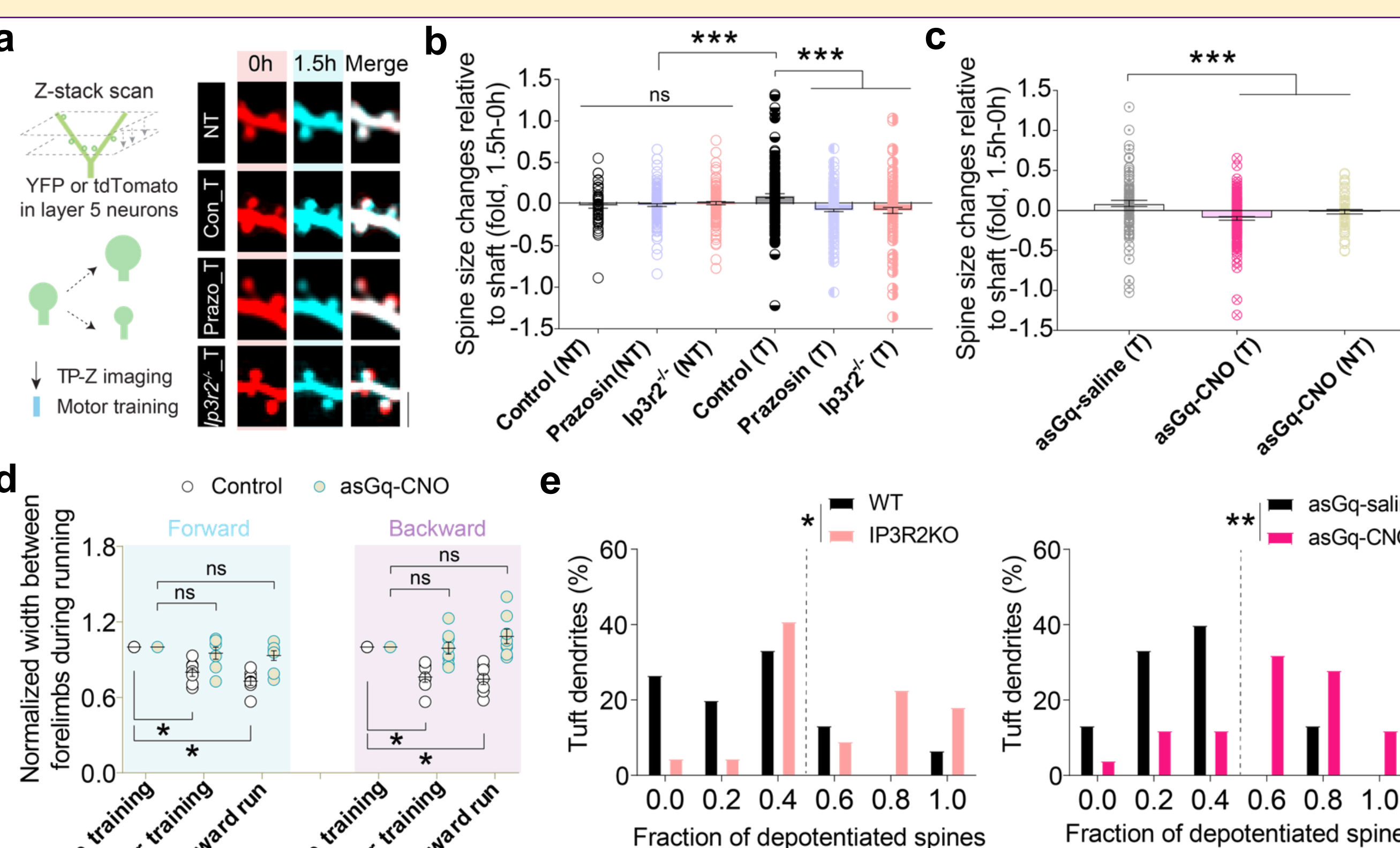
Motor training-induced astrocyte Ca²⁺ transient is dependent on IP3R-mediated Ca²⁺ release from the endoplasmic reticulum



a-b. Images (up) and average amplitude of motor training-induced astrocytic Ca²⁺ transients with or without prazosin (adrenergic $\alpha 1$ receptor antagonist, a), or in WT and IP3R2 KO mice (b). Data are mean ± s.e.m. ***P<0.001, Mann-Whitney U test. Scale bar, 20µm.

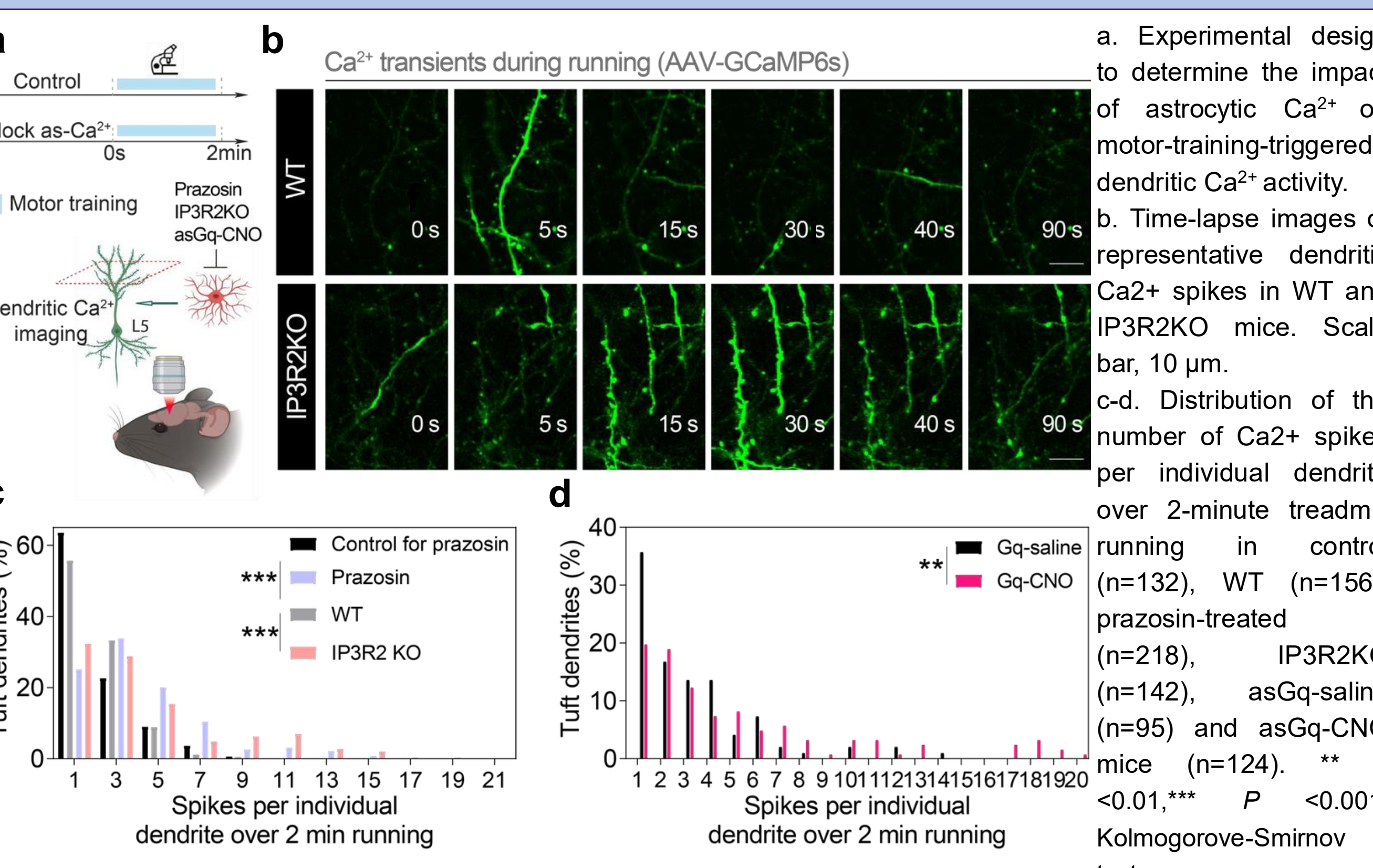
c. Images (up) and heatmaps of astrocytes expressing hM3Dq (asGq) and cytosolic GCaMP6f during resting and treadmill running with saline or CNO treatment. The color bar represents mean gray values within ROI; d. Images of astrocytic ER Ca²⁺ before and after CNO treatment under running conditions. Scale bar, 20µm.

Reducing astrocytic Ca²⁺ transient leads to spine size decrease during motor learning and impairs performance improvement



a. Dendritic spine images of layer 5 pyramidal neurons at 0h (in red) and 1.5h (in blue) under no training (NT), training (control_T, prazosin_T and IP3R2KO_T) conditions. Scale bar, 1 µm. b-c. Spine size changes induced by NT over 1.5h in control (n=54), prazosin (n=145), IP3R2KO (n=132) and asGq-CNO (n=61), and motor training over 1.5h in control (n=157), prazosin (n=221), IP3R2KO (n=110) and asGq-saline (n=107) asGq-CNO (n=142). d. Width changes between forelimbs after Forward/Backward training in control (7 mice) and asGq-CNO mice (8 mice). e. The percentage of dendrites with overall reduction of spine sizes after training is higher in mice with reduced astrocytic Ca²⁺ (WT=4 mice and IP3R2KO=6 mice; asGq-saline=4 mice and asGq-CNO=4 mice); Data are mean ± s.e.m. ns P>0.05, *P<0.05, **P<0.01, ***P<0.001, Mann-Whitney U test.

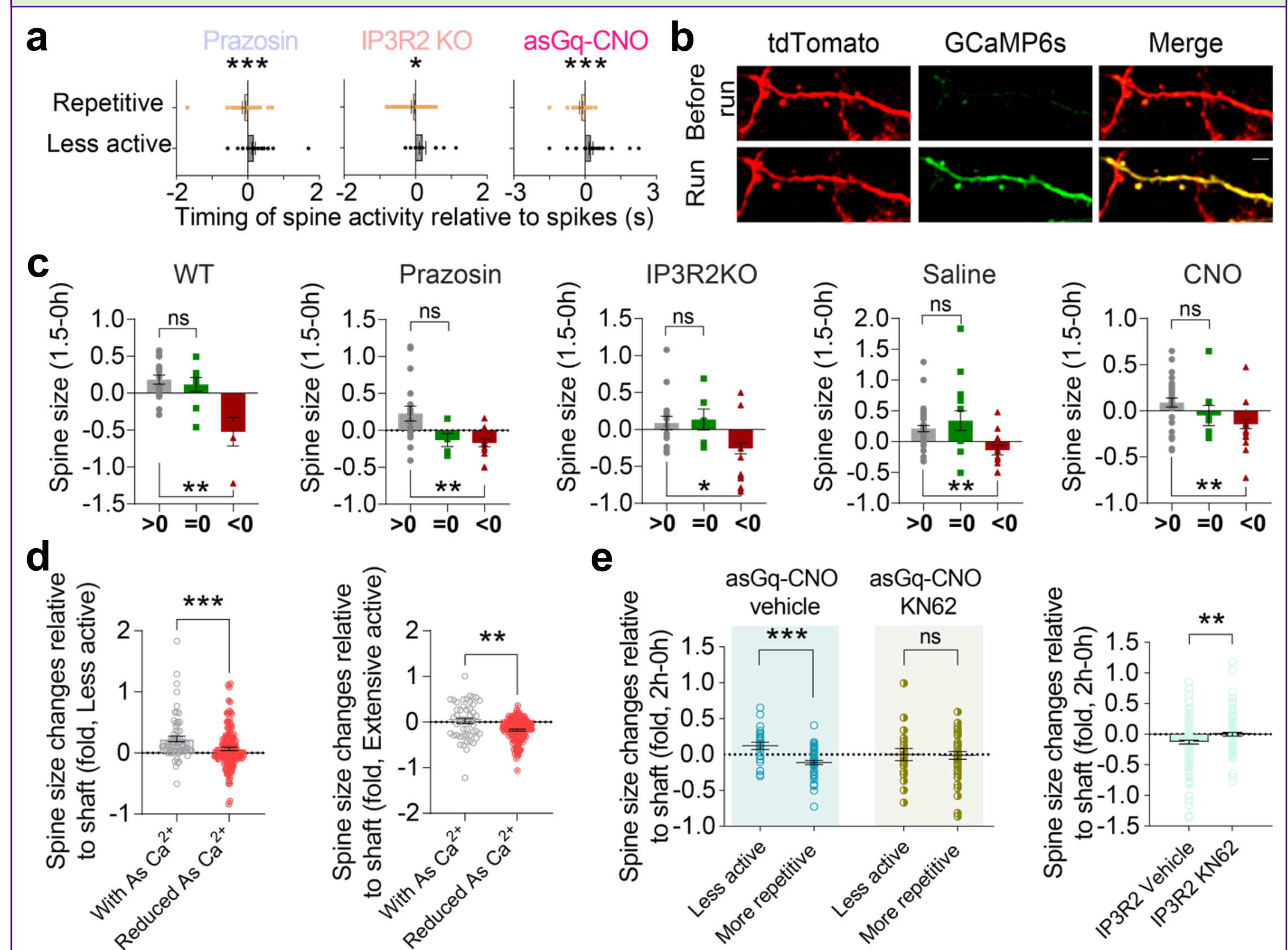
Reduction of astrocytic Ca²⁺ increases repetitive dendritic Ca²⁺ activity 10-30s after the start of motor training



a. Experimental design to determine the impact of astrocytic Ca²⁺ on motor-training-triggered dendritic Ca²⁺ activity. b. Time-lapse images of representative dendritic Ca²⁺ spikes in WT and IP3R2KO mice. Scale bar, 10 µm. c-d. Distribution of the number of Ca²⁺ spikes per individual dendrite over 2-minute treadmill running in control (n=132), WT (n=156), prazosin-treated (n=218), IP3R2KO (n=95), asGq-saline (n=142) and asGq-CNO mice (n=124). ** P<0.01, *** P<0.001, Kolmogorov-Smirnov test.

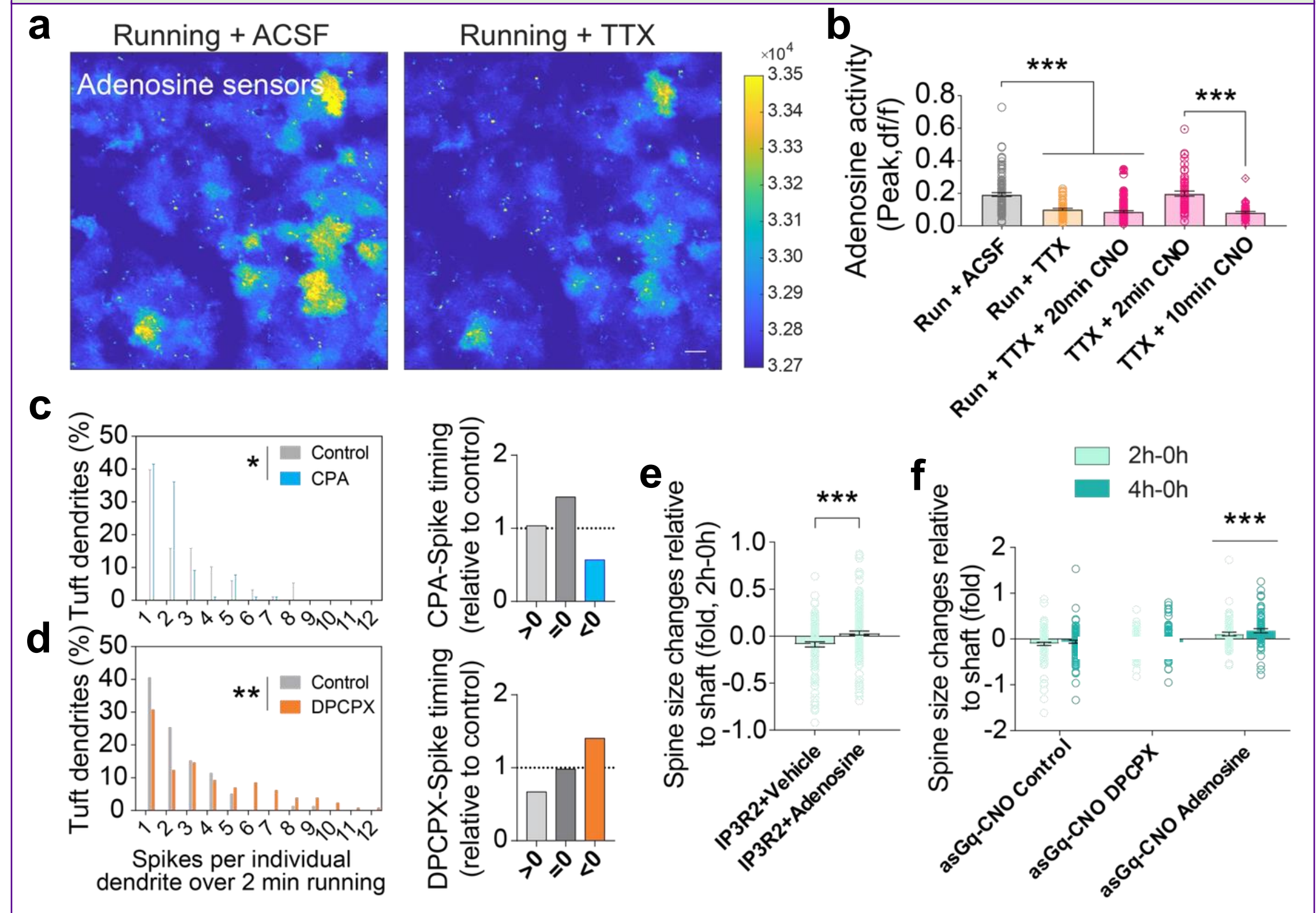
e. The overall spine size shrinkage at 2h was prevented by local application of adenosine in IP3R2KO mice (control: n=102, adenosine: n=185). f. Adenosine prevented overall spine size shrinkage at 2h and 4h in asGq-CNO mice (control: n=73, DPCPX: n=87, adenosine: n=70). Data are mean ± s.e.m. *P<0.05, **P<0.01, ***P<0.001, Mann-Whitney U test.

Spine size decrease on dendrites with repetitive Ca²⁺ is mediated by spike-timing-dependent mechanism



a. The latency from the peak of spine Ca²⁺ activity to the peak of dendritic Ca²⁺ activity on dendrites with repetitive Ca²⁺ or less active in prazosin (n=111), IP3R2KO (n=102) and asGq-CNO (n=80). Mann-Whitney test. b. Co-expression of tdTomato and GCaMP6s in dendrites of layer 5 pyramidal neurons. Scale bar, 4µm. c. The average spine size changes according to different averaged spike-timing over 1.5h motor training in control (4 mice), prazosin (3 mice), IP3R2KO (6 mice), asGq-saline (3 mice) and asGq-CNO mice (3 mice). Kruskal-Wallis test. d. Synaptic depotentiation occurred on dendrites with repetitive Ca²⁺ activity when astrocytic Ca²⁺ was reduced. e. Motor training-induced spine size changes over 2h in asGq-CNO vehicle (n=72), asGq-CNO KN62 (n=142), IP3R2 vehicle (n=154) and KN62 (n=99). Data are mean ± s.e.m. ns P>0.05, *P<0.05, **P<0.01, ***P<0.001, Mann-Whitney U test.

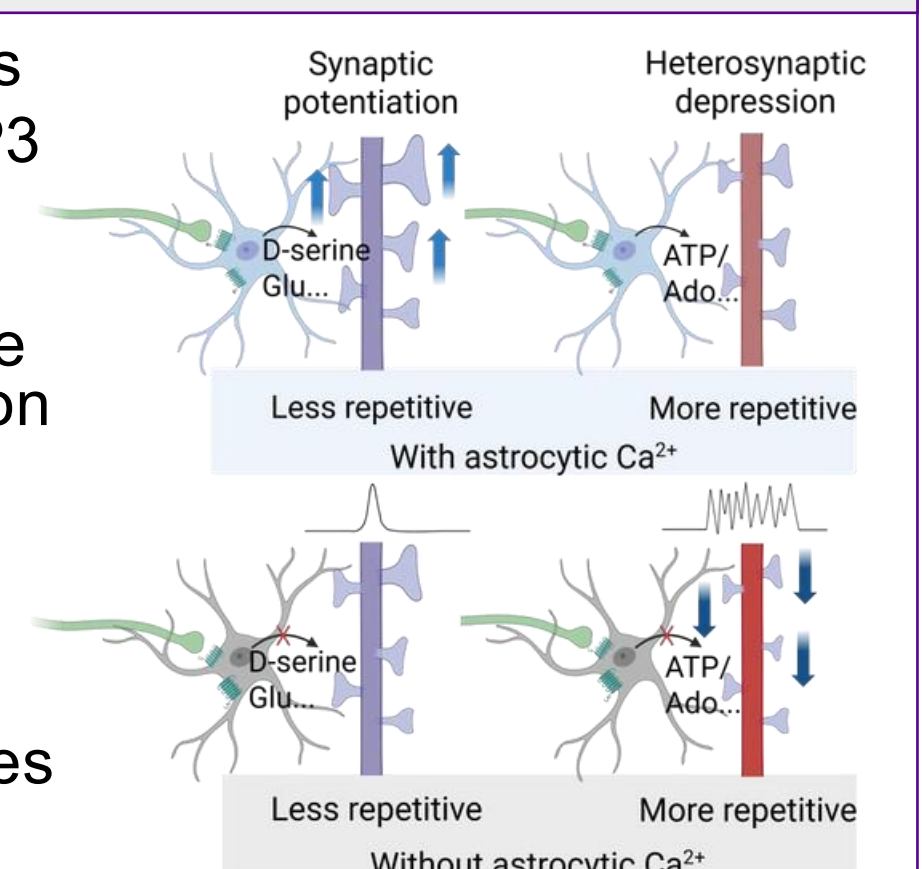
Adenosine signaling is involved in astrocytic Ca²⁺-dependent synaptic plasticity



a. Representative images of astrocytes expressing genetically encoded GPCR-Activation-Based (GRAB) adenosine sensors during motor training treated with ACSF followed by TTX. The color bar represents the mean gray value. Scale bar, 20µm. b. The average peak of extracellular adenosine changes under running conditions with ACSF (n=104), TTX (n=70), TTX+20min post-CNO injection (n=84), and under resting conditions with TTX followed by CNO injection for 2 min (n=54) and 10 min (n=54). c-d. Local application of CPA (adenosine A1 agonist, c) and DPCPX (adenosine A1 antagonist, d) changed the number of dendrites with repetitive Ca²⁺ and the number of spines with their Ca²⁺ peak occurring before the peak of dendritic Ca²⁺ activity. e. The overall spine size shrinkage at 2h was prevented by local application of adenosine in IP3R2KO mice (control: n=102, adenosine: n=185). f. Adenosine prevented overall spine size shrinkage at 2h and 4h in asGq-CNO mice (control: n=73, DPCPX: n=87, adenosine: n=70). Data are mean ± s.e.m. *P<0.05, **P<0.01, ***P<0.001, Mann-Whitney U test.

SUMMARY

- A surge of Ca²⁺ activity in astrocytes occurs during motor training, and it relies on $\alpha 1$ -IP3 dependent ER Ca²⁺ release.
- Reduced astrocytic Ca²⁺ leads to spine size reduction during motor learning on a fraction of dendrites with repetitive dendritic Ca²⁺ activity.
- CaMKII and adenosine regulate astrocyte-dependent synaptic strength changes during motor learning.



ACKNOWLEDGEMENTS

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