Supportive information to
2018-BJP-0934-RP
Title: The impact of dihydropyridine derivatives on the cerebral blood flow response to somatosensory stimulation and spreading depolarization
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During the review process, the suggestion has been formulated that the selective elimination of astrocytes may support the proposal in the Discussion that nimodipine may increase functional hyperemia to whisker stimulation by acting on astrocytes.
The selective elimination of astrocytes in these additional experiments was achieved by the application of fluorocitrate, which disrupts the citrate cycle in astrocytes, and achieves the selective elimination of astrocytes within hours (Swanson and Graham, 1994). Importantly, the chemical was previously used in the context of studying astrocyte function during SD (Lian and Stringer, 2004). The time window of drug effect was favorable to our experiments, which were acute by nature.

A pilot study consisting of 6 additional animals was initiated, matching the experimental procedures for the ischemia group of *Series 2* presented in the body of the article. The animals were assigned to either nimodipine (n=3) or vehicle treatment (n=3), given exactly as stated in the article. The experimental protocol was complemented by the topical administration of fluorocitrate (Sigma), a glia toxin, into the recording cranial window (0.1 mM, 2.5 hours), prior to the application of nimodipine or vehicle (Fig. S1).

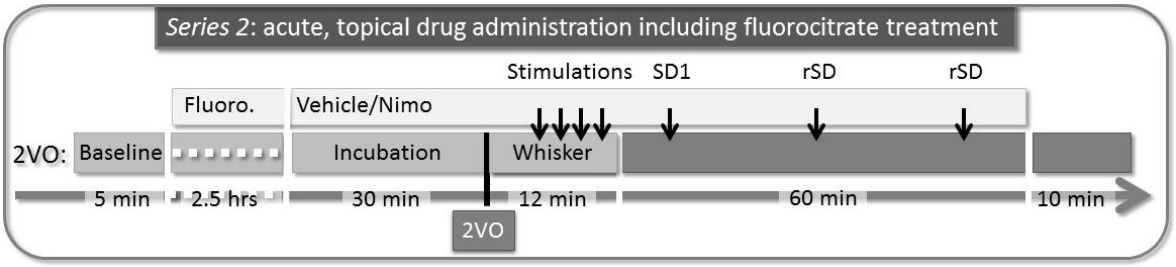

Figure S1. Time line of the protocols implemented. Abbreviations: 2VO, permanent, bilateral occlusion of the common carotid arteries ("two-vessel occlusion"); Fluoro: fluorocitrate; Nimo, nimodipine; rSD, recurrent spreading depolarization; SD, spreading depolarization; SD1, first spreading depolarization in a train.

Following fluorocitrate incubation, complex neurophysiological and cerebrovascular changes were observed. In the context of neurovascular coupling initiated by somatosensory stimulation, the amplitude of evoked field potentials (EFP) appeared to be greater after fluorocitrate application than in the control condition (164±24 vs. 108±19 μV, fluorocitrate+ vehicle vs. control+vehicle), and reduced significantly with respect to vehicle, in case nimodipine was co-administered with fluorocitrate (117±23 vs. 164±24 μV, fluorocitrate+nimodipine vs. fluorocitrate+vehicle) (Fig. S2A-B).

CBF baseline directly preceding whisker stimulation was significantly lower in the fluorocitrate experiments (50±7 vs. 79±11 %, fluorocitrate+ vehicle vs. control+vehicle), but the relative amplitude of functional hyperemia increased (9.2±8.2 vs, 2.9±6.7 pp,

fluorocitrate+ vehicle vs. control+vehicle), in agreement with the increased EFP amplitude (Fig. S2C-D). Finally, nimodipine in combination with fluorocitrate failed to increase the magnitude of functional hyperemia – in fact, functional hyperemia under this condition could not be discriminated from baseline CBF oscillation reliably (Fig. S2C). Thus, the CBF response to whisker stimulation was compromised under nimodipine treatment co-applied with fluorocitrate, compared to nimodipine given alone (Fig. S2D).

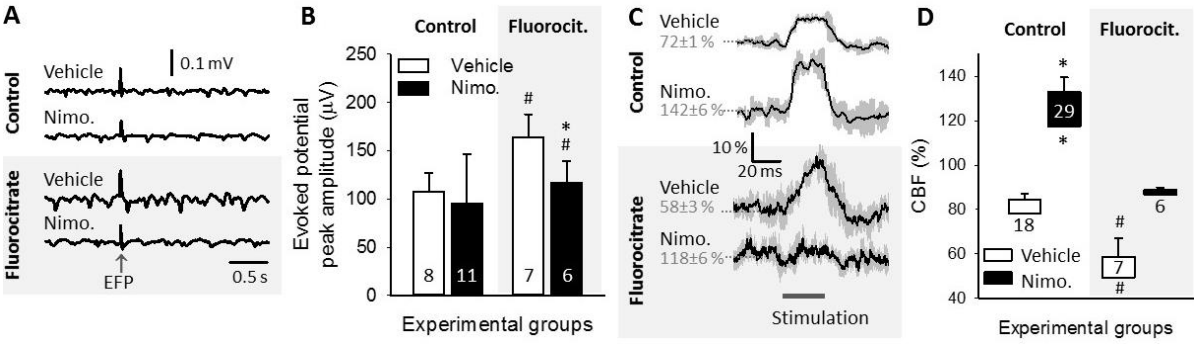

Figure S2. The impact of pharmacological treatments on evoked potentials and the coupled cerebral blood flow (CBF) response during whisker stimulation after bilateral common carotid artery occlusion (2VO) in Series 2. **A,** Representative local field potential (LFP) traces display somatosensory evoked field potentials (EFPs) during whisker stimulation. Traces in front of light gray background were taken from animals control to fluorocitrate treatment, while the trace in front of dark gray background represents EFPs under fluorocitrate treatment. **B,** The peak amplitude of evoked potentials. **C,** Traces (each is the mean of 4 stimulations in an animal representative of each condition) show the CBF response to whisker stimulation. **D,** The amplitude of the CBF response to whisker stimulation. The base of each bar in the chart is set to the CBF level directly preceding whisker stimulation. In B and D, data are given as mean±stdev; sample size (i.e. the number of events analyzed) is indicated in each bar. Statistical analysis relied on a two-way analysis of variance (ANOVA) paradigm (factors: 2VO and treatment). The level of significance was defined as $p<0.05$*. A Games-Howell post hoc test was applied for group comparisons ($p<0.05$* vs. vehicle; $p<0.05^{\#}$ vs. control). Significance symbols under the bars represent differences of baseline, while symbols over the bars stand for the difference between functional hyperemia amplitude.

Although it may be tempting to conclude that the reduction of the CBF response to whisker stimulation under nimodipine treatment co-applied with fluorocitrate (compared to nimodipine alone) indicates nimodipine action on astrocytes, this may be an oversimplistic approach. Astrocytes are known to finely tune synaptic activity, remove surplus glutamate and potassium from the extracellular space, feed neurons with lactate and glutamine, and mediate the CBF response to neuronal activation (Verkhratsky and Nedergaard). The selective elimination of astrocytes is thus expected to impact on all these functions. The target of nimodipine (the L-type voltage gated calcium channel) is known to be present on cerebrovascular smooth muscle cells, neurons, as well as astrocytes, which complicates data interpretation further. Therefore, it is difficult to posit, that the results obtained here are the primary consequence of the lack of astrocytic L-type voltage gated calcium channels. The elimination of astrocytes must substantially alter neuronal activity, which creates a profoundly different neurophysiological environment exposed to nimodipine.

References for the supporting information:

Lian XY, Stringer JL. Astrocytes contribute to regulation of extracellular calcium and potassium in the rat cerebral cortex during spreading depression. Brain Res. 2004 Jun 25;1012(1-2):177-84.

Swanson RA, Graham SH. Fluorocitrate and fluoroacetate effects on astrocyte metabolism in vitro. Brain Res. 1994 Nov 21;664(1-2):94-100.

Verkhratsky A, Nedergaard M. Physiology of Astroglia. Physiol Rev. 2018 Jan 1;98(1):239-389.