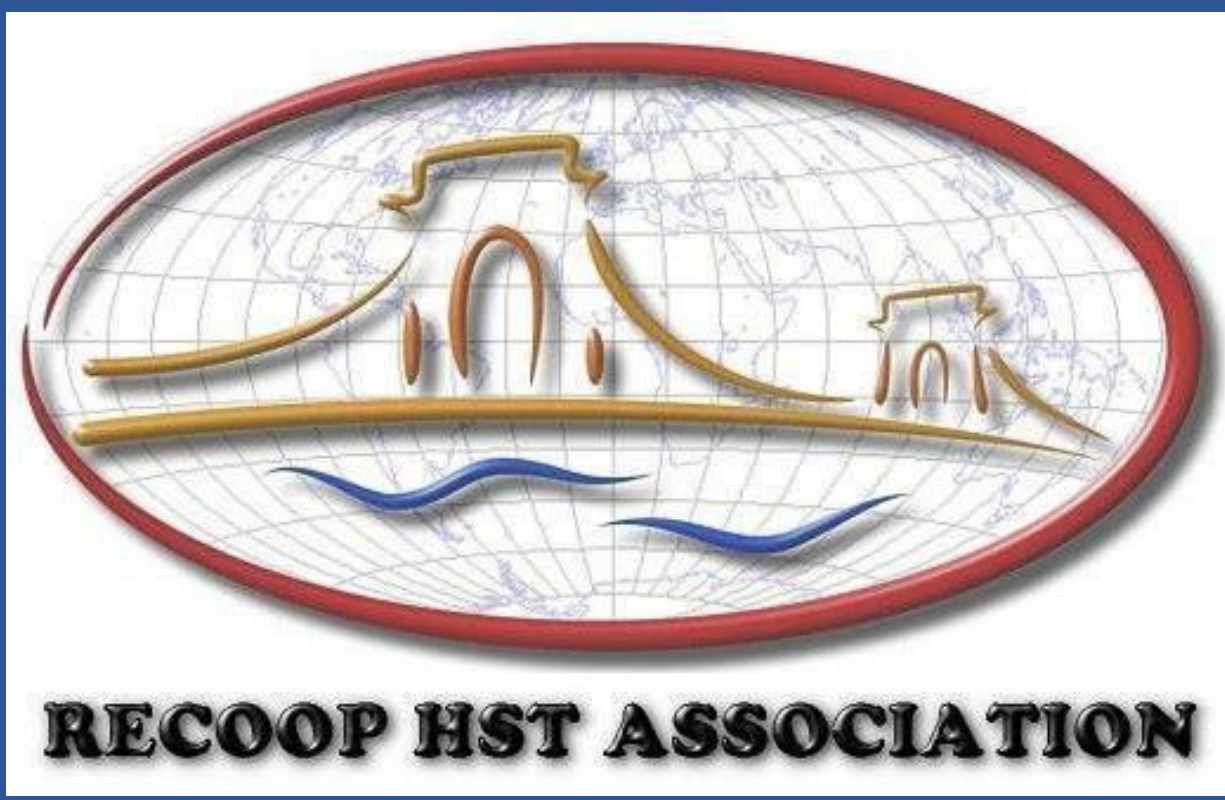




The potassium wave of spreading depolarization initiates vasoconstriction in the mouse cerebral cortex

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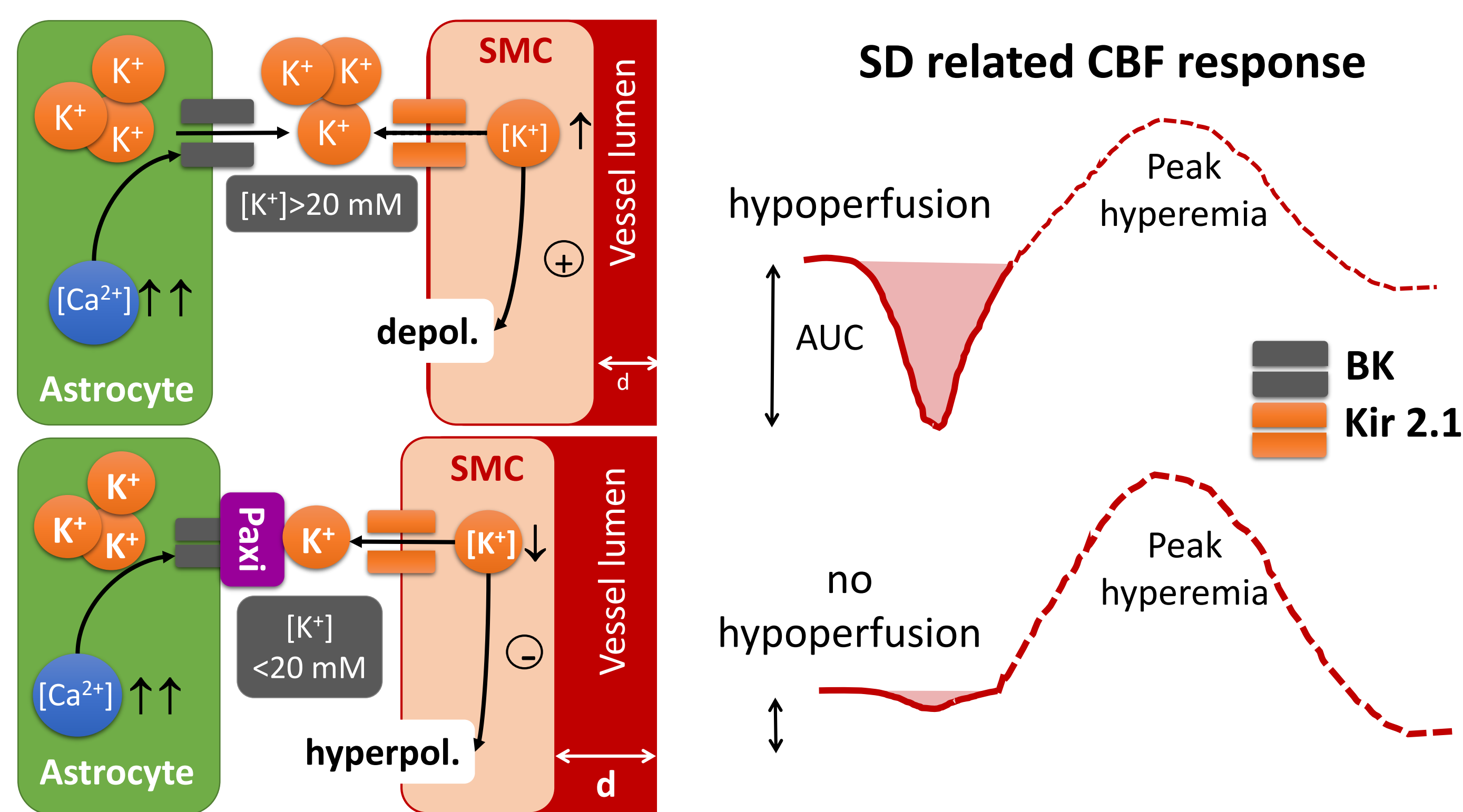
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INTRODUCTION

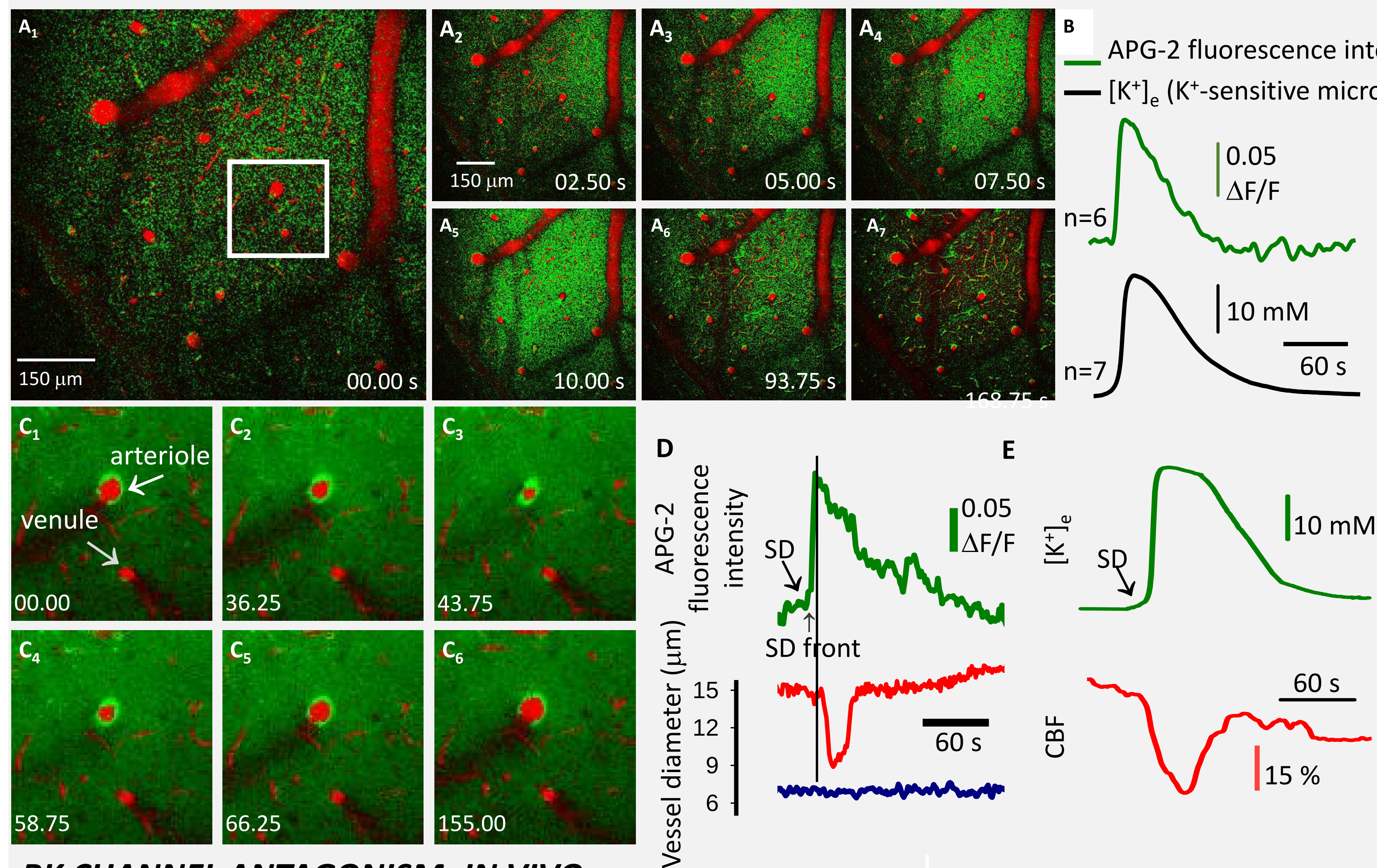
Recurrent spreading depolarizations (SD) of the ischemic brain are the near complete, spontaneously occurring local depolarization of neurons and glia cells, which propagate from the core of the injury to the adjacent cell populations of the intact cerebral gray matter. Neuronal depolarization causes the release of K^+ from the cells and the accumulation of the ion in the extracellular space (20-65 mM), which further depolarizes the surrounding nervous tissue. Spreading depolarizations exacerbate the primary damage to the brain through generating widespread necrosis mediated by insufficient perfusion, since the SD associated functional hyperemia is overruled by adverse hypoperfusion under ischemic conditions. The increase of extracellular K^+ with the SD reaches concentrations (>20 mM) at which K^+ acts as a potent vasoconstrictor, in contrast to vasodilator effects of a mild elevation of $[K^+]_e$ (<20 mM). We formulated the hypothesis that the SD associated elevation of $[K^+]_e$ over 20 mM might play a central role in the SD-related vasoconstriction. Furthermore, we suggest the involvement of astrocyte large-conductance Ca^{2+} -activated potassium channels (BK channels) in the generation of vasoconstriction.

EXPERIMENTAL HYPOTHESIS

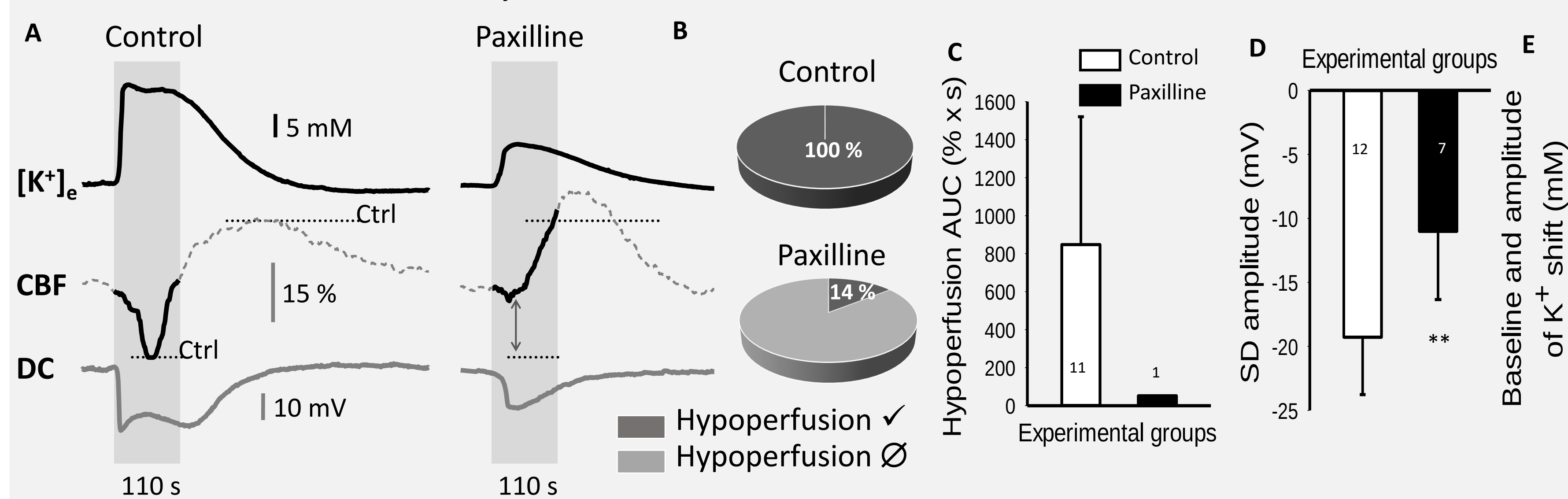


RESULTS

EXTRACELLULAR POTASSIUM IMAGING



BK CHANNEL ANTAGONISM; IN VIVO



CONCLUSIONS

We set up a novel technology based on two-photon imaging and the use of a K^+ sensitive fluorescent dye, suitable for the assessment of changes of $[K^+]_e$ with SD in the mouse cerebral cortex. Our results indicate that the $[K^+]_e$ elevates over the vasoconstrictive threshold with SD, and thus SD causes remarkable vasoconstriction. Longer duration of the K^+ shift with SD appears to predict a greater magnitude of the associated hypoperfusion transient. Lastly, astrocytic BK channels are implicated in the generation of SD-related arterial vasoconstriction.

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MATERIALS AND METHODS

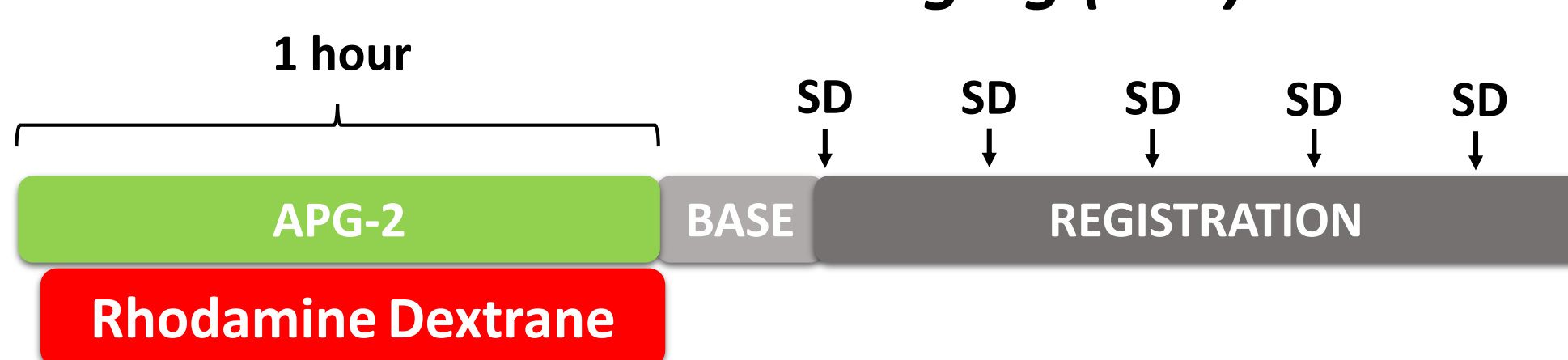
Extracellular potassium imaging

Animals: C57BL/6 mice (n=6)
Anesthesia: Avertin (4mg/kg)
Two craniotomies:
• Caudal: imaging (closed)
• Rostral: SD elicitation (open)
SD elicitation: 2 μ l 1M KCl
2-photon excitation: 810 nm
Femto 2D, 690-1024 nm sapphire laser
 K^+ labeling: **Assante Potassium Green-2** (topical application)
Cerebral vessel labeling: **Rhodamine Dextran** (i.v. application)

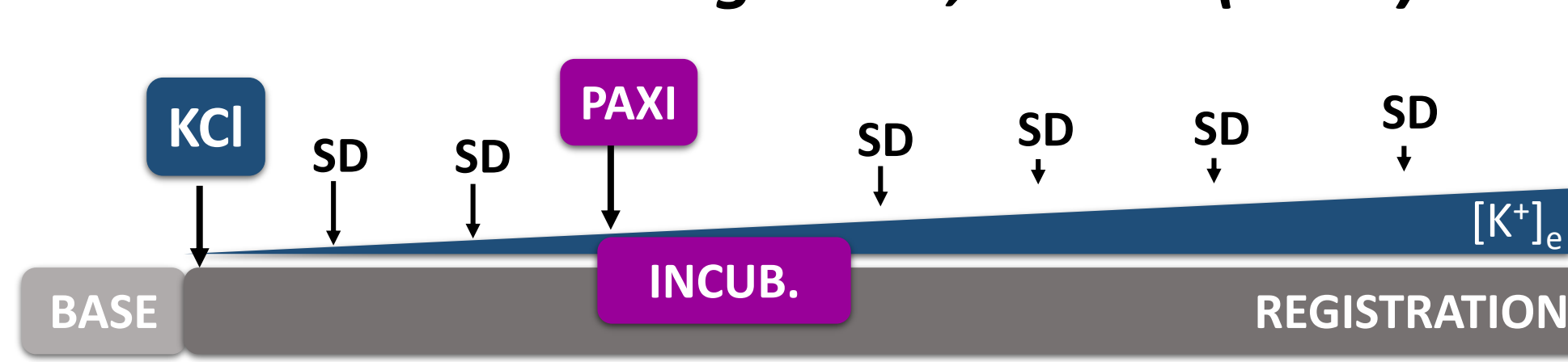
BK channel antagonism; in vivo

Animals: C57BL/6 mice (n=8)
Anesthesia: Isoflurane (1.5-2%) in $N_2O:O_2$
Two craniotomies (Figure)
SD elicitation: 1M KCl
Acquired parameters:
• DC potential (microelectrode)
• extracellular K^+ (K^+ sensitive microelectrode)
• CBF (laser Doppler flowmeter)
Same set-up for group treated with 500 nM paxilline (n=6).

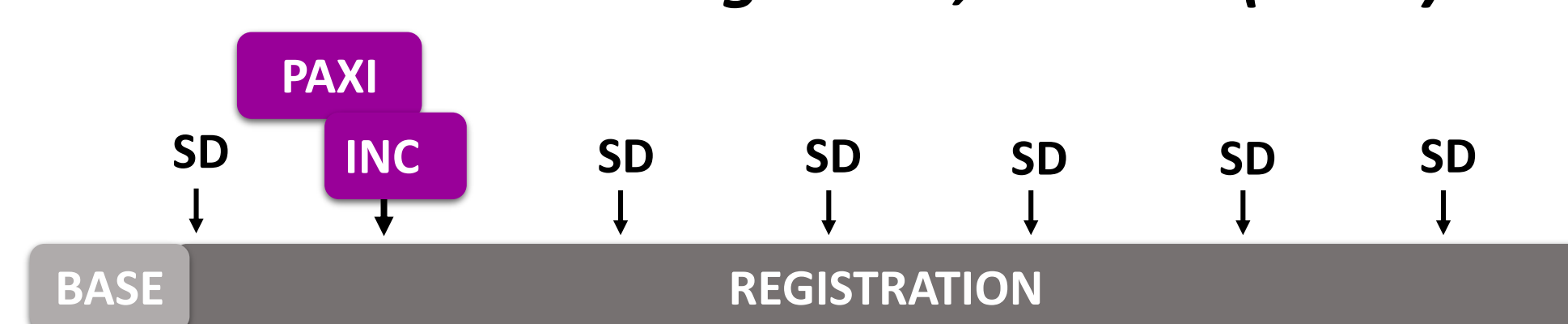
Extracellular K^+ imaging (n=6)



BK channel antagonism; in vivo (n=14)



BK channel antagonism; in vitro (n=20)

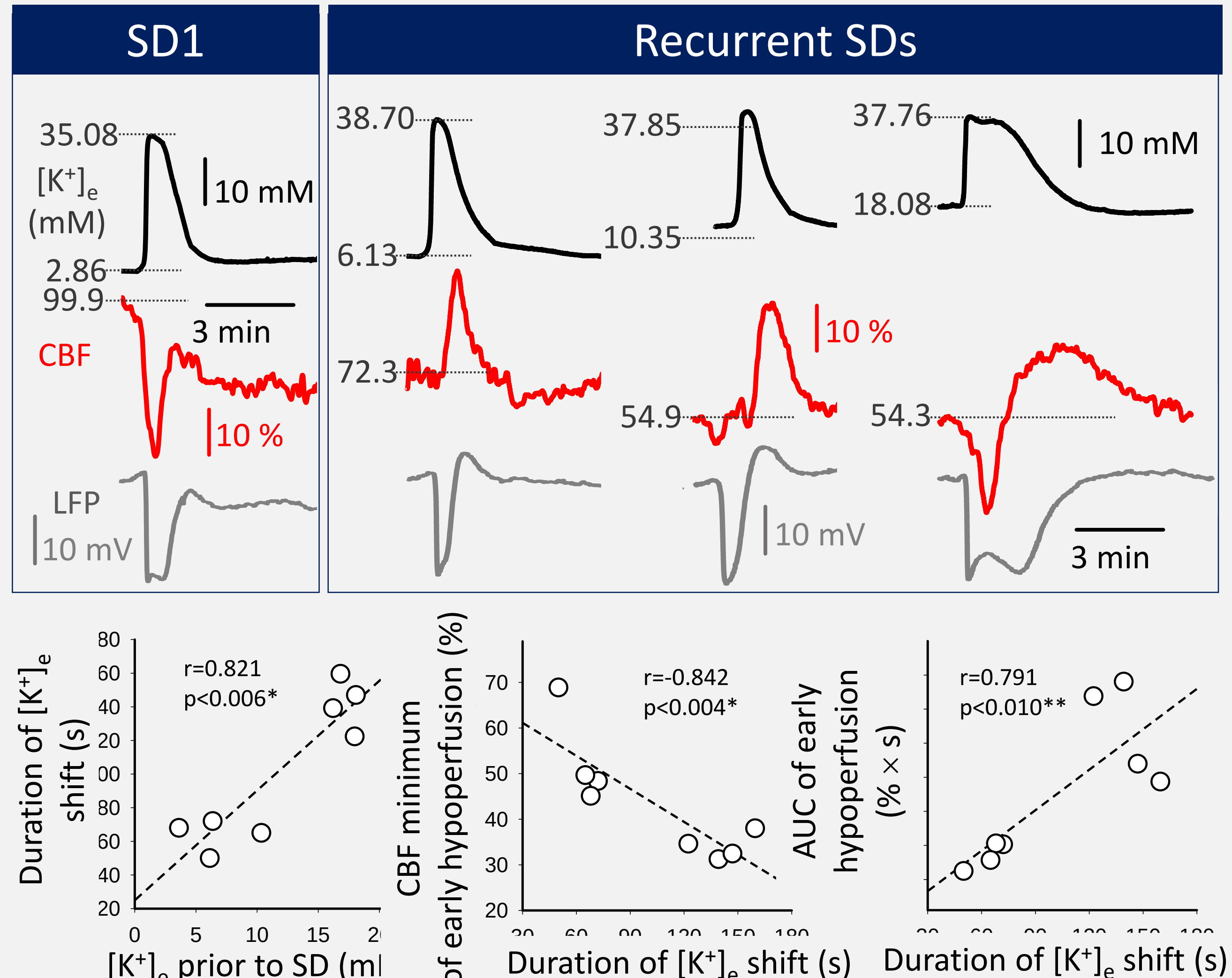


BK channel antagonism; in vitro

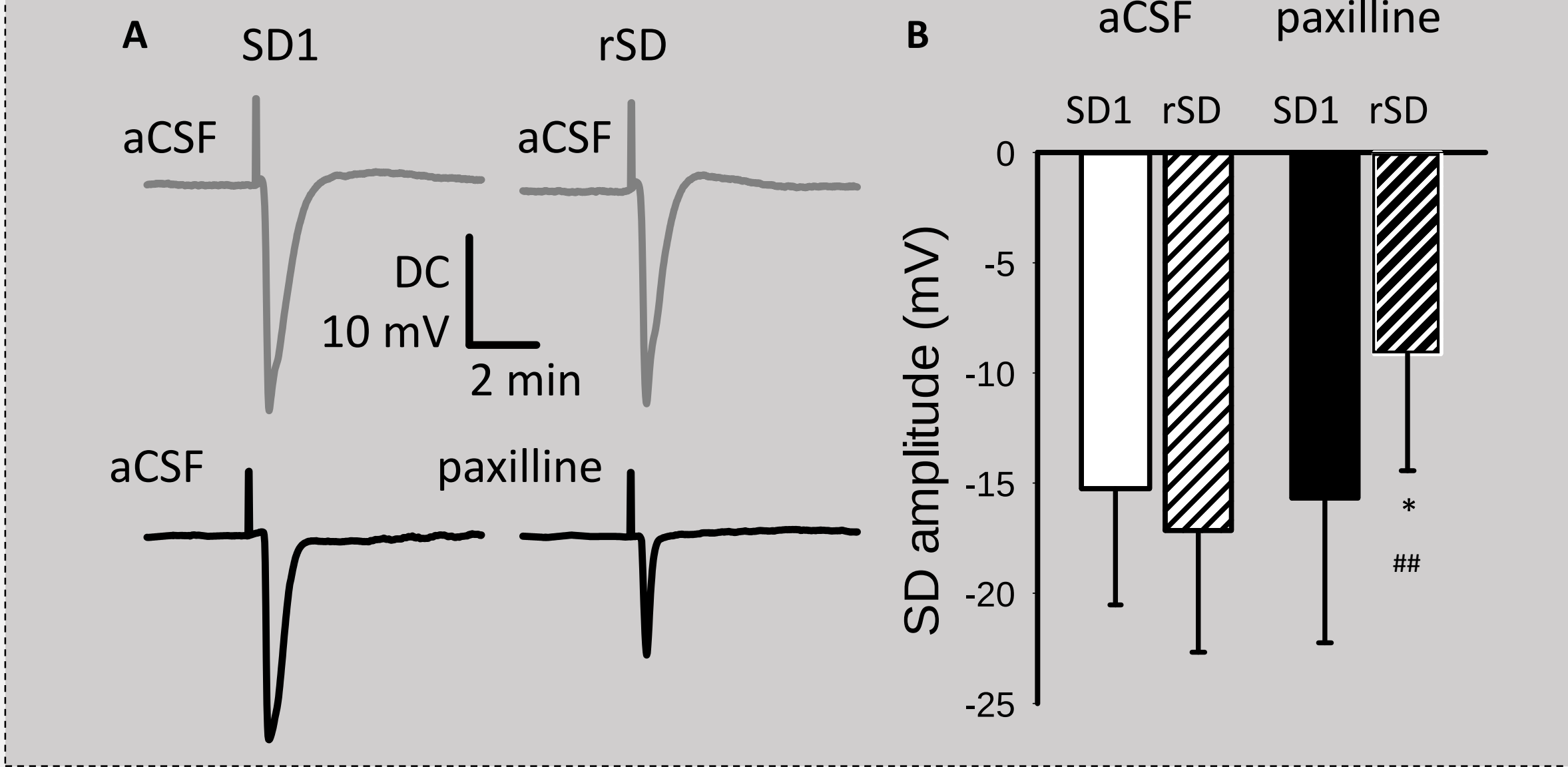
Animals: male Sprague-Dawley rats
Decapitation under Isoflurane anesthesia
Brain slices: 350 μ m (n=5, SD: n=20)
SD elicitation: electric stimulation, 50-1500 μ C

Acquired parameters:
• DC potential (microelectrode) 800-1000 μ m from stimulating electrode
Paxilline treatment: 500 nM

EXTRACELLULAR ELECTROPHYSIOLOGY



BK CHANNEL ANTAGONISM IN VITRO



FUNDING

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