Supplemental material:

BG Ca^{2+} transient second phase is not mediated by α1 receptors or nitric oxide (NO)

It was reported previously that high frequency stimulation in the granule cell layer evokes $[Ca^{2+}]_i$ increases in BG mediated by activation of α1 adrenergic receptors by synaptically-released norepinephrine (Kulik et al, 1999). We therefore tested the effect of prazosin, an α1 receptor antagonist, on the second phase of the BG Ca^{2+} transients. Prazosin, at a concentration of 10 μM, did not affect BG Ca^{2+} transients and currents (107.2 ± 12.2 % and 103.6 ±7.6 % of control values, p=0.67 and 0.69 respectively, n=4, fig. S1).

In mice, electrical stimulation of parallel fibers induces transient and localized $[Ca^{2+}]_i$ elevations in BG processes and somata that are mediated by NO (Grosche et al, 1999; Matyash et al, 2001). We thus investigated the involvement of NO in the late phase of BG Ca^{2+} transients by using L-NAME, an NO synthase inhibitor. Incubation in 2 mM L-NAME for 15-20 minutes did not affect the amplitude of the second phase of BG Ca^{2+} transients and the currents (109.4 ± 19.7 % and 100.2 ± 12.9 % of control values, p=0.82 and 0.98 respectively, n=4, fig. S1).

Reactive blue 2 (RB-2) decreases the second phase of BG Ca^{2+} transients

Because PPADS may have effects on signaling cascades independently of P2 receptor binding (Shehnaz et al, 2000; see also Singaravelu and Deitmer 2006), we tested the effect of a different P2 receptor antagonist. The P2Y receptor antagonist RB-2 (50 μM) decreased the amplitude of BG Ca^{2+} transients (66.2 ± 8.4 % of control, p=0.001, n=5) without significantly altering the current amplitude (89.9 ± 4.5 %, p=0.12; Fig. S2). RB-2 also decreased the amplitude of BG Ca^{2+} transients elicited by 10 ms pressure applications of 1mM ATP onto BG processes (30.1 ± 6.5 % of control, p=0.005, n=5; Fig. S2).

mGluR5 do not participate to the generation of BG Ca^{2+} transients

It was reported recently that mGluR5 activation induces $[Ca^{2+}]_i$ increases in astrocytes of the barrel cortex *in vivo* (Wang et al, 2006). These receptors could thus play a role in the generation of BG Ca^{2+} transients. We found, however, that MPEP (10 μM), a specific mGluR5 antagonist, did not significantly affect the second phase of BG Ca^{2+} signals (82.2 ± 6.0 % of control, p=0.1, n=4, Fig. S3). We then tested whether glutamate transporters prevented activation of these receptors, as is the case for mGluR1 (see Fig. 3). Application of 50 μM TBOA in the presence of NBQX increased the amplitude of BG Ca^{2+} transients to 382.0 ± 41.2 % of control. Subsequent application of MPEP in the presence of TBOA did not alter BG Ca^{2+} transients significantly (345.0 ± 134.9 % of control, p= 0.69, n=4, Fig. S3). Therefore, mGluR5 do not participate in the generation of BG Ca^{2+} transients.

NBQX and R-CPP do not significantly alter BG Ca^{2+} transients

We tested whether activation of AMPA and NMDA receptors contributed to the generation of BG Ca^{2+} transients evoked by distal PF stimulation. NBQX 20 μM and R-CPP 10 μM, AMPA and NMDA receptor antagonists respectively, did not significantly alter the second phase of BG Ca^{2+} transients (89.2 ± 4.7 % of control, p=0.17; Fig. S4). The first phase and the currents, however, were inhibited (1^{st} phase: -2.6 ± 13.2 % of control, p=0.015; current: 49.6 ± 9.0 % of control, p=0.022; n=4; Fig. S4).

References

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Figure S1 legend

Supplemental figure S1: α1 adrenoceptor and NO do not mediate the second phase of BG Ca^{2+} transients.

A,B: Prazosin, an α1 adrenoceptor antagonist (A) and L-NAME, an NO synthase inhibitor (B) had no effect on BG Ca^{2+} transients. **C:** Summary histogram of the effect of prazosin and L-NAME on BG currents and Ca^{2+} transient second phase (n = 4 for each condition). I=BG current.

Figure S2 legend

Supplemental figure S2: Reactive Blue 2 (RB-2), a P2Y antagonist, decreases the amplitude of BG Ca^{2+} transients.

A: Sample traces showing the effect of 50 μM RB-2 on BG currents and Ca^{2+} transients. **B:** Example traces illustrating the effect of 50 μM RB-2 on BG Ca^{2+} transients elicited by pressure application of 1mM ATP. **C:** Summary histogram of the effect of RB-2 on BG currents and Ca^{2+} transients synaptically evoked (n=5) and on Ca^{2+} transients evoked by pressure application of ATP (n=5). ** $p<0.01$.

Figure S3 legend

Supplemental figure S3: mGluR5 do not mediate BG Ca^{2+} transients.

A: Sample traces showing the lack of effect of MPEP on BG Ca^{2+} transients. **B:** Summary histogram of the effect of MPEP (n = 4), of TBOA and of MPEP in the presence of TBOA(n = 5) on BG Ca^{2+} transients.

Figure S4 legend

Supplemental figure S4: AMPA and NMDA receptors do not mediate the second phase of BG Ca^{2+} transients evoked by distal PF stimulation.

A: Example traces showing the effect of NBQX and R-CPP on the BG Ca^{2+} transient first and second phase. **B:** Summary histogram of the effect of the two drugs on the 1^{st} and 2^{nd} phases of BG Ca^{2+} transients and on associated currents. * $p<0.05$.