N-Methyl-D-Aspartate Receptors are the primary source of striatal calcium *in* vivo and modulate continuous learning

Abstract:

Introduction:

Calcium is a ubiquitous molecule in neurons.

Striatal neurons contain multiple sources of calcium, including voltage-gated calcium channels, NMDARs, calcium-permeable AMPARs, and intracellular calcium stores.

In a previous study, we demonstrated that bulk calcium changes primarily reflect non-somatic calcium changes and only have a moderate relationship with action potential-driven activity. These results suggested that most calcium changes come from non-somatic sources. Yet, the physiological source of these calcium changes is unknown.

Systemic antagonism of NMDARs nearly fully abolishes all calcium signaling

*Ex vivo* studies have shown that N-Methyl-D-Aspartate receptors (NMDARs) are the main contributor to dendritic and spine calcium (Sabatini paper). Given the dense dendritic arbor of striatal medium spiny neurons (MSNs), we hypothesized that NMDARs are the primary contributors of non-somatic calcium changes, and thus, of bulk calcium signals. To test this, we expressed GCaMP8f in the dorsomedial striatum (DMS) of X mice (Y males, Z females) and implanted an optical fiber for fiber photometry recordings. We recorded bulk calcium activity using fiber photometry during an open field. After a baseline recording period, we injected intra-peritoneally (i.p.) the NMDAR non-competitive antagonist MK-801 (0.3mg/Kg) or saline. Surprisingly, blocking NMDARs with MK-801 diminished almost all calcium signaling, significantly reducing the number of spontaneous transients compared to a baseline injection (Figure 1c). To further characterize the effect of MK-801, we expressed GCaMP8f selectively in Drd1-expressing and Drd2-expressing medium spiny neurons (D1-MSNs and D2-MSNs), using a Cre-lox strategy. We injected MK-801 (0.3mg/Kg). To better understand the time course of MK-801, we quantified the number of transients before and after injection in five minute bins with respect to the first 5 minutes, including the data from saline and mice expressing non-selective GCaMP in panels 1c,d. We observed a decrease in transient over time, including the saline group. However, mice injected with MK-801 showed a significantly more pronounced decrease in transients. During the last five minutes, mice injected with saline had a transient rate of 60% with respect of the first 5 minutes of the recording. In contrast, all mice injected with MK-801 had close to zero transients (Non-selective GCaMP = 5%, D1-MSNs GCaMP = 7%, D2-MSNs GCaMP = 12%). MK-801 is a non-competitive NMDAR antagonist, which works as an open-channel pore blocker and should be an irreversible manipulation, potentially requiring a NMDAR turnover to recover. Thus, to test whether the effects of MK-801 on calcium signaling follow this, we again non-selectively expressed GCaMP8f in the DMS of ?? mice. We injected either saline, MK-801 or the competitive NMDAR antagonist CPP, whose pharmacokinetics have been well characterized (CPP MS paper). We then quantified calcium transients 30 minutes, 1 hour, 4 hours, 24 hours, 72 hours, and one week after injection.

Systemic antagonism of NMDARs disproportionately diminishes calcium signaling compared to spiking activity.

In our previous study, we showed that changes in striatal bulk calcium only have moderate correlations with spiking activity. Thus, here we tested whether systemic antagonism of NMDARs would have distinct effects on bulk calcium and action potential-driven activity. We expressed GCaMP8f in the DMS of 6 mice (#males, #females, age) and implanted an implant containing a fiber photometry fiber surrounded by 32-microwire electrodes for simultaneous *in vivo* electrophysiology and fiber photometry recordings. We recorded bulk calcium and spiking activity for a 20-minute baseline period. Then, we systemically injected 0.3mg/Kg of MK-801 and continued recording for 40 additional minutes. To compare the effects of MK-801 in spiking and bulk calcium activity, we first averaged the single unit and multi-unit activity (see methods). Then we detected peaks in activity in both calcium (calcium transients) and in the average spiking activity (spiking bursts, see methods). We observed a significant reduction in both calcium transients and spiking bursts. However, calcium transients were significantly more strongly diminished than spiking bursts. In the last 20 minutes of the recording, calcium transients were reduced to 15% compared to baseline (avg stats), while spiking bursts were reduced to 50% compared to baseline. This suggests that NMDAR antagonism disrupt spiking and bulk calcium activity distinctly, with stronger effects on calcium activity. To further compare the difference in effect of MK-801 on spiking and calcium activity, we found spiking bursts of similar amplitude in the baseline and post-MK801 period. We then assessed the change in calcium around these spiking bursts. We observed an increase in calcium around spiking bursts both in the baseline and post-MK801 period. However, spiking bursts of similar amplitude had diminished concurrent calcium after MK-801 injections. More generally, after MK-801 injections, the amplitude of spiking bursts had a weaker influence on the amplitude of calcium transients. This demonstrates that NMDAR antagonism diminishes both spiking and bulk calcium activity, with a significantly larger diminishment in calcium activity. Moreover, these results further support our previous findings, showing that bulk calcium activity and spiking activity reflect distinct cellular phenomena which, although correlated, are not fully dependent on each other.

Intra-striatal antagonism of NMDARs diminishes striatal calcium signaling in a dose-dependent manner.

A limitation of using systemic antagonism of NMDARs is the reduction in striatal calcium activity cannot be exclusively attributed to striatal NMDARs, given that all NMDARs are antagonized. To test whether the effects of MK-801 were specifically due to antagonism of striatal NMDARs, we unilaterally expressed GCaMP8f in the DMS of 10 mice. We then implanted these mice with a bundle consisting of an optical fiber and an infusion cannula in the DMS (Figure X). Using this approach, we were able to deliver MK-801 intra-striatally, approximately in the same volume to the field of view of the optical fiber. We recorded calcium activity in a 30 min baseline period. Then, we infused saline or different doses of MK-801 (0.1-4 mg/mL) over 10 minutes. Fifteen minutes after infusion we again recorded calcium activity for an additional hour. We first identified peaks in both the baseline period and the post-infusion period. After saline infusion,

NMDARs drive striatal calcium signaling during reward-related behaviors.

Intra-striatal antagonism of NMDARs impairs learning from rewarded trials in a two-armed bandit task.

Our results show that NMDARs is the main driver of calcium activity of the striatum both in spontaneous and reward-related behavior, and that calcium activity is independent of spiking activity. However, these results do not clarify what the role of NMDAR-driven striatal calcium is. Previous studies have shown that systemic antagonism of NMDARs decreases acquisition and performance of operant learning tasks, including reversal learning. Moreover, antagonism of NMDARs in the striatum has been shown to disrupt acquisition, but not maintenance of a fixed-ratio one (FR1) operant task. However, it is unknown whether striatal NMDARs are necessary for operant task that require continuous learning, such as a two-armed bandit task. To test whether intra-striatal infusion of MK-801 would disrupt performance in a two-armed bandit task, we first implanted bilateral cannulas in the DMS of mice and then trained these mice on the two-armed bandit task in their home cage using the FED3 devices until they were proficient in the task, as defined by a performance threshold (see methods). Twenty four hours before the test day, we removed the FED3 device from the mice’s home cages to induce mild food deprivation. We then infused saline or MK-801 into the striatum of mice and put the devices back in their cages and tested their performance on the task for the following 8 hours.