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.

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

**Results:**

Systemic antagonism of NMDARs abolishes most calcium signaling in the striatum, indepependently from changes in spiking activity.

*Ex vivo* studies have shown that N-Methyl-D-Aspartate receptors (NMDARs) are the main contributor to dendritic and spine calcium (Sabatini paper). Whether this holds *in vivo*, and what the relative contribution of NMDAR-driven calcium to the totality of calcium signaling is unclear. To test this, we expressed GCaMP8f in the dorsomedial striatum (DMS), either in all neurons, or in D1-expressing spiny projection neurons (SPNs) or D2-expressing SPNs (6 wild-type mice, A males, B females; 5 D1-Cre mice, A males, B females; 5 A2a-Cre mice, A males B females), and implanted an optical fiber for fiber photometry recordings. We recorded bulk calcium activity using fiber photometry during an open field. To test the contribution of NMDARs to bulk calcium signaling in striatal neurons, we first recorded a 15-minute baseline period, after which we in we injected intra-peritoneally (i.p.) the NMDAR non-competitive antagonist MK-801 (0.3mg/Kg), or saline as a control. Surprisingly, blocking NMDARs with MK-801 diminished the majority of calcium signaling. After injection, mice injected with MK801 showed a decrease in spontaneous calcium transients, with a sharper decrease 10 minutes after the injection (Figure 1C). After 30 minutes, mice injected with MK801 displayed a transient rate lower than 20% of their baseline in the three groups, while a transient rate of over 60% was observed after a saline injection (Figure 1C,D). This suggests that NMDARs are the primary contributor of, and are necessary for, calcium signaling in the striatum *in vivo*.

In the striatum, NMDARs also play an important role facilitating in DOWN to UP state transitions and spontaneous firing *in vivo* (refs). Therefore, the observed decrease in calcium signaling induced by NMDAR antagonism may be caused indirectly, through the reduction action potentials, which may lead to a decrease in calcium influx by voltage-gated calcium channels. To test this possibility, we performed *in vivo,* simultaneous action potential and bulk calcium activity recordings. We expressed GCaMP8f in the DMS of X mice (A males, B females) and implanted an array consisting of 32-micro wire electrodes surrounding an optical fiber in this region. Similar to the previous experiment, after a baseline recording period, we injected i.p. MK801 and measured changes in both average firing rate and bulk calcium activity. We identified peaks in average firing rate and calcium activity (hereon spiking bursts and calcium transients, respectively). We observed a reduction in the frequency of both spiking bursts and calcium transients. However, the reduction in calcium transients was significantly stronger, as the transient rate was less than 20% (specific #s) to that of baseline, while the frequency of spiking bursts was maintained to 50% (specific #s) (Figure 1G). This shows that although NMDARs are important for both striatal action potential and calcium activity, they play a much more substantial role in calcium activity.

In our previous study, we reported than most calcium transients do not occur concurrently with spiking bursts and vice versa. Yet, there is a consistent, moderate relationship between these two signals. To test whether this relationship is altered after antagonism of NMDARs, we first assessed calcium activity around spiking bursts of similar amplitude (see methods) in both the baseline period and after MK801 injections. We observed that spiking bursts of similar amplitude had diminished concurrent calcium after MK-801 injections (Figure 1H,I). *DECIDE WHETHER WE ARE GOING TO ADD THE OTHER GRAPHS OR NOT.*

All together, these results show that NMDARs are necessary for spontaneous striatal calcium signaling and likely are the primary source of striatal calcium.

NMDARs antagonism diminishes behaviorally-evoked calcium changes.

Intro paragraph

Paradoxically, NMDAR antagonism induces hyperlocomotion and does not prevent initiation of actions, including reward-related behaviors. While the previous experiments showed that NMDAR antagonism reduces spontaneous calcium activity, it is possible that behaviorally evoked calcium remains intact, which would be consistent with these behavioral observations. To test this, we first assessed how the relationship between locomotor properties such as speed and acceleration changed after NMDAR antagonism. To do this, we expressed GCaMP8f in the DMS of X D1-Cre mice (A males and B females) and X A2a-Cre (A males and B females) and implanted an optical fiber in the same region.

We recorded locomotor and calcium activity. After a 20-minute baseline period, we i.p. injected either saline or 0.3mg/Kg of MK801. As previously reported, injection of MK801, but not of saline, led to hyperlocomotion (Figure 2). To assess the effect MK801 injection on the relationship between locomotor properties we first assessed the average (normalized) calcium levels at different speeds. After a saline injection, we observed a nonlinear relationship between speed and calcium levels (Figure 2B, rho-value), consistent with a previous study from our group. In contrast,

After a saline injection, we observed SOME SORT OF RELATIONSHIP BETWEEN SPEED AND PHOTOMETRY. In contrast, after MK801, there was no relationship between speed and calcium levels. We further analyzed calcium activity around acceleration peaks. Consistent with previous literature, there was an increase in calcium activity around acceleration peaks after a saline injection. This calcium activity increase was substantially diminished after MK801 injection. Together, these results show that NMDAR disrupts the relationship between locomotor parameters and calcium activity.

Next, we assessed the calcium response around the fixed-ratio 1 (FR1) task.

All together, these experiments demonstrate that systemic antagonism of NMDARs diminishes both spontaneous and behaviorally evoked calcium activity, without preventing reward-based behavior, action initiation or locomotion.

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

A limitation of using systemic antagonism of NMDARs is that observed effects in striatal calcium signaling cannot directly be attributed to striatal NMDARs. For instance, the decrease in calcium activity may be caused by an overall reduction of excitatory drive in the entire brain. To directly address this possibility, we infused MK801 directly into the DMS of mice and recorded bulk calcium activity in this same region. To do this, expressed GCaMP8f in the DMS of 10 mice. We then implanted these mice with a bundle consisting of an optical fiber coupled to an infusion cannula in the DMS (Figure X). Using this approach, we delivered different doses of MK-801 (1uL at 0.1, 1, 2, or 4 mg/mL, see methods) or saline intra-striatally, approximately in the same volume to the field of view of the optical fiber. We observed that saline infusion reduced the amplitude of the signals detected by the fiber, likely due to changes in lights scattering caused by the solution. However, the dynamics and signal-to-noise ratio and transient rate remained unchanged.

These results demonstrate that striatal NDMARs are the primary drivers of striatal calcium signaling and that antagonism of these receptors

Intra-striatal antagonism of striatal NMDARs disrupts learning from rewards.

Our previous experiments demonstrate that NMDARs are the primary source of calcium in striatal neurons. An intriguing corollary to our results is the observation that NMDAR-driven calcium changes is highly dynamic, with many transient increases in activity.

NMDARs have widely been implicated in both synaptic plasticity and learning in the striatum. Yet, finding mechanisms that plausibly bridge the sub-second timescales of synaptic plasticity events and to those of behavioral learning events has proved to be challenging. One hypothesis is that NMDAR driven calcium influx work.

Under this hypothesis, NMDAR driven intracellular calcium allows learning by keeping a record of recent actions and restricting dopamine-driven plasticity to recently activated neurons. If this hypothesis is true, then disrupting NMDAR signaling should disrupt learning in a moment-to-moment timescale. For instance, in a trial-based task where optimal performance requires learning from previous trials, disruption of NMDAR signaling should disrupt reinforcement of previously rewarded action and thus decrease the performance in the task.

To test this hypothesis, we turned to a two-armed bandit task, where mice had to choose between a left or a right nose-poke to obtain a food reward. Each side was associated with either an 80% probability or a 20% probability of obtaining a reward (heron “bandit task”). These probabilities reversed every time mouse obtained 30 pellets. We trained 15 mice on this bandit task for at least two weeks in their home-cage, until they had reached pre-established performance criteria (see methods). Then, we implanted bilateral cannula implants into the DMS of 15 mice. After surgery recovery (see methods), we food deprived mice for a test 8-hr session (see methods). Prior to the beginning of the test session, we infused into the striatum saline or MK-801 (1uL per hemisphere at a 4mg/mL concentration). We then evaluated performance and learning metrics on the test session. First, we found no difference in the number of pellets mice obtained in the 8-hour session after infusion of MK-801 versus saline (Figure 4? stats here), showing the mice were able to properly perform the task. The number of pokes was slightly higher after MK-801 infusion, although this difference was not significant (Figure 4?, stats here).

To test whether performance and learning in the task was different after MK-801 vs saline infusion, we first measured the accuracy prior to the reversal of reward p

**Discussion:**

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.