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, independently 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 unknown. 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.

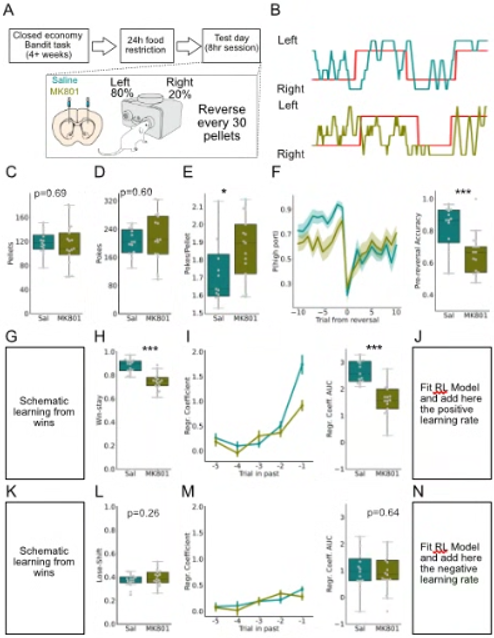
These results demonstrate that striatal NDMARs are the primary drivers of striatal calcium signaling and that antagonism of these receptors

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 the reward (heron “bandit task”). These probabilities reversed every time mouse obtained 30 pellets. We trained 15 mice (X males, Y females) 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 these mice. After surgery recovery (see methods), we food deprived the 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 found no difference in the number of pellets mice obtained in the 8-hour session after infusion of MK-801 versus saline, nor in the number of pokes the mice made (Figure 4C,D stats here), showing the mice were able to properly perform the task.

**Figure 4.** Antagonism of striatal NMDARs disrupt learning from rewarded actions. (A) Experimental design. Animals were trained for (B). Example behavior in the 8-hr test session of the same mouse after a saline (top) or an MK-801 (bottom) infusion. Red lines indicate true probability of reward, cyan and green lines indicate mouse behavior. (C-D) Number of pellets obtained (C), and pokes made (D) in the test session. (E) Ratio of pokes per pellets. (F) (Left) Peri-event histogram around probability reversals. Trial zero depicts first trial after reversal. (Right) Average accuracy (probability of poking on the high probability side) in the ten trials prior to the reversal. (G) Schematic of rewarded trials. (H) Quantification of win-stay behavior in test session (I) Influence of past rewarded trials in . (Left) Regressor coefficients of each of past 5 trials. (Right) Sum of the regressor coefficients. (J) Estimated learning rate from rewarded trials from a fitted RL model. (K) Schematic of unrewarded trials. (L) Lose-shift. (M) Same as I but for unrewarded trials. (N) Same as J but for unrewarded trials.

To test whether performance and learning in the task was different after MK-801 versos saline infusion, we first tested how many pokes it took, on average, to obtain a pellet. A higher number in this metric suggests worse performance and vice versa, with a value of 2 being chance level and a value of 1 being perfect performance. After MK-801 infusion, despite having similar number of pokes and pellets, it took mice more pokes to obtain a pellet than after a saline infusion (p-value<0.05).

To further investigate changes in performance and learning metrics, we assessed how well mice performed around the trials where the probabilities associated which each side were reversed. We observed that after a saline infusion, mice displayed an increasing accuracy as they approached the criterion (obtaining 30 pellets) for reversal of probabilities. In contrast, after an MK-801 infusion, mice did not show an increase in accuracy as the reversal of probabilities approached (Figure 4F, left). Overall, saline mice had a significantly higher average accuracy in the 10 trials prior probability reversal (Figure 4F, right). Interestingly, there was no difference in the average accuracy post-reversal (stats). There are important differences between the pre-reversal and post-reversal periods. Prior to the reversal, mice display high accuracy, with few mistakes made, and thus it would appear that mice are learning mostly from rewarded actions. In contrast, in the post-reversal period accuracy is low, with mice learning primarily from unrewarded actions. Prior studies have suggested that mice learn from rewarded and unrewarded actions at different rates (REFs), which suggest these learning strategies may be dissociable. Given the decrease in pre-reversal accuracy but not in post-reversal accuracy after MK-801 infusion and the prior literature, we investigated more in depth the effect of NMDAR antagonism in these two strategies.

First, we assessed differences in learning from rewarded trials (Figure 4G-J). After MK-801 infusion, mice displayed a significantly lower *win-stay* behavior (Figure 4H). Next, to expand on this analysis, we used logistic regression to determine how much influence the past 5 trials, if and only if they were rewarded, had on the next choice. The higher the regressor coefficient of a trial, the more influence it had on the next choice. In agreement with previous studies (REFS), we found that the most recent trial had the highest influence on choice, with trials farther in the past showing decreasing influence (Figure 4I, left). Notably, after MK-801 infusion, mice previously rewarded actions had significantly less influence on choice compared to saline infusions (Figure 4I, right). Finally, we estimated the learning rate from rewarded trials, using a well-established reinforcement learning model (*risk-sensitive reinforcement learning,* REFs) that uses two distinct learning rate parameters for learning from wins (positive learning rate) and losses (negative learning rate). We found that the estimated positive learning rate was significantly lower after an MK-801 infusion compared to the saline infusion. Overall,

Next, we assessed differences in learning from unrewarded trials (Figure 4K-N). We observed no difference in *lose-shift* behavior after MK-801 infusion compared to saline infusion (Figure 4L). However, we did observe that *lose-shift* behavior was below 0.5 in both, meaning that mice tend to repeat actions even after “losing”, consistent with previously described “sticky” behavior (REF). Next, we used logistic regression to measure the influence of past unrewarded trials on choice. We found that losses had smaller influence on choice compared to wins, and that antagonizing NMDARs had no effect in this influence (Figure 4M). Similarly, the estimated negative learning rate was not different after MK-801 infusion compared to saline (Figure 4N). Overall, these results demonstrate that the antagonism of striatal NMDARs leads to a robust decrease in learning from rewarded actions, but not unrewarded actions, suggesting that NMDAR-driven calcium signal is essential for moment-to-moment learning from rewarded actions.

Importantly, the effects of NMDAR antagonism were dose-dependent, as a lower concentration of MK-801 infusion (2mg/mL) led to similar effects, but with a lower effect size (Figure S8). Additionally, i.p. injections of MK-801 (1mg/Kg) using the same experimental paradigm led to similar, yet stronger effects (Figure S7), with, for instance, pre-reversal accuracy being no higher than chance levels in i.p. MK-801 injected mice (Figure S7F). This suggests that the volume restriction of local drug injections using cannulas leads to only partial, yet substantial, effects on learning. Yet, antagonism of all (or at least a majority of) striatal NMDARs through systemic delivery of antagonists, with likely additional contributions from NMDAR in other brain regions, leads to a very strong reduction of learning from rewarded actions.

Finally, we evaluated the possibility that these effects were due to an increase in non-goal directed behavior. We home-cage trained 6 mice (X males, Y females) on a Fixed Ratio 1 task (FR1 task), such that a left-side poke was associated with a 100% of probability of a food reward (active), while a right-side poke was associated with a 0% of reward (inactive), with no further change in these probabilities. We followed the same experimental paradigm as in the bandit task experiment, with training until proficiency was reached, bilateral cannula implantation in DMS, and a test session after surgery recovery. During the test session, mice infused with MK-801 (1uL per hemisphere, at 4mg/mL) obtained poked significantly more and obtained more pellets (although the increase in pellets obtained was not statistically significant, at p=0.07). Importantly, the ratio of active pokes and inactive pokes, and *win-stay* behavior was not different between MK-801 and saline infusion (Figure S8). This result also held for mice where MK-801 was injected i.p. (Figure S9). These results suggest that the decrease in performance and learning observed in the bandit task after striatal NMDAR antagonism was not due to an increase in non-goal directed actions but rather by a decrease in the capacity of learning from rewards.

**Discussion:**

Here, we demonstrated that NMDARs are the primary driver of striatal calcium *in vivo*. Moreover, we showed that NMDAR-driven calcium is necessary for moment-to-moment learning from rewarded actions.

Our previous study showed that most calcium changes in striatum come from non-somatic sources (REF), most likely driven by dendritic activity. However, MSN dendrites contain multiple calcium sources including NMDARs, different types of voltage-gated calcium channels, and calcium-permeable AMPARs. *Ex vivo*, it has been shown that NMDARs are the primary source of dendritic calcium. Here, we show that antagonism of NMDARs diminishes most striatal calcium signaling, supporting that NMDARs are the primary driver of calcium signaling *in vivo*. Importantly, we observed that NMDAR antagonism also decreased action potentials in striatal neurons, consistent with previous studies (REFs). […]

Thus, calcium signaling diminishment by NMDAR antagonism is at least partially independent from changes in action potential activity.

Thus, calcium influx through NMDARs appear to have an orthogonal function in addition to the facilitation of bursts of action potentials.

We interpret calcium influx through NMDARs as having two orthogonal functions: First, facilitating bursts of action potentials. Second, “tagging” recently active neurons, which presumably correlate with specific actions (REFS), making them eligible for dopamine-driven plasticity. This is consistent and partially explains the increase in activity of both D1-MSNs and D2-MSNs neurons upon reward-related behaviors and movement onset. When