N-Methyl-D-Aspartate Receptors drive striatal calcium signaling *in* vivo and modulate continuous learning.

Abstract.

Here, we studied the subcellular source and function of striatal calcium signaling *in vivo* using a combination of bulk calcium recordings, pharmacology and behavioral assessment. We report that systemic and local antagonism of striatal NMDARs strongly diminish spontaneous and behaviorally evoked calcium, in addition to disrupting learning from rewarded actions on a moment-to-moment basis. These results provide novel insight into the role and mechanisms of striatal calcium signaling.

Main text.

In a previous study, we showed that *in vivo* bulk calcium recordings in the striatum primarily reflect non-somatic changes in calcium (REF). However, the subcellular source of these changes and their function remained unknown. To answer these questions, we first turned to the literature and found that NMDARs are the primary source of dendritic calcium changes *ex vivo*. Therefore, we first tested whether NMDARs substantially contribute to the non-somatic calcium changes detected by bulk calcium recordings. To do this, we expressed the calcium sensor GCaMP8f in the dorsomedial striatum of A mice (x females, y males). Specifically, we expressed GCaMP8f in Drd1-expressing spiny projection neurons (D1-SPNs) of D1-Cre mice (X females, Y males) or Drd2-expressing SPNs (D2-SPNs) of A2a-Cre mice (X females, Y males) using a Cre-Lox viral strategy, or non-selectively in neurons of wild-type mice (X females, Y males, see Online Methods). To test the contribution of NMDARs to bulk calcium signaling in striatal neurons, we first recorded a 15-minute baseline period, and then we injected intra-peritoneally (i.p.) the NMDAR non-competitive antagonist MK-801 (0.3mg/Kg), or saline (in wild-type mice) as control. Mice injected with MK-801 displayed hyperlocomotion (Figure 1?), consistent with previous reports (REFs).

To test whether calcium signaling was disrupted by NMDAR antagonism, we first evaluated the rate of spontaneous calcium transients after an MK-801 or saline injection (see methods for detection strategy). Strikingly, mice injected with MK801, but not saline-injected mice, showed a very strong decrease in spontaneous calcium transients after 10 minutes. After 30 minutes, mice injected with MK801 displayed a transient rate lower than 20% compared to their baseline period, while a transient rate of over 60% was observed after a saline injection (Figure 1C,D). We also observed a significantly stronger decrease in the power of the calcium signal after an MK-801 injection compared to the saline injection (Figure 1?). No difference was observed in the effect of MK-801 in the D1-SPN, D2-SPN, and all-neurons groups, suggesting that this effect is not isolated to a specific neuronal population.

Additionally, after a saline injection, we observed a correlation between speed and calcium levels (Figure 1?), consistent with previous studies (REFs). After MK-801 injection, the relationship coefficient between speed and calcium levels was maintained, yet the range of calcium level changes was significantly reduced (Figure 1C, statistics), suggesting a decrease in calcium changes.