DYNAMIC CONTRIBUTIONS OF NUCLEUS ACCUMBENS D1- AND D2-MSNS TO CUE-OUTCOME ASSOCIATIVE LEARNING AND EXTINCTION

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Background/rationale: Adaptive behavior requires learning associations between environmental cues and motivationally relevant outcomes. The nucleus accumbens (NAc) shell is central to this process, yet the specific contributions of its two major neuronal subtypes – D1- and D2-expressing medium spiny neurons (MSNs) – remain unresolved. While classical models assign reward to D1-MSNs and aversion to D2-MSNs, emerging data suggest both populations may encode appetitive and aversive stimuli. However, how these populations differentially contribute to cue–outcome associative learning and extinction remains unclear.

Research questions: (1) Do NAc D1- and D2-MSNs differentially encode appetitive and aversive stimuli and cue-outcome learning? (2) Are these neuronal populations differentially engaged during extinction? (3) Is activity in either population causally required for extinction learning?

Methods: We used microendoscopic calcium imaging in D1-cre or A2A-cre (marking D2-MSNs) mice, expressing GCaMP6f, to track population activity in the NAc shell during exposure to appetitive (sucrose) and aversive (foot shock) unconditioned stimuli, as well as during appetitive and aversive Pavlovian conditioning (cue-outcome associations). Optogenetic inhibition of D2-MSNs was employed during extinction sessions of an aversive cue–shock association to test causal involvement.

Analysis: Analyses were not pre-registered, and results represent final findings. Sample size for calcium imaging was 6 D1-cre and 6 A2A-cre mice, with an average number of neurons per animal of 20. Neurons were classified as “responsive” based on significant deviation from baseline (permutation test, $p < 0.05$). Population-level decoders (support vector machine) were trained on activity during USs; peristimulus and population trajectory analyses characterized responses across trials. Sample size for optogenetics was 19 D1-YFP, 19 D1-eNpHR, 10 A2A-YFP and 18 A2A-eNpHR. Statistical comparisons of behavioral performance in the optogenetic experiments used unpaired *t-tests*, ANOVAs, and appropriate *post-hoc* tests were applied.

Results: Both D1- and D2-MSN populations discriminated between appetitive (sucrose) and aversive (shock) unconditioned stimuli: decoders achieved on average 86% accuracy for D1-MSNs and 77% accuracy for D2-MSNs. A majority of tracked neurons responded to both types of US: 67% of D1-MSNs and 78% of D2-MSNs were responsive to both sucrose and shock; responses were largely inhibitory to sucrose and excitatory to shock. During Pavlovian conditioning (appetitive and aversive), both populations were co-recruited: functional clustering revealed that D1- and D2-MSNs formed similar neuronal clusters under both appetitive and aversive conditions. In extinction of an aversive cue-shock association (i.e. repeated cue presentations without shock), there

was an asymmetric shift in neuronal responses with D2-MSNs maintaining robust CS-evoked activity across extinction days, whereas D1-MSN responses changed more dynamically (e.g. proportion of CS-excited D1-MSNs decreased over days; D1-inhibited cells increased over extinction). Critically, optogenetic inhibition of D2-MSNs during cue presentation across extinction sessions delayed extinction of conditioned aversive behavior ($F_{1,209}$=57.8, p<0.0001), providing causal evidence that D2-MSN activity is required for extinction of aversive associations.

Discussion: These findings challenge the classical dichotomy that assigns positive valence to D1-MSNs and negative valence to D2-MSNs. Instead, both neuronal subpopulations in the NAc shell reliably encode appetitive and aversive unconditioned stimuli and are co-recruited during associative learning. However, during extinction – when contingencies change – D2-MSNs are preferentially engaged, and their activity is necessary for extinguishing aversive associations. This supports a model in which D1- and D2-MSNs cooperate during learning but assume distinct roles when associations must be updated. These insights refine our understanding of NAc shell function in motivated behavior, with potential implications for disorders involving maladaptive learning (e.g., anxiety, addiction).

Keywords: Nucleus accumbens, Reward, Aversion, Associative learning

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