

Coding of behaviorally relevant and irrelevant cue features in the nucleus accumbens

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1 Abstract

2 to do

3 Significance Statement (120 words)

4 to do

5 Introduction

6 Theories of nucleus accumbens (NAc) function generally agree that this brain structure contributes to moti-
7 vated behavior, with some emphasizing a role in learning from reward prediction errors (Joel, Doya, Schultz;
8 see also the addiction literature on the effects of drug rewards; Nestler, Kalivas; Carelli) and others a role in
9 the modulation of ongoing behavior through stimuli associated with motivationally relevant outcomes (in-
10 vigorating, directing; Nicola, Floresco, Salamone). These proposals echo similar ideas on the functions of
11 the neuromodulator dopamine (Schultz, Berridge, Maia/Frank, Cools), with which the NAc is tightly linked
12 functionally as well as anatomically (Haber, Sesack, Takahashi).

13 Much of our understanding of NAc function comes from studies of how cues that predict motivationally
14 relevant outcomes (e.g. reward) influence behavior and neural activity in the NAc. Task designs that asso-
15 ciate such cues with rewarding outcomes provide a convenient access point eliciting conditioned responses
16 such as sign-tracking and goal-tracking (Robinson), pavlovian-instrumental transfer (Balleine) and enhanced
17 response vigor (Niv; McGinty), which tend to be affected by NAc manipulations (Flagel, Balleine, Chang;
18 although not always straightforwardly: Hauber, Chang). Similarly, analysis of reward prediction errors typ-
19 ically proceeds by establishing an association between a cue and subsequent reward, with NAc responses
20 transferring from outcome to the cue with learning (Schultz, Schoenbaum, Carelli). WHAT ABOUT HU-
21 MAN WORK

22 Surprisingly, although substantial work has been done on the coding of outcomes predicted by such cues
23 (e.g. reward value; Hollerman/Schultz, Roesch, Day; reward identity; Cooch), much less is known about
24 how reward-predictive cues themselves are encoded in the NAc (Hayden from primate realm). This is an
25 important issue for at least two reasons. First, in reinforcement learning, motivationally relevant outcomes
26 are typically temporally delayed relative to the cues that predict them. In order to solve the problem of as-
27 signing credit (or blame) across such temporal gaps, some trace of preceding activity needs to be maintained
28 (Maia?). Since NAc is a primary target of DA signals interpretable as RPEs, and NAc lesions impair RPEs

29 related to timing, its activity trace will help determine what can be learned when RPEs arrive (Takahashi).

30 Second, for ongoing behavior, the relevance of cues typically depends on context. In experimental settings,
31 context may include the identity of a preceding cue (occasion setter, Holland, Kesner), spatial or configural
32 arrangements (Good/Honey, Eichenbaum), and unsignaled rules as occurs in set shifting and other cognitive
33 control tasks (CITE). In such situations, the question arises how selective, context-dependent processing of
34 reward-predictive cues is implemented. For instance, is there a gate prior to NAc, or are all cues represented
35 in NAc but their current values dynamically updated (FitzGerald; WHAT ARE SOME THEORETICAL
36 POSITIONS ON THIS)?

37 Thus, both from a learning and a flexible performance perspective, it is of interest to determine how cue iden-
38 tity is represented in the brain, with NAc of particular interest given its anatomical and functional position
39 at the center of motivational systems. We sought to determine whether cue features signalling identity are
40 represented in the NAc, and if cue identity is represented alongside other motivationally relevant variables,
41 such as cue value (Figure 1). To address this question, we designed an experiment in which multiple, distinct
42 sets of cues predicted the same outcome. We recorded the activity of NAc neurons as rats performed this
43 task.

44 [Figure 1 about here.]

45 **Methods**

46 **Subjects:**

47 Adult male Long-Evans rats ($n = 4$, Charles River, Saint Constant, QC) were used as subjects. Rats were in-

48 individually housed with a 12/12-h light-dark cycle, and tested during the light cycle. Rats were food deprived
49 to 85-90% of their free feeding weight (weight at time of implantation was 440 - 470 g), and water restricted
50 4-6 hours before testing. All experimental procedures were approved by the the University of Waterloo An-
51 imal Care Committee (protocol# 11-06) and carried out in accordance with Canadian Council for Animal
52 Care (CCAC) guidelines.

53 **Overall timeline:**

54 Each subject was first handled for seven days where they were exposed to the running room, the sucrose
55 solution, and the click of the valves upon approach to the receptacles. They were then shaped to run on the
56 task for seven days where they were restricted to running in the clockwise direction by presenting a physical
57 barrier to running counterclockwise. Rats underwent hyperdrive implantation after showing discrimination
58 of approach behavior for rewarded and unrewarded cues for three consecutive days according to a chi square
59 test. Rats were allowed to recover for a minimum of five days before being retrained on the task, and
60 recording began once performance returned to pre-surgery levels. Upon completion of recording, animals
61 were sacrificed and recording sites were histologically confirmed.

62 **Behavioral task and training:**

63 Rats were trained to run clockwise on an elevated, square-shaped track (100x100 cm) containing four possi-
64 ble reward locations (Figure 2). Rats initiated a trial by running down the length of an arm, and triggering a
65 photobeam located 24 cm from the start of each arm. Upon trial initiation, a cue was presented that signaled
66 the presence or absence of a 12% sucrose water reward (0.1 mL) at the upcoming site. A trial was classified
67 as an approach trial if the rat turned left at the decision point and made a nosepoke at the reward receptacle
68 (40 cm from the decision point), while trials were classified as a skip trial if the rat instead turned right at the
69 decision point and triggered the photobeam to initiate the following trial. There was a 1 second delay from
70 a rewarded nosepoke and reward delivery. Each day rats were trained in both a light and sound block for

71 100 trials each. Within a block, one cue signaled reward was available on that trial, while the other signaled
72 reward was not available. Light block cues were a flashing white light, and a constant yellow light. Sound
73 block cues were a 2 kHz sine wave and a 8 kHz sine wave whose amplitude was modulated from 0 to maxi-
74 mum by a 2 Hz sine wave. Reward-cue associations were counterbalanced across rats. Cue presentation was
75 pseudorandomized so that the same cue could not be presented more than twice in a row. Block order within
76 each day was also pseudorandomized, such that the rat could not start with the block within a session more
77 than two days in a row. Each training or testing day consisted of a 5 minute pre-session period on a pedestal,
78 followed by the first block, then the second block, then a 5 minute post-session period on the pedestal. Ac-
79 curacy was determined by the proportion of trials a rat approached each cue. Perfect performance would be
80 100% approach on approach trials (reward available), and 0% approach on skip trials (no reward available).
81 Trial length was determined by measuring the length of time from cue onset until nosepoke or the start of the
82 following trial. Rats were trained daily until they could distinguish between the rewarded and unrewarded
83 cues for both light and sound blocks for three consecutive days according to a chi-square test, at which point
84 they underwent surgery. Furthermore, we generated linear mixed effects models to look at the relationships
85 between cue type and our behavioral variables. Cue type was used as a fixed effect, and we had an intercept
86 for rat identity as a random effect. Average proportion of trials approached and trial length for a session
87 were used as response variables. Contribution of cue type to behavior was determined by comparing the full
88 model to a model with cue type removed for each behavioral variable.

89 [Figure 2 about here.]

90 **Surgery:**

91 Surgical procedures were as described previously (Malhotra et al., 2015). Briefly, animals were anesthetized
92 with isoflurane, induced with 5% in medical grade oxygen and maintained at 2% throughout the surgery
93 (0.8 L/min). Rats were then chronically implanted with a hyperdrive consisting of 16 independently drivable
94 tetrodes, either all 16 targeted for the right NAc (AP +1.4 mm and ML +1.6 mm, relative to bregma; Paxinos

95 and Watson, 2005), or 12 in the right NAc and 4 targeted at the mPFC (AP +3.0 mm and ML +0.6 mm,
96 relative to bregma; only data from NAc tetrodes were analyzed). Following surgery, all animals were given
97 a least five days to recover and lower tetrodes to the target (DV -6.0 mm) before being reintroduced to the
98 behavioral task.

99 **Data acquisition and preprocessing:**

100 After recovery, rats were placed back on the task for recording. NAc signals were acquired at 20 kHz with
101 a RHA2132 v0810 preamplifier (Intan) and a KJE-1001/KJD-1000 data acquisition system (Amplipex).
102 Signals were referenced against a tetrode placed in the corpus callosum above the NAc.

103 Candidate spikes for sorting into putative single units were obtained by band-pass filtering the data between
104 600-9000 Hz, thresholding and aligning the peaks (UltraMegaSort2k, Hull et al., 2011). Spike waveforms
105 were then clustered with KlustaKwik using energy and the first derivative of energy as features (peak, valley,
106 peak index, wave PC1, time were used as extra features, does this need to be included?), and manually
107 sorted into units (MClust 3.5, A.D. Redish et al.). Isolated units containing a minimum of 200 spikes within
108 a session were included for subsequent analysis. Units were classified as high firing neurons if they had high
109 tonic firing rates marked by an absence of interspike intervals (ISIs) > 2 s, while medium spiny neurons had
110 a combination of ISIs > 2 s and phasic activity with shorter ISIs (Barnes 2005, Atallah 2014).

111 **Data analysis:**

112 Average firing rates for a session were generated for the 1 s preceding cue-onset, and the 1 s following cue-
113 onset. Single units were considered to be cue-responsive if both the mean firing rate difference between pre-
114 and post-cue onset was within the lower or upper 2.5% of a shuffled distribution, and a Wilcoxon signed-rank
115 test comparing pre- and post-cue firing was $p < .01$. Units where a Mann-Whitney U test revealed a drift
116 in firing rate between the first and second half of the trials in either task block were excluded from analysis.

117 Cue-modulated responses were classified as either increasing or decreasing if the post-cue activity was higher
118 or lower than the pre-cue activity, respectively. A stepwise general linear model (GLM) was then fit to cue-
119 responsive units using cue modality, cue location, cue outcome, approach behavior, trial length, trial number,
120 and trial history as predictors, and 1 s post-cue firing rate as the response variable. Units were classified as
121 being modulated by a given task parameter if addition of the parameter significantly improvement model
122 fit using deviance as the criterion ($p < .01$). A comparison of the R-squared value between the complete
123 model and the model minus the predictor of interest was used to determine the amount of firing rate variance
124 explained by the addition of that predictor. Spike trains were convolved with a Gaussian kernel (Matt: do I
125 need to show or say something that convolving the spike trains did not interfere too much with the response
126 around stimulus onset?), and peri-event time histograms (PETHs) were generated by taking the average of the
127 convolved spike trains across trials for a given task condition. To analyze population-level responses for cue
128 features, convolved spike trains for all cells where a specified cue feature explained a significant portion of
129 firing rate variance were z-scored. Normalized spike trains were separated according to preferred and non-
130 preferred cue condition, and averaged across cells to generate population-level averages. STATISTICAL
131 TEST? To visualize NAc representation of task space within cue conditions, normalized spike trains for all
132 cells were ordered by the location of their maximum or minimum firing rate for a specified cue condition. To
133 compare representation of task space across cue conditions, the ordering of cells taken from one condition
134 was then applied to the normalized spike trains from the condition to be compared. STATISTICAL TEST?
135 The same cells identified as being responsive to cue-onset were also analyzed at the time of nosepoke into a
136 reward receptacle using similar methods. All analyses were completed in MATLAB R2015a, and the code
137 is available on GitHub.

138 **Histology:**

139 Upon completion of the experiment, rats were anesthetized with 5% isoflurane, then asphyxiated with carbon
140 dioxide. Transcardial perfusions were performed, and brains were fixed and removed. Brains were sliced in
141 50 um coronal sections and stained with thionin. Slices were visualized under light microscopy, and tetrode

¹⁴² placement was determined (Figure 3).

¹⁴³ [Figure 3 about here.]

¹⁴⁴ Results

¹⁴⁵ Behavior

¹⁴⁶ Rats were trained to discriminate between cues signaling the availability and absence of reward on a square
¹⁴⁷ track with four identical arms for two distinct sets of cues. An example learning curve is seen in Figure
¹⁴⁸ 4A,B. All four rats learned to discriminate between the rewarded and unrewarded cue for both the light and
¹⁴⁹ sound blocks as determined by reaching significance ($p < .05$) on a daily chi-square test comparing approach
¹⁵⁰ behavior for rewarded and unrewarded cues for each block, for at least three consecutive days. Linear mixed
¹⁵¹ effects models revealed that cue type had an influence on the likelihood of a rat to make an approach ($p <$
¹⁵² $.001$), but not for the length of time taken to complete a trial ($p = .13$)(Figure 4C,D).

¹⁵³ [Figure 4 about here.]

¹⁵⁴ NAc neurons encode behaviorally relevant and irrelevant cue features

¹⁵⁵ General responses to cue:

¹⁵⁶ A total of 443 units were recorded in the NAc from 4 rats over 57 sessions (Table 1). The activity of 133

157 (30%) of these was modulated by the cue, with more units showing a decrease in firing ($n = 124$) than an
158 increase ($n = 47$) around the time of cue-onset (Table 2). Within this group, 32 were classified as HFNs, while
159 139 were classified as SPNs. Fitting a GLM to each unit revealed that a variety of task parameters accounted
160 for a significant portion of firing rate variance in NAc cue-modulated units (Figures ??, 6). Notably, there
161 were units that discriminated between whether the rat was performing in the light or sound block, which arm
162 the rat was currently on, and whether the rat was engaged in a rewarded or unrewarded trial (Figure 5A-F).
163 Interactions between multiple cue features appeared as significant predictors of firing rate variance for 9%
164 cue-modulated units, although this effect was relatively modest (Figure 5G,H). Fitting a GLM to all recorded
165 units...*Jimmie finish this* (data not shown).

166 [Table 1 about here.]

167 [Table 2 about here.]

168 [Figure 5 about here.]

169 [Figure 6 about here.]

170 **Population level responses:**

171 To get a sense of how cue information was encoded at the population level, firing activity for each unit
172 modulated by a cue feature was z-scored, then the population average for a cue feature was aligned to cue-
173 onset was generated (Fig 7). This visualization of the data revealed units whose activity was modulated by
174 cue modality showed a difference in firing rate across blocks that extended beyond the transient response to
175 the cue (Figure 7A,B). Additionally, units whom had exhibited a decrease in firing in response to the cue and
176 whose activity was modulated by cue outcome, showed a sustained response that extended beyond cue-onset

¹⁷⁷ (Figure 7F).

¹⁷⁸ [Figure 7 about here.]

¹⁷⁹ **NAc units segment the task:**

¹⁸⁰ NAc neurons have been shown to have correlates across an entire task space. To look at the distribution of
¹⁸¹ responses throughout our task space and see if this distribution is modulated by cue features, we z-scored the
¹⁸² firing rate of each unit and plotted the normalized firing rates of all units aligned to cue-onset and according
¹⁸³ to peak firing rate. We did this separately for both the light and sound blocks, and found a nearly uniform
¹⁸⁴ distribution of firing fields in task space that was not limited to alignment to the cue (Figure 8). Furthermore,
¹⁸⁵ to see if this population level activity was similar across blocks, we also organized firing during the sound
¹⁸⁶ blocks according to the ordering derived from the light blocks. This revealed, that the overall firing was
¹⁸⁷ qualitatively different across the two blocks. Additionally, given that the majority of our units showed an
¹⁸⁸ inhibitory response to the cue, we also plotted the firing rates according to the lowest time in firing. This
¹⁸⁹ process was repeated for cue location and cue outcomes.

¹⁹⁰ [Figure 8 about here.]

¹⁹¹ **Encoding of cue features is not limited to cue-onset:**

¹⁹² In order to be useful for learning, a trace of the cue must be maintained until the outcome. Fitting a GLM
¹⁹³ to the firing rates of cue-modulated units at the time of a nosepoke response showed that a variety of units
¹⁹⁴ still discriminated firing according to various cue features, but not other task parameters (Table 3, Figures
¹⁹⁵ 9, 10). Fitting a GLM to all recorded units...*Jimmie finish this* (data not shown). Furthermore, aligning
¹⁹⁶ normalized peak firing rates to nosepoke onset, revealed a clustering of responses around reward receipt

197 (Figure 11). Alongside this, fitting a GLM to the firing rates of cue-modulated units at the time of reward
198 receipt, revealed 10 units (8%) where cue outcome accounted for an average of 32% of firing rate variance
199 (data not shown).

200 [Table 3 about here.]

201 [Figure 9 about here.]

202 [Figure 10 about here.]

203 [Figure 11 about here.]

204 Discussion

205 The present study found evidence for coding of multiple identifying features of motivationally relevant stim-
206 uli; the sensory modality of the presented cue, as well as its physical location within the track. Furthermore,
207 this coding was both independent, and intermixed with coding for the associated outcome of the cue as well
208 as motivational vigor, measured by time to complete the trial. At the population level, a tiling of task struc-
209 ture was observed such that all points within our analyzed task space was accounted for by the ordered peak
210 firing rates of all cells, and this tiling differed between blocks where sound or light cues were presented.
211 Cells that discriminated across blocks were not simply due to drifting of the signal across trials, as cells that
212 showed a drift in firing between the first and second half within a block were excluded from the analysis.
213 Furthermore, even though actions were stereotyped during correct trials, such that the rat always turned left
214 at the decision point to approach for reward, and right to skip the receptacle and initiate the next trial, cells
215 that were modulated by the expected value of the cue maintained their specific firing patterns even during

216 error trials where the rat turned left after presentation of the unrewarded cue, suggesting that these signals
217 did not represent action values. Additionally, NAc signals have been shown to be modulated by response
218 vigor, to detangle this from our results we included the trial length as a predictor in our GLMs, and found
219 cells with correlates independent of trial length.

220 **Cue modality:**

221 Our finding that ventral striatal units can discriminate between cues from different sensory modalities ex-
222 pands upon an extensive literature examining neural correlates of conditioned stimuli. Perhaps the most
223 comparable work in rodents comes from a study that found distinct coding for an odor when it predicted
224 separate but equally valued rewards (Cooch). The present work is complementary to this as it shows that
225 ventral striatal cells have representations of identifiable aspects of the cue itself, in addition to the reward
226 it predicts. Another study paired separate cues with appetitive or aversive outcomes, and found separate
227 populations of cells that encode each cue, with many switching selectivity after reversal of the associations
228 between the cues and outcomes, providing evidence that the NAc encodes the biological significance of
229 stimuli. Once again, our study was different as we recorded neural responses to distinct cues encoding the
230 same anticipated outcome, suggesting that even when the biological relevance of these stimuli is similar, the
231 NAc dissociates their representations at the level of the single-unit (Setlow). Another possibility is that these
232 modality specific cells were encoding the context, rule, or sequence within a session as some cells responded
233 similar for both rewarded and unrewarded cues within a block. This interpretation is in alignment with a
234 recent paper from the primate realm that recorded ventral striatal responses during the Wisconsin Card Sort-
235 ing Task (WCST), a common set-shifting task used in both the laboratory and clinic, and found cells that
236 preferred firing to stimuli when a certain rule, or rule category was currently active (Sleazeer). Indeed, an
237 encoding of the current strategy could be an explanation as to why differentially tiling of task structure was
238 observed across blocks in the current study. Further support for a modulation of NAc responses by strategy
239 comes from an fMRI study that examined BOLD levels during a set-shifting task (FitzGerald et al., 2014). In
240 this task, participants learned two sets of stimuli-reward contingencies, a visual set and auditory set. During

241 testing they were presented with both simultaneously, and the stimulus dimension that was relevant was peri-
242 odically shifted between the two. Here, they found that bilateral NAc activity reflected value representations
243 of whatever the currently relevant stimulus dimension was, and not the irrelevant stimulus. The current find-
244 ing of separate, but overlapping, populations of cells encoding cue modality and expected value, suggests
245 that the fMRI finding is generated by the combined activity of several different functional cell types.

246 A caveat of the current study is that rats were never presented with both sets of cues simultaneously, and
247 thus never had to switch strategies, although extrapolating the data from the primate study, suggests that the
248 activity of the cue modality cells would be modulated by relevance. Keeping along this theme, the current
249 data set is unable to identify precisely what the modality-sensitive neurons were encoding, that is were they
250 tracking representations of stimulus identity, a preferred context, or even a macroscale representation of
251 progress through the session. Furthermore, their relevance for ongoing behavior is also uncertain. NAc core
252 lesions have been shown to impair shifting between different behavioral strategies, and it is possible that
253 selectively silencing the cells that prefer responding for a given modality or rule would impair performance
254 when the animal is required to use that information, or artificial enhancement of those cells would cause
255 them to use the rule when it is the inappropriate strategy.

256 **Encoding of position:**

257 Our finding that cue-evoked activity was modulated by cue location sides with some of the literature (Lavoie,
258 1994; Tabuchi, 2000; Strait, 2016). An alternative explanation for a pure spatial representation, is that
259 these are task segmentation correlates, keeping track of where in the task the rat is. A previous non-human
260 primate paper has shown that when reward is contingent upon completion of a series of trials, separate
261 populations of NAc neurons signal the start of a schedule, subsequent trials in the schedule, and the first
262 trial in extended schedules (Shidara et al., 1998). This signalling of position within a sequence has been
263 observed in subsequent studies, and it is possible that the our rats were keeping track of which specific arm
264 they were in as part of a sequence of arms, and not just strictly a spatial representation (Mulder, 2004 and

265 2005; Khamassi et al., 2008; Berke, 2009). Also, given that our task is pseudo-random, it is possible that the
266 rats learned which cue to anticipate, and the neural activity could reflect this. However, this is unlikely as
267 including a previous trial variable in the analysis did not explain a significant amount of firing rate variance
268 in response to the cue for the vast majority of cells..

269 **Mixed selectivity:**

270 Several other papers have reported unit profiles that integrate different task-related variables. These papers
271 report integrated coding between expected value and subsequent motor responses, expected value and iden-
272 tity of a reward, and a combination of spatial-, movement-, and reward-related features (Roesch, Lavoie,
273 Cooch). However, our study is the first to show mixed selectivity among identifying features of a cue and
274 expected outcome or behavior. The presence of mixed selectivity responses confers a larger number of input-
275 output relationships that are available to a given neuron. A possible functional consequence of this attribute
276 of NAc units, is the combination and transformation of various motivationally relevant features into a sig-
277 nal informing downstream decoders such as the ventral pallidum about appropriate behaviors in obtaining
278 motivationally relevant goals and biasing action selection towards these behaviors. Mixed selectivity in the
279 NAc could be a consequence of synaptic integration from a variety of anatomically distinct inputs, as seen
280 in experiments examining the convergence of various NAc afferents at the level of synaptic transmission and
281 stimulation-induced firing (Goto and Grace 2008). In one such experiment it was shown that NAc cells that
282 responded to stimulation of either the fornix, amygdala, or PFC, typically responded to stimulation from all
283 inputs (ODonnell and Grace, 1995). Furthermore, an interaction between these inputs was observed such
284 that PFC stimulation failed to elicit spiking in the NAc neurons unless they were in a depolarized UP-state, a
285 state induced by hippocampal stimulation and was dependent on an intact fornix. Hippocampal-induced sup-
286 pression of other inputs has also been observed for the BLA (Mulder et al., 1998). Recently, it has also been
287 shown that train stimulation of PFC afferents reduces hippocampal-evoked NAc responses, suggesting that
288 there is competition between various inputs (Calhoon and ODonnell, 2013). These studies suggest that the
289 integration of the variables we saw could be the result of this gating observed in behaviorally-independent

290 preparations. However, given that we did not systematically manipulate these various limbic and cortical
291 afferents, comments on the anatomical origins of the observed mixed selectivity responses are speculative at
292 this point.

293 Integrating cue identity and value, as seen in the present study, could be one neural instantiation of how
294 value is associated with the appropriate predictive stimuli (credit assignment), keeping in mind that value
295 encoding is distributed, redundantly in some aspects, across various structures (Hayden Nat Neuro opinion).
296 Indeed, lesions of the NAc impair the ability to learn changes in reward value or identity in an unblocking
297 experiment, as well as disrupting dopamine RPEs generated by modification of timing of reward (McDannald
298 2011, Takahashi 2016). Would be interesting to see if uncoupling the integrated coding of stimulus features
299 and predictive properties of a cue has an effect on the ability of a rat to use reward-predictive cues to pursue
300 the associated reward.

301 **Tiling of task structure:**

302 Additionally, we found that the population of recorded units had a relatively uniform distribution of firing
303 fields within our task space, similar to what has been reported previously (Shidara, 1998; Berke, 2009;
304 Lansink, 2012). Uniquely, we found that this representation differed according to whether the rat was cur-
305 rently engaged in the light or sound block, suggesting that this could be a possible neural correlate for
306 encoding the currently relevant strategy in the NAc. During progress through a predictable trial series, neu-
307 rons represented state value of cue (Shidara 1998). Single-unit responses allowed the monkey to know how
308 it was progressing throughout the task. Likewise, the tiling we saw could be a consequence of upstream cor-
309 tical or limbic inputs informing the striatum of the current task rules. Another possibility is that the NAc not
310 only pays attention to progress throughout a task within a trial, but also higher-order task information, like
311 blocks. Cue location was a behaviorally irrelevant variable in the current experiment, but it is possible that
312 if this tiling is dependent on hippocampal input, or related to a state value representation, that making cue
313 location a relevant variable by adding positional contingencies such as only alternating arms are rewarded

314 in one block, would result in a further separation of the mapping within a block between the rewarded and
315 unrewarded arms. Furthermore, dopamine levels in the NAc fluctuate through a trial, and it is possible that
316 the observed tiling could be a NAc-representation of state value related to this temporally evolving dopamine
317 signal. Future experiments should monitor this mapping of task structure during the application of dopamine
318 antagonists. Finally, the presence of functional correlates not evident when looking at single-unit responses
319 time-locked to salient task events emphasizes the need to employ ensemble level analyses across all aspects
320 of a task.

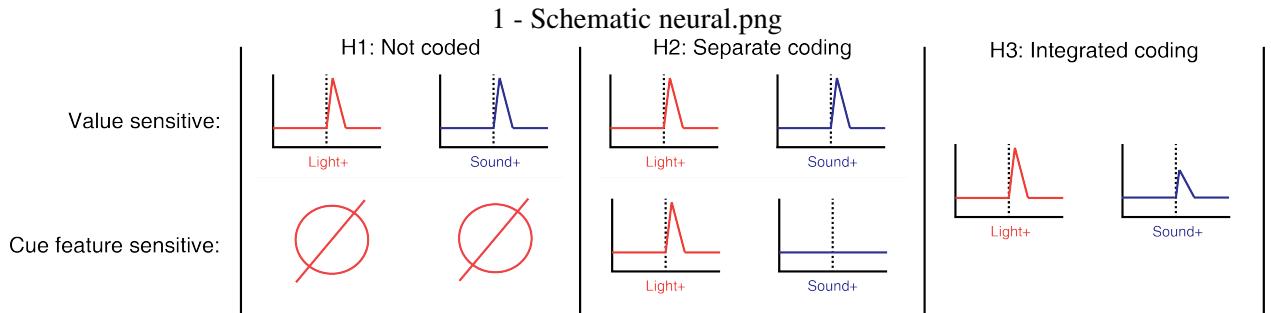


Figure 1: Schematic of potential coding strategies employed by single units in the NAc. Coding of identifying cue features could occur either be absent in the NAc (left), occur independently of encoding of motivationally relevant variables like expected value or subsequent vigor (middle), and/or be integrated with coding of other motivationally relevant variables (right).

2 - Schematic task.png

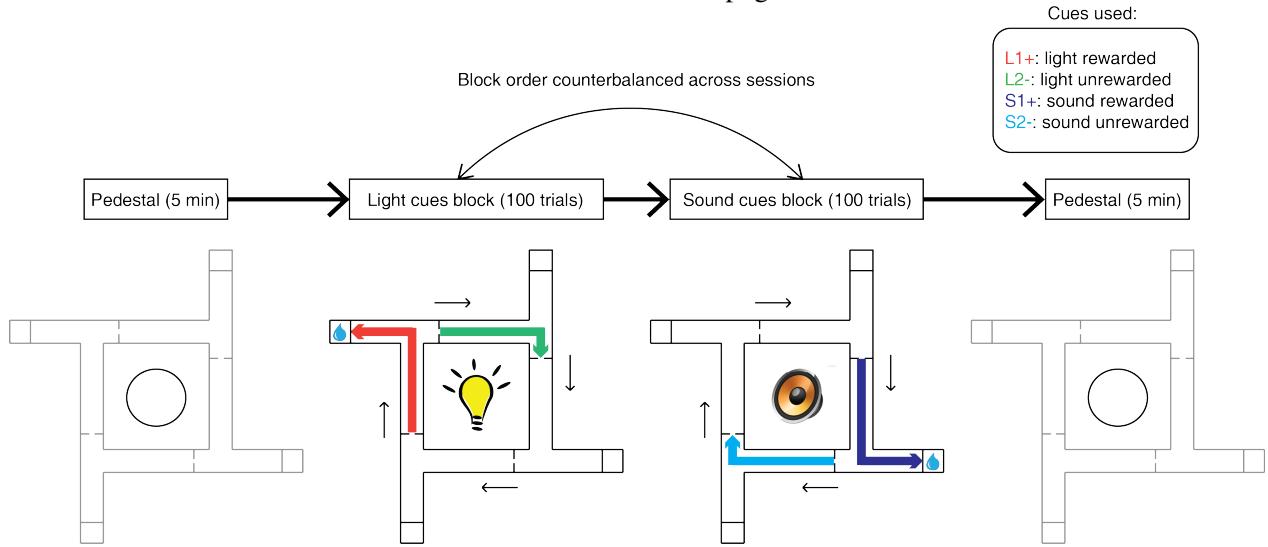


Figure 2: Schematic of behavioral task and procedure used in current study. A session was started with a 5 minute recording period on a pedestal place in the center of the apparatus (left). Rats then underwent two blocks of a cue discrimination task on a square track where they had to dissociate between a rewarded and unrewarded cue for a set of light cues and a set of sound cues for a target of 100 trials each (middle). Rats ran clockwise on the square track, and upon presentation of the cue had to turn left at the choice point to receive reward for a rewarded cue (approach trial; red and navy blue in figure), or turn right at the choice point to initiate the following trial for an unrewarded cue (skip trial; green and cyan in figure). Ordering of the light and sound blocks was counterbalanced across sessions. A session ended with another 5 minute recording period on the pedestal (right).

3 - Histology.png

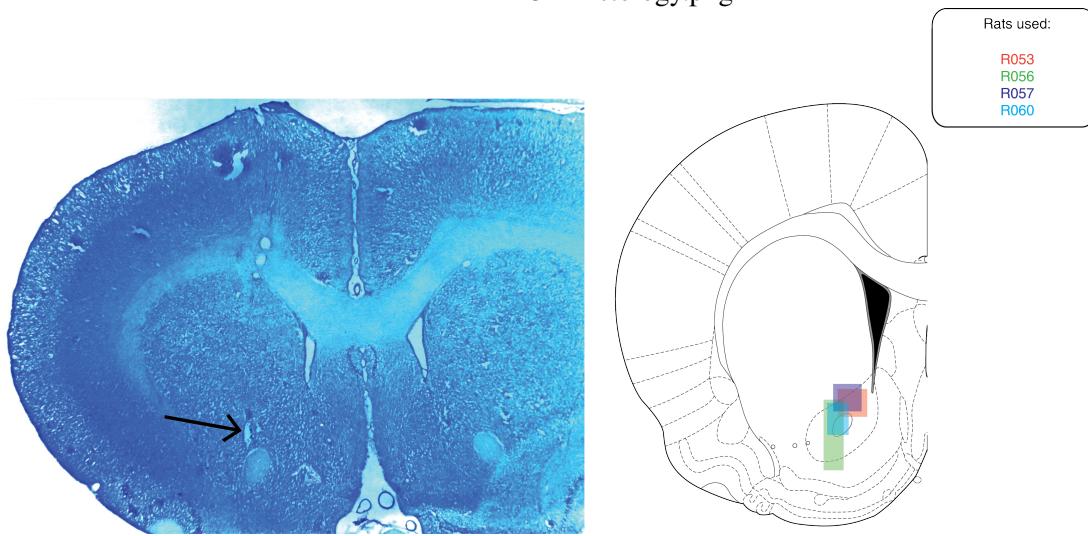


Figure 3: Histological verification of recording sites. Upon completion of experiments, brains were sliced and tetrode placement was confirmed. A. Example section from R060 showing a recording site in the NAc core. B. Schematic showing recording areas for the four rats used in the present study.

4 - Behavioral results.png

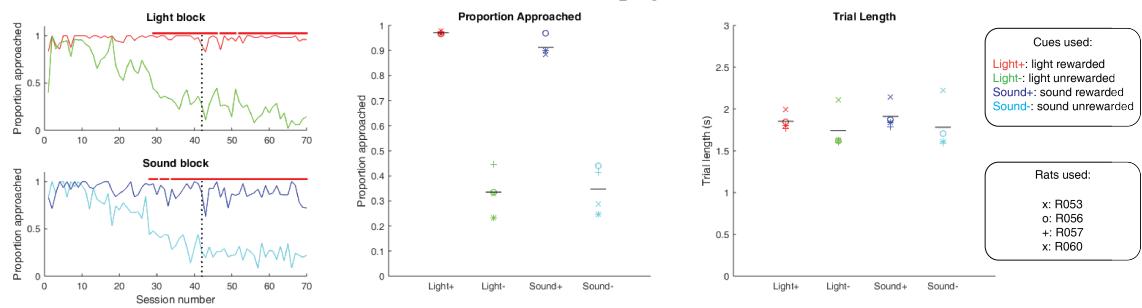


Figure 4: Performance on the behavioral task. A-B. Example learning curves from R060 showing acquisition and maintenance of performance in the light (A) and sound (B) blocks. Dependent measure is proportion of trials approached within a session for a given cue, with a value of 1 being perfect performance for rewarded cues, and 0 being perfect performance for unrewarded cues. Red bars indicate days in which a rat statistically discriminated between rewarded and unrewarded cues, determined by a chi square test. Dashed line indicates time of implantation. C-D. Summary of performance during recording sessions for each rat. C. All rats learned to discriminate between the rewarded and unrewarded cues within a block as measured by a comparison of linear mixed effects models, interpreted as a decreased likelihood to make an approach for an unrewarded cue. D. Average length of time to complete a trial for each cue. Rats on average showed a comparable length of time to complete a trial regardless of cue.

5 - Neural examples.png

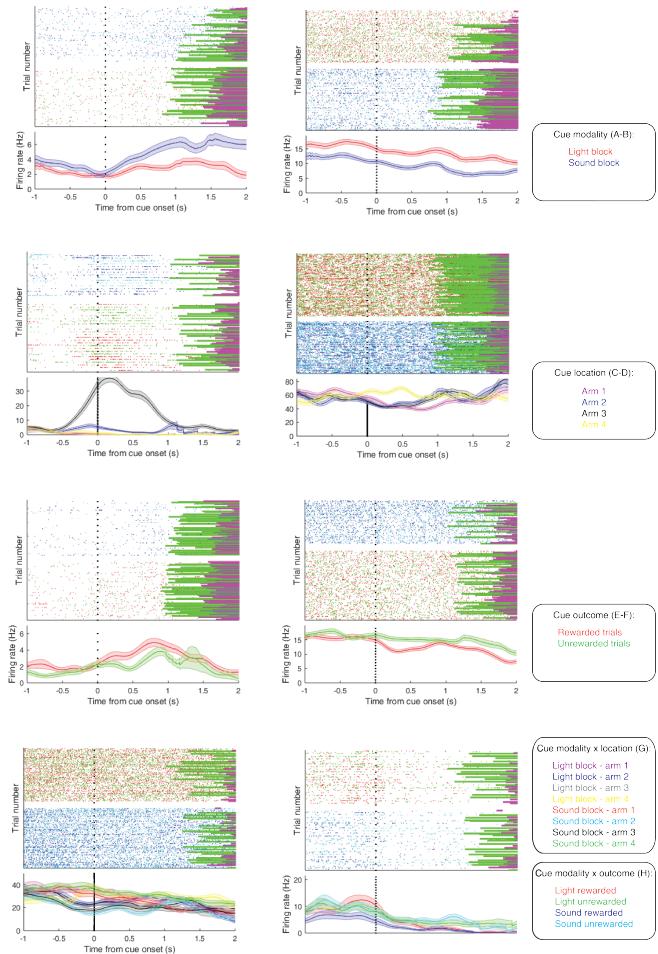


Figure 5: Individual examples of NAc units influenced by various task parameters. A. Example of a NAc unit that showed an increase in firing in response to the cue, and whose activity was influenced by which block the rat was in. Top: rasterplot showing the spiking activity across all trials aligned to cue-onset. Spikes across trials are color coded according to cue type, using the same scheme as in previous figures. Green and magenta bars indicate trial termination when a rat initiated the next trial or made a nosepoke, respectively. Bottom: PETHs showing the average smoothed firing rate for the unit for light (red) and sound (blue) blocks, aligned to cue-onset. B. Same as A for a unit that showed a decrease in firing. C-D. Same as A-B for cue location, each color in the PETHs represents average firing response for a different cue location. E-F. Same as A-B for cue outcome, with the PETHs comparing rewarded (red) and unrewarded (green) trials. G-H. Example of units who were modulated by both cue modality and location (G) and cue modality and outcome (H) using similar color schemes as the other examples.

6 - GLM.png

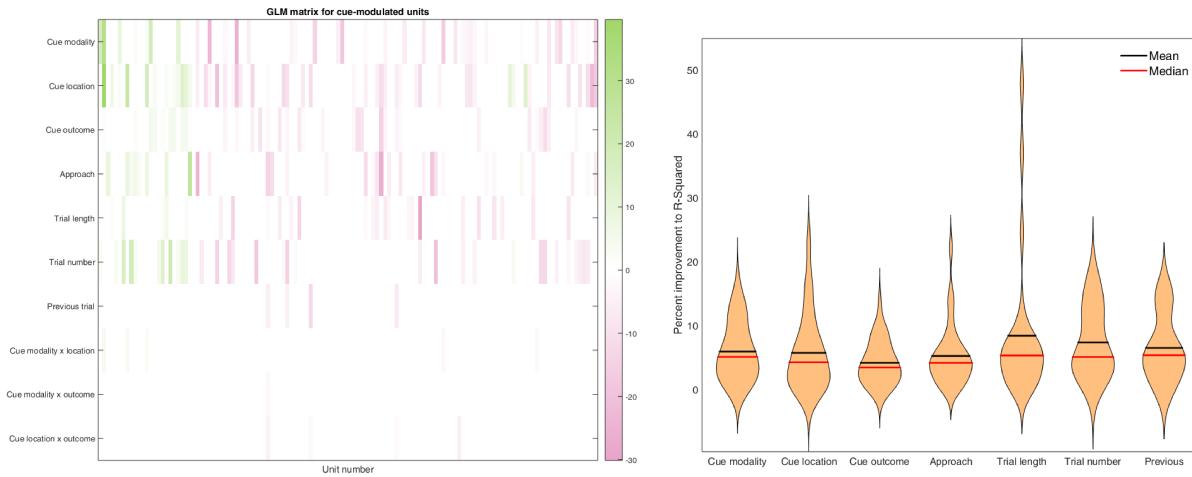


Figure 6: Summary of influence of various task parameters of cue-modulated NAc units after cue-onset. A. GLM matrix demonstrating impact of various task parameters on NAc firing rates. A GLM was fit to each unit that showed evidence of cue modulation by a Wilcoxon signed-rank test. Each row represents a given task parameter, and the x axis shows the influence of the task parameters for each unit, organized from left to right for MSNs that increased firing in response to the cue (green left), MSNs with a decreasing response (red left), FSIs with an increasing response (green right), FSIs with a decreasing response (red right). Response variable is how much of the firing rate variance an individual predictor contributed to the model, as measured by differences in R-squared between the final model and the model minus the predictor of interest. B. Bar graph demonstrating average change in R-squared value with the addition of each of the individual predictors.

7 - Population averages.png

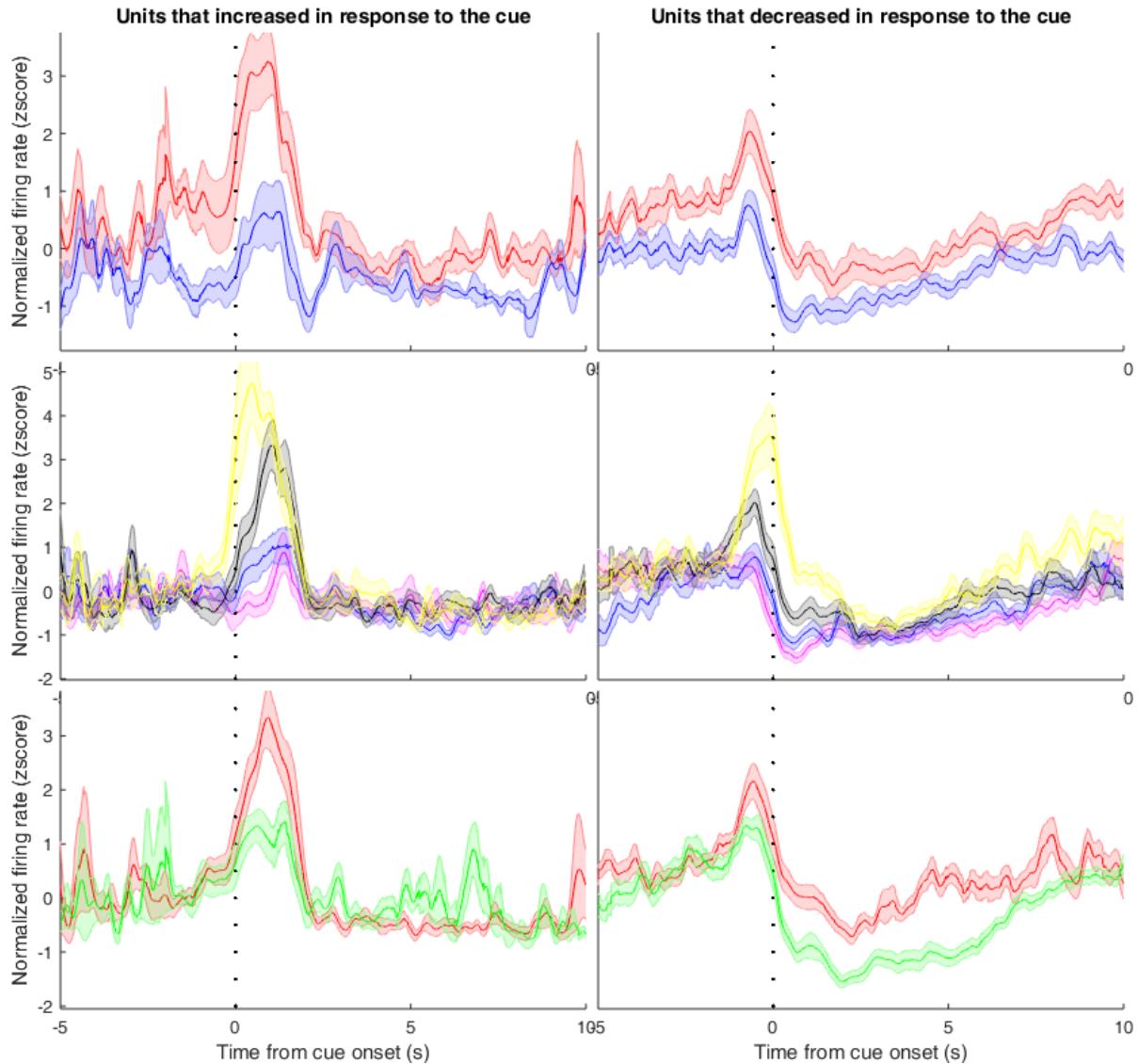


Figure 7: Population-level averages of cue feature sensitive NAc units. A. Average normalized activity for cue-modulated units where cue modality was a significant predictor in the GLM, aligned to cue-onset. Activity is plotted for preferred block (red) and nonpreferred block (blue). B. Same as A-B but for units that decreased in firing. C-D. Same as A-B for cue location. Activity is plotted from most preferred arm (yellow), in decreasing order to least preferred arm (black, navy blue, magenta, respectively). E-F. Same as A-B for cue outcome. Activity is plotted for preferred expected outcome (red), and nonpreferred outcome (green).

8 - Task tiling.png

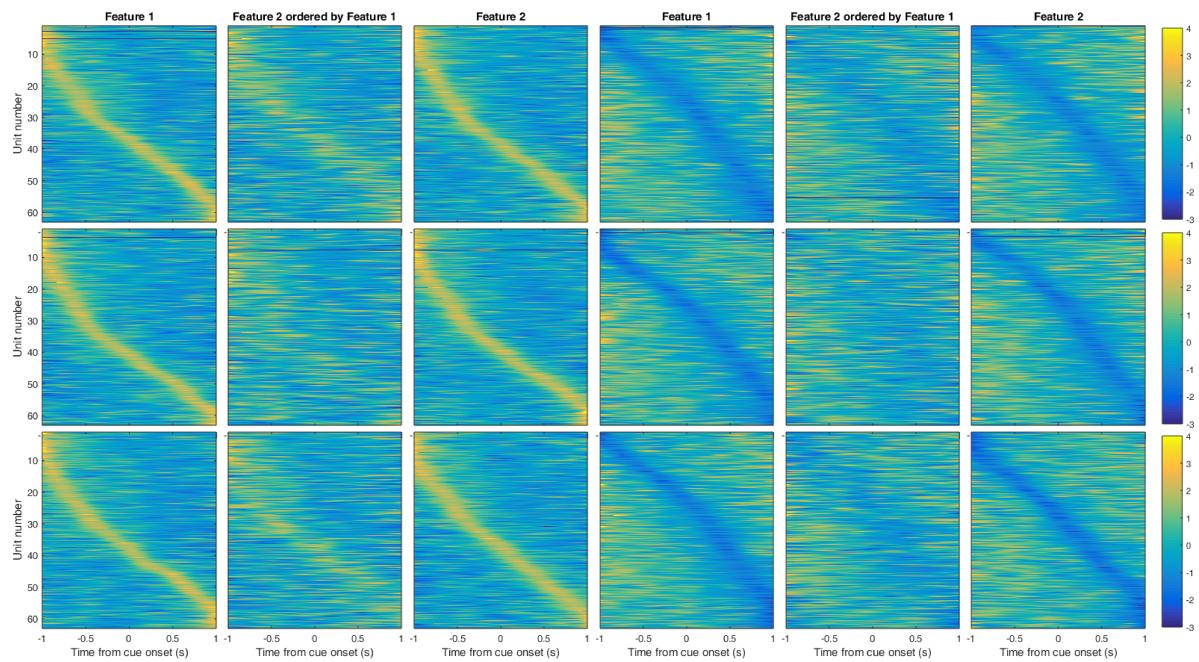


Figure 8: Distribution of NAc firing rates across task space surrounding cue-onset. A. Firing rates for all recorded units were normalized and ordered according to peak firing rates for light block (left) and sound block (right), aligned to cue-onset. Middle: Distribution of sound block firing rates using ordering for light block. B. Same as A but ordered according to minimum firing rate. C-D. Same as A-B for a comparison of two cue locations. E-F Same as A-B for comparison of rewarded and unrewarded cues.

9 - NP GLM.png

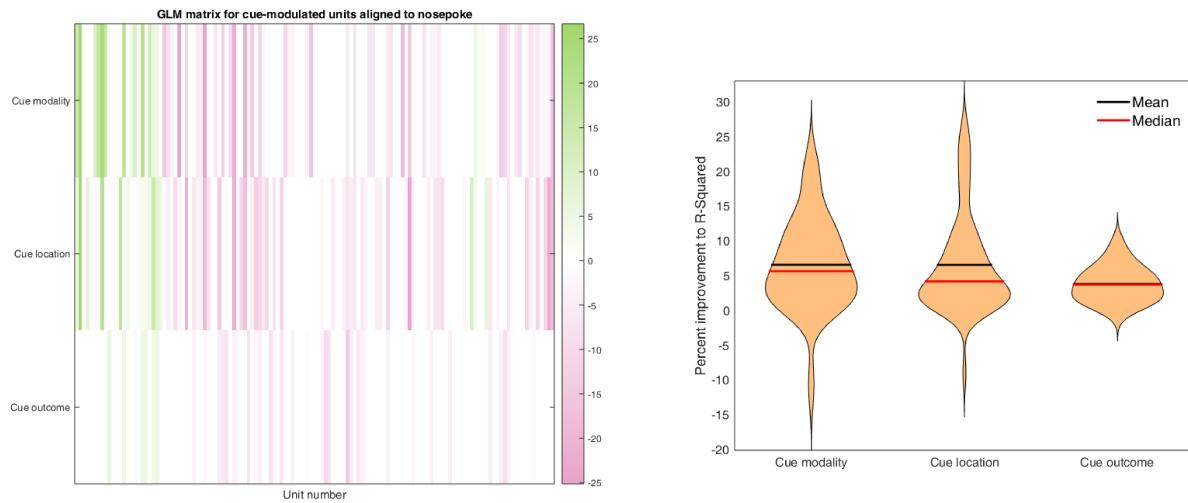


Figure 9: Summary of influence of various task parameters of cue-modulated NAc units during nosepoke. A. GLM matrix demonstrating impact of various task parameters on NAc firing rates. A GLM was fit to each unit that showed evidence of cue modulation by a Wilcoxon signed-rank test. Each row represents a given task parameter, and the x axis shows the influence of the task parameters for each unit, organized from left to right for MSNs that increased firing in response to the cue (green left), MSNs with a decreasing response (red left), FSIs with an increasing response (green right), FSIs with a decreasing response (red right). Response variable is how much of the firing rate variance an individual predictor contributed to the model, as measured by differences in R-squared between the final model and the model minus the predictor of interest. B. Bar graph demonstrating average change in R-squared value with the addition of each of the individual predictors.

10 - NP population averages.png

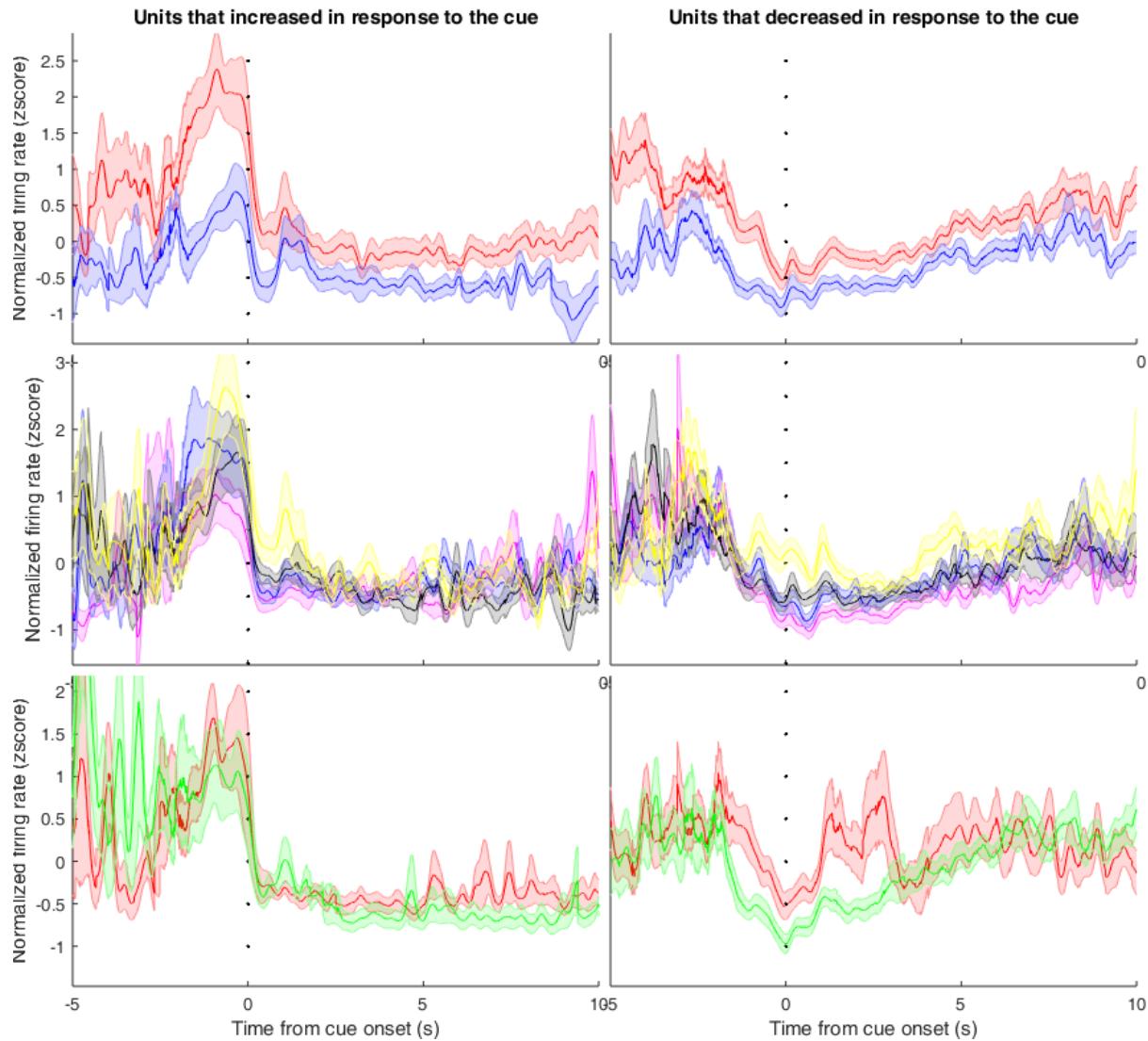


Figure 10: Population-level averages of cue feature sensitive NAc units during a nosepoke. A. Average normalized activity for cue-modulated units where cue modality was a significant predictor in the GLM, aligned to nosepoke with reward delivery occurring 1 s after nosepoke. Activity is plotted for preferred block (red) and nonpreferred block (blue). B. Same as but for units that decreased in firing. C-D. Same as A-B for cue location. Activity is plotted from most preferred arm (cyan), in decreasing order to least preferred arm (navy blue, green, red, respectively). E-F. Same as A-B for cue outcome. Activity is plotted for preferred expected outcome (red), and nonpreferred outcome (green).

11 - NP task tiling.png

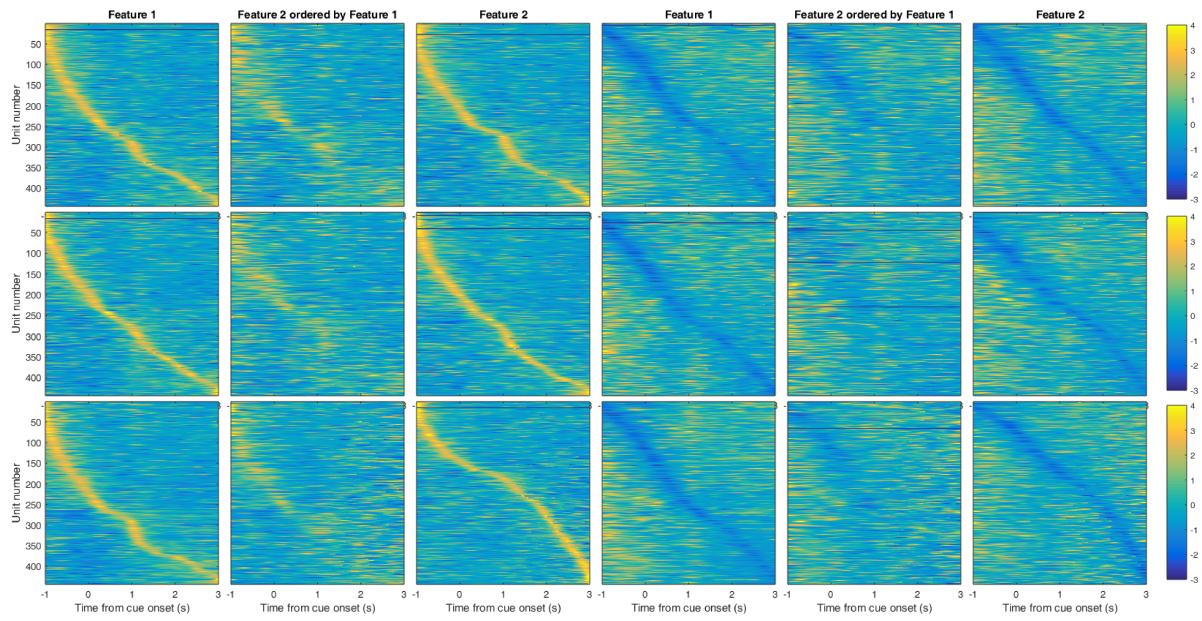


Figure 11: Distribution of NAc firing rates across task space during approach trials. A. Firing rates for all recorded units were normalized and ordered according to peak firing rates for light block (left) and sound block (right), aligned to nosepoke with reward delivery occurring 1 s after nosepoke. Middle: Distribution of sound block firing rates using ordering for light block. B. Same as A but ordered according to minimum firing rate. C-D. Same as A-B for a comparison of two cue locations. E-F Same as A-B for comparison of rewarded and unrewarded cues.

| Rat | Total | MSN (increasing) | MSN (decreasing) | FSI (increasing) | FSI (decreasing) |
|------|-------|------------------|------------------|------------------|------------------|
| R053 | 145 | 51 | 79 | 4 | 11 |
| R056 | 70 | 12 | 13 | 17 | 28 |
| R057 | 136 | 55 | 75 | 3 | 3 |
| R060 | 92 | 37 | 49 | 3 | 3 |

Table 1: Cells from each rat

| Task parameter | Total | MSN (increasing) | MSN (decreasing) | FSI (increasing) | FSI (decreasing) |
|-----------------------------|-------|------------------|------------------|------------------|------------------|
| All cells | 443 | 155 | 216 | 27 | 45 |
| Cue modulated | 133 | 24 | 85 | 6 | 18 |
| Cue modality | 37 | 7 | 21 | 1 | 8 |
| Cue location | 50 | 13 | 27 | 3 | 7 |
| Cue outcome | 34 | 10 | 18 | 0 | 6 |
| Approach behavior | 31 | 8 | 18 | 1 | 4 |
| Trial length | 25 | 5 | 18 | 0 | 2 |
| Trial number | 32 | 11 | 12 | 1 | 8 |
| Recent trial history | 5 | 0 | 5 | 0 | 0 |
| Cue x cue interactions | 11 | 3 | 7 | 0 | 1 |
| Cue x behavior interactions | ? | ? | ? | ? | ? |

Table 2: Cells from GLM

| Task parameter | Total | MSN (increasing) | MSN (decreasing) | FSI (increasing) | FSI (decreasing) |
|-----------------------------|-------|------------------|------------------|------------------|------------------|
| Cue modality | 66 | 14 | 36 | 2 | 14 |
| Cue location | 66 | 14 | 40 | 3 | 9 |
| Cue outcome | 42 | 8 | 29 | 0 | 5 |
| Trial length | 0 | 0 | 0 | 0 | 0 |
| Trial number | 0 | 0 | 0 | 0 | 0 |
| Recent trial history | 0 | 0 | 0 | 0 | 0 |
| Cue x cue interactions | 0 | 0 | 0 | 0 | 0 |
| Cue x behavior interactions | 0 | 0 | 0 | 0 | 0 |

Table 3: Cells from nosepoke GLM