

# **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 pos-  
64 sible reward locations (Figure 2). Rats initiated a trial by running down the length of an arm, and triggering  
65 a photobeam located 24 cm from the start of each arm. Upon trial initiation, a light or sound cue was pre-  
66 sented that signaled the presence of absence of a 12% sucrose water reward (0.1 mL) at the upcoming site.  
67 A trial was classified as an approach trial if the rat turned left at the decision point and made a nosepoke at  
68 the reward receptacle (40 cm from the decision point), while trials were classified as a skip trial if the rat  
69 instead turned right at the decision point and triggered the photobeam to initiate the following trial. There  
70 was a 1 second delay from between a rewarded nosepoke and subsequent reward delivery. Trial length was

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

93 [Figure 2 about here.]

94 **Surgery:**

95 Surgical procedures were as described previously (Malhotra et al., 2015). Briefly, animals were anesthetized  
96 with isoflurane, induced with 5% in medical grade oxygen and maintained at 2% throughout the surgery  
97 (0.8 L/min). Rats were then chronically implanted with a hyperdrive consisting of 16 independently drivable  
98 tetrodes, either all 16 targeted for the right NAc (AP +1.4 mm and ML +1.6 mm, relative to bregma; Paxinos  
99 and Watson, 2005), or 12 in the right NAc and 4 targeted at the mPFC (AP +3.0 mm and ML +0.6 mm,  
100 relative to bregma; only data from NAc tetrodes were analyzed). Following surgery, all animals were given  
101 a least five days to recover and lower tetrodes to the target (DV -6.0 mm) before being reintroduced to the  
102 behavioral task.

103 **Data acquisition and preprocessing:**

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

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

115 **Data analysis:**

116 ~~Average firing rates~~ To investigate the contribution of various cue features on NAc firing rates we first

117 determined whether firing rates for a unit were modulated by the onset of a cue by collapsing across all cues  
118 and comparing the firing rates for a session were generated for the 1 s preceding cue-onset, and with the 1 s  
119 following cue-onset. Single units were considered to be cue-responsive if both a Wilcoxon signed-rank test  
120 comparing pre- and post-cue firing had a  $p < .01$ . (Excluded: the mean firing rate difference between pre-  
121 and post-cue onset was within the lower or upper 2.5% of a shuffled distribution, and a Wilcoxon signed-rank  
122 test comparing pre- and post-cue firing was  $p < .01$ . Units where a Mann-Whitney U test revealed a drift  
123 in firing rate between the first and second half of the trials in either task block were excluded from analysis,  
124 as there is redundancy using both, and I did both because I was paranoid about just using one.). Cue-  
125 modulated responses were units were then classified as either increasing or decreasing in response to the cue  
126 if the post-cue activity was higher or lower than the pre-cue activity, respectively. A-

127 To determine the relative contribution of various task parameters to firing rate variance for units whose firing  
128 was modulated by cue-onset (as in Figures 5,6), a forward selection stepwise general linear model (GLM)  
129 was then fit to fit to each cue-responsive units using cue-unit. Cue modality, cue location, cue outcome,  
130 approach behavior, trial length, trial number, and trial history were used as predictors, and the 1 s post-cue  
131 firing rate as the response variable. Units were classified as being modulated by a given task parameter if  
132 addition of the parameter significantly improvement improved model fit using deviance as the criterion ( $p <$   
133  $.01$ ). A comparison of the R-squared value between the complete final model and the final model minus the  
134 predictor of interest was used to determine the amount of firing rate variance explained by the addition of  
135 that predictor. Spike for a given unit.

136 To better visualize responses to cues and enable subsequent population level analyses (as in Figures 5,7,8),  
137 spike trains were convolved with a Gaussian kernel (Matt: do I need to show or say something that convolving  
138 the spike trains did not interfere too much with the response around stimulus onset?), and peri-event time  
139 histograms (PETHs) were generated by taking the average of the convolved spike trains across trials for  
140 a given task condition. To analyze For analysis of population-level responses for cue features (Figure 7),  
141 convolved spike trains for all cells where a specified cue feature units where cue modality, cue location, or

142 cue outcome explained a significant portion of firing rate variance were z-scored. Normalized Within a given  
143 cue feature, normalized spike trains were then separated according to the preferred and non-preferred cue  
144 condition (e.g. light vs sound block), and averaged across cells units to generate population-level averages.  
145 STATISTICAL TEST?

146 To visualize NAc representation of task space within cue conditions, normalized spike trains for all cells  
147 units were ordered by the location of their maximum or minimum firing rate for a specified cue condition  
148 (Figure 8). To compare representation of task space across cue conditions for a cue feature, the ordering  
149 of cells taken from one condition units derived for one condition (e.g. light block) was then applied to the  
150 normalized spike trains from the condition to be compared. STATISTICAL TEST? The same cells identified  
151 as being responsive to cue-onset were also analyzed for the other condition (e.g. sound block).

152 To identify the responsivity of units to different cue features at the time of a nosepoke into a reward recepta-  
153 cle using similar methods., the same cue-responsive units from the cue-onset analyses were analyzed at the  
154 time of nosepoke using identical analysis techniques (Figures 9,10,11).

155 Given that some of our analyses compare firing rates across time, particularly comparisons across blocks,  
156 we sought to exclude units with unstable firing rates that would generate spurious results reflecting a drift  
157 in firing rate over time unrelated to our task. To do this we ran a Mann-Whitney U test comparing the  
158 cue-evoked firing rates for the first and second half of trials within a block, and excluded units from analysis  
159 that showed a significant change for either block. All analyses were completed in MATLAB R2015a, and  
160 the code is available on GitHub.

161 **Histology:**

162 Upon completion of the experiment, rats were anesthetized with 5% isoflurane, then asphyxiated with carbon  
163 dioxide. Transcardial perfusions were performed, and brains were fixed and removed. Brains were sliced in

<sup>164</sup> 50 um coronal sections and stained with thionin. Slices were visualized under light microscopy, ~~and~~tetrode  
<sup>165</sup> placement was determined, and electrodes with recording locations in the NAc were analyzed (Figure 3).

<sup>166</sup> [Figure 3 about here.]

## <sup>167</sup> **Results**

### <sup>168</sup> **Behavior**

<sup>169</sup> Rats were trained to discriminate between cues signaling the availability and absence of reward on a square  
<sup>170</sup> track with four identical arms for two distinct sets of cues. An example learning curve is seen in Figure  
<sup>171</sup> 4A,B. All four rats learned to discriminate between the rewarded and unrewarded cue for both the light and  
<sup>172</sup> sound blocks as determined by reaching significance ( $p < .05$ ) on a daily chi-square test comparing approach  
<sup>173</sup> behavior for rewarded and unrewarded cues for each block, for at least three consecutive days. Linear mixed  
<sup>174</sup> effects models revealed that cue type had an influence on the likelihood of a rat to make an approach ( $p <$   
<sup>175</sup> .001), but not for the length of time taken to complete a trial ( $p = .13$ )(Figure 4C,D).

<sup>176</sup> [Figure 4 about here.]

### <sup>177</sup> **NAc neurons encode behaviorally relevant and irrelevant cue features**

#### <sup>178</sup> **General responses to cue:**

179 A total of 443 units were recorded in the NAc from 4 rats over 57 sessions (Table 1). The activity of 133  
180 (30%) of these was modulated by the cue, with more units showing a decrease in firing ( $n = 124$ ) than an  
181 increase ( $n = 47$ ) around the time of cue-onset (Table 2). Within this group, 32 were classified as HFNs, while  
182 139 were classified as SPNs. Fitting a GLM to each unit revealed that a variety of task parameters accounted  
183 for a significant portion of firing rate variance in NAc cue-modulated units (Figure 6, ??Figures ??, 6).  
184 Notably, there were units that discriminated between whether the rat was performing in the light or sound  
185 block, which arm the rat was currently on, and whether the rat was engaged in a rewarded or unrewarded  
186 trial (Figure 5A-F). Interactions between multiple cue features appeared as significant predictors of firing  
187 rate variance for 9% cue-modulated units, although this effect was relatively modest (Figure 5G,H). Fitting  
188 a GLM to all recorded units...\*Jimmie finish this\* (data not shown).

189 [Table 1 about here.]

190 [Table 2 about here.]

191 [Figure 5 about here.]

192 [Figure 6 about here.]

193 ~~Average improvement to R-Squared for addition of each parameter. Matt: Was thinking of merging this with~~  
194 ~~the prior GLM matrix, is this worth taking up figure space?~~

195 **Population level responses:**

196 To get a sense of how cue information was encoded at the population level, firing activity for each unit  
197 modulated by a cue feature was z-scored, then the population average for a cue feature was aligned to cue-

198 onset was generated (Fig 7). This visualization of the data revealed units whose activity was modulated by  
199 cue modality showed a difference in firing rate across blocks that extended beyond the transient response to  
200 the cue (Figure 7A,B). Additionally, units whom had exhibited a decrease in firing in response to the cue and  
201 whose activity was modulated by cue outcome, showed a sustained response that extended beyond cue-onset  
202 (Figure 7F).

203 [Figure 7 about here.]

204 **NAc units segment the task:**

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

215 [Figure 8 about here.]

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

217 In order to be useful for learning, a trace of the cue must be maintained until the outcome. Fitting a GLM

218 to the firing rates of cue-modulated units at the time of a nosepoke response showed that a variety of units  
219 still discriminated firing according to various cue features, but not other task parameters (Table 3, Figures 9,  
220 22, 10). Fitting a GLM to all recorded units...\*Jimmie finish this\* (data not shown). Furthermore, aligning  
221 normalized peak firing rates to nosepoke onset, revealed a clustering of responses around reward receipt  
222 (Figure 11). Alongside this, fitting a GLM to the firing rates of cue-modulated units at the time of reward  
223 receipt, revealed 10 units (8%) where cue outcome accounted for an average of 32% of firing rate variance  
224 (data not shown).

225 [Table 3 about here.]

226 [Figure 9 about here.]

227 [Figure 10 about here.]

228 [Figure 11 about here.]

## 229 Discussion

230 The present study found evidence for coding of multiple identifying features of motivationally relevant stim-  
231 uli; the sensory modality of the presented cue, as well as its physical location within the track. Furthermore,  
232 this coding was both independent, and intermixed with coding for the associated outcome of the cue as well  
233 as motivational vigor, measured by time to complete the trial. At the population level, a tiling of task struc-  
234 ture was observed such that all points within our analyzed task space was accounted for by the ordered peak  
235 firing rates of all cells, and this tiling differed between blocks where sound or light cues were presented.  
236 Cells that discriminated across blocks were not simply due to drifting of the signal across trials, as cells that

237 showed a drift in firing between the first and second half within a block were excluded from the analysis.  
238 Furthermore, even though actions were stereotyped during correct trials, such that the rat always turned left  
239 at the decision point to approach for reward, and right to skip the receptacle and initiate the next trial, cells  
240 that were modulated by the expected value of the cue maintained their specific firing patterns even during  
241 error trials where the rat turned left after presentation of the unrewarded cue, suggesting that these signals  
242 did not represent action values. Additionally, NAc signals have been shown to be modulated by response  
243 vigor, to detangle this from our results we included the trial length as a predictor in our GLMs, and found  
244 cells with correlates independent of trial length.

245 **Cue modality:**

246 Our finding that ventral striatal units can discriminate between cues from different sensory modalities ex-  
247 pands upon an extensive literature examining neural correlates of conditioned stimuli. Perhaps the most  
248 comparable work in rodents comes from a study that found distinct coding for an odor when it predicted  
249 separate but equally valued rewards (Cooch). The present work is complementary to this as it shows that  
250 ventral striatal cells have representations of identifiable aspects of the cue itself, in addition to the reward  
251 it predicts. Another study paired separate cues with appetitive or aversive outcomes, and found separate  
252 populations of cells that encode each cue, with many switching selectivity after reversal of the associations  
253 between the cues and outcomes, providing evidence that the NAc encodes the biological significance of  
254 stimuli. Once again, our study was different as we recorded neural responses to distinct cues encoding the  
255 same anticipated outcome, suggesting that even when the biological relevance of these stimuli is similar, the  
256 NAc dissociates their representations at the level of the single-unit (Setlow). Another possibility is that these  
257 modality specific cells were encoding the context, rule, or sequence within a session as some cells responded  
258 similar for both rewarded and unrewarded cues within a block. This interpretation is in alignment with a  
259 recent paper from the primate realm that recorded ventral striatal responses during the Wisconsin Card Sort-  
260 ing Task (WCST), a common set-shifting task used in both the laboratory and clinic, and found cells that  
261 preferred firing to stimuli when a certain rule, or rule category was currently active (Sleazer). Indeed, an

262 encoding of the current strategy could be an explanation as to why differentially tiling of task structure was  
263 observed across blocks in the current study. Further support for a modulation of NAc responses by strategy  
264 comes from an fMRI study that examined BOLD levels during a set-shifting task (FitzGerald et al., 2014). In  
265 this task, participants learned two sets of stimuli-reward contingencies, a visual set and auditory set. During  
266 testing they were presented with both simultaneously, and the stimulus dimension that was relevant was peri-  
267 odically shifted between the two. Here, they found that bilateral NAc activity reflected value representations  
268 of whatever the currently relevant stimulus dimension was, and not the irrelevant stimulus. The current find-  
269 ing of separate, but overlapping, populations of cells encoding cue modality and expected value, suggests  
270 that the fMRI finding is generated by the combined activity of several different functional cell types.

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

281 **Encoding of position:**

282 Our finding that cue-evoked activity was modulated by cue location sides with some of the literature (Lavoie,  
283 1994; Tabuchi, 2000; Strait, 2016). An alternative explanation for a pure spatial representation, is that  
284 these are task segmentation correlates, keeping track of where in the task the rat is. A previous non-human  
285 primate paper has shown that when reward is contingent upon completion of a series of trials, separate

286 populations of NAc neurons signal the start of a schedule, subsequent trials in the schedule, and the first  
287 trial in extended schedules (Shidara et al., 1998). This signalling of position within a sequence has been  
288 observed in subsequent studies, and it is possible that the our rats were keeping track of which specific arm  
289 they were in as part of a sequence of arms, and not just strictly a spatial representation (Mulder, 2004 and  
290 2005; Khamassi et al., 2008; Berke, 2009). Also, given that our task is pseudo-random, it is possible that the  
291 rats learned which cue to anticipate, and the neural activity could reflect this. However, this is unlikely as  
292 including a previous trial variable in the analysis did not explain a significant amount of firing rate variance  
293 in response to the cue for the vast majority of cells..

294 **Mixed selectivity:**

295 Several other papers have reported unit profiles that integrate different task-related variables. These papers  
296 report integrated coding between expected value and subsequent motor responses, expected value and iden-  
297 tity of a reward, and a combination of spatial-, movement-, and reward-related features (Roesch, Lavoie,  
298 Cooch). However, our study is the first to show mixed selectivity among identifying features of a cue and  
299 expected outcome or behavior. The presence of mixed selectivity responses confers a larger number of input-  
300 output relationships that are available to a given neuron. A possible functional consequence of this attribute  
301 of NAc units, is the combination and transformation of various motivationally relevant features into a sig-  
302 nal informing downstream decoders such as the ventral pallidum about appropriate behaviors in obtaining  
303 motivationally relevant goals and biasing action selection towards these behaviors. Mixed selectivity in the  
304 NAc could be a consequence of synaptic integration from a variety of anatomically distinct inputs, as seen  
305 in experiments examining the convergence of various NAc afferents at the level of synaptic transmission and  
306 stimulation-induced firing (Goto and Grace 2008). In one such experiment it was shown that NAc cells that  
307 responded to stimulation of either the fornix, amygdala, or PFC, typically responded to stimulation from all  
308 inputs (ODonnell and Grace, 1995). Furthermore, an interaction between these inputs was observed such  
309 that PFC stimulation failed to elicit spiking in the NAc neurons unless they were in a depolarized UP-state, a  
310 state induced by hippocampal stimulation and was dependent on an intact fornix. Hippocampal-induced sup-

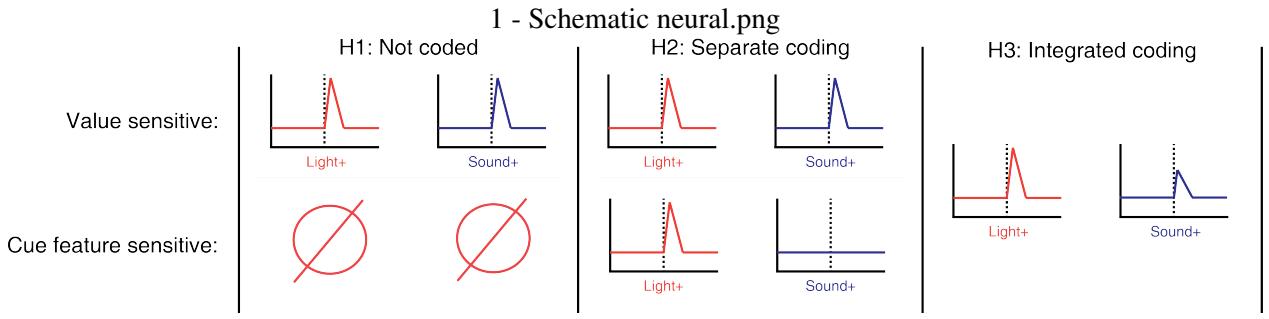
311 pressure of other inputs has also been observed for the BLA (Mulder et al., 1998). Recently, it has also been  
312 shown that train stimulation of PFC afferents reduces hippocampal-evoked NAc responses, suggesting that  
313 there is competition between various inputs (Calhoon and O'Donnell, 2013). These studies suggest that the  
314 integration of the variables we saw could be the result of this gating observed in behaviorally-independent  
315 preparations. However, given that we did not systematically manipulate these various limbic and cortical  
316 afferents, comments on the anatomical origins of the observed mixed selectivity responses are speculative at  
317 this point.

318 Integrating cue identity and value, as seen in the present study, could be one neural instantiation of how  
319 value is associated with the appropriate predictive stimuli (credit assignment), keeping in mind that value  
320 encoding is distributed, redundantly in some aspects, across various structures (Hayden Nat Neuro opinion).  
321 Indeed, lesions of the NAc impair the ability to learn changes in reward value or identity in an unblocking  
322 experiment, as well as disrupting dopamine RPEs generated by modification of timing of reward (McDannald  
323 2011, Takahashi 2016). Would be interesting to see if uncoupling the integrated coding of stimulus features  
324 and predictive properties of a cue has an effect on the ability of a rat to use reward-predictive cues to pursue  
325 the associated reward.

326 **Tiling of task structure:**

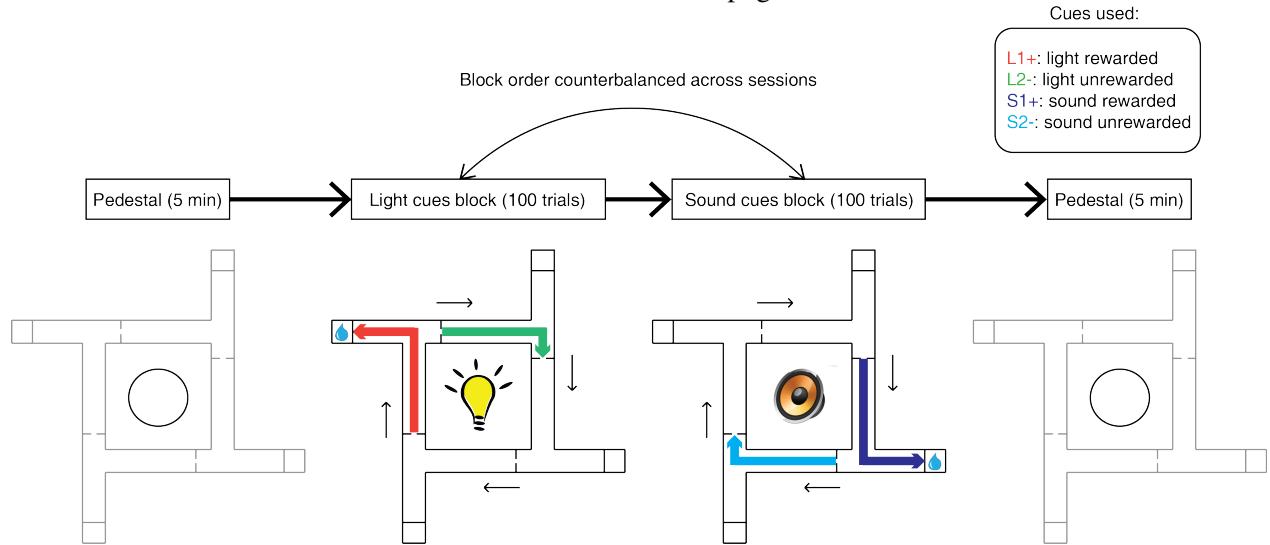
327 Additionally, we found that the population of recorded units had a relatively uniform distribution of firing  
328 fields within our task space, similar to what has been reported previously (Shidara, 1998; Berke, 2009;  
329 Lansink, 2012). Uniquely, we found that this representation differed according to whether the rat was cur-  
330 rently engaged in the light or sound block, suggesting that this could be a possible neural correlate for  
331 encoding the currently relevant strategy in the NAc. During progress through a predictable trial series, neu-  
332 rons represented state value of cue (Shidara 1998). Single-unit responses allowed the monkey to know how  
333 it was progressing throughout the task. Likewise, the tiling we saw could be a consequence of upstream cor-  
334 tical or limbic inputs informing the striatum of the current task rules. Another possibility is that the NAc not

only pays attention to progress throughout a task within a trial, but also higher-order task information, like blocks. Cue location was a behaviorally irrelevant variable in the current experiment, but it is possible that if this tiling is dependent on hippocampal input, or related to a state value representation, that making cue location a relevant variable by adding positional contingencies such as only alternating arms are rewarded in one block, would result in a further separation of the mapping within a block between the rewarded and unrewarded arms. Furthermore, dopamine levels in the NAc fluctuate through a trial, and it is possible that the observed tiling could be a NAc-representation of state value related to this temporally evolving dopamine signal. Future experiments should monitor this mapping of task structure during the application of dopamine antagonists. Finally, the presence of functional correlates not evident when looking at single-unit responses time-locked to salient task events emphasizes the need to employ ensemble level analyses across all aspects of a task.



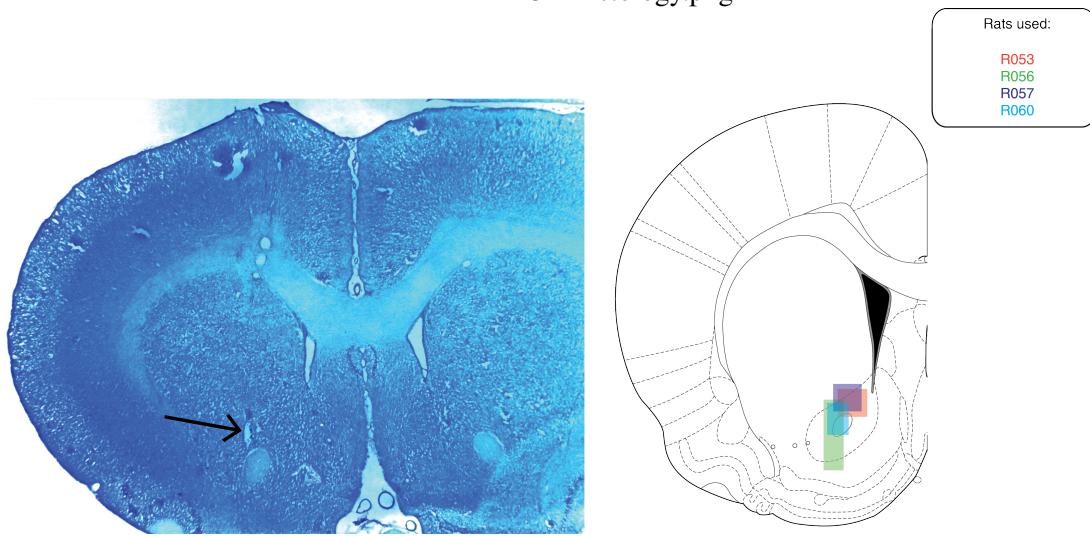
**Figure 1: Neural schematic** Schematic of potential coding strategies employed by single units in the NAc. Coding of identifying cue features could occur either be absent in the NAc (left), occur independently of encoding of motivationally relevant variables like expected value or subsequent vigor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

2 - Schematic task.png



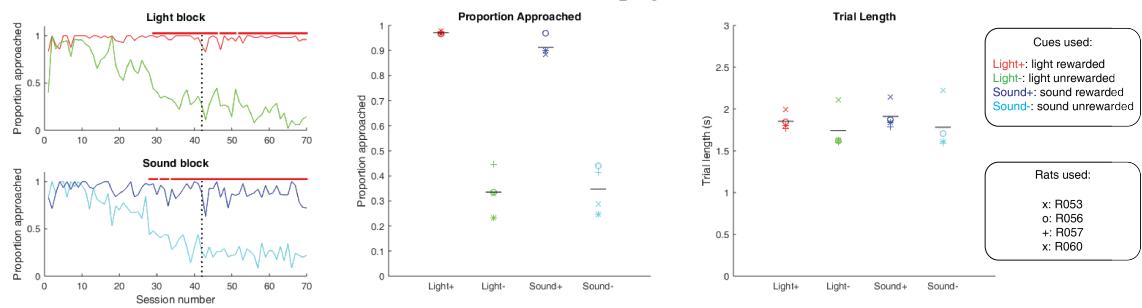
**Figure 2: Task schematic** Schematic of behavioral task and procedure used in current study. A session was started with a 5 minute recording period on a pedestal place in the center of the apparatus (left). Rats then underwent two blocks of a cue discrimination task on a square track where they had 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



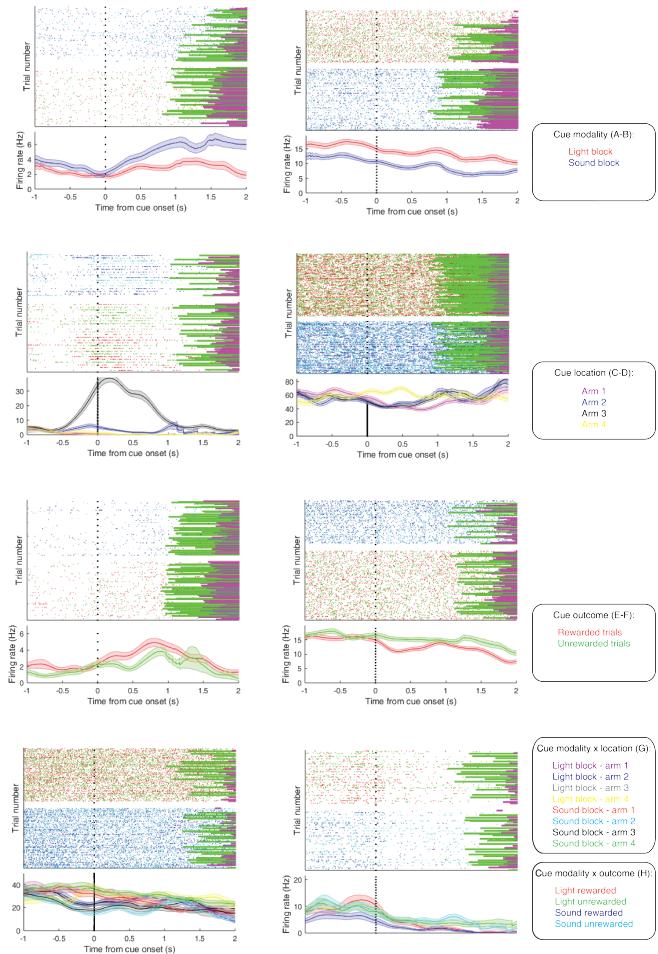
**Figure 3: Histology**Histological verification of recording sites. Upon completion of experiments, brains were sliced and tetrode placement was confirmed. A. Example section from R060 showing a recording site in the NAc core. B. Schematic showing recording areas for the four rats used in the present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

4 - Behavioral results.png

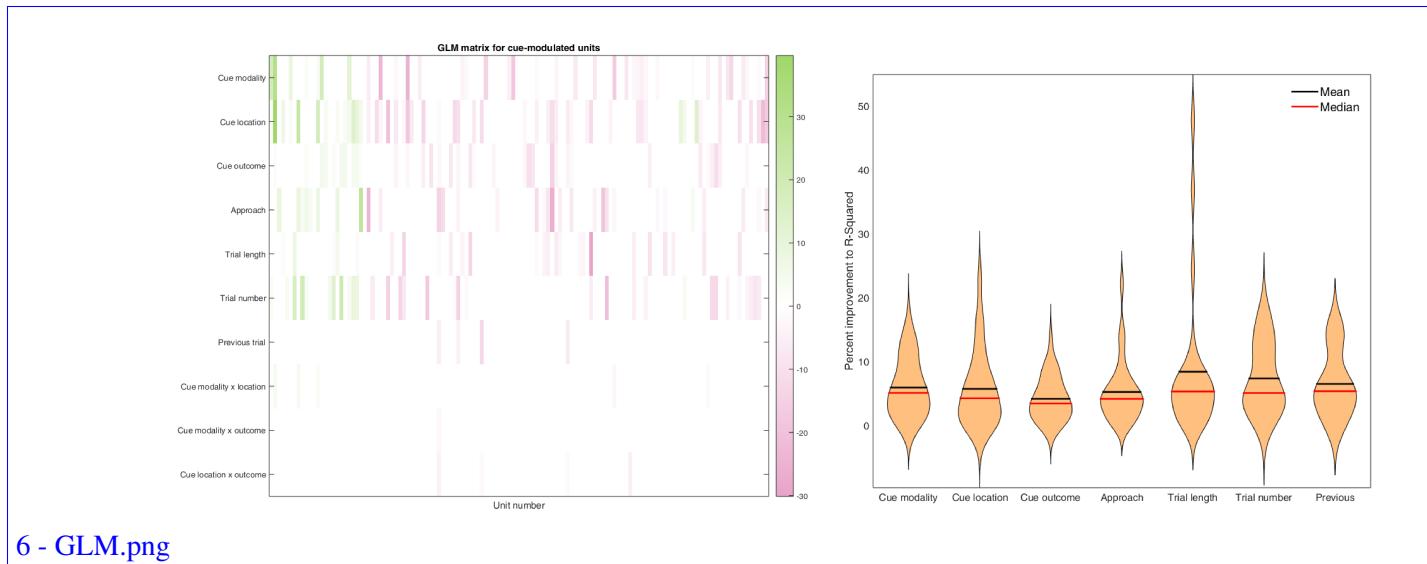


**Figure 4: Behavioral results** Performance on the behavioral task. A-B. Example learning curves from R060 showing acquisition and maintenance of performance in the light (A) and sound (B) blocks. Dependent measure is proportion of trials approached within a session for a given cue, with a value of 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



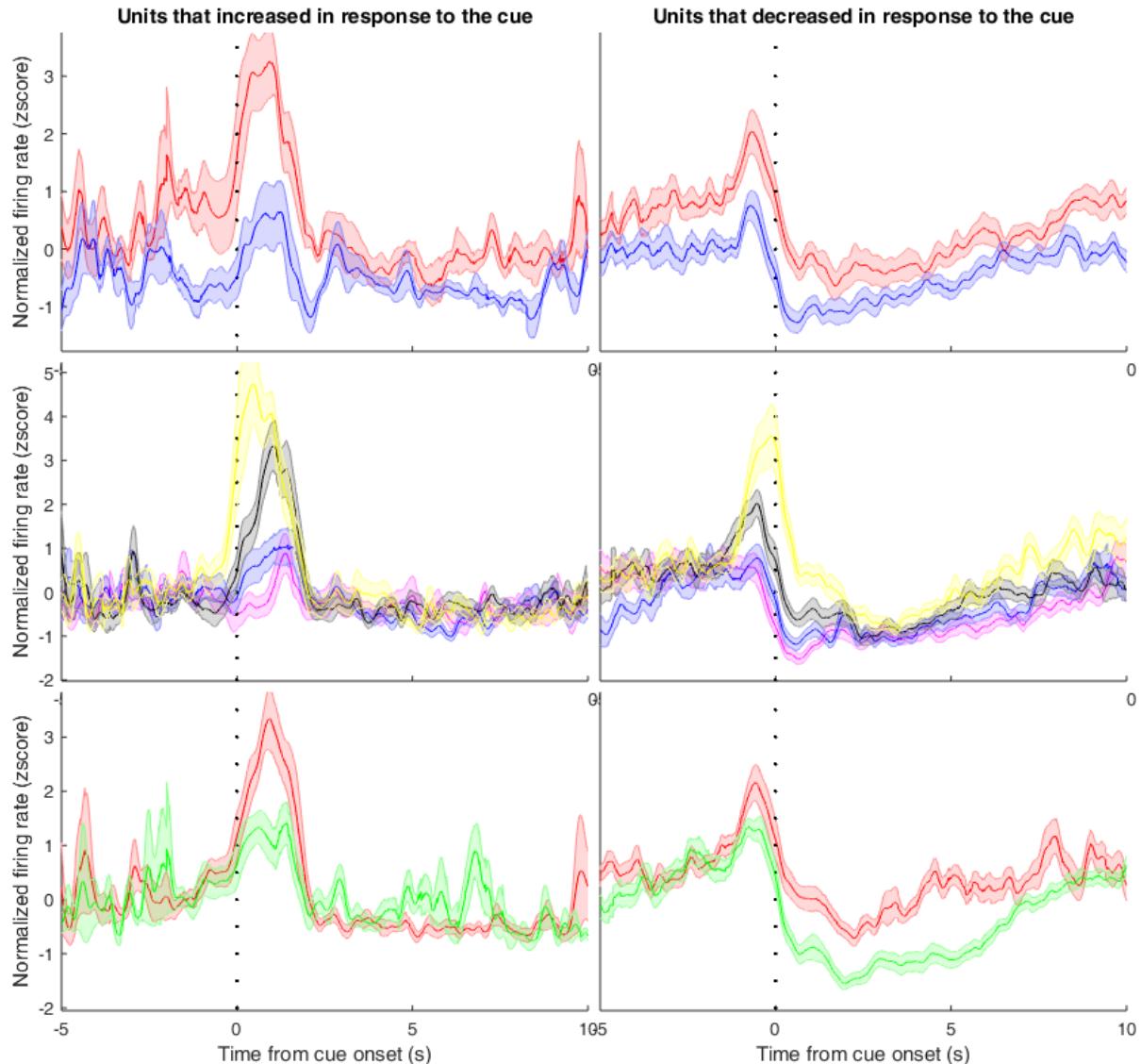
**Figure 5: Neural** Individual examples of NAc units influenced by various task parameters. A. Example of a NAc unit that showed an increase in firing in response to the cue, and whose activity was influenced by which block the rat was in. Top: rasterplot showing the spiking activity across all trials 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

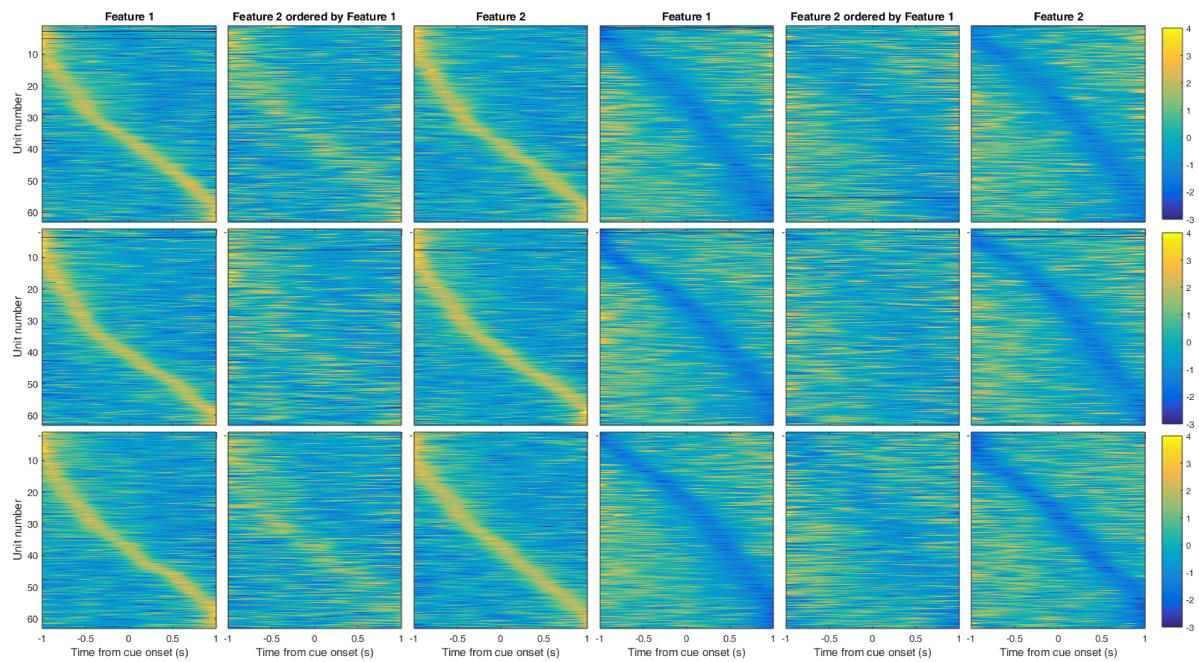
**Figure 6:** Summary of influence of various task parameters of cue-modulated NAc units after cue-onset. A. GLM matrix demonstrating impact of various task parameters on NAc firing rates. A GLM was fit to each unit that showed evidence of cue modulation by a Wilcoxon signed-rank test. Each row represents 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

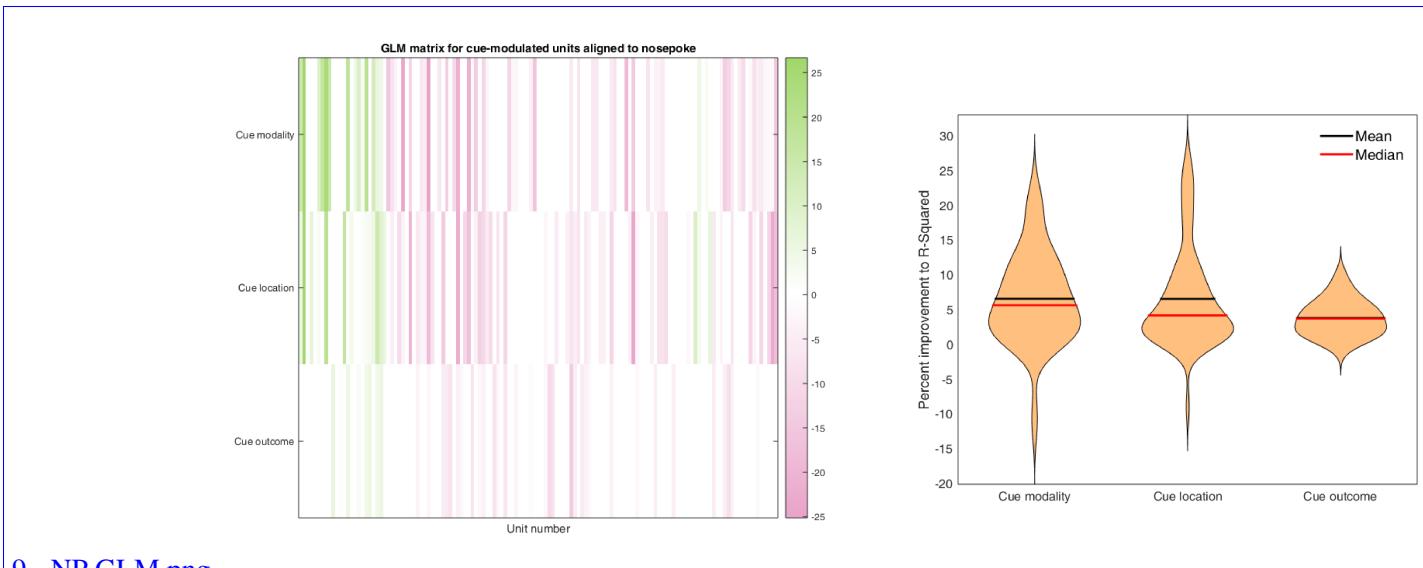


**Figure 7:** ~~Population~~ Population-level averages of cue feature sensitive NAc units. A. Average normalized activity for cue-modulated units where cue modality was a significant predictor in the GLM, aligned to cue-onset. Activity is plotted for preferred block (red) and nonpreferred block (blue). B. Same as 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



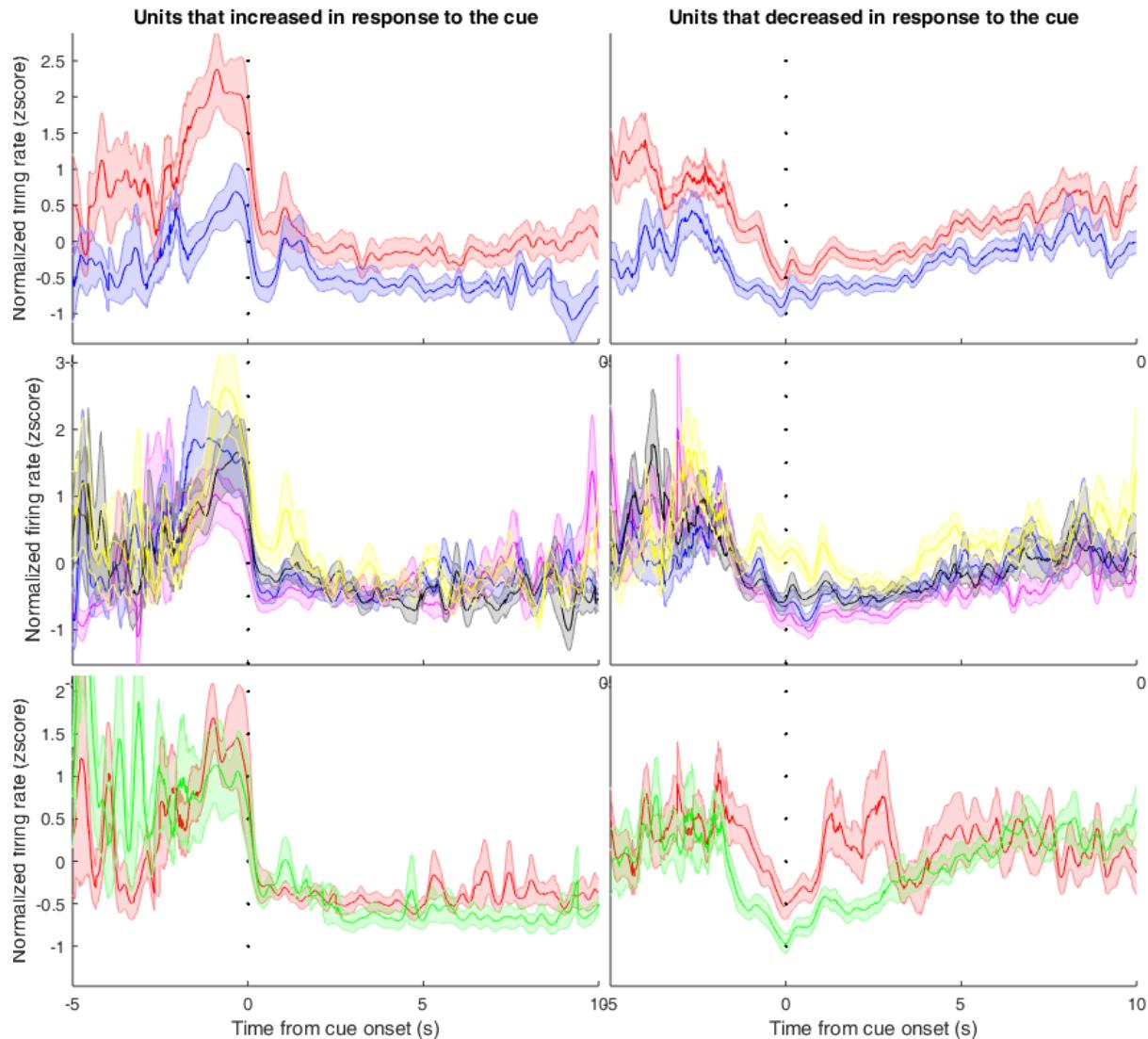
**Figure 8: Task tiling** Distribution of NAc firing rates across task space surrounding cue-onset. A. Firing rates for all recorded units were normalized and ordered according to peak firing rates for light block (left) and sound block (right), aligned to cue-onset. Middle: Distribution of sound block 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

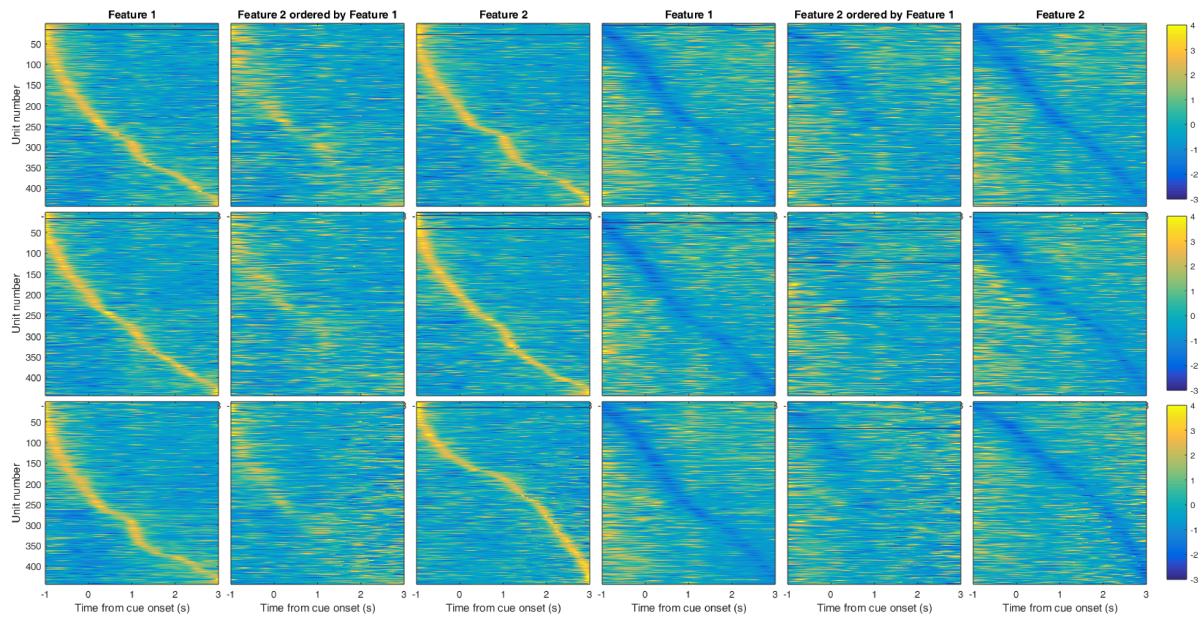
**Figure 9: NP - Summary of influence of various task parameters of cue-modulated NAc units during nosepoke.** A. GLM matrix demonstrating impact of various task parameters on NAc firing rates. A GLM was fit to each unit that showed evidence of cue modulation by a Wilcoxon signed-rank test. Each row 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.

**NP-RSquared**  
10 - NP population averages.png



**Figure 10:** NP 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 A-B 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: NP** Distribution of NAc firing rates across task tiling 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