

# **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 light or sound cue was presented  
66 that signaled the presence of absence of a 12% sucrose water reward (0.1 mL) at the upcoming site. A trial  
67 was classified as an approach trial if the rat turned left at the decision point and made a nosepoke at the  
68 reward receptacle (40 cm from the decision point), while trials were classified as a skip trial if the rat instead  
69 turned right at the decision point and triggered the photobeam to initiate the following trial. There was a 1  
70 second delay between a rewarded nosepoke and subsequent reward delivery. Trial length was determined

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

91 [Figure 2 about here.]

92 **Surgery:**

93 Surgical procedures were as described previously (Malhotra et al., 2015). Briefly, animals were anesthetized  
94 with isoflurane, induced with 5% in medical grade oxygen and maintained at 2% throughout the surgery

95 ( 0.8 L/min). Rats were then chronically implanted with a hyperdrive consisting of 16 independently drivable  
96 tetrodes, either all 16 targeted for the right NAc (AP +1.4 mm and ML +1.6 mm, relative to bregma; Paxinos  
97 and Watson, 2005), or 12 in the right NAc and 4 targeted at the mPFC (AP +3.0 mm and ML +0.6 mm,  
98 relative to bregma; only data from NAc tetrodes were analyzed). Following surgery, all animals were given  
99 a least five days to recover and lower tetrodes to the target (DV -6.0 mm) before being reintroduced to the  
100 behavioral task.

101 **Data acquisition and preprocessing:**

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

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

112 **Data analysis:**

113 To investigate the contribution of various cue features on NAc firing rates we first determined whether firing  
114 rates for a unit were modulated by the onset of a cue by collapsing across all cues and comparing the firing  
115 rates for the 1 s preceding cue-onset with the 1 s following cue-onset. Single units were considered to be  
116 cue-responsive if a Wilcoxon signed-rank test comparing pre- and post-cue firing had a p < .01. (Excluded:

117 the mean firing rate difference between pre- and post-cue onset was within the lower or upper 2.5% of a  
118 shuffled distribution, as there is redundancy using both, and I did both because I was paranoid about just  
119 using one. ). Cue-modulated units were then classified as either increasing or decreasing in response to the  
120 cue if the post-cue activity was higher or lower than the pre-cue activity, respectively.

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

129 To better visualize responses to cues and enable subsequent population level analyses (as in Figures 5,7,8),  
130 spike trains were convolved with a Gaussian kernel, and peri-event time histograms (PETHs) were generated  
131 by taking the average of the convolved spike trains across trials for a given task condition. For analysis  
132 of population-level responses for cue features (Figure 7), convolved spike trains for all units where cue  
133 modality, cue location, or cue outcome explained a significant portion of firing rate variance were z-scored.  
134 Within a given cue feature, normalized spike trains were then separated according to the preferred and non-  
135 preferred cue condition (e.g. light vs sound block), and averaged across units to generate population-level  
136 averages.

137 To visualize NAc representation of task space within cue conditions, normalized spike trains for all units  
138 were ordered by the location of their maximum or minimum firing rate for a specified cue condition (Figure  
139 8). To compare representation of task space across cue conditions for a cue feature, the ordering of units  
140 derived for one condition (e.g. light block) was then applied to the normalized spike trains for the other

<sup>141</sup> condition (e.g. sound block).

<sup>142</sup> To identify the responsivity of units to different cue features at the time of a nosepoke into a reward receptacle, the same cue-responsive units from the cue-onset analyses were analyzed at the time of nosepoke using  
<sup>144</sup> identical analysis techniques (Figures 9,10,11).

<sup>145</sup> Given that some of our analyses compare firing rates across time, particularly comparisons across blocks,  
<sup>146</sup> we sought to exclude units with unstable firing rates that would generate spurious results reflecting a drift  
<sup>147</sup> in firing rate over time unrelated to our task. To do this we ran a Mann-Whitney U test comparing the cue-  
<sup>148</sup> evoked firing rates for the first and second half of trials within a block, and excluded units from analysis that  
<sup>149</sup> showed a significant change for either block. All analyses were completed in MATLAB R2015a, and the  
<sup>150</sup> code is available on GitHub.

<sup>151</sup> **Histology:**

<sup>152</sup> Upon completion of the experiment, rats were anesthetized with 5% isoflurane, then asphyxiated with carbon  
<sup>153</sup> dioxide. Transcardial perfusions were performed, and brains were fixed and removed. Brains were sliced  
<sup>154</sup> in 50 um coronal sections and stained with thionin. Slices were visualized under light microscopy, tetrode  
<sup>155</sup> placement was determined, and electrodes with recording locations in the NAc were analyzed (Figure 3).

<sup>156</sup> [Figure 3 about here.]

157 **Results**

158 **Behavior**

159 Rats were trained to discriminate between cues signaling the availability and absence of reward on a square  
160 track with four identical arms for two distinct sets of cues. An example learning curve is seen in Figure  
161 4A,B. All four rats learned to discriminate between the rewarded and unrewarded cue for both the light and  
162 sound blocks as determined by reaching significance ( $p < .05$ ) on a daily chi-square test comparing approach  
163 behavior for rewarded and unrewarded cues for each block, for at least three consecutive days. Linear mixed  
164 effects models revealed that cue type had an influence on the likelihood of a rat to make an approach ( $p <$   
165  $.001$ ), but not for the length of time taken to complete a trial ( $p = .13$ )(Figure 4C,D).

166 [Figure 4 about here.]

167 **NAc neurons encode behaviorally relevant and irrelevant cue features**

168 **General responses to cue:**

169 A total of 443 units were recorded in the NAc from 4 rats over 57 sessions (Table 1). The activity of 133  
170 (30%) of these was modulated by the cue, with more units showing a decrease in firing ( $n = 124$ ) than an  
171 increase ( $n = 47$ ) around the time of cue-onset (Table 2). Within this group, 32 were classified as HFNs, while  
172 139 were classified as SPNs. Fitting a GLM to each unit revealed that a variety of task parameters accounted  
173 for a significant portion of firing rate variance in NAc cue-modulated units (Figures ??, 6). Notably, there  
174 were units that discriminated between whether the rat was performing in the light or sound block, which arm  
175 the rat was currently on, and whether the rat was engaged in a rewarded or unrewarded trial (Figure 5A-F).

176 Interactions between multiple cue features appeared as significant predictors of firing rate variance for 9%  
177 cue-modulated units, although this effect was relatively modest (Figure 5G,H). Fitting a GLM to all recorded  
178 units...\*Jimmie finish this\* (data not shown).

179 [Table 1 about here.]

180 [Table 2 about here.]

181 [Figure 5 about here.]

182 [Figure 6 about here.]

183 **Population level responses:**

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

191 [Figure 7 about here.]

192 **NAc units segment the task:**

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

203 [Figure 8 about here.]

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

205 In order to be useful for learning, a trace of the cue must be maintained until the outcome. Fitting a GLM  
206 to the firing rates of cue-modulated units at the time of a nosepoke response showed that a variety of units  
207 still discriminated firing according to various cue features, but not other task parameters (Table 3, Figures  
208 9, 10). Fitting a GLM to all recorded units...\*Jimmie finish this\* (data not shown). Furthermore, aligning  
209 normalized peak firing rates to nosepoke onset, revealed a clustering of responses around reward receipt  
210 (Figure 11). Alongside this, fitting a GLM to the firing rates of cue-modulated units at the time of reward  
211 receipt, revealed 10 units (8%) where cue outcome accounted for an average of 32% of firing rate variance  
212 (data not shown).

213 [Table 3 about here.]

214 [Figure 9 about here.]

215

[Figure 10 about here.]

216

[Figure 11 about here.]

217 **Discussion**

218 The present study found evidence for coding of multiple identifying features of motivationally relevant stim-  
219 uli; the sensory modality of the presented cue, as well as its physical location within the track. Furthermore,  
220 this coding was both independent, and intermixed with coding for the associated outcome of the cue as well  
221 as motivational vigor, measured by time to complete the trial. At the population level, a tiling of task struc-  
222 ture was observed such that all points within our analyzed task space was accounted for by the ordered peak  
223 firing rates of all cells, and this tiling differed between blocks where sound or light cues were presented.  
224 Cells that discriminated across blocks were not simply due to drifting of the signal across trials, as cells that  
225 showed a drift in firing between the first and second half within a block were excluded from the analysis.  
226 Furthermore, even though actions were stereotyped during correct trials, such that the rat always turned left  
227 at the decision point to approach for reward, and right to skip the receptacle and initiate the next trial, cells  
228 that were modulated by the expected value of the cue maintained their specific firing patterns even during  
229 error trials where the rat turned left after presentation of the unrewarded cue, suggesting that these signals  
230 did not represent action values. Additionally, NAc signals have been shown to be modulated by response  
231 vigor, to detangle this from our results we included the trial length as a predictor in our GLMs, and found  
232 cells with correlates independent of trial length.

233 **Cue modality:**

234 Our finding that ventral striatal units can discriminate between cues from different sensory modalities ex-

235 pands upon an extensive literature examining neural correlates of conditioned stimuli. Perhaps the most  
236 comparable work in rodents comes from a study that found distinct coding for an odor when it predicted  
237 separate but equally valued rewards (Coch). The present work is complementary to this as it shows that  
238 ventral striatal cells have representations of identifiable aspects of the cue itself, in addition to the reward  
239 it predicts. Another study paired separate cues with appetitive or aversive outcomes, and found separate  
240 populations of cells that encode each cue, with many switching selectivity after reversal of the associations  
241 between the cues and outcomes, providing evidence that the NAc encodes the biological significance of  
242 stimuli. Once again, our study was different as we recorded neural responses to distinct cues encoding the  
243 same anticipated outcome, suggesting that even when the biological relevance of these stimuli is similar, the  
244 NAc dissociates their representations at the level of the single-unit (Setlow). Another possibility is that these  
245 modality specific cells were encoding the context, rule, or sequence within a session as some cells responded  
246 similar for both rewarded and unrewarded cues within a block. This interpretation is in alignment with a  
247 recent paper from the primate realm that recorded ventral striatal responses during the Wisconsin Card Sort-  
248 ing Task (WCST), a common set-shifting task used in both the laboratory and clinic, and found cells that  
249 preferred firing to stimuli when a certain rule, or rule category was currently active (Sleazeer). Indeed, an  
250 encoding of the current strategy could be an explanation as to why differentially tiling of task structure was  
251 observed across blocks in the current study. Further support for a modulation of NAc responses by strategy  
252 comes from an fMRI study that examined BOLD levels during a set-shifting task (FitzGerald et al., 2014). In  
253 this task, participants learned two sets of stimuli-reward contingencies, a visual set and auditory set. During  
254 testing they were presented with both simultaneously, and the stimulus dimension that was relevant was peri-  
255 odically shifted between the two. Here, they found that bilateral NAc activity reflected value representations  
256 of whatever the currently relevant stimulus dimension was, and not the irrelevant stimulus. The current find-  
257 ing of separate, but overlapping, populations of cells encoding cue modality and expected value, suggests  
258 that the fMRI finding is generated by the combined activity of several different functional cell types.

259 A caveat of the current study is that rats were never presented with both sets of cues simultaneously, and  
260 thus never had to switch strategies, although extrapolating the data from the primate study, suggests that the

261 activity of the cue modality cells would be modulated by relevance. Keeping along this theme, the current  
262 data set is unable to identity precisely what the modality-sensitive neurons were encoding, that is were they  
263 tracking representations of stimulus identity, a preferred context, or even a macroscale representation of  
264 progress through the session. Furthermore, their relevance for ongoing behavior is also uncertain. NAc core  
265 lesions have been shown to impair shifting between different behavioral strategies, and it is possible that  
266 selectively silencing the cells that prefer responding for a given modality or rule would impair performance  
267 when the animal is required to use that information, or artificial enhancement of those cells would cause  
268 them to use the rule when it is the inappropriate strategy.

269 **Encoding of position:**

270 Our finding that cue-evoked activity was modulated by cue location sides with some of the literature (Lavoie,  
271 1994; Tabuchi, 2000; Strait, 2016). An alternative explanation for a pure spatial representation, is that  
272 these are task segmentation correlates, keeping track of where in the task the rat is. A previous non-human  
273 primate paper has shown that when reward is contingent upon completion of a series of trials, separate  
274 populations of NAc neurons signal the start of a schedule, subsequent trials in the schedule, and the first  
275 trial in extended schedules (Shidara et al., 1998). This signalling of position within a sequence has been  
276 observed in subsequent studies, and it is possible that the our rats were keeping track of which specific arm  
277 they were in as part of a sequence of arms, and not just strictly a spatial representation (Mulder, 2004 and  
278 2005; Khamassi et al., 2008; Berke, 2009). Also, given that our task is pseudo-random, it is possible that the  
279 rats learned which cue to anticipate, and the neural activity could reflect this. However, this is unlikely as  
280 including a previous trial variable in the analysis did not explain a significant amount of firing rate variance  
281 in response to the cue for the vast majority of cells..

282 **Mixed selectivity:**

283 Several other papers have reported unit profiles that integrate different task-related variables. These papers

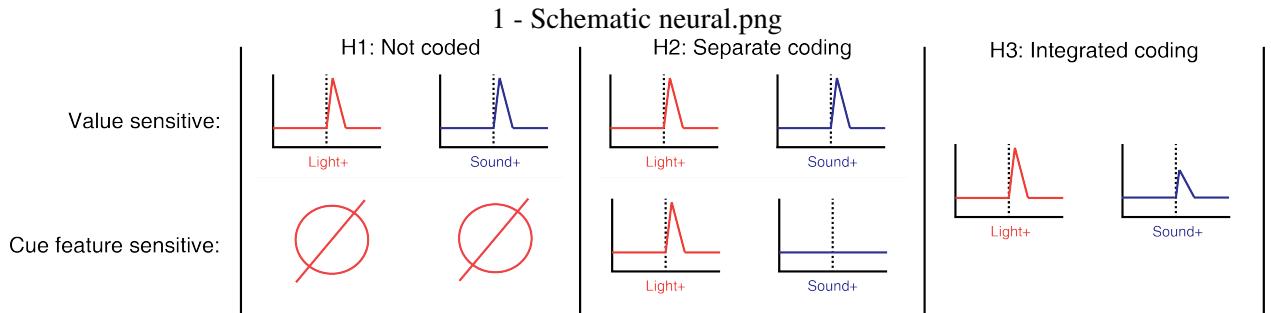
284 report integrated coding between expected value and subsequent motor responses, expected value and iden-  
285 tity of a reward, and a combination of spatial-, movement-, and reward-related features (Roesch, Lavoie,  
286 Cooch). However, our study is the first to show mixed selectivity among identifying features of a cue and  
287 expected outcome or behavior. The presence of mixed selectivity responses confers a larger number of input-  
288 output relationships that are available to a given neuron. A possible functional consequence of this attribute  
289 of NAc units, is the combination and transformation of various motivationally relevant features into a sig-  
290 nal informing downstream decoders such as the ventral pallidum about appropriate behaviors in obtaining  
291 motivationally relevant goals and biasing action selection towards these behaviors. Mixed selectivity in the  
292 NAc could be a consequence of synaptic integration from a variety of anatomically distinct inputs, as seen  
293 in experiments examining the convergence of various NAc afferents at the level of synaptic transmission and  
294 stimulation-induced firing (Goto and Grace 2008). In one such experiment it was shown that NAc cells that  
295 responded to stimulation of either the fornix, amygdala, or PFC, typically responded to stimulation from all  
296 inputs (ODonnell and Grace, 1995). Furthermore, an interaction between these inputs was observed such  
297 that PFC stimulation failed to elicit spiking in the NAc neurons unless they were in a depolarized UP-state, a  
298 state induced by hippocampal stimulation and was dependent on an intact fornix. Hippocampal-induced sup-  
299 pression of other inputs has also been observed for the BLA (Mulder et al., 1998). Recently, it has also been  
300 shown that train stimulation of PFC afferents reduces hippocampal-evoked NAc responses, suggesting that  
301 there is competition between various inputs (Calhoon and ODonnell, 2013). These studies suggest that the  
302 integration of the variables we saw could be the result of this gating observed in behaviorally-independent  
303 preparations. However, given that we did not systematically manipulate these various limbic and cortical  
304 afferents, comments on the anatomical origins of the observed mixed selectivity responses are speculative at  
305 this point.

306 Integrating cue identity and value, as seen in the present study, could be one neural instantiation of how  
307 value is associated with the appropriate predictive stimuli (credit assignment), keeping in mind that value  
308 encoding is distributed, redundantly in some aspects, across various structures (Hayden Nat Neuro opinion).  
309 Indeed, lesions of the NAc impair the ability to learn changes in reward value or identity in an unblocking

310 experiment, as well as disrupting dopamine RPEs generated by modification of timing of reward (McDannald  
311 2011, Takahashi 2016). Would be interesting to see if uncoupling the integrated coding of stimulus features  
312 and predictive properties of a cue has an effect on the ability of a rat to use reward-predictive cues to pursue  
313 the associated reward.

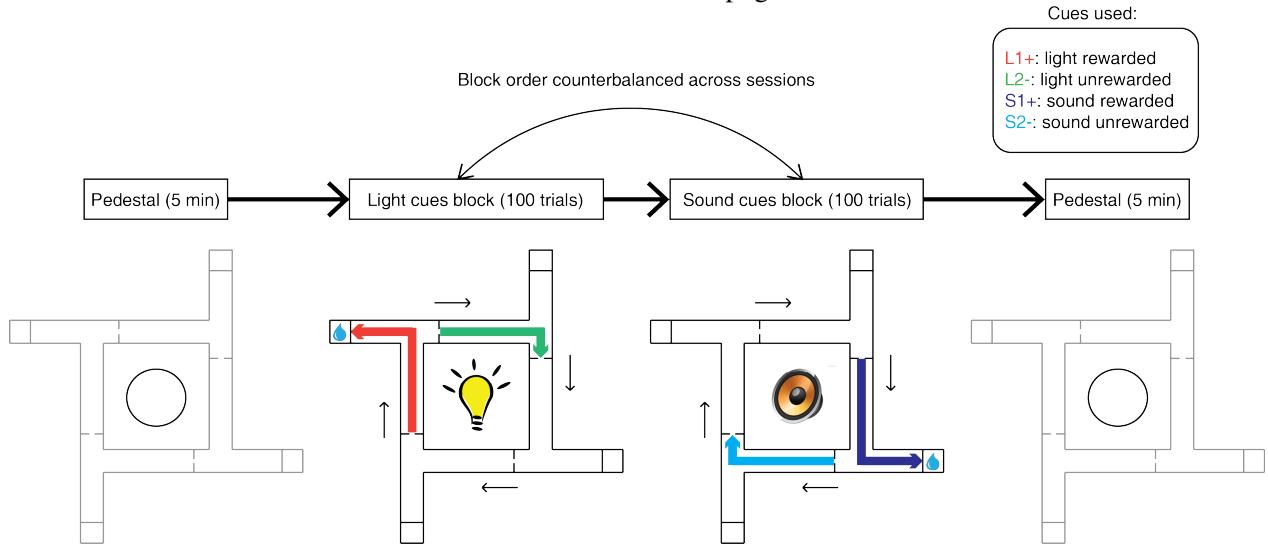
314 **Tiling of task structure:**

315 Additionally, we found that the population of recorded units had a relatively uniform distribution of firing  
316 fields within our task space, similar to what has been reported previously (Shidara, 1998; Berke, 2009;  
317 Lansink, 2012). Uniquely, we found that this representation differed according to whether the rat was cur-  
318 rently engaged in the light or sound block, suggesting that this could be a possible neural correlate for  
319 encoding the currently relevant strategy in the NAc. During progress through a predictable trial series, neu-  
320 rons represented state value of cue (Shidara 1998). Single-unit responses allowed the monkey to know how  
321 it was progressing throughout the task. Likewise, the tiling we saw could be a consequence of upstream cor-  
322 tical or limbic inputs informing the striatum of the current task rules. Another possibility is that the NAc not  
323 only pays attention to progress throughout a task within a trial, but also higher-order task information, like  
324 blocks. Cue location was a behaviorally irrelevant variable in the current experiment, but it is possible that  
325 if this tiling is dependent on hippocampal input, or related to a state value representation, that making cue  
326 location a relevant variable by adding positional contingencies such as only alternating arms are rewarded  
327 in one block, would result in a further separation of the mapping within a block between the rewarded and  
328 unrewarded arms. Furthermore, dopamine levels in the NAc fluctuate through a trial, and it is possible that  
329 the observed tiling could be a NAc-representation of state value related to this temporally evolving dopamine  
330 signal. Future experiments should monitor this mapping of task structure during the application of dopamine  
331 antagonists. Finally, the presence of functional correlates not evident when looking at single-unit responses  
332 time-locked to salient task events emphasizes the need to employ ensemble level analyses across all aspects  
333 of a task.



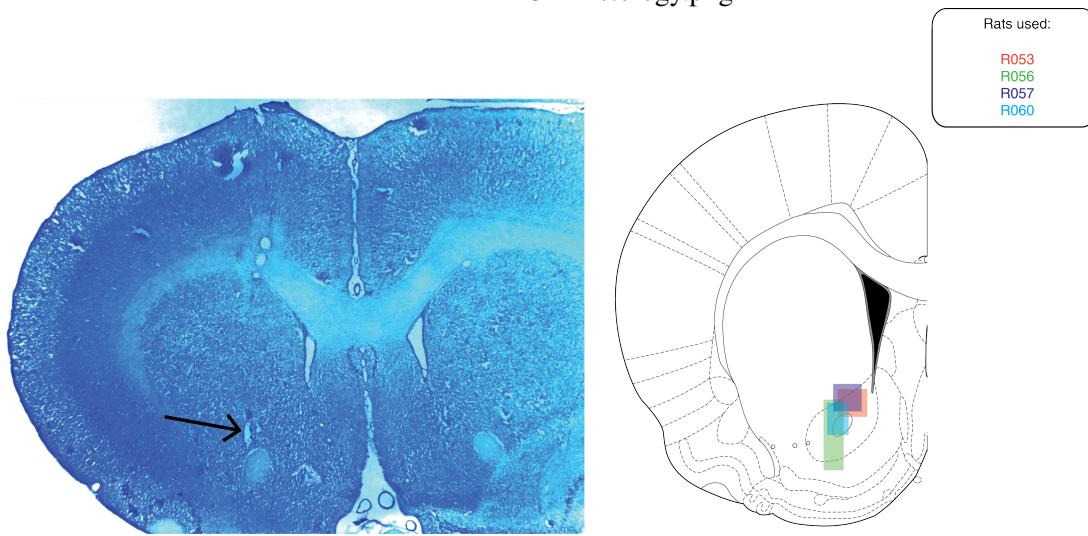
**Figure 1:** Schematic of potential coding strategies employed by single units in the NAc. Coding of identifying cue features could occur either be absent in the NAc (left), occur independently of encoding of motivationally relevant variables like expected value or subsequent vigor (middle), and/or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

2 - Schematic task.png



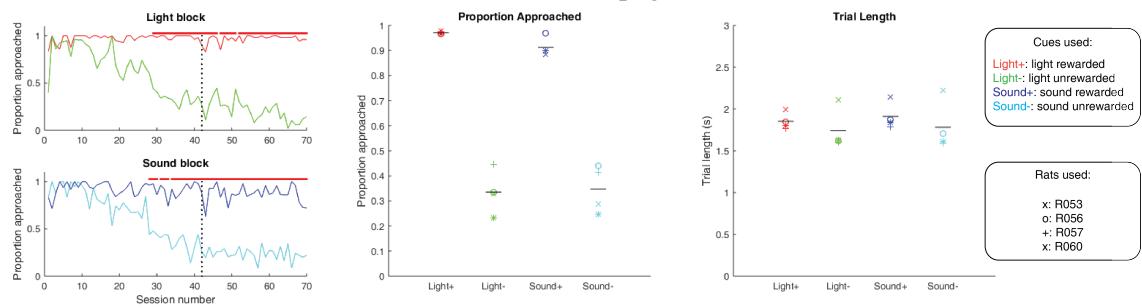
**Figure 2:** Schematic of behavioral task and procedure used in current study. A session was started with a 5 minute recording period on a pedestal place in the center of the apparatus (left). Rats then underwent two blocks of a cue discrimination task on a square track where they had to dissociate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



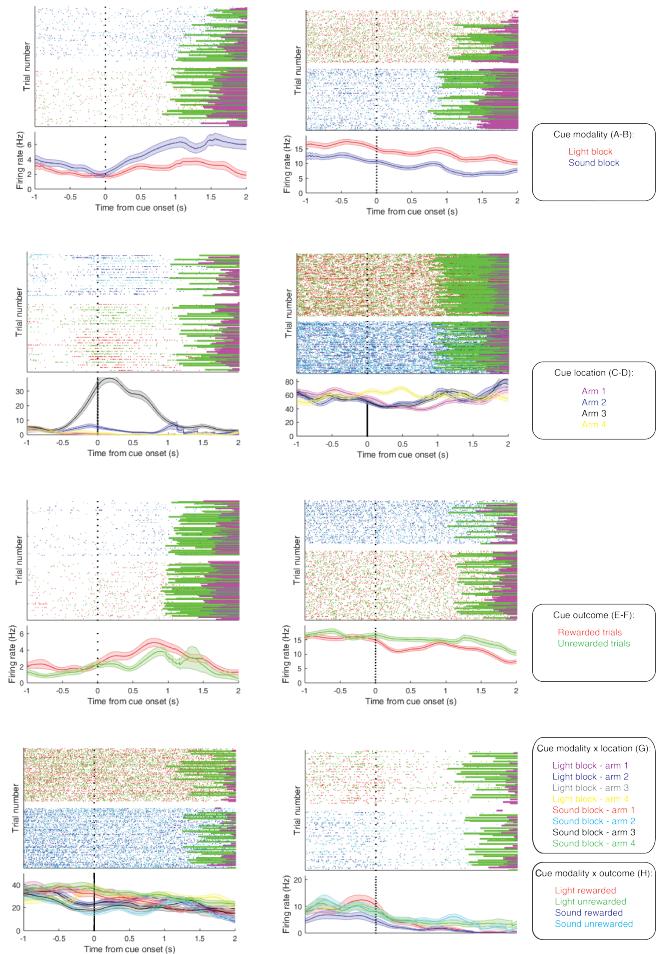
**Figure 3:** Histological verification of recording sites. Upon completion of experiments, brains were sliced and tetrode placement was confirmed. A. Example section from R060 showing a recording site in the NAc core. B. Schematic showing recording areas for the four rats used in the present study.

4 - Behavioral results.png



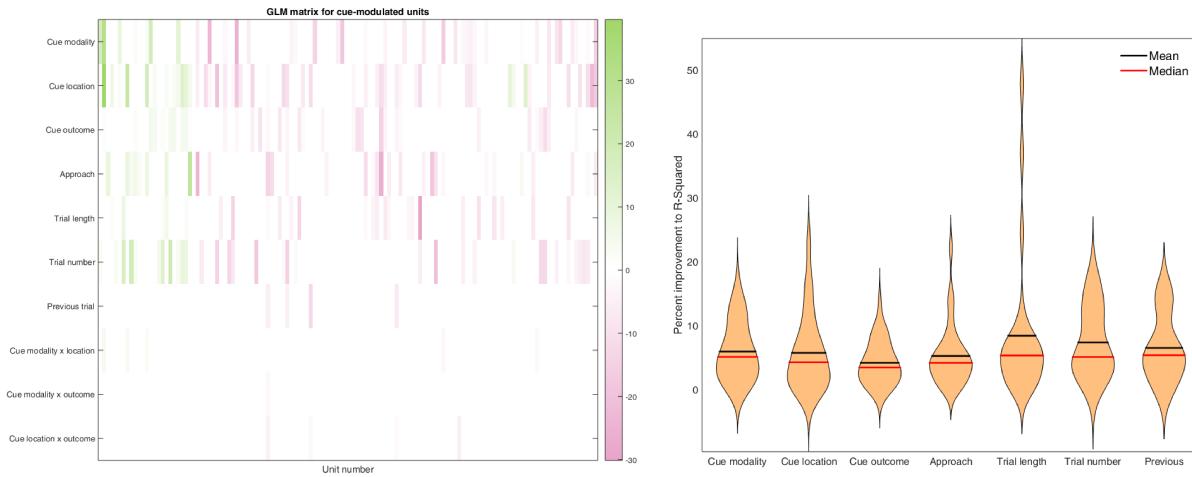
**Figure 4:** Performance on the behavioral task. A-B. Example learning curves from R060 showing acquisition and maintenance of performance in the light (A) and sound (B) blocks. Dependent measure is proportion of trials approached within a session for a given cue, with a value of 1 being perfect performance 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



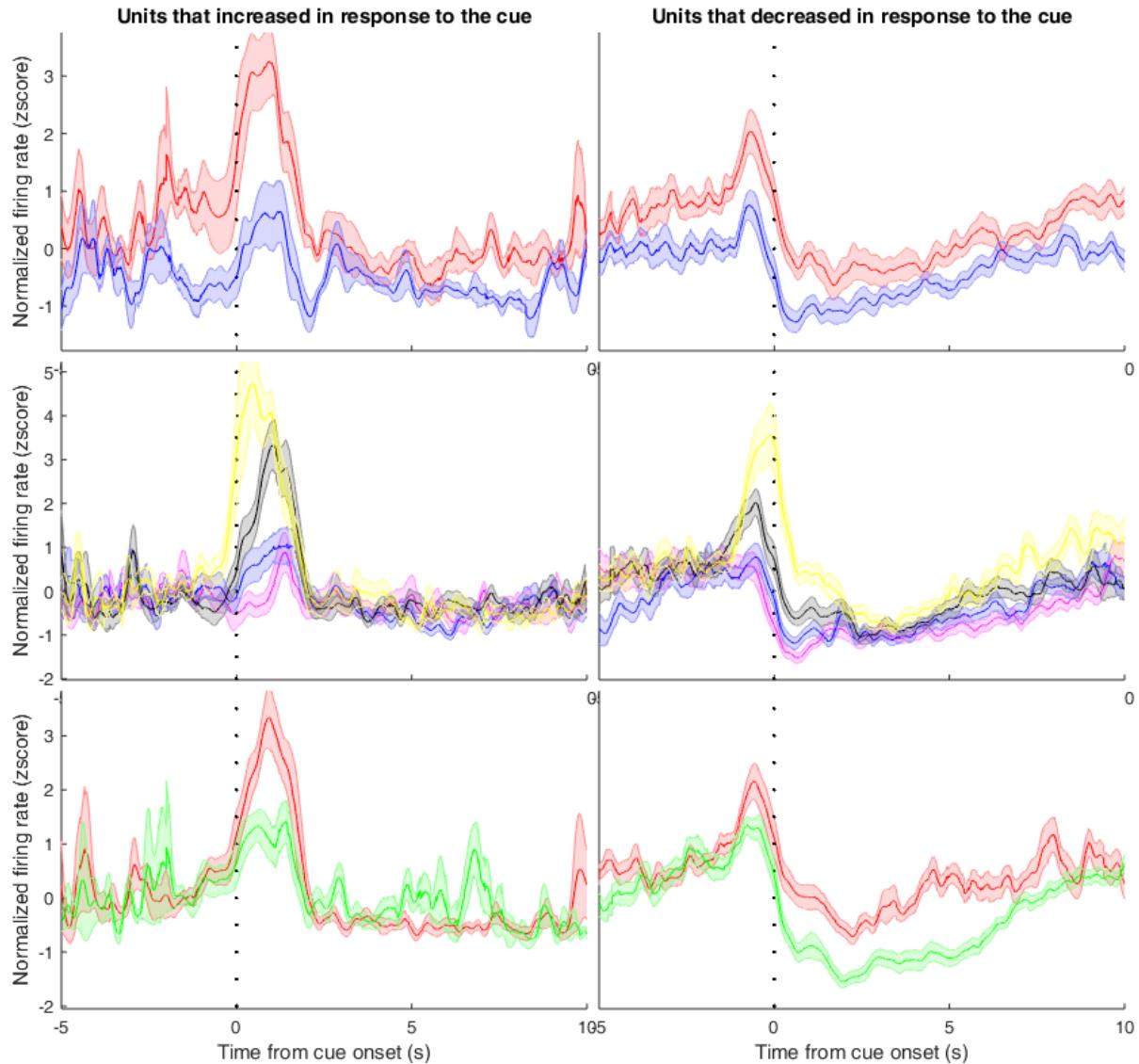
**Figure 5:** Individual examples of NAc units influenced by various task parameters. A. Example of a NAc unit that showed an increase in firing in response to the cue, and whose activity was influenced by which block the rat was in. Top: rasterplot showing the spiking activity across all trials aligned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



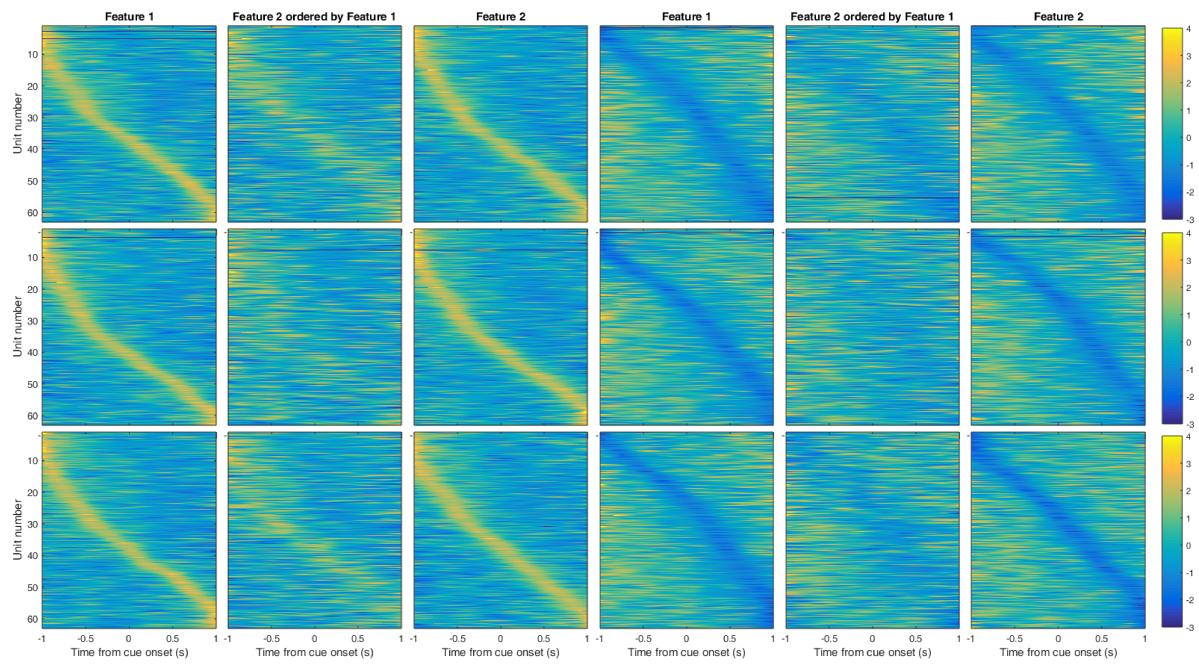
**Figure 6:** Summary of influence of various task parameters of cue-modulated NAc units after cue-onset. A. GLM matrix demonstrating impact of various task parameters on NAc firing rates. A GLM was fit to each unit that showed evidence of cue modulation by a Wilcoxon signed-rank test. Each row represents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



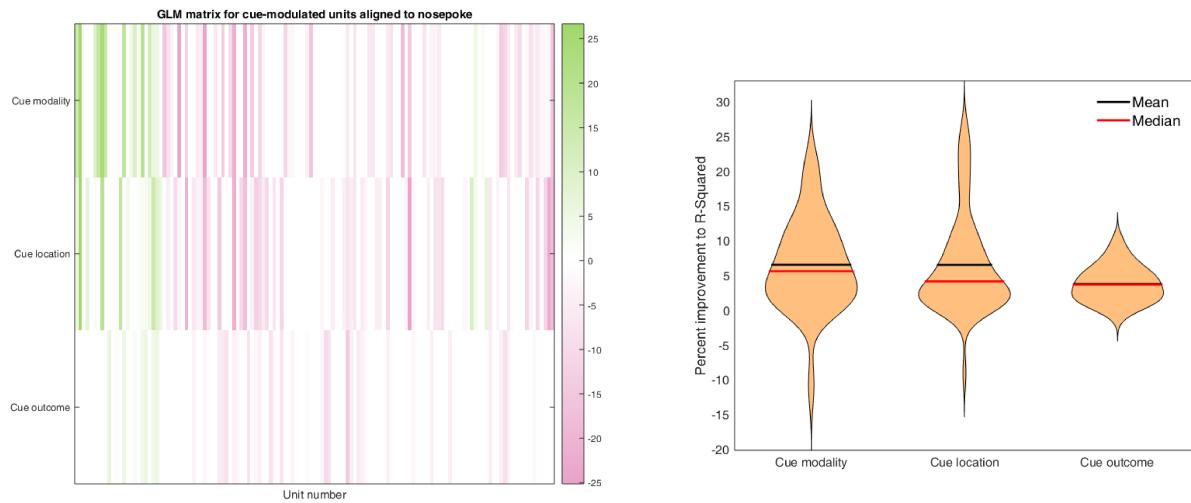
**Figure 7:** Population-level averages of cue feature sensitive NAc units. A. Average normalized activity for cue-modulated units where cue modality was a significant predictor in the GLM, aligned to cue-onset. Activity is plotted for preferred block (red) and nonpreferred block (blue). B. Same as A-B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



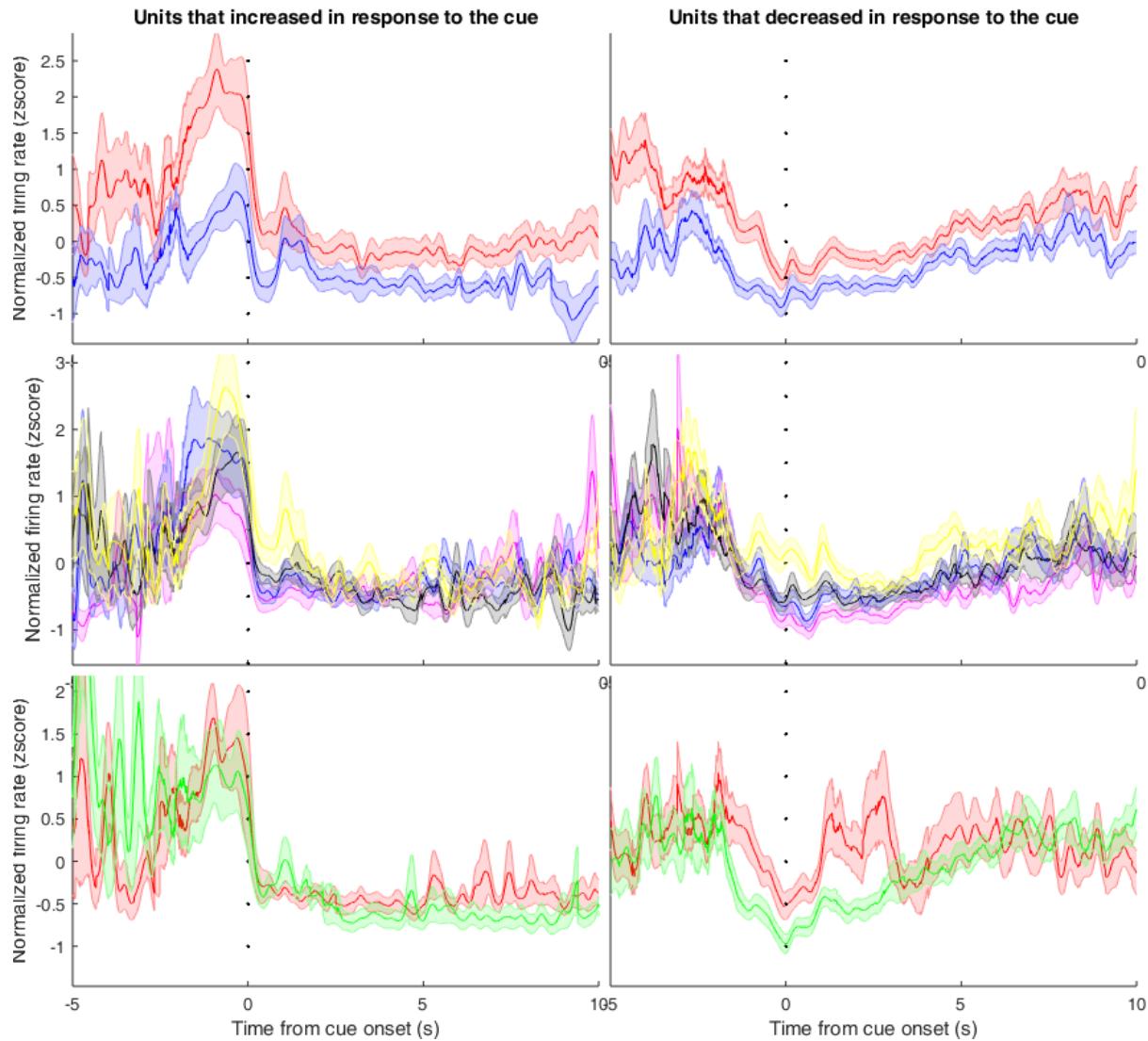
**Figure 8:** Distribution of NAc firing rates across task space surrounding cue-onset. A. Firing rates for all recorded units were normalized and ordered according to peak firing rates for light block (left) and sound block (right), aligned to cue-onset. Middle: Distribution of sound block firing rates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



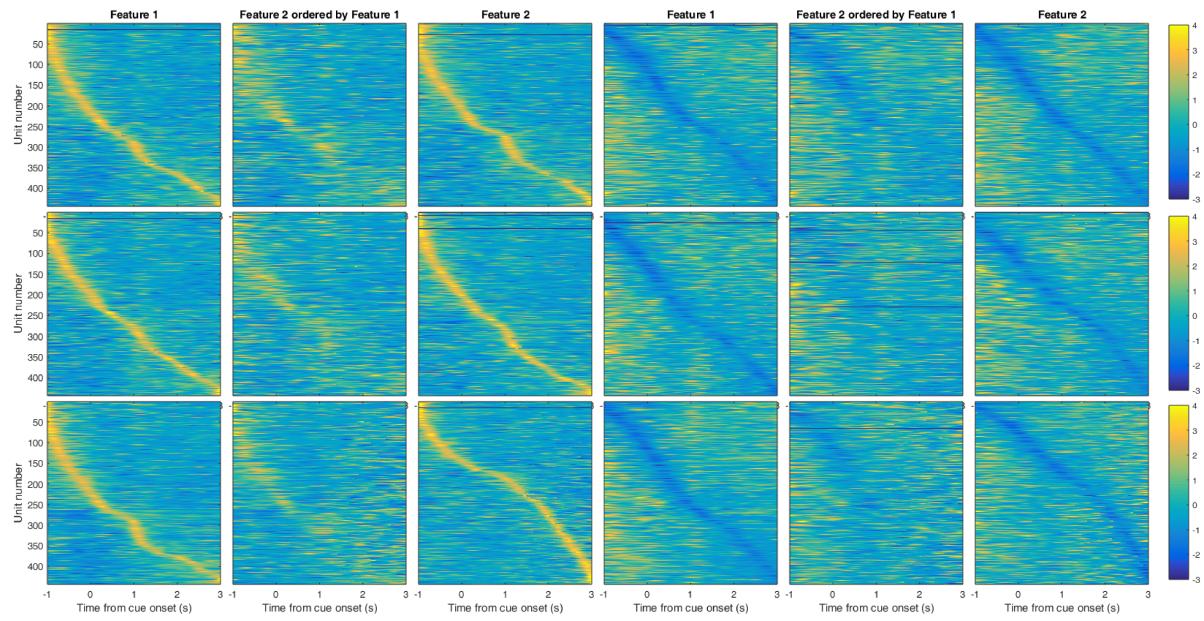
**Figure 9:** Summary of influence of various task parameters of cue-modulated NAc units during nosepoke. A. GLM matrix demonstrating impact of various task parameters on NAc firing rates. A GLM was fit to each unit that showed evidence of cue modulation by a Wilcoxon signed-rank test. Each row represents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