

Maintained coding of environmental features alongside outcome-predictive signals in the NAc (old: 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 ([Lee et al., 2012](#)) (Maia?). Since NAc is a primary target of DA signals interpretable as RPEs, and NAc

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

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

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

45 [Figure 1 about here.]

46 **Methods**

47 **Subjects:**

48 Adult male Long-Evans rats ($n = 4$, Charles River, Saint Constant, QC) were used as subjects. Rats were in-
49 dividually housed with a 12/12-h light-dark cycle, and tested during the light cycle. Rats were food deprived
50 to 85-90% of their free feeding weight (weight at time of implantation was 440 - 470 g), and water restricted
51 4-6 hours before testing. All experimental procedures were approved by the the University of Waterloo An-
52 imal Care Committee (protocol# 11-06) and carried out in accordance with Canadian Council for Animal
53 Care (CCAC) guidelines.

54 **Overall timeline:**

55 Each subject was first handled for seven days where during which they were exposed to the running room,
56 the sucrose solution used as a reinforcer, and the click of the valves upon approach to the receptacles. They
57 were then shaped to run on the task for seven days where they were restricted to running in the clock-
58 wise direction by presenting a physical barrier to running counterclockwise. Rats underwent hyperdrive
59 implantation after showing discrimination of approach behavior for rewarded and unrewarded then continued
60 to be trained each day for 100 trials in both a light and sound block until approach behavior discriminated
61 between reward-available and reward-unavailable cues for three consecutive days according to a chi square
62 test, at which point they underwent hyperdrive implantation targeted at the NAc. Rats were allowed to re-
63 cover for a minimum of five days before being retrained on the task, and recording began once performance
64 returned to pre-surgery levels. Upon completion of recording, animals were sacrificed and recording sites
65 were histologically confirmed.

66 **Behavioral task and training:**

67 Rats were trained to run clockwise on an elevated, square-shaped track (100x100 cm) containing four pos-
68 sible reward locations (Figure 2). Rats initiated a trial by running down the length of an arm, and triggering
69 a photobeam located 24 cm from the start of each arm. Upon trial initiation, a light or sound cue was pre-
70 sented that signaled the presence of absence (reward-available trial) or absence (reward-unavailable trial)

71 of a 12% sucrose water reward (0.1 mL) at the upcoming site. A trial was classified as an approach trial
72 if the rat turned left at the decision point and made a nosepoke at the reward receptacle (40 cm from the
73 decision point), while trials were classified as a skip trial if the rat instead turned right at the decision point
74 and triggered the photobeam to initiate the following trial. ~~There~~In reward-available trials there was a 1
75 second delay between a ~~rewarded~~-nosepoke and subsequent reward delivery. Trial length was determined
76 by measuring the length of time from cue onset until nosepoke (for approach trials), or from cue onset until
77 the start of the following trial (for skip trials). Trials could only be initiated through clockwise progression
78 through the series of arms, and each entry into the subsequent arm on the track counted as a trial. ~~Each day~~
79 To get enough trials for neural analysis rats were trained each day in both a light and sound block for 100
80 trials each. Within a block, one cue signaled reward was available on that trial, while the other signaled
81 reward was not available. Light block cues were a flashing white light, and a constant yellow light. Sound
82 block cues were a 2 kHz sine wave and a 8 kHz sine wave whose amplitude was modulated from 0 to max-
83 imum by a 2 Hz sine wave. Reward-cue associations were counterbalanced across rats. Cue presentation
84 was pseudorandomized so that the same cue could not be presented more than twice in a row. Block order
85 within each day was also pseudorandomized, such that the rat could begin a session with the same block
86 for more than two days in a row. Each training or testing day consisted of a 5 minute pre-session period
87 on a pedestal, followed by the first block, then the second block, then a 5 minute post-session period on
88 the pedestal. ~~Accuraey~~ Performance was determined by the proportion of trials a rat approached each cue.
89 Perfect performance would be 100% approach on ~~approach trials(reward available)~~reward-available trials,
90 and 0% approach on ~~skip trials(no reward available)~~reward-unavailable trials. Rats were trained daily un-
91 til they could distinguish between the ~~rewarded and unrewarded~~reward-available and reward-unavailable
92 cues for both light and sound blocks for three consecutive days, according to a chi-square test rejecting the
93 null hypothesis of equal approaches for reward-available and reward-unavailable trials, at which point they
94 underwent surgery. ~~Furthermore, we generated linear mixed effects models to investigate the relationships~~
95 ~~between cue type and our behavioral variables, with cue type as a fixed effect, and the addition of an intercept~~
96 ~~for rat identity as a random effect. Average proportion of trials approached and trial length for a session were~~
97 ~~used as response variables. Contribution of cue type to behavior was determined by comparing the full model~~
98 ~~to a model with cue type removed for each behavioral variable.~~

[Figure 2 about here.]

100 **Surgery:**

101 Surgical procedures were as described previously (Malhotra et al., 2015). Briefly, animals were anesthetized
102 with isoflurane, induced with 5% in medical grade oxygen and maintained at 2% throughout the surgery
103 (0.8 L/min). Rats were then chronically implanted with a hyperdrive consisting of 16 independently drivable
104 tetrodes, either all 16 targeted for the right NAc (AP +1.4 mm and ML +1.6 mm, relative to bregma; Paxinos
105 and Watson, 2005), or 12 in the right NAc and 4 targeted at the mPFC (AP +3.0 mm and ML +0.6 mm,
106 relative to bregma; only data from NAc tetrodes were analyzed). Following surgery, all animals were given
107 ~~a-at~~ least five days to recover and lower tetrodes to the target (DV -6.0 mm) before being reintroduced to the
108 behavioral task.

109 **Data acquisition and preprocessing:**

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

113 Candidate spikes for sorting into putative single units were obtained by band-pass filtering the data between
114 600-9000 Hz, thresholding and aligning the peaks (UltraMegaSort2k, Hull et al., 2011). Spike waveforms
115 were then clustered with KlustaKwik using energy and the first derivative of energy as features, and manually
116 sorted into units (MClust 3.5, A.D. Redish et al.). Isolated units containing a minimum of 200 spikes within
117 a session were included for subsequent analysis. Units were classified as ~~high firing neurons if they had high~~
118 ~~tonic firing rates marked fast spiking interneurons (FSIs)~~ by an absence of interspike intervals (ISIs) > 2 s,
119 while medium spiny neurons (MSNs) had a combination of ISIs > 2 s and phasic activity with shorter ISIs
120 (Barnes 2005, Atallah 2014).

121 **Data analysis:**

122 For behavioral performance we generated linear mixed effects models to investigate the relationships between
123 cue type and our behavioral variables, with cue type as a fixed effect, and the addition of an intercept for rat
124 identity as a random effect. Average proportion of trials approached and trial length for a session were used
125 as response variables. Contribution of cue type to behavior was determined by comparing the full model to
126 a model with cue type removed for each behavioral variable.

127 To investigate the contribution of various cue features on NAc firing rates we first determined whether firing
128 rates for a unit were modulated by the onset of a cue by collapsing across all cues and comparing the firing
129 rates for the 1 s preceding cue-onset with the 1 s following cue-onset. Single units were considered to be
130 cue-responsive if a Wilcoxon signed-rank test comparing pre- and post-cue firing had a $p < .01$. (Excluded:
131 the mean firing rate difference between pre- and post-cue onset was within the lower or upper 2.5% of a
132 shuffled distribution, as there is redundancy using both, and I did both because I was paranoid about just
133 using one.). Cue-modulated units were then classified as either increasing or decreasing in response to the
134 cue if the post-cue activity was higher or lower than the pre-cue activity, respectively.

135 To determine the relative contribution of various task parameters to firing rate variance for units whose firing
136 was modulated by cue-onset (as in Figures 5,6), a forward selection stepwise general linear model (GLM)
137 was fit to each cue-responsive unit. Cue ~~modality, cue location, cue outcome, identity (light block, sound~~
138 ~~block), cue location (arm 1, arm 2, arm 3, arm 4), cue outcome (reward-available, reward-unavailable),~~
139 approach behavior, trial length, trial number, and trial history were used as predictors, and the 1 s post-cue
140 firing rate as the response variable. Units were classified as being modulated by a given task parameter
141 if addition of the parameter significantly improved model fit using deviance as the criterion ($p < .01$). A
142 comparison of the R-squared value between the final model and the final model minus the predictor of
143 interest was used to determine the amount of firing rate variance explained by the addition of that predictor
144 for a given unit.

145 To better visualize responses to cues and enable subsequent population level analyses (as in Figures 5,7,8),
146 spike trains were convolved with a Gaussian kernel ($\sigma = 100$ ms), and peri-event time histograms (PETHs)
147 were generated by taking the average of the convolved spike trains across trials for a given task condition. For
148 analysis of population-level responses for cue features (Figure 7), convolved spike trains for all units where
149 cue modality identity, cue location, or cue outcome explained a significant portion of firing rate variance were
150 z-scored. Within a given cue feature, normalized spike trains were then separated according to the preferred
151 and non-preferred cue condition (e.g. light vs sound block), and averaged across units to generate population-
152 level averages. To account for separation that would result from any random selection of units, cell identity
153 was shuffled and the shuffled average for preferrend and non-preferred cue conditions was generated for
154 1000 shuffles.

155 To visualize NAc representation representations of task space within cue conditions, normalized spike trains
156 for all units were ordered by the location of their maximum or minimum firing rate for a specified cue
157 condition (Figure 8). To compare representation representations of task space across cue conditions for a cue
158 feature, the ordering of units derived for one condition (e.g. light block) was then applied to the normalized
159 spike trains for the other condition (e.g. sound block). For control comparisons within cue conditions, half
160 of the trials for a condition were compared against the other half. To look at the correlation of firing rates of
161 all cells within and across various cue conditions, trials for each cue condition for a cell were shuffled and
162 divided into two averages, and averages within and across cue conditions were correlated. A linear mixed
163 effects model was run for each cue condition to determine if correlations of firing rates within cue conditions
164 were more similar than correlations across cue conditions.

165 To identify the responsivity of units to different cue features at the time of a nosepoke into a reward re-
166 ceptacle, and subsequent reward delivery, the same cue-responsive units from the cue-onset analyses were
167 analyzed at the time of nosepoke and reward receipt using identical analysis techniques (Figures 9,10,11,12).

168 Given that some of our analyses compare firing rates across time, particularly comparisons across blocks,

169 we sought to exclude units with unstable firing rates that would generate spurious results reflecting a drift
170 in firing rate over time unrelated to our task. To do this we ran a Mann-Whitney U test comparing the cue-
171 evoked firing rates for the first and second half of trials within a block, and excluded 99 units from analysis
172 that showed a significant change for either block. All analyses were completed in MATLAB R2015a, and
173 the code is available on GitHub—our public GitHub repository (<http://github.com/vandermeelab/papers>),
174 and the data can be accessed through DataLad.

175 **Histology:**

176 Upon completion of the experiment, rats were anesthetized with 5% isoflurane, then asphyxiated with carbon
177 dioxide. Transcardial perfusions were performed, and brains were fixed and removed. Brains were sliced in
178 50 um coronal sections and stained with thionin. Slices were visualized under light microscopy, tetrode
179 placement was determined, and electrodes with recording locations in the NAc were analyzed (Figure 3).

180 [Figure 3 about here.]

181 **Results**

182 **Behavior**

183 Rats were trained to discriminate between cues signaling the availability and absence of reward on a square
184 track with four identical arms for two distinct sets of cues. During each session, rats were presented
185 sequentially with two behavioral blocks containing ~~different sensory cues in sequential order~~ cues from
186 different sensory modalities, a light and a sound block, with each block containing a cue that ~~predicted~~
187 ~~reward~~ signalled the availability of reward (reward-available), and a cue that signalled the absence of reward

188 (reward-unavailable). An example learning curve is seen in Figure 4A,B. All four rats learned to discrimi-
189 nate between the rewarded and unrewarded cue reward-available and reward-unavailable cues for both the
190 light and sound blocks as determined by reaching significance ($p < .05$) on a daily chi-square test compar-
191 ing approach behavior for rewarded and unrewarded reward-available and reward-unavailable cues for each
192 block, for at least three consecutive days (range for time to criterion: 22 - 57 days). Maintenance of
193 behavioral performance during recording sessions was assessed using linear mixed effects models for both
194 proportion of trials where the rat approached the receptacle, and trial length. Analyses revealed that cue type
195 had an influence on the likelihood of a rat to make an approach (was influenced by which of the cues was
196 presented (Percentage approached: light reward-available = 97%; light reward-unavailable = 34%; sound
197 reward-available = 91%; sound reward-unavailable 35%; $p < .001$), but not for that cue identity did not
198 significantly affect the length of time taken to complete a trial (Trial length: light reward-available = 1.85
199 s; light reward-unavailable = 1.74 s; sound reward-available = 1.91 s; sound reward-unavailable 1.78 s; p
200 = .13)(Figure 4C,D). Thus, during recording, rats successfully discriminated the various cues according to
201 whether or not they signalled the availability of reward at the reward receptacle.

202 [Figure 4 about here.]

203 NAc neurons encode behaviorally relevant and irrelevant cue features

204 General responses to cue Single unit responses discriminate cue features:

205 We sought to address which parameters of our task were encoded by NAc activity, specifically whether
206 the NAc encodes aspects of motivationally relevant cues not directly tied to reward, such as the identity
207 and location of the cue, and whether this coding is independent or integrated with coding of cue outcome.
208 To do this we recorded a total of 443 units with > 200 spikes in the NAc from 4 rats over 57 sessions
209 while they performed on the square track a cue discrimination task (Table 1). Units that had less than 200

210 spikes, or exhibited a drift in firing rate over the course of either block were excluded from further analysis,
211 leaving 344 units for further analysis. The activity of 133 (30%) of these was 344 units were modulated
212 by the cue, with more units showing a decrease in firing ($n = 124$) than an increase ($n = 47$) around
213 the time of cue-onset (Table 2). Within this group, 32 were classified as HFNs, while 139 FSiS, while
214 109 were classified as SPNs. To determine Upon visual inspection, we observed several patterns of firing
215 activity, including units that; discriminated firing upon cue-onset across various cue conditions, showed
216 sustained differences in firing across cue conditions, had transient responses to the cue, showed a ramping
217 of activity starting at cue-onset, and showed elevated activity immediately preceding cue-onset, for example
218 (Figure 5). To characterize more formally whether these cue-evoked responses were modulated by various
219 aspects of the task, we fit a GLM to each cue-modulated unit. Fitting GLMs revealed that a variety of task
220 parameters accounted for a significant portion of firing rate variance in NAc cue-modulated units ,with
221 all task parameters except trial history being predictors in 19-38% of cue-modulated units, and explaining
222 4-9% on average, although there was substantial variability across cells (Figures 5, (Figure 6, Table 2).
223 Notably, there were units that discriminated between whether the rat was performing in the light or sound
224 block ($n = 37 / 133$ of cue-modulated units, accounting for 6% of variance on average), which arm the
225 rat was currently on ($n = 50 / 133$ of cue-modulated units, accounting for 6% of variance on average),
226 and whether the rat was engaged in a rewarded or unrewarded trial ($n = 34 / 133$ the common portion of a
227 reward-available or reward-unavailable trial (26% of cue-modulated units, accounting for 4% of variance
228 on average), suggesting that the NAc independently encodes features of reward-predictive cues separate
229 from expected outcome (Figure 5A-F). Furthermore, interactions between multiple cue features appeared
230 as significant predictors of firing rate variance for 9%-8% of cue-modulated units($n = 11 / 133$), although
231 this effect was modest (~1-2% variance explained), suggesting a weak effect for integrated coding across
232 various aspects of a cue (Figure 5G,H). Fitting a GLM to all recorded units ...*Jimmie finish this* revealed
233 a similar pattern of results (data not shown). Together, these findings show that various cue features are
234 represented in the NAc, and that this coding has some overlap, but is mostly distinct from expected outcome
235 (Figure 1; H2,H3).

236

[Table 1 about here.]

237

[Table 2 about here.]

238

[Figure 5 about here.]

239

[Figure 6 about here.]

240 **Population level ~~responses averages reveal characteristic response profiles:~~**

241 ~~NAc units do not work in isolation, and some information about the cue may be hidden in single unit~~
 242 ~~responses. To get a sense of how NAc output encoded cue information at the population level, A variety of~~
 243 ~~single unit response profiles were observed around the time of cue-onset. To investigate whether these firing~~
 244 ~~rate patterns were related to the cue information they represented, we looked at population level averages~~
 245 ~~for units that were modulated by each feature. To do this we normalized firing activity for each unit that~~
 246 ~~was modulated by a given cue feature, such as light block, then generated the cue-onset aligned population~~
 247 ~~average firing rate for each of the cue features (Fig Figure 7). This analyses revealed overall that cells that~~
 248 ~~showed an increase upon cue presentation had stronger responses for the preferred cue condition (Figure~~
 249 ~~7A,C,E). Interestingly, units that were classified as decreasing in response to the cue showed a biphasic re-~~
 250 ~~sponse at the population level, with a small peak at a time in alignment with entry into the arm, followed~~
 251 ~~by a sustained dip after cue-onset (Figure 7B,D,F). Units that were modulated by cue ~~modality showed by~~~~
 252 ~~identity showed~~ a stronger increase in response to the preferred ~~cue modality task block~~, as well as a higher
 253 tonic firing rate to the preferred ~~cue modality task block~~, most notably in units that decreased in firing rate to
 254 the cue (Figure 7A,B). Units that were modulated by cue location showed a graded response to locations of
 255 decreasing preference, with peak firing occurring around cue-onset (Figure 7C,D). ~~Units that were modulated~~
 256 ~~by cue outcome showed a ramping of activity after cue-onset for their preferred cue type.~~ Additionally, units
 257 ~~whom had that~~ exhibited a decrease in firing in response to the cue and whose activity was modulated by

258 cue outcome, showed a sustained discriminatory response to ~~rewarded and unrewarded reward-available and~~
259 ~~reward-unavailable~~ cues that extended beyond cue-onset (Figure 7F). Together, these visualizations of the
260 averaged population responses revealed nuanced differences in the way NAc units are modulated by cue
261 conditions across cue features.

262 [Figure 7 about here.]

263 **NAc units dynamically segment the task:**

264 ~~Another method to get a sense of how the NAc is behaving at the population level is to analyze NAc unit~~
265 ~~activity across time, and NAc neurons have been shown to have correlates across an entire task space~~
266 ~~Given the varied time courses and response profiles of NAc units to various aspects of the cue, the NAc may be~~
267 ~~computing a temporally evolving state value signal (Pennartz., 2011). If this is the case, then the recruitment~~
268 ~~of NAc units should vary alongside changes in the environment.~~ To look at the distribution of responses
269 throughout our task space and see if this distribution is modulated by cue features, we z-scored the firing rate
270 of each unit and plotted the normalized firing rates of all units aligned to cue-onset and ~~according to sorted~~
271 ~~them according to the time of~~ peak firing rate (Figure 8). We did this separately for both the light and sound
272 blocks, and found a nearly uniform distribution of firing fields in task space that was not limited to alignment
273 to the cue (Figure 8A). Furthermore, to ~~see determine~~ if this population level activity was similar across
274 blocks, we also organized firing during the sound blocks according to the ordering derived from the light
275 blocks. This revealed that while there was some preservation of order, the overall firing was qualitatively
276 different across the two blocks. ~~To control for the possibility that any comparison of trials would produce~~
277 ~~this effect, we did a within block comparison, comparing half of the trials in the light block against the other~~
278 ~~half. This comparison looked similar to our test comparison of sound block trials ordered by light block~~
279 ~~trials.~~ Additionally, given that the majority of our units showed an inhibitory response to the cue, we also
280 plotted the firing rates according to the lowest time in firing, and again found some maintenance of order, but
281 largely different ordering across the two blocks, and the within block comparison (Figure 8B). To further test

282 this, we divided each block into two halves and looked at the correlation of the average smoothed firing rates
283 across various combinations of these halves across our cue-aligned centered epoch (Table 3). A linear mixed
284 effects model revealed that within block correlations (e.g. one half of light trials vs other half of light trials)
285 were higher and more similar than across block correlations (e.g. half of light trials vs half of sound trials),
286 suggesting that activity in the NAc discriminates across various cue conditions. This process was repeated for
287 cue location and cue ~~outcomes~~outcome, showing that NAc segmentation of the task is qualitatively different
288 ~~across various cue conditions~~ even during those parts of the task not immediately associated with a specific
289 cue, action, or outcome, although the within condition comparison of reward-unavailable trials was less
290 correlated than reward-available trials, and more similar to the across condition comparisons (Figure 8C-F).

291 [Figure 8 about here.]

292 [Table 3 about here.]

293 **Encoding of cue features is ~~not limited to cue-onset~~maintained until outcome:**

294 In order to be useful for ~~reinforcement~~ learning, a trace of the cue must be maintained until the outcome.
295 ~~Fitting to link the outcome to the outcome-predictive cue. To test whether representations of cue features~~
296 ~~were maintained post-approach until the outcome was revealed, we fit a GLM to the post-approach fir-~~
297 ~~ing rates of cue-modulated units at aligned to the time of a nosepoke response nosepoke into the reward~~
298 ~~receptacle. This analysis~~ showed that a variety of units still discriminated firing according to various cue
299 features, but not other task parameters, showing that NAc activity discriminates various cue conditions well
300 into a trial (Table 3, ~~Figure 1, Figures 9,10~~). Additionally, these units were a mix between those that encoded
301 ~~cue features at cue-onset, and those that did not previously have a cue feature as a predictor (29, 48, and 30~~
302 ~~units encoded both time points for cue identity, location, and outcome, respectively)~~. Fitting a GLM to all
303 recorded units ...*Jimmie finish this* ~~revealed a similar pattern~~ (data not shown). Population level averages
304 for units that increased to cue-onset showed a ramping up of activity that peaked upon nosepoke, whereas

305 units that decreased to cue-onset showed a gradual reduction of firing activity that reached a minimum upon
306 nosepoke (Figure 11). Additionally, a peak is seen for preferred cue outcome in decreasing units at 1 second
307 post cue-onset when reward was received, demonstrating an integration of expected and received reward
308 (Figure 11F). Furthermore, aligning normalized peak firing rates to nosepoke onset, revealed a clustering
309 of responses around reward receipt for all cue conditions where the rat would have received reward (Figure
310 12). Alongside this, fitting a GLM To determine whether coding of cue features was maintained after the
311 outcome was revealed, a GLM was fit to the firing rates of cue-modulated units at the time of reward receipt,
312 during which the cue was still present. Fitting a GLM revealed 10 units (8%) where cue outcome accounted
313 for an average of 32% of firing rate variance (Table 1, data not shown). However, an absence of cue identity
314 or cue location coding was observed, suggesting that the NAc does not maintain a representation of these
315 cue features once the rat receives behavioral feedback for its decision.

316 [Figure 9 about here.]

317 ~~Task parameter Total MSN (increasing) MSN (decreasing) FSI (increasing) FSI (decreasing) Cue modality~~
318 ~~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~~
319 ~~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~~

320 Cells from nosepoke GLM

321 [Figure 10 about here.]

322 [Figure 11 about here.]

323 [Figure 12 about here.]

324 **Discussion**

325 The present study found evidence for coding of multiple identifying features of motivationally relevant stim-
326 uli in addition to expected outcome; the sensory modality of the presented cue, as well as its physical lo-
327 cation within the track, and that this coding was ~~still present maintained~~ on approach trials ~~when the rat~~
328 ~~nosepoked during a delay period where the rat held a nosepoke until receipt of outcome~~. Furthermore, this
329 coding was both independent, and intermixed across cue features and behavioral measures. Coding at the
330 population level revealed qualitatively distinct comparison profiles across cue features, such as cells that
331 encoded cue modality tended to sustain the discriminatory firing outside of cue presentation, while a subset
332 cells that discriminated ~~rewarded from unrewarded~~ reward-available from reward-unavailable cues main-
333 tained this discrimination until after reward receipt. Across all recorded cells, a tiling of task structure was
334 observed such that all points within our analyzed task space was accounted for by the ordered peak firing
335 rates of all cells, and this tiling differed between various conditions with a cue feature, such as light versus
336 sound blocks. Cells that discriminated across blocks were not simply due to drifting of the signal across
337 trials, as cells that showed a drift in firing between the first and second half within a block were excluded
338 from the analysis. Furthermore, even though actions were stereotyped during correct trials, such that the rat
339 always turned left at the decision point to approach for reward, and right to skip the receptacle and initiate the
340 next trial, cells that were modulated by the expected value of the cue maintained their specific firing patterns
341 even during error trials where the rat turned left after presentation of the ~~unrewarded reward-unavailable~~ cue,
342 suggesting that these signals did not represent action values. Additionally, NAc signals have been shown to
343 be modulated by response vigor, to detangle this from our results we included the trial length as a predictor
344 in our GLMs, and found cells with correlates independent of trial length.

345 **Cue modality**Potential functional consequences of maintained coding:

346 NAc has been shown to encode stimulus-outcome associations. In reinforcement learning, these associations
347 are commonly framed as a value estimate tied to the reward-predicting stimulus. This view, while informative,

348 may not be complete in the sense that these value estimates may be more complex than just an agnostic value
349 signal, for instance, the NAc is tuned to the identity of a reward. Our goal was to expand our understanding of
350 the informational content of these outcome-predictive signals to include aspects of this environmental signal
351 expected to be encoded by the NAc based on its corticolimbic inputs (discrete cues - amygdala input, spatial
352 context - hpc input, task rules/set, behavioral strategy - pfc input). Having an enriched (multidimensional?)
353 representation that includes details about the environmental context the animal finds itself and is maintained
354 online until the outcome is revealed could be useful for assigning credit of a reward to the appropriate
355 elements of an environment, to develop an accurate value function and optimize long term acquisition of
356 reward (2012 Lee - eligibility trace). From a motivated behavior standpoint, the presence of these enriched
357 representations can help inform action selection involving Pavlovian behaviors (conditioned approach), and
358 after a decision has been made the maintained representation can help to hold the response in the face of
359 competing alternatives while waiting for an outcome (Floresco 2015 summarizes this literature).

360 If these representations function as an eligibility trace, then there should be a relationship between the
361 content and robustness of these representations and the degree to which organisms form adaptive associations
362 between reinforcers and their environment, which could contribute to the presence of prediction errors in the
363 NAc in those that learned an experimental task in humans versus those that did not (Schonberg 2007). In
364 the present experiment, understanding what information about the environment the NAc is keeping track of
365 when reward arrives could be useful for potentially determining what representations are being reinforced.
366 Would be interesting to see if selectively reward an animal when certain representations are active (e.g. arm
367 1), would help shape their assignment of reward to that arm. Could be useful for altering a maladaptive
368 preference back to a more adaptive one, as the loss of certain aspects of coding via BLA and PFC inputs
369 leads to the loss of outcome-predictive activity to discrete cues in the NAc. Furthermore, non-discriminatory
370 coding in the NAc could hypothetically be correlated to over-generalization of situations, in which the animal
371 may not recognize it is in a different situation and perform actions that are inefficient or maladaptive for
372 reward procurement.

373 **Cue identity:**

374 Our finding that NAc units can discriminate between outcome-predictive stimuli with similar biological
375 significance, in the current study cues from different sensory modalities, expands upon an extensive literature
376 examining neural correlates of conditioned stimuli. Perhaps the most comparable work in rodents comes
377 from a study that found distinct coding for an odor when it predicted separate but equally valued rewards
378 (Cooch). The present work is complementary to this as it shows that NAc cells have representations of
379 identifiable aspects of the cue itself, in addition to the reward it predicts. Another study paired separate
380 cues with appetitive or aversive outcomes, and found separate populations of cells that encode each cue,
381 with many switching selectivity after reversal of the associations between the cues and outcomes, providing
382 evidence that the NAc encodes the biological significance of stimuli. Once again, our study was different
383 as we recorded neural responses to distinct cues encoding the same anticipated outcome, suggesting that
384 even when the biological relevance of these stimuli is similar, the NAc dissociates their representations
385 at the level of the single-unit (Setlow), although this doesn't explain the difference in population averaged
386 firing rate for the epochs when the cue is not present. Another possibility is that these modality specific
387 cells were encoding the context, rule, or sequence within a session as some cells responded similar for both
388 ~~rewarded and unrewarded~~ reward-available and reward-unavailable cues within a block. This interpretation
389 is in alignment with a recent paper from the primate realm non-human primate literature that recorded NAc
390 responses during the Wisconsin Card Sorting Task (WCST), a common set-shifting task used in both the
391 laboratory and clinic, and found cells that preferred firing to stimuli when a certain rule, or rule category
392 was currently active (Sleazer). Indeed, an encoding of the current strategy could be an explanation as to why
393 a sustained difference in population averaged firing was seen across stimulus blocks, as well as a potential
394 explanation for the differentially tiling of task structure across blocks in the current study. Further support for
395 a modulation of NAc responses by strategy comes from an fMRI study that examined BOLD levels during
396 a set-shifting task (FitzGerald et al., 2014). In this task, participants learned two sets of ~~stimuli-reward~~
397 stimulus-outcome contingencies, a visual set and auditory set. During testing they were presented with
398 both simultaneously, and the stimulus dimension that was relevant was periodically shifted between the two.

399 Here, they found that bilateral NAc activity reflected value representations of whatever the currently relevant
400 stimulus dimension was, and not the irrelevant stimulus. The current finding of separate, but overlapping,
401 populations of cells encoding cue modality and expected value, suggests that the fMRI finding is generated
402 by the combined activity of several different functional cell types.

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

413 **Encoding of position:**

414 Our finding that cue-evoked activity was modulated by cue location sides with some of the literature (Lavoie,
415 1994; Tabuchi, 2000; Strait, 2016). An alternative explanation for a pure spatial representation, is that
416 these are task segmentation correlates, keeping track of where in the task the rat is. A previous non-human
417 primate paper has shown that when reward is contingent upon completion of a series of trials, separate
418 populations of NAc neurons signal the start of a schedule, subsequent trials in the schedule, and the first
419 trial in extended schedules (Shidara et al., 1998). This signalling of position within a sequence has been
420 observed in subsequent studies, and it is possible that the our rats were keeping track of which specific arm
421 they were in as part of a sequence of arms, and not just strictly a spatial representation (Mulder, 2004 and
422 2005; Khamassi et al., 2008; Berke, 2009). Also, given that our task is pseudo-random, it is possible that the

423 rats learned which cue to anticipate, and the neural activity could reflect this. However, this is unlikely as
424 including a previous trial variable in the analysis did not explain a significant amount of firing rate variance
425 in response to the cue for the vast majority of cells..

426 **Mixed selectivity:**

427 Several other papers have reported unit profiles that integrate different task-related variables. These papers
428 report integrated coding between expected value and subsequent motor responses, expected value and iden-
429 tity of a reward, and a combination of spatial-, movement-, and reward-related features (Roesch, Lavoie,
430 Cooch). However, our study is the first to show mixed selectivity among identifying features of a cue and
431 expected outcome or behavior. The presence of mixed selectivity responses confers a larger number of input-
432 output relationships that are available to a given neuron. A possible functional consequence of this attribute
433 of NAc units, is the combination and transformation of various motivationally relevant features into a sig-
434 nal informing downstream decoders such as the ventral pallidum about appropriate behaviors in obtaining
435 motivationally relevant goals and biasing action selection towards these behaviors.

436 Mixed selectivity in the NAc could be a consequence of synaptic integration from a variety of anatomically
437 distinct inputs, as seen in experiments examining the convergence of various NAc afferents at the level of
438 synaptic transmission and stimulation-induced firing (Goto and Grace 2008). In one such experiment it
439 was shown that NAc cells that responded to stimulation of either the fornix, amygdala, or PFC, typically
440 responded to stimulation from all inputs (ODonnell and Grace, 1995). Furthermore, an interaction between
441 these inputs was observed such that PFC stimulation failed to elicit spiking in the NAc neurons unless they
442 were in a depolarized UP-state, a state induced by hippocampal stimulation and was dependent on an intact
443 fornix. Hippocampal-induced suppression of other inputs has also been observed for the BLA (Mulder et al.,
444 1998). Recently, it has also been shown that train stimulation of PFC afferents reduces hippocampal-evoked
445 NAc responses, suggesting that there is competition between various inputs (Calhoon and ODonnell, 2013).
446 These studies suggest that the integration of the variables we saw could be the result of this gating observed

447 in behaviorally-independent preparations. However, given that we did not systematically manipulate these
448 various limbic and cortical afferents, comments on the anatomical origins of the observed mixed selectivity
449 responses are speculative at this point.

450 Integrating cue identity and value, as seen modestly in the present study, could be one neural instantiation of
451 how value is associated with the appropriate predictive stimuli (credit assignment), keeping in mind that value
452 encoding is distributed, redundantly in some aspects, across various structures (Hayden Nat Neuro opinion).
453 Indeed, lesions of the NAc impair the ability to learn changes in reward value or identity in an unblocking
454 experiment, as well as disrupting dopamine RPEs generated by modification of timing of reward (McDannald
455 2011, Takahashi 2016). Would be interesting to see if uncoupling the integrated coding of stimulus features
456 and predictive properties of a cue has an effect on the ability of a rat to use reward-predictive cues to pursue
457 the associated reward.

458 **Tiling of task structure:**

459 Additionally, we found that the population of recorded units had a relatively uniform distribution of firing
460 fields within our task space, similar to what has been reported previously (Shidara, 1998; Berke, 2009;
461 Lansink, 2012). Uniquely, we found that this representation differed across conditions of a cue features,
462 such according to whether the rat was currently engaged in the light or sound block, suggesting that this
463 could be a possible neural correlate for encoding the currently relevant strategy in the NAc. It has been
464 previously shown that during progress through a predictable trial series, neurons represented state value
465 of cue (Shidara 1998), and that single-unit responses allowed the monkey to know how it was progressing
466 throughout the task. Likewise, the tiling we saw could be a consequence of upstream cortical or limbic inputs
467 informing the striatum of the current task rules. Another possibility is that the NAc not only pays attention
468 to progress throughout a task within a trial, but also higher-order task information, like blocks. Furthermore,
469 dopamine levels in the NAc fluctuate through a trial, and it is possible that the observed tiling could be a
470 NAc-representation of state value related to this temporally evolving dopamine signal. Future experiments

471 should monitor this mapping of task structure during the application of dopamine antagonists. Finally, the
472 presence of functional correlates not evident when looking at single-unit responses time-locked to salient
473 task events emphasizes the need to employ ensemble level analyses across all aspects of a task.

474 **References**

- 475 Lee, D., Seo, H., and Jung, M. W. (2012). Neural Basis of Reinforcement Learning and Decision Making. *Annu. Rev. Neurosci*,
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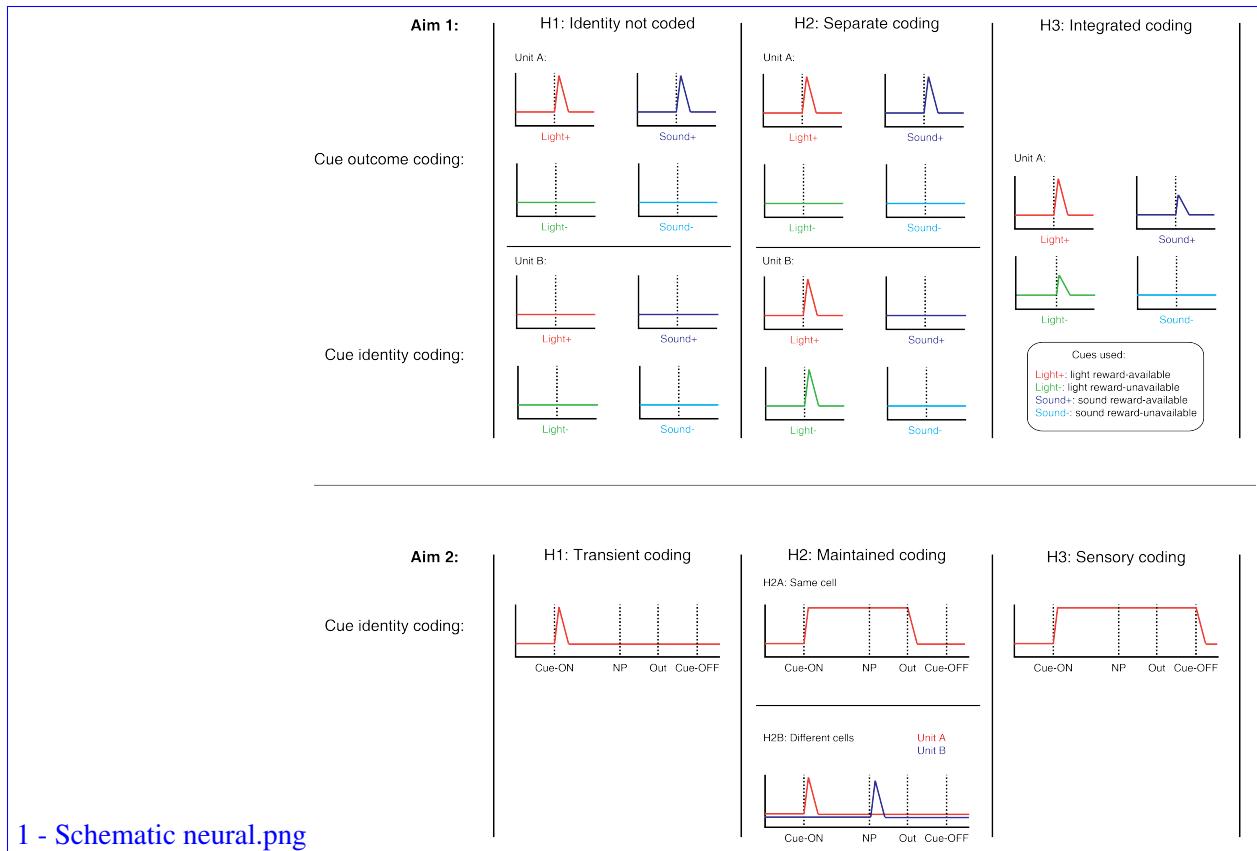


Figure 1: Schematic of potential coding strategies employed by single units in the NAc. **Aim 1:** Displayed are schematic PETHs illustrating putative responses to different cues under different hypotheses of how cue identity and value are coded. H1 (left panel): Coding of **identifying cue features identity** is absent in the NAc. Top: Unit A responds to a motivationally relevant variable, such as expected **value outcome**, similarly across other cue features, such as stimulus modality or physical location. Hypothetical plot is firing rate across time. Light+ (red) signifies a **rewarded reward-available** light cue, Sound+ (navy blue) a **rewarded reward-available** sound cue, Light- (green) **an unrewarded a reward-unavailable** light cue, Sound- (light blue) **an unrewarded a reward-unavailable** sound cue. Dashed line indicates onset of cue. Bottom: No units within the NAc discriminate their firing **across various conditions of these according to cue features identity**. H2 (middle panel): Coding of cue **features identity** occurs independently of encoding of motivationally relevant variables like expected **value outcome** or subsequent vigor. Top: Same as H1, with unit A discriminating between **rewarded reward-available** and **unrewarded reward-unavailable** cues. Bottom: Unit B discriminates firing across **conditions of a cue feature stimulus modalities**, depicted here as firing to light cues but not sound cues. H3 (right panel): Coding of cue **features identity** is integrated with coding of other motivationally relevant variables. Hypothetical example demonstrating a unit that responds to reward-predictive cues, but firing rate is also modulated by stimulus modality, firing most for the **rewarded reward-available** light cue. **Aim 2:** Displayed are schematic PETHs illustrating potential ways in which cue identity is represented. H1 (left panel): Cue-onset triggers a transient response to a unit that codes for cue identity. Dashed lines indicate time of a behavioral or environmental event. Cue-ON signifies onset of cue, NP signifies when the rat holds a nosepoke at a reward receptacle, Out signifies when the outcome is revealed, Cue-OFF signifies when the cue turns off. H2 (middle panel): Coding of cue identity is maintained during a nosepoke hold period until outcome is revealed. Coding can either be maintained by the same unit as during cue-onset (H2A) or by a sequence of units (H2B). H3 (right panel): Coding of cue identity is maintained during entire duration of cue, including after outcome is revealed. Same hypotheses applies to other information-containing aspects of the environment when the cue is presented, such as the physical location of the cue.

2 - Schematic task.png

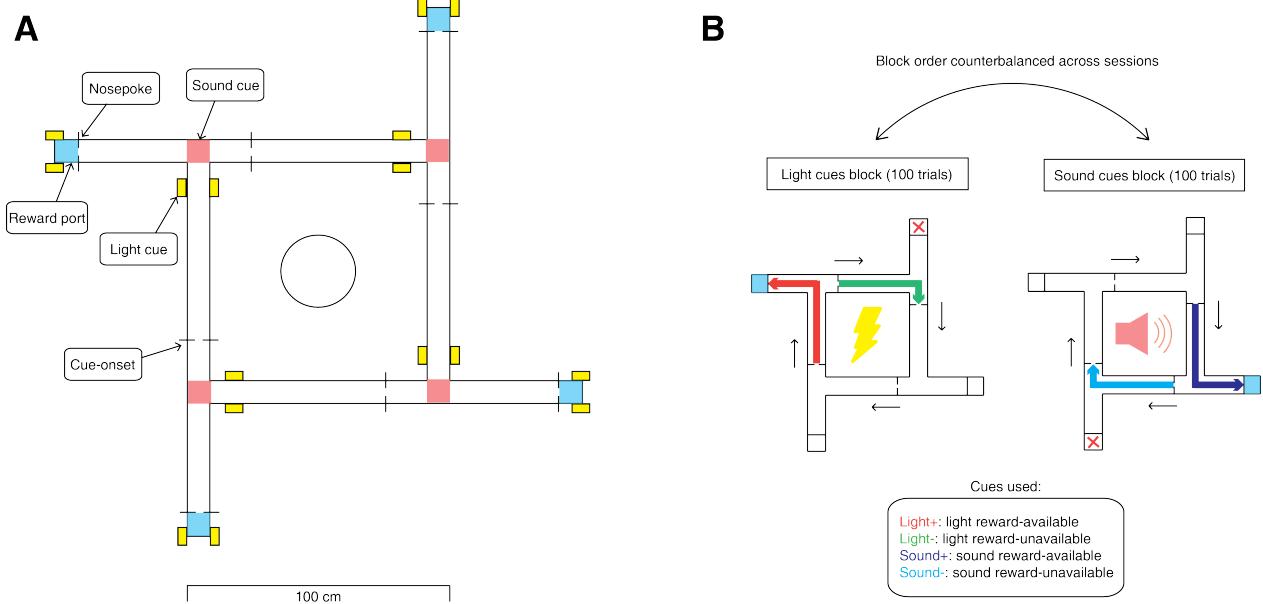


Figure 2: Schematic of behavioral task. A. To scale depiction of square track consisting of multiple identical T-choice points. Dashed lines indicate location of photobeams. Photobeams on the center, square portion of the track triggered the start of a trial and onset of a cue, whereas photobeams at the ends of the arms by the receptacles registered nosepokes. Rectangular boxes surrounded by light bulbs with yellow fill indicate location of LEDs used for light cues. Speakers for tone cues were placed underneath the choice points, indicated by the speaker images magenta fill on track. Water droplets Light blue fill on ends of arms indicate location of receptacles where sucrose was dispensed on rewarded trials. Circle in the center indicates location of pedestal during pre- and post-records. Scale bar is located beneath the track. B. Progression of a recording session. ~~Left:~~ A session was started with a 5 minute recording period on a pedestal placed in the center of the apparatus. ~~Here the square track is greyed out and used as a reference point to contextualize the position of the pedestal, indicated here by the black circle.~~ ~~Middle:~~ Rats then ~~underwent performed~~ two blocks of a cue discrimination task on a square track where they had to dissociate between a ~~rewarded reward-available~~ and ~~unrewarded reward-unavailable~~ cue for a set of light cues and a set of sound cues for a target of 100 trials each. Rats ran clockwise on the square track, and upon presentation of the cue ~~had to could either~~ turn left at the choice point to ~~receive check if reward for a rewarded cue was available at the reward receptacle~~ (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 light blue in figure). ~~Rewarded Reward-available~~ and ~~unrewarded reward-unavailable~~ cues were presented pseudo-randomly, such that not more than two of the same type of cue could be presented in a row. Location of the cue on the track was irrelevant for behavior, all cue locations ~~were equally rewarded contained an equal amount of reward-available~~ and ~~unrewarded reward-unavailable trials~~. ~~Middle-left Left~~ in figure depicts a light block, showing an example trajectory for a ~~rewarded correct reward-available~~ (red) and ~~unrewarded reward-unavailable~~ (green) trial. ~~Middle-right Right~~ in figure depicts a sound block, with a ~~rewarded reward-available~~ (navy blue) and ~~unrewarded reward-unavailable~~ (light blue) trial. Ordering of the light and sound blocks was counterbalanced across sessions. Dashed line within track indicates photobeam that triggered cue onset. Solid line within track indicates photobeam that registered a nosepoke into the reward receptacles. Receptacles containing sucrose solution are present within the squares at the end of each arm. ~~Water droplet Light blue fill~~ signifies reward receipt on a correct ~~reward-available~~ trial. Arrows outside of track indicate correct running direction. ~~Right:~~ A session ended with another 5 minute recording period on the pedestal.

3 - Histology.png

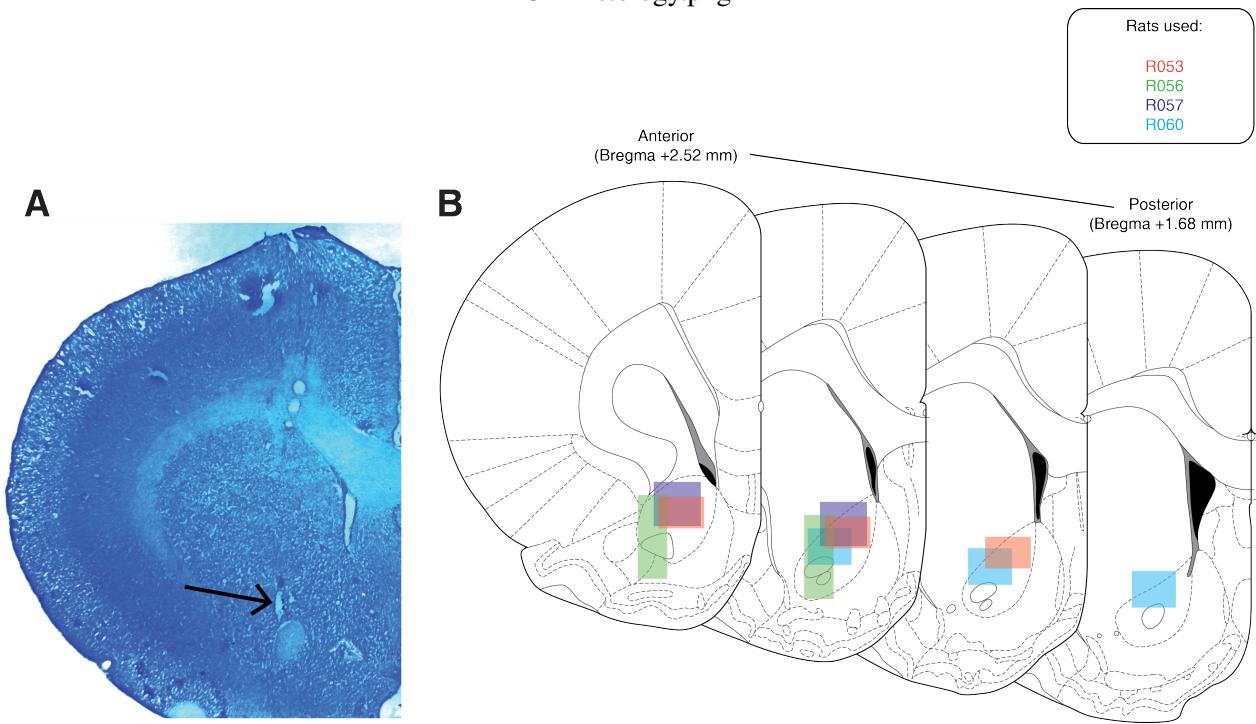


Figure 3: Histological verification of recording sites. Upon completion of experiments, brains were sectioned and tetrode placement was confirmed. A. Example section from R060 showing a recording site in the NAc core just dorsal to the anterior commissure (white circle). B. Schematic showing recording areas for the four rats used in the present study..

4 - Behavioral results.png

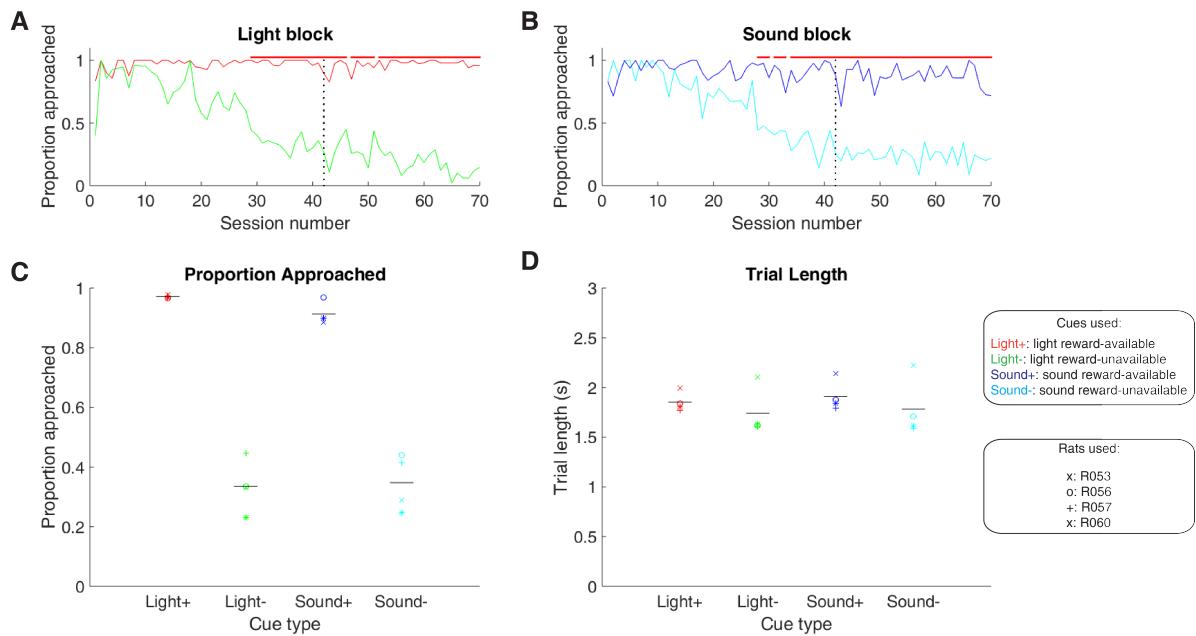


Figure 4: Performance on the behavioral task. A-B. Example learning curves across sessions from a single subject (R060) showing the proportion approached for rewarded reward-available (red line for light block, navy blue line for sound block) and unrewarded reward-unavailable trials (green line for light block, light blue line for sound block). Fully correct performance corresponds to approach proportion of 1 for rewarded reward-available trials and 0 for unrewarded reward-unavailable trials. **Note that this subject starts** Rats initially start out approaching on both rewarded reward-available and unrewarded reward-unavailable trials, and learns learn with experience to skip non-rewarded trials. Red bars indicate days in which a rat statistically discriminated between rewarded reward-available and unrewarded reward-unavailable cues, determined by a chi square test. Dashed line indicates time of electrode implant surgery. C-D. Summary of performance during recording sessions for each rat. C. Proportion approached for all rats, averaged across all recording sessions. Different columns indicate the different cues (rewarded reward-available (+red) and unrewarded reward-unavailable (-green) light cues, rewarded reward-available (navy blue) and unrewarded reward-unavailable (light blue) sound cues). Different symbols correspond to individual subjects; horizontal black line shows the mean. All rats learned to discriminate between rewarded reward-available and unrewarded reward-unavailable cues, as indicated by the clear difference of proportion approached between rewarded reward-available (0.9x ~90% approached) and unrewarded reward-unavailable cues (0.3x ~30% approached), for both blocks (see Results for statistics). D. Average trial length for each cue. Note that the time to complete a trial was comparable for the different cues.

5 - Neural examples.png

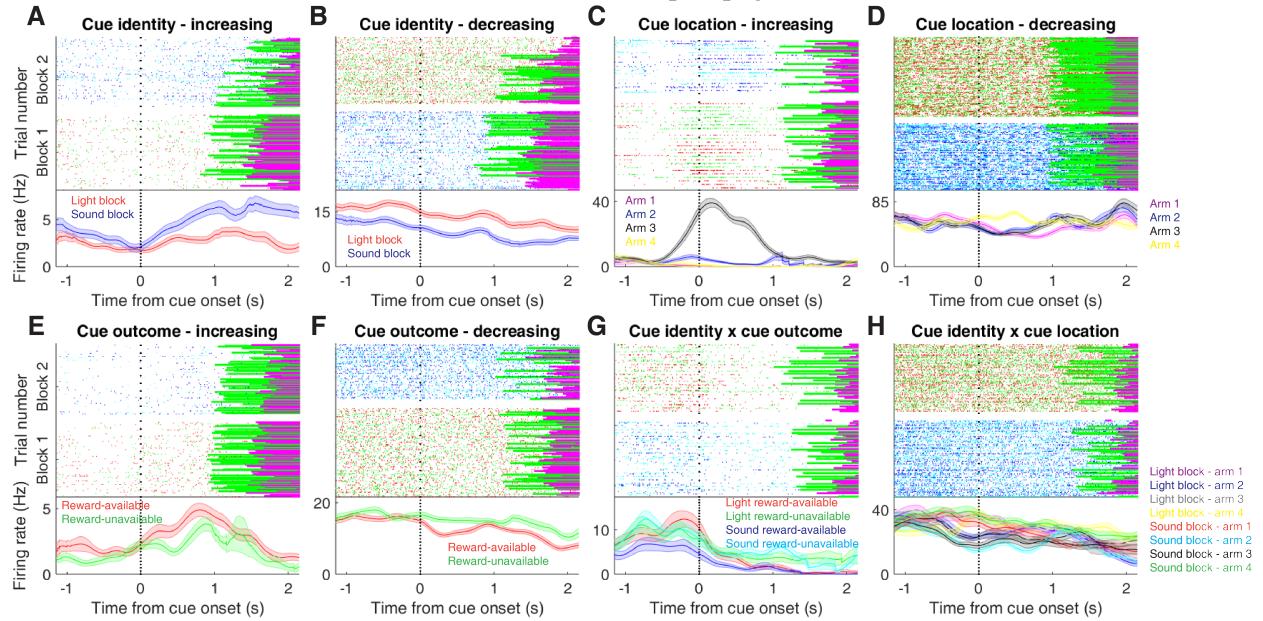


Figure 5: Examples of different cue-modulated NAc units influenced by various task parameters. A. Example of a cue-modulated NAc unit that showed an increase in firing in response to the cue, and encoded cue **modality identity**. Top: rasterplot showing the spiking activity across all trials aligned to cue-onset. Spikes across trials are color coded according to cue type (red: **rewarded reward-available** light; green: **unrewarded reward-unavailable** light; navy blue: **rewarded reward-available** sound; light blue: **unrewarded reward-unavailable** sound). Green and magenta bars indicate trial termination when a rat initiated the next trial or made a nosepoke, respectively. White space halfway up the rasterplot indicates switching from one block to the next. Dashed line indicates cue onset. Bottom: PETHs showing the average smoothed firing rate for the unit for trials during light (red) and sound (blue) blocks, aligned to cue-onset. Lightly shaded area indicates standard error of the mean. Note this unit showed a larger increase in firing to sound cues. B. An example of a unit that was responsive to cue **modality identity** as in A, but for a unit that showed a decrease in firing to the cue. Note the sustained higher firing rate during the light block. C-D. Cue-modulated units that encoded cue location, each color in the PETHs represents average firing response for a different cue location. C. This firing rate of this unit only changed on arm 3 of the task. D. Firing decreased for this unit on all arms but arm 4. E-F. Cue-modulated units that encoded cue outcome, with the PETHs comparing **rewarded reward-available** (red) and **unrewarded reward-unavailable** (green) trials. E. This unit showed a slightly higher response during presentation of **rewarded reward-available** cues. F. This unit showed a dip in firing when presented with **rewarded reward-available** cues. G-H. Examples of cue-modulated units that encoded multiple cue features. G. This unit integrated cue **modality identity** and outcome. H. An example of a unit that integrated cue **modality identity** and location.

6 - GLM.png

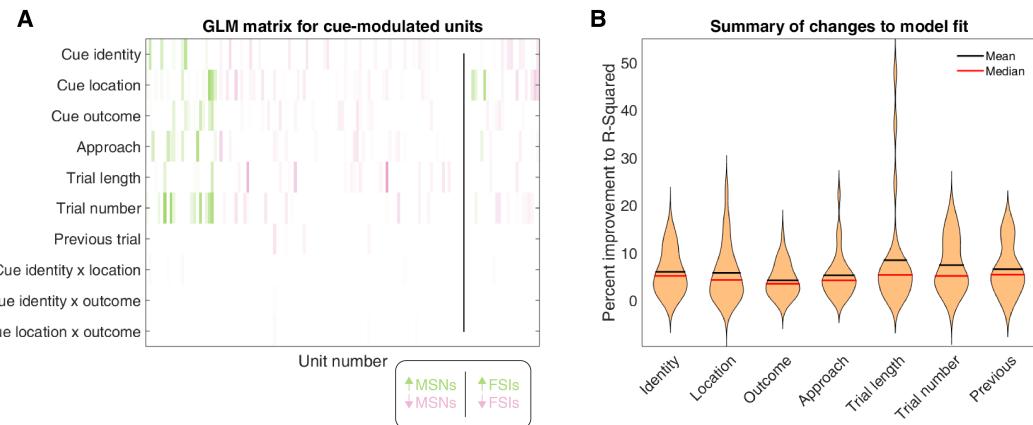


Figure 6: Summary of influence of various task parameters on cue-modulated NAc units after cue-onset. A. GLM matrix demonstrating impact of various task parameters on NAc firing rates. A stepwise GLM was fit to each unit that showed evidence of cue modulation by a Wilcoxon signed-rank test. Each row represents a given task parameter, and each column is the influence of that task parameter on a given unit. 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. Ordering from left to right: MSNs that increased firing in response to the cue (green, left of line), MSNs with a decreasing response (red, left of line), FSIs with an increasing response (green, right of line), FSIs with a decreasing response (red, right of line). Darker shades indicate more firing rate variance explained by a given predictor. Black line indicates separation of MSNs and FSIs. B. Violin plots demonstrating changes in R-squared values with the addition of each of the individual predictors. The mean, median, and distribution of changes in R-squared values is plotted for each of the seven task parameters used in the GLM.

7 - Population averages.png

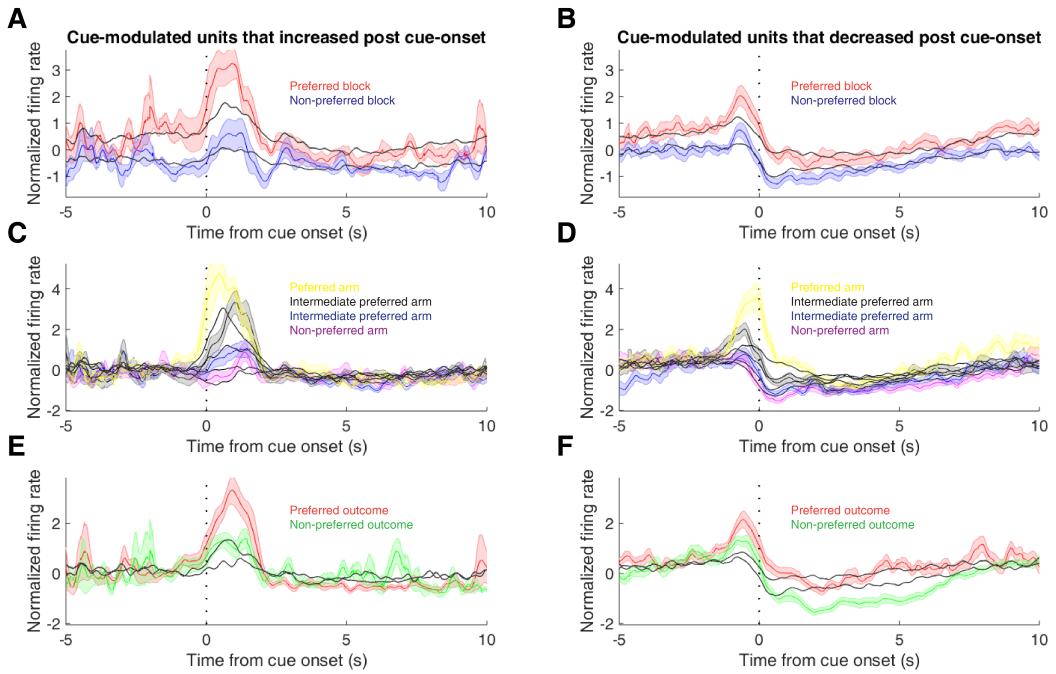
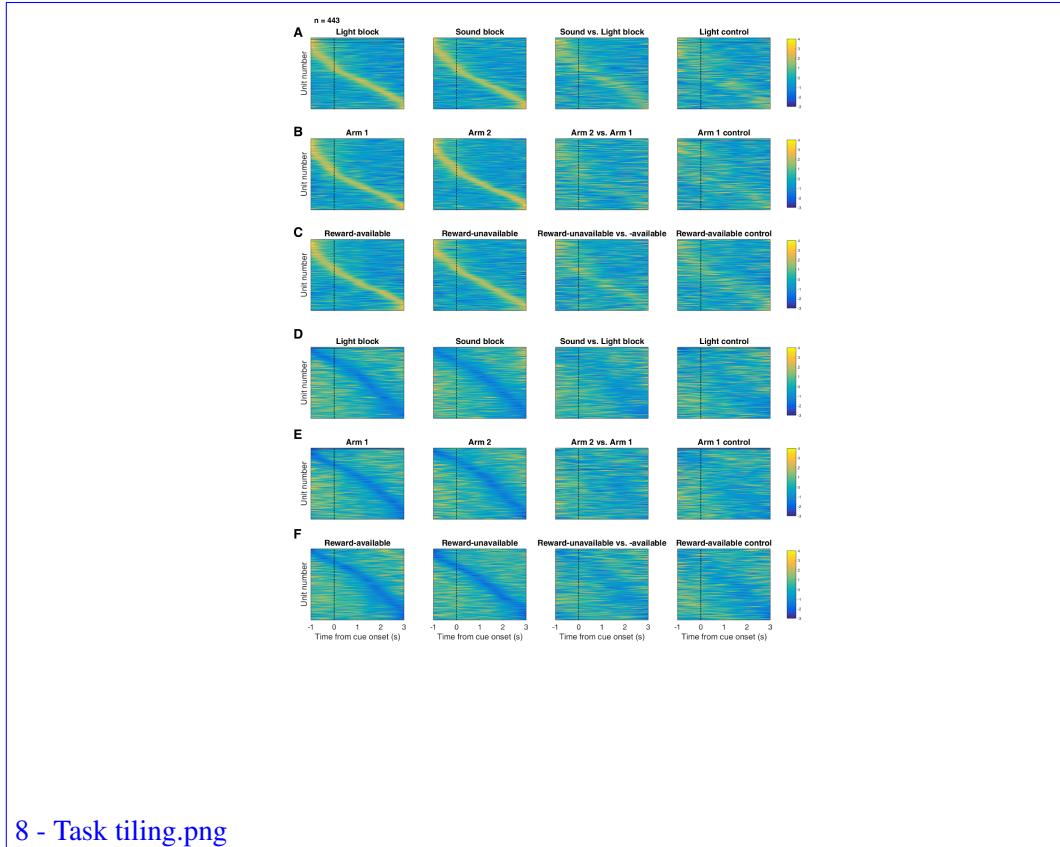
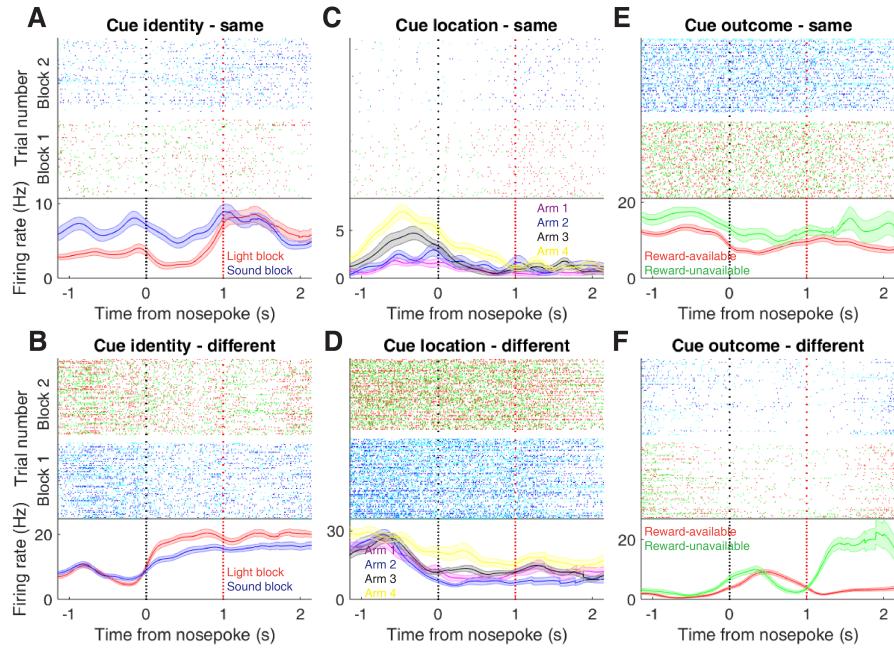


Figure 7: Population-level averages of cue feature sensitive NAc units. A. Average smoothed normalized ([zscoredz-score](#)) activity for cue-modulated units where cue [modality_identity](#) was a significant predictor in the GLM, aligned to cue-onset. Activity is plotted for preferred stimulus block (red) and nonpreferred stimulus block (blue). Dashed line indicates onset of cue. Lightly shaded area indicates standard error of the mean. Note larger increase to preferred stimulus block to nonpreferred stimulus block. [Black lines indicate the average of 1000 rounds of random sampling of units from the non-drifting population for the preferred and non-preferred blocks.](#) B. Same as A but for units that decreased in firing. Note population level activity reveals cells classified as decreasing in response to cue show a biphasic response at the population level, with a transient increase around the time the rat starts on the arm, followed by a minimum after cue onset. Also, note the sustained difference in firing between the two blocks. 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). Note the graded response to arms of decreasing preference. E-F. Same as A-B for cue outcome. Activity is plotted for preferred expected outcome (red), and nonpreferred outcome (green). Note the larger increase to the cue representing the cells preferred outcome (E), and the sustained decrease to the nonpreferred outcome (F).



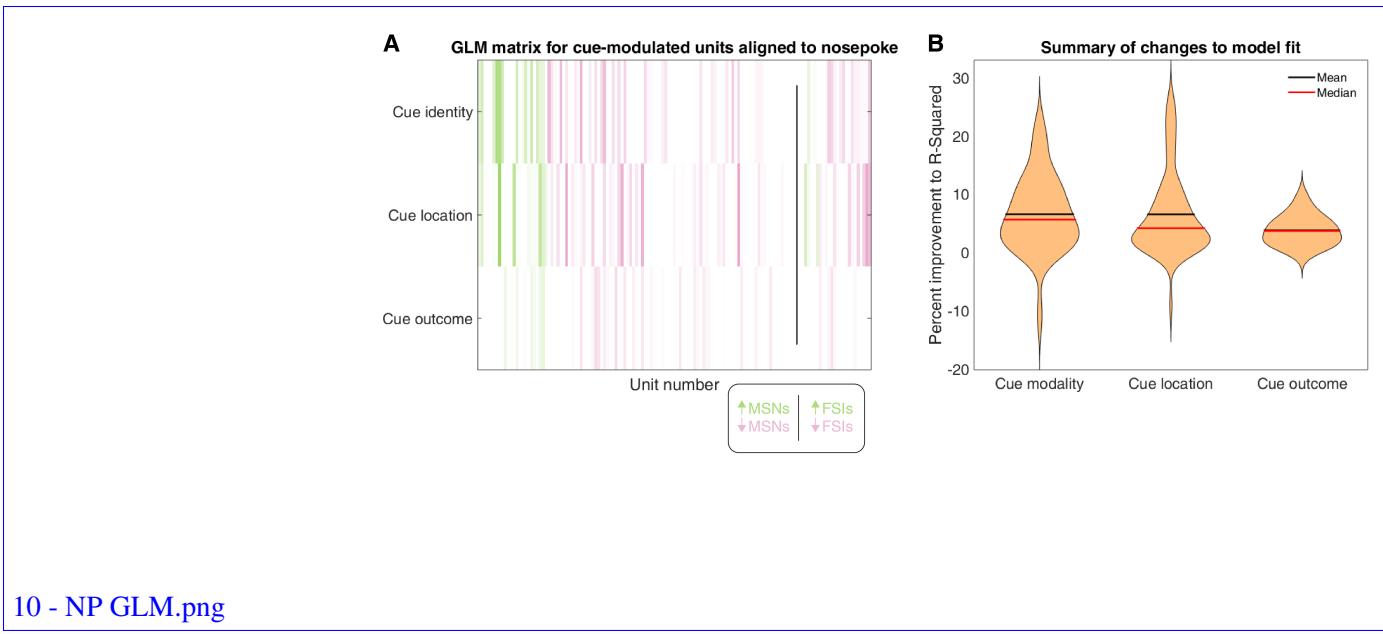
8 - Task tiling.png

Figure 8: Distribution of NAc firing rates across time surrounding cue onset. Each panel shows normalized ([zscored z-score](#)) firing rates for all recorded NAc units (each row corresponds to one unit) as a function of time (time 0 indicates cue onset), averaged across all trials for a specific cue type, indicated by text labels. A-C. Heat plots aligned to normalized peak firing rates. A, far left: Heat plot showing smoothed normalized firing activity of all recorded NAc units ordered according to the time of their peak firing rate during the light block. Each row is a units average activity across time to the light block. Dashed line indicates cue onset. Notice the yellow band across time, indicating all aspects of visualized task space were captured by the peak firing rates of various units. A, middle left: Same units ordered according to the time of the peak firing rate during the sound block. Note that for both blocks, units tile time approximately uniformly with a clear diagonal of elevated firing rates. A, middle right: Unit firing rates taken from the sound block, ordered according to peak firing rate taken from the light block. Note that a weaker but still discernible diagonal persists, indicating partial similarity between firing rates in the two blocks. A, far right: Unit firing rates taken from half of the light block, ordered according to peak firing rate taken from the other half of light trials. Note similar pattern to middle right suggests that reordering any two sets of trials produces this partial similarity, however correlations within blocks are more similar than correlations across blocks (see text). B. Same layout as in A, except that the panels now compare two different locations on the track instead of two cue modalities. As for the different cue modalities, NAc units clearly discriminate between locations, but also maintain some similarity across locations, as evident from the visible diagonal in the right panel. Two example locations were used for display purposes; other location pairs showed a similar pattern. C. Same layout as in A, except that panels now compare rewarded reward-available and unrewarded reward-unavailable trials. D-F. Heat plots aligned to normalized minimum firing rates. D. Responses during different stimulus blocks as in A, but with units ordered according to the time of their minimum firing rate. E. Responses during trials on different arms as in B, but with units ordered by their minimum firing rate. F. Responses during cues signalling different outcomes as in C, but with units ordered by their minimum firing rate. Overall, NAc units "tiled" experience on the task, as opposed to being confined to specific task events only. Units from all sessions and animals were pooled for this analysis.



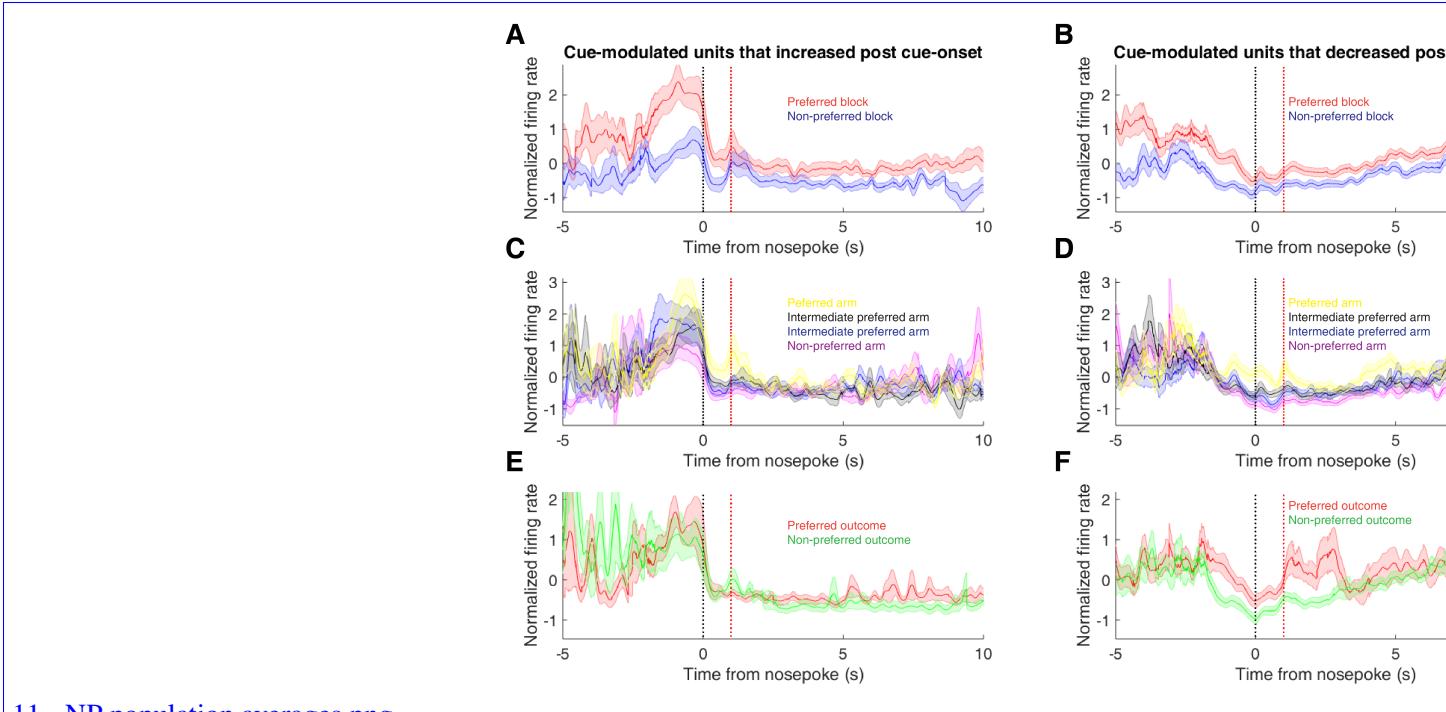
9 - NP Neural examples.png

Figure 9: Examples of cue-modulated NAc units influenced by various task parameters at time of nosepoke. A. Example of a cue-modulated NAc unit that encoded cue identity at both cue-onset and during nosepoke hold. Top: rasterplot showing the spiking activity across all trials aligned to nosepoke. Spikes across trials are color coded according to cue type (red: reward-available light; green: reward-unavailable light; navy blue: reward-available sound; light blue: reward-unavailable sound). White space halfway up the rasterplot indicates switching from one block to the next. Black dashed line indicates nosepoke. Red dashed line indicates receipt of outcome. Bottom: PETHs showing the average smoothed firing rate for the unit for trials during light (red) and sound (blue) blocks, aligned to nosepoke. Lightly shaded area indicates standard error of the mean. Note this unit showed a sustained increase to sound cues during the trial. B. An example of a unit that was responsive to cue identity at time of nosepoke but not cue-onset. C-D. Cue-modulated units that encoded cue location, at both cue-onset and nosepoke (C), and only nosepoke (D). Each color in the PETHs represents average firing response for a different cue location. E-F. Cue-modulated units that encoded cue outcome, at both cue-onset and nosepoke (E), and only nosepoke (F), with the PETHs comparing reward-available (red) and reward-unavailable (green) trials.



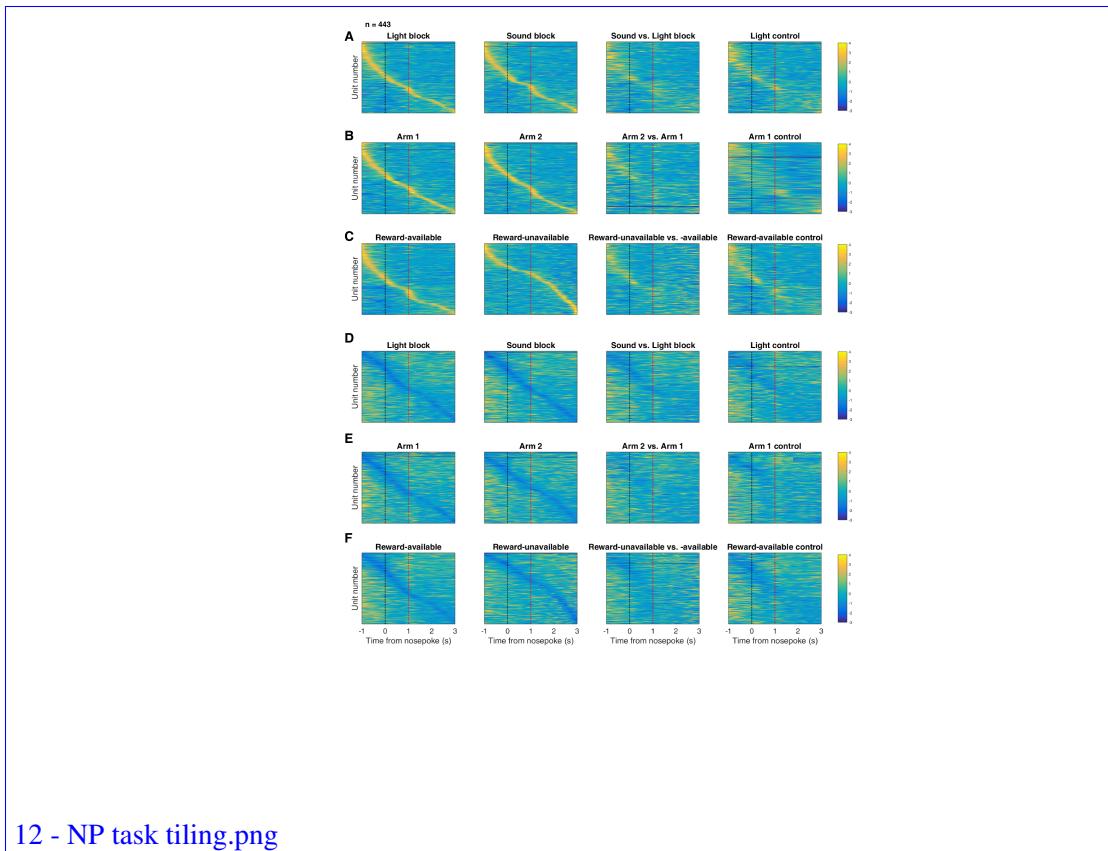
10 - NP GLM.png

Figure 10: 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 stepwise 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 each column is the influence of that task parameter on a given unit. 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. Ordering from left to right: MSNs that increased firing in response to the cue (green, left of line), MSNs with a decreasing response (red, left of line), FSIs with an increasing response (green, right of line), FSIs with a decreasing response (red, right of line). Darker shades indicate more firing rate variance explained by a given predictor. Black line indicates separation of MSNs and FSIs. B. Violin plots demonstrating changes in R-squared values with the addition of each of the individual predictors. The mean, median, and distribution of changes in R-squared values is plotted for each of the three task parameters that were significant predictors in the GLM.



11 - NP population averages.png

Figure 11: Population-level averages of cue feature sensitive NAc units during a nosepoke. A. Average smoothed normalized activity for cue-modulated units where cue modality identity was a significant predictor in the GLM, aligned to nosepoke with reward delivery occurring 1 s after nosepoke. Activity is plotted for preferred stimulus block (red) and nonpreferred stimulus block (blue). Dashed Black dashed line indicates nosepoke. Red dashed line indicates reward delivery occurring 1 s after nosepoke for reward-available trials. Lightly shaded area indicates standard error of the mean. Note larger increase leading up to nosepoke to preferred stimulus block to nonpreferred stimulus block. B. Same as A but for units that decreased in firing. Note the sustained difference in firing between the two blocks. 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). Note the subtle but graded response to arms of decreasing preference. E-F. Same as A-B for cue outcome. Activity is plotted for preferred expected outcome (red), and nonpreferred outcome (green). Note the peak after reward receipt for preferred outcome in decreasing cells (F).



12 - NP task tiling.png

Figure 12: Distribution of NAc firing rates across task space during approach trials. Each panel shows normalized firing rates for all recorded NAc units (each row corresponds to one unit) as a function of time (time 0 indicates nosepoke), averaged across all approach trials for a specific cue type, indicated by text labels. A, far left: Heat plot showing smoothed normalized firing activity of all recorded NAc units ordered according to the time of their peak firing rate during the light block. Each row is a units average activity across time to the light block. Dashed Black dashed line indicates nosepokewith. Red dashed line indicates reward delivery occurring 1 s after nosepoke for rewarded reward-available trials. Notice the yellow band across time, indicating all aspects of visualized task space were captured by the peak firing rates of various units. A, middle left: Same units ordered according to the time of the peak firing rate during the sound block. Note that for both blocks, units tile time approximately uniformly with a clear diagonal of elevated firing rates, and a clustering around reward receipt. A, middle right: Unit firing rates taken from the sound block, ordered according to peak firing rate taken from the light block. Note that a weaker but still discernible diagonal persists, indicating partial similarity between firing rates in the two blocks. A, far right: Unit firing rates taken from half of the light block, ordered according to peak firing rate taken from the other half of light trials. Note similar pattern to middle right suggests that reordering any two sets of trials produces this partial similarity, however correlations within blocks are more similar than correlations across blocks (see text). B: Same layout as in A, but with units ordered according to the time of their minimum firing rate. C: Same layout as in A, except that the panels now compare two different locations on the track instead of two cue modalities. As for the different cue modalities, NAc units clearly discriminate between locations, but also maintain some similarity across locations, as evident from the visible diagonal in the right panel. Two example locations were used for display purposes; other location pairs showed a similar pattern. D: As for C, but with units ordered by their minimum firing rate.³⁵ E: Same layout as in A, except that panels now compare rewarded reward-available and unrewarded reward-unavailable trials. Notice the absence of a cluster of tiling around reward receipt for unrewarded reward-unavailable trials. F: As for A, but with units ordered by their minimum firing rate. Overall, NAc units "tiled" experience on the task, as opposed to being confined to specific task events only. Units from all sessions and animals were pooled for this analysis.

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

Table 1: Cells from each rat

Task parameter	Total	MSN (increasing) ↑ MSN	MSN (decreasing) ↓ MSN	FSN
All <u>cells-units</u>	443	155	216	
<u>Cue modulated Analyzed units</u>	<u>344</u>	<u>?</u>	<u>?</u>	
<u>Cue modulated units</u>	133	24	85	
<u>Cue modality GLM aligned to cue-onset</u>				
<u>Cue identity</u>	37	7	21	
Cue location	50	13	27	
Cue outcome	34	10	18	
Approach behavior	31	8	18	
Trial length	25	5	18	
Trial number	32	11	12	
Recent trial history	5	0	5	
Cue x cue interactions	11	3	7	
Cue x behavior interactions	?	?	?	
<u>GLM aligned to nosepoke</u>				
<u>Cue identity</u>	<u>66</u>	<u>14</u>	<u>36</u>	
<u>Cue location</u>	<u>66</u>	<u>14</u>	<u>40</u>	
<u>Cue outcome</u>	<u>42</u>	<u>8</u>	<u>29</u>	
<u>GLM aligned to outcome</u>				
<u>Cue outcome</u>	<u>10</u>	<u>0</u>	<u>6</u>	

Table 2: Cells from GLM

Task parameter	Within condition 1	Within condition 2	Across conditions 1	Across conditions 2
Cue identity	.383	.379	.343	.338
Cue location	.369	.350	.290	.286
Cue outcome	.429	.261	.258	.253

Table 3:
Firing rate correlations