

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 im-
25 portant issue for at least two reasons. First, in reinforcement learning, motivationally relevant outcomes are
26 typically temporally delayed relative to the cues that predict them. In order to solve the problem of assigning
27 credit (or blame) across such temporal gaps, some trace of preceding activity needs to be maintained (Lee
28 et al., 2012) (Maia?). Since NAc is a primary target of DA signals interpretable as RPEs, and NAc lesions

29 impair RPEs related to timing, its activity trace will help determine what can be learned when RPEs arrive
30 (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 during which they were exposed to the running room, the
56 sucrose solution used as a reinforcer, and the click of the valves upon approach to the receptacles. They were
57 then shaped to run on the task for seven days where they were restricted to running in the clockwise direction
58 by presenting a physical barrier to running counterclockwise. Rats then continued to be trained each day for
59 100 trials in both a light and sound block until approach behavior discriminated between reward-available
60 and reward-unavailable cues for three consecutive days according to a chi square test, at which point they
61 underwent hyperdrive implantation targeted at the NAc. Rats were allowed to recover for a minimum of five
62 days before being retrained on the task, and recording began once performance returned to pre-surgery levels.
63 Upon completion of recording, animals were sacrificed and recording sites were histologically confirmed.

64 **Behavioral task and training:**

65 Rats were trained to run clockwise on an elevated, square-shaped track (100x100 cm) containing four possi-
66 ble reward locations (Figure 2). Rats initiated a trial by running down the length of an arm, and triggering a
67 photobeam located 24 cm from the start of each arm. Upon trial initiation, a light or sound cue was presented
68 that signaled the presence (reward-available trial) or absence (reward-unavailable trial) of a 12% sucrose wa-
69 ter reward (0.1 mL) at the upcoming site. A trial was classified as an approach trial if the rat turned left at
70 the decision point and made a nosepoke at the reward receptacle (40 cm from the decision point), while trials

71 were classified as a skip trial if the rat instead turned right at the decision point and triggered the photobeam
72 to initiate the following trial. In reward-available trials there was a 1 second delay between a nosepoke and
73 subsequent reward delivery. Trial length was determined by measuring the length of time from cue onset
74 until nosepoke (for approach trials), or from cue onset until the start of the following trial (for skip trials).
75 Trials could only be initiated through clockwise progression through the series of arms, and each entry into
76 the subsequent arm on the track counted as a trial. To get enough trials for neural analysis rats were trained
77 each day in both a light and sound block for 100 trials each. Within a block, one cue signaled reward was
78 available on that trial, while the other signaled reward was not available. Light block cues were a flashing
79 white light, and a constant yellow light. Sound block cues were a 2 kHz sine wave and a 8 kHz sine wave
80 whose amplitude was modulated from 0 to maximum by a 2 Hz sine wave. Reward-cue associations were
81 counterbalanced across rats. Cue presentation was pseudorandomized so that the same cue could not be
82 presented more than twice in a row. Block order within each day was also pseudorandomized, such that the
83 rat could begin a session with the same block for more than two days in a row. Each training or testing day
84 consisted of a 5 minute pre-session period on a pedestal, followed by the first block, then the second block,
85 then a 5 minute post-session period on the pedestal. Performance was determined by the proportion of trials
86 a rat approached each cue. Perfect performance would be 100% approach on reward-available trials, and
87 0% approach on reward-unavailable trials. Rats were trained daily until they could distinguish between the
88 reward-available and reward-unavailable cues for both light and sound blocks for three consecutive days,
89 according to a chi-square test rejecting the null hypothesis of equal approaches for reward-available and
90 reward-unavailable trials, at which point they underwent surgery.

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 at 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 by an absence
110 of interspike intervals (ISIs) > 2 s, while medium spiny neurons had a combination of ISIs > 2 s and phasic
111 activity with shorter ISIs (Barnes 2005, Atallah 2014).

112 **Data analysis:**

113 For behavioral performance we generated linear mixed effects models to investigate the relationships be-
114 tween cue type and our behavioral variables, with cue type as a fixed effect, and the addition of an intercept
115 for rat identity as a random effect. Average proportion of trials approached and trial length for a session
116 were used as response variables. Contribution of cue type to behavior was determined by comparing the full

117 model to a model with cue type removed for each behavioral variable.

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

126 To determine the relative contribution of various task parameters to firing rate variance for units whose firing
127 was modulated by cue-onset (as in Figures 5,6), a forward selection stepwise general linear model (GLM)
128 was fit to each cue-responsive unit. Cue modality (light block, sound block), cue location (arm 1, arm 2, arm
129 3, arm 4), cue outcome (reward-available, reward-unavailable), approach behavior, trial length, trial number,
130 and trial history were used as predictors, and the 1 s post-cue firing rate as the response variable. Units were
131 classified as being modulated by a given task parameter if addition of the parameter significantly improved
132 model fit using deviance as the criterion ($p < .01$). A comparison of the R-squared value between the final
133 model and the final model minus the predictor of interest was used to determine the amount of firing rate
134 variance explained by the addition of that predictor for a given unit.

135 To better visualize responses to cues and enable subsequent population level analyses (as in Figures 5,7,8),
136 spike trains were convolved with a Gaussian kernel ($\sigma = 100$ ms), and peri-event time histograms (PETHs)
137 were generated by taking the average of the convolved spike trains across trials for a given task condition.
138 For analysis of population-level responses for cue features (Figure 7), convolved spike trains for all units
139 where cue modality, cue location, or cue outcome explained a significant portion of firing rate variance
140 were z-scored. Within a given cue feature, normalized spike trains were then separated according to the

¹⁴¹ preferred and non-preferred cue condition (e.g. light vs sound block), and averaged across units to generate
¹⁴² population-level averages.

¹⁴³ To visualize NAc representation of task space within cue conditions, normalized spike trains for all units
¹⁴⁴ were ordered by the location of their maximum or minimum firing rate for a specified cue condition (Figure
¹⁴⁵ 8). To compare representation of task space across cue conditions for a cue feature, the ordering of units
¹⁴⁶ derived for one condition (e.g. light block) was then applied to the normalized spike trains for the other
¹⁴⁷ condition (e.g. sound block).

¹⁴⁸ To identify the responsivity of units to different cue features at the time of a nosepoke into a reward recepta-
¹⁴⁹ cle, the same cue-responsive units from the cue-onset analyses were analyzed at the time of nosepoke using
¹⁵⁰ identical analysis techniques (Figures 9,10,11).

¹⁵¹ Given that some of our analyses compare firing rates across time, particularly comparisons across blocks,
¹⁵² we sought to exclude units with unstable firing rates that would generate spurious results reflecting a drift
¹⁵³ in firing rate over time unrelated to our task. To do this we ran a Mann-Whitney U test comparing the cue-
¹⁵⁴ evoked firing rates for the first and second half of trials within a block, and excluded 99 units from analysis
¹⁵⁵ that showed a significant change for either block. All analyses were completed in MATLAB R2015a, the
¹⁵⁶ code is available on our public GitHub repository (<http://github.com/vandermeerlab/papers>), and the data
¹⁵⁷ can be accessed through DataLad.

¹⁵⁸ **Histology:**

¹⁵⁹ Upon completion of the experiment, rats were anesthetized with 5% isoflurane, then asphyxiated with carbon
¹⁶⁰ dioxide. Transcardial perfusions were performed, and brains were fixed and removed. Brains were sliced
¹⁶¹ in 50 μ m coronal sections and stained with thionin. Slices were visualized under light microscopy, tetrode
¹⁶² placement was determined, and electrodes with recording locations in the NAc were analyzed (Figure 3).

163

[Figure 3 about here.]

164 **Results**

165 **Behavior**

166 Rats were trained to discriminate between cues signaling the availability and absence of reward on a square
167 track with four identical arms for two distinct sets of cues. During each session, rats were presented with
168 two behavioral blocks containing different sensory cues in sequential order, a light and a sound block, with
169 each block containing a cue that predicted reward, and a cue that signalled the absence of reward. An
170 example learning curve is seen in Figure 4A,B. All four rats learned to discriminate between the rewarded
171 and unrewarded cue for both the light and sound blocks as determined by reaching significance ($p < .05$)
172 on a daily chi-square test comparing approach behavior for rewarded and unrewarded cues for each block,
173 for at least three consecutive days (range for time to criterion: 22 - 57 days). Maintenance of behavioral
174 performance during recording sessions was assessed using linear mixed effects models for both proportion
175 of trials where the rat approached the receptacle, and trial length. Analyses revealed that the likelihood of a
176 rat to make an approach was influenced by which of the cues was presented (Percentage approached: light
177 rewarded = 97%; light unrewarded = 34%; sound rewarded = 91%; sound unrewarded 35%; $p < .001$),
178 but that cue identity did not significantly affect the length of time taken to complete a trial (Trial length:
179 light rewarded = 1.85 s; light unrewarded = 1.74 s; sound rewarded = 1.91 s; sound unrewarded 1.78 s; p
180 = .13)(Figure 4C,D). Thus, during recording, rats successfully discriminated the various cues according to
181 whether or not they signalled the availability of reward at the reward port.

182

[Figure 4 about here.]

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

184 **General responses to cue:**

185 We sought to address which parameters of our task were encoded by NAc activity, specifically whether the
186 NAc encodes aspects of motivationally relevant cues not directly tied to reward, such as the identity and
187 location of the cue, and whether this coding is independent or integrated with coding of cue outcome. To do
188 this we recorded a total of 443 units in the NAc from 4 rats over 57 sessions while they performed on the
189 square track (Table 1). Units that had less than 200 spikes, or exhibited a drift in firing rate over the course
190 of either block were excluded from further analysis. The activity of 133 (30%) of these was modulated by
191 the cue, with more units showing a decrease in firing ($n = 124$) than an increase ($n = 47$) around the time
192 of cue-onset (Table 2). Within this group, 32 were classified as HFNs, while 139 were classified as SPNs.
193 To determine whether these cue-evoked responses were modulated by various aspects of the task, we fit a
194 GLM to each cue-modulated unit. Fitting GLMs revealed that a variety of task parameters accounted for a
195 significant portion of firing rate variance in NAc cue-modulated units, with all task parameters except trial
196 history being predictors in 19-38% of cue-modulated units, and explaining 4-9% on average, although there
197 was substantial variability across cells (Figures 5, 6, Table 2). Notably, there were units that discriminated
198 between whether the rat was performing in the light or sound block ($n = 37 / 133$), which arm the rat was
199 currently on ($n = 50 / 133$), and whether the rat was engaged in a rewarded or unrewarded trial ($n = 34 / 133$),
200 suggesting that the NAc independently encodes features of reward-predictive cues separate from expected
201 outcome (Figure 5A-F). Furthermore, interactions between multiple cue features appeared as significant
202 predictors of firing rate variance for 9% cue-modulated units ($n = 11 / 133$), although this effect was modest
203 (1-2% variance explained), suggesting a weak effect for integrated coding across various aspects of a cue
204 (Figure 5G,H). Fitting a GLM to all recorded units...*Jimmie finish this* (data not shown).

205

[Table 1 about here.]

206

[Table 2 about here.]

207

[Figure 5 about here.]

208

[Figure 6 about here.]

209 **Population level responses:**

210 NAc units do not work in isolation, and some information about the cue may be hidden in single unit re-
211 sponse. To get a sense of how NAc output encoded cue information at the population level, we normalized
212 firing activity for each unit that was modulated by a given cue feature, then generated the cue-onset aligned
213 population average firing rate for each of the cue features (Fig 7). This analyses revealed overall that cells
214 that showed an increase upon cue presentation had stronger responses for the preferred cue condition (Fig-
215 ure 7A,C,E). Interestingly, units that were classified as decreasing in response to the cue showed a biphasic
216 response at the population level, with a small peak at a time in alignment with entry into the arm, followed
217 by a sustained dip after cue-onset (Figure 7B,D,F). Units that were modulated by cue modality showed by
218 a stronger increase in response to the preferred cue modality, as well as a higher tonic firing rate to the
219 preferred cue modality, most notably in units that decreased in firing rate to the cue (Figure 7A,B). Units
220 that were modulated by cue location showed a graded response to locations of decreasing preference (Figure
221 7C,D). Additionally, units whom had exhibited a decrease in firing in response to the cue and whose activity
222 was modulated by cue outcome, showed a sustained discriminatory response to rewarded and unrewarded
223 cues that extended beyond cue-onset (Figure 7F). Together, these visualizations of the averaged population
224 responses revealed nuanced differences in the way NAc units are modulated by cue conditions across cue
225 features.

226

[Figure 7 about here.]

227 **NAc units segment the task:**

228 Another method to get a sense of how the NAc is behaving at the population level is to analyze NAc unit
229 activity across time, and NAc neurons have been shown to have correlates across an entire task space. To
230 look at the distribution of responses throughout our task space and see if this distribution is modulated by cue
231 features, we z-scored the firing rate of each unit and plotted the normalized firing rates of all units aligned
232 to cue-onset and according to peak firing rate (Figure 8). We did this separately for both the light and sound
233 blocks, and found a nearly uniform distribution of firing fields in task space that was not limited to alignment
234 to the cue (Figure 8A). Furthermore, to see if this population level activity was similar across blocks, we
235 also organized firing during the sound blocks according to the ordering derived from the light blocks. This
236 revealed that while there was some preservation of order, the overall firing was qualitatively different across
237 the two blocks. Additionally, given that the majority of our units showed an inhibitory response to the cue,
238 we also plotted the firing rates according to the lowest time in firing, and again found some maintenance
239 of order, but largely different ordering across the two blocks (Figure 8B). This process was repeated for
240 cue location and cue outcomes, showing that NAc segmentation of the task is qualitatively different across
241 various cue conditions (Figure 8C-F).

242 [Figure 8 about here.]

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

244 In order to be useful for learning, a trace of the cue must be maintained until the outcome. Fitting a GLM
245 to the firing rates of cue-modulated units at the time of a nosepoke response showed that a variety of units
246 still discriminated firing according to various cue features, but not other task parameters, showing that NAc
247 activity discriminates various cue conditions well into a trial (Table 3, Figure 9). Fitting a GLM to all
248 recorded units...*Jimmie finish this* (data not shown). Population level averages for units that increased
249 to cue-onset showed a ramping up of activity that peaked upon nosepoke, whereas units that decreased to

250 cue-onset showed a gradual reduction of firing activity that reached a minimum upon nosepoke (Figure
251 10). Additionally, a peak is seen for preferred cue outcome in decreasing units at 1 second post cue-onset
252 when reward was received, demonstrating an integration of expected and received reward (Figure 10F).
253 Furthermore, aligning normalized peak firing rates to nosepoke onset, revealed a clustering of responses
254 around reward receipt for all cue conditions where the rat would have received reward (Figure 11). Alongside
255 this, fitting a GLM to the firing rates of cue-modulated units at the time of reward receipt, revealed 10 units
256 (8%) where cue outcome accounted for an average of 32% of firing rate variance (data not shown).

257 [Table 3 about here.]

258 [Figure 9 about here.]

259 [Figure 10 about here.]

260 [Figure 11 about here.]

261 Discussion

262 The present study found evidence for coding of multiple identifying features of motivationally relevant stim-
263 uli in addition to expected outcome; the sensory modality of the presented cue, as well as its physical location
264 within the track, and that this coding was still present on approach trials when the rat nosepoked. Further-
265 more, this coding was both independent, and intermixed across cue features and behavioral measures. Cod-
266 ing at the population level revealed qualitatively distinct comparison profiles across cue features, such as
267 cells that encoded cue modality tended to sustain the discriminatory firing outside of cue presentation, while
268 a subset cells that discriminated rewarded from unrewarded cues maintained this discrimination until after

reward receipt. Across all recorded cells, a tiling of task structure was observed such that all points within our analyzed task space was accounted for by the ordered peak firing rates of all cells, and this tiling differed between various conditions with a cue feature, such as light versus sound blocks. Cells that discriminated across blocks were not simply due to drifting of the signal across trials, as cells that showed a drift in firing between the first and second half within a block were excluded from the analysis. Furthermore, even though actions were stereotyped during correct trials, such that the rat always turned left at the decision point to approach for reward, and right to skip the receptacle and initiate the next trial, cells that were modulated by the expected value of the cue maintained their specific firing patterns even during error trials where the rat turned left after presentation of the unrewarded cue, suggesting that these signals did not represent action values. Additionally, NAc signals have been shown to be modulated by response vigor, to detangle this from our results we included the trial length as a predictor in our GLMs, and found cells with correlates independent of trial length.

281 Cue modality:

Our finding that NAc units can discriminate between cues from different sensory modalities expands upon an extensive literature examining neural correlates of conditioned stimuli. Perhaps the most comparable work in rodents comes from a study that found distinct coding for an odor when it predicted separate but equally valued rewards (Cooch). The present work is complementary to this as it shows that NAc cells have representations of identifiable aspects of the cue itself, in addition to the reward it predicts. Another study paired separate cues with appetitive or aversive outcomes, and found separate populations of cells that encode each cue, with many switching selectivity after reversal of the associations between the cues and outcomes, providing evidence that the NAc encodes the biological significance of stimuli. Once again, our study was different as we recorded neural responses to distinct cues encoding the same anticipated outcome, suggesting that even when the biological relevance of these stimuli is similar, the NAc dissociates their representations at the level of the single-unit (Setlow), although this doesn't explain the difference in population averaged firing rate for the epochs when the cue is not present. Another possibility is that these modality specific

294 cells were encoding the context, rule, or sequence within a session as some cells responded similar for both
295 rewarded and unrewarded cues within a block. This interpretation is in alignment with a recent paper from
296 the primate realm that recorded NAc responses during the Wisconsin Card Sorting Task (WCST), a common
297 set-shifting task used in both the laboratory and clinic, and found cells that preferred firing to stimuli when a
298 certain rule, or rule category was currently active (Sleazeer). Indeed, an encoding of the current strategy could
299 be an explanation as to why a sustained difference in population averaged firing was seen across stimulus
300 blocks, as well as a potential explanation for the differentially tiling of task structure across blocks in the
301 current study. Further support for a modulation of NAc responses by strategy comes from an fMRI study
302 that examined BOLD levels during a set-shifting task (FitzGerald et al., 2014). In this task, participants
303 learned two sets of stimuli-reward contingencies, a visual set and auditory set. During testing they were
304 presented with both simultaneously, and the stimulus dimension that was relevant was periodically shifted
305 between the two. Here, they found that bilateral NAc activity reflected value representations of whatever the
306 currently relevant stimulus dimension was, and not the irrelevant stimulus. The current finding of separate,
307 but overlapping, populations of cells encoding cue modality and expected value, suggests that the fMRI
308 finding is generated by the combined activity of several different functional cell types.

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

319 **Encoding of position:**

320 Our finding that cue-evoked activity was modulated by cue location sides with some of the literature (Lavoie,
321 1994; Tabuchi, 2000; Strait, 2016). An alternative explanation for a pure spatial representation, is that
322 these are task segmentation correlates, keeping track of where in the task the rat is. A previous non-human
323 primate paper has shown that when reward is contingent upon completion of a series of trials, separate
324 populations of NAc neurons signal the start of a schedule, subsequent trials in the schedule, and the first
325 trial in extended schedules (Shidara et al., 1998). This signalling of position within a sequence has been
326 observed in subsequent studies, and it is possible that the our rats were keeping track of which specific arm
327 they were in as part of a sequence of arms, and not just strictly a spatial representation (Mulder, 2004 and
328 2005; Khamassi et al., 2008; Berke, 2009). Also, given that our task is pseudo-random, it is possible that the
329 rats learned which cue to anticipate, and the neural activity could reflect this. However, this is unlikely as
330 including a previous trial variable in the analysis did not explain a significant amount of firing rate variance
331 in response to the cue for the vast majority of cells..

332 **Mixed selectivity:**

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

342 Mixed selectivity in the NAc could be a consequence of synaptic integration from a variety of anatomically
343 distinct inputs, as seen in experiments examining the convergence of various NAc afferents at the level of
344 synaptic transmission and stimulation-induced firing (Goto and Grace 2008). In one such experiment it
345 was shown that NAc cells that responded to stimulation of either the fornix, amygdala, or PFC, typically
346 responded to stimulation from all inputs (ODonnell and Grace, 1995). Furthermore, an interaction between
347 these inputs was observed such that PFC stimulation failed to elicit spiking in the NAc neurons unless they
348 were in a depolarized UP-state, a state induced by hippocampal stimulation and was dependent on an intact
349 fornix. Hippocampal-induced suppression of other inputs has also been observed for the BLA (Mulder et al.,
350 1998). Recently, it has also been shown that train stimulation of PFC afferents reduces hippocampal-evoked
351 NAc responses, suggesting that there is competition between various inputs (Calhoon and ODonnell, 2013).
352 These studies suggest that the integration of the variables we saw could be the result of this gating observed
353 in behaviorally-independent preparations. However, given that we did not systematically manipulate these
354 various limbic and cortical afferents, comments on the anatomical origins of the observed mixed selectivity
355 responses are speculative at this point.

356 Integrating cue identity and value, as seen modestly in the present study, could be one neural instantiation of
357 how value is associated with the appropriate predictive stimuli (credit assignment), keeping in mind that value
358 encoding is distributed, redundantly in some aspects, across various structures (Hayden Nat Neuro opinion).
359 Indeed, lesions of the NAc impair the ability to learn changes in reward value or identity in an unblocking
360 experiment, as well as disrupting dopamine RPEs generated by modification of timing of reward (McDannald
361 2011, Takahashi 2016). Would be interesting to see if uncoupling the integrated coding of stimulus features
362 and predictive properties of a cue has an effect on the ability of a rat to use reward-predictive cues to pursue
363 the associated reward.

364 **Tiling of task structure:**

365 Additionally, we found that the population of recorded units had a relatively uniform distribution of firing

366 fields within our task space, similar to what has been reported previously (Shidara, 1998; Berke, 2009;
367 Lansink, 2012). Uniquely, we found that this representation differed according to whether the rat was cur-
368 rently engaged in the light or sound block, suggesting that this could be a possible neural correlate for
369 encoding the currently relevant strategy in the NAc. It has been previously shown that during progress
370 through a predictable trial series, neurons represented state value of cue (Shidara 1998), and that single-unit
371 responses allowed the monkey to know how it was progressing throughout the task. Likewise, the tiling
372 we saw could be a consequence of upstream cortical or limbic inputs informing the striatum of the current
373 task rules. Another possibility is that the NAc not only pays attention to progress throughout a task within
374 a trial, but also higher-order task information, like blocks. Furthermore, dopamine levels in the NAc fluc-
375 tuate through a trial, and it is possible that the observed tiling could be a NAc-representation of state value
376 related to this temporally evolving dopamine signal. Future experiments should monitor this mapping of
377 task structure during the application of dopamine antagonists. Finally, the presence of functional correlates
378 not evident when looking at single-unit responses time-locked to salient task events emphasizes the need to
379 employ ensemble level analyses across all aspects of a task.

380 **References**

381 Lee, D., Seo, H., and Jung, M. W. (2012). Neural Basis of Reinforcement Learning and Decision Making. *Annu. Rev. Neurosci.*,
382 35:287–308.

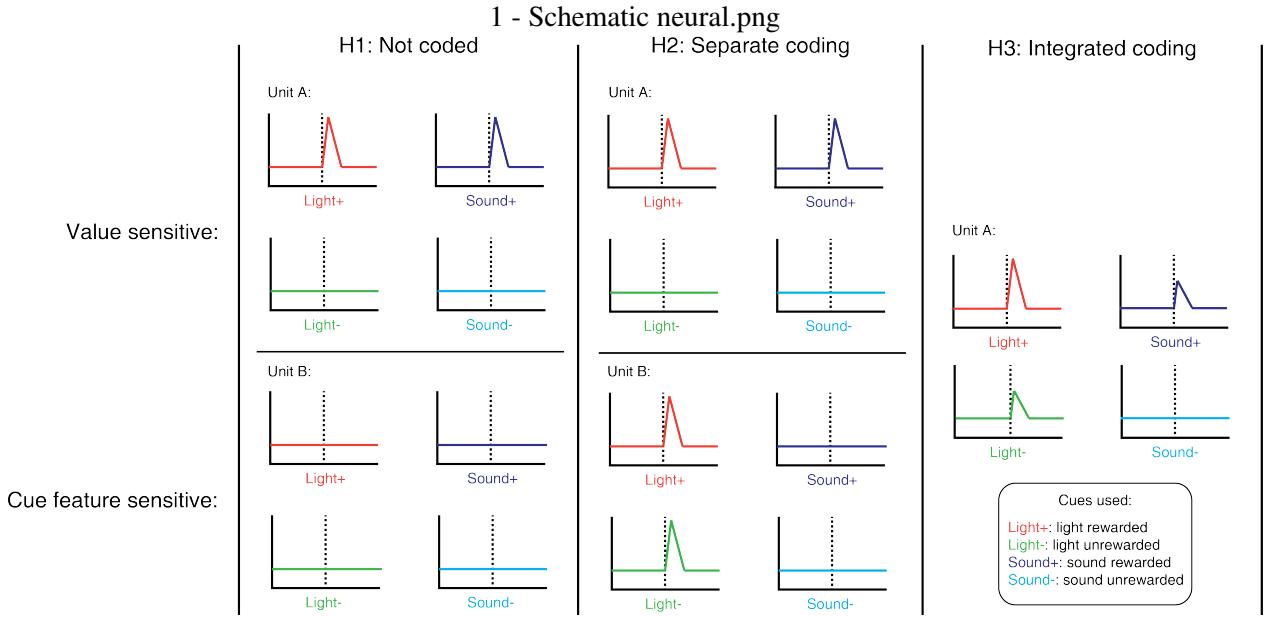


Figure 1: Schematic of potential coding strategies employed by single units in the NAc. Displayed are schematic PETHs illustrating putative responses to different cues under different hypotheses of how cue identity and value are coded. H1 (left panel): Coding of identifying cue features is absent in the NAc. Top: Unit A responds to a motivationally relevant variable, such as expected value, similarly across other cue features, such as stimulus modality or physical location. Hypothetical plot is firing rate across time. Light+ (red) signifies a rewarded light cue, Sound+ (navy blue) a rewarded sound cue, Light- (green) an unrewarded light cue, Sound- (light blue) an unrewarded sound cue. Dashed line indicates onset of cue. Bottom: No units within the NAc discriminate their firing across various conditions of these cue features. H2 (middle panel): Coding of cue features occurs independently of encoding of motivationally relevant variables like expected value or subsequent vigor. Top: Same as H1, with unit A discriminating between rewarded and unrewarded cues. Bottom: Unit B discriminates firing across conditions of a cue feature, depicted here as firing to light cues but not sound cues. H3 (right panel): Coding of cue features 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 light cue.

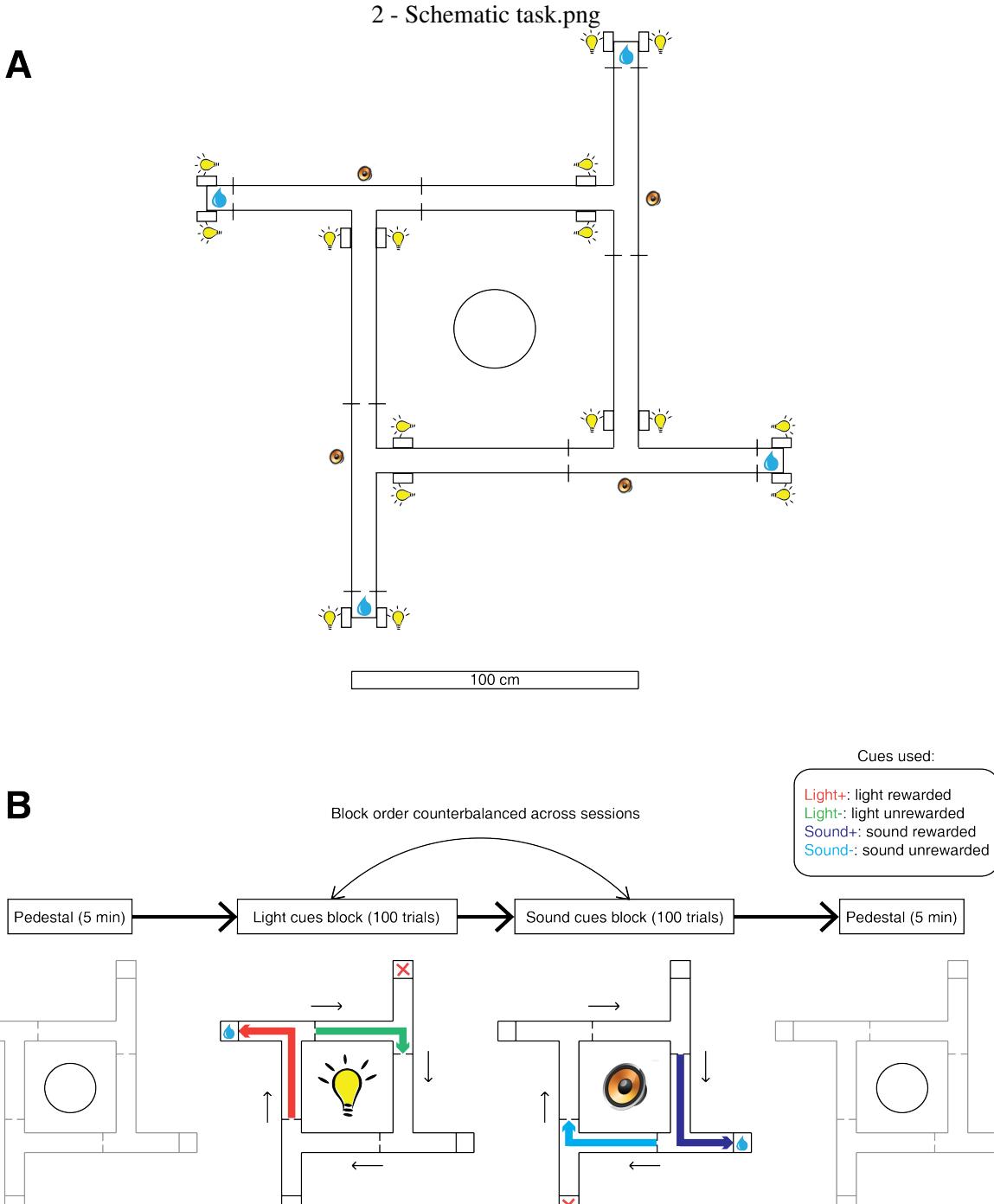
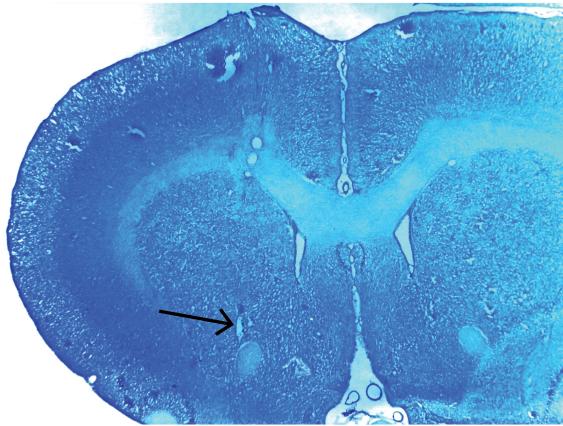


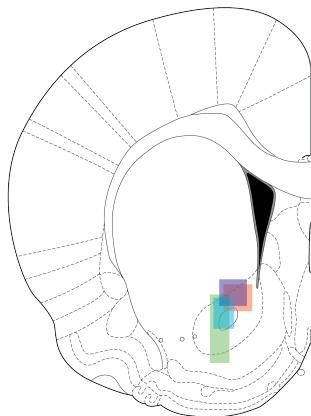
Figure 2: Schematic of behavioral task. A. To scale depiction of square track consisting of multiple identical T-choice points. Dashed lines indicate location of photobeams. Photobeams on the center, square portion of the track triggered the start of a trial and onset of a cue, whereas photobeams at the ends of the arms by the receptacles registered nosepokes. Rectangular boxes surrounded by light bulbs indicate location of LEDs used for light cues. Speakers for tone cues were placed underneath the choice points, indicated by the speaker images. Water droplets 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 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. Rats ran clockwise on the square track, and

3 - Histology.png

A



B



Rats used:

R053
R056
R057
R060

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

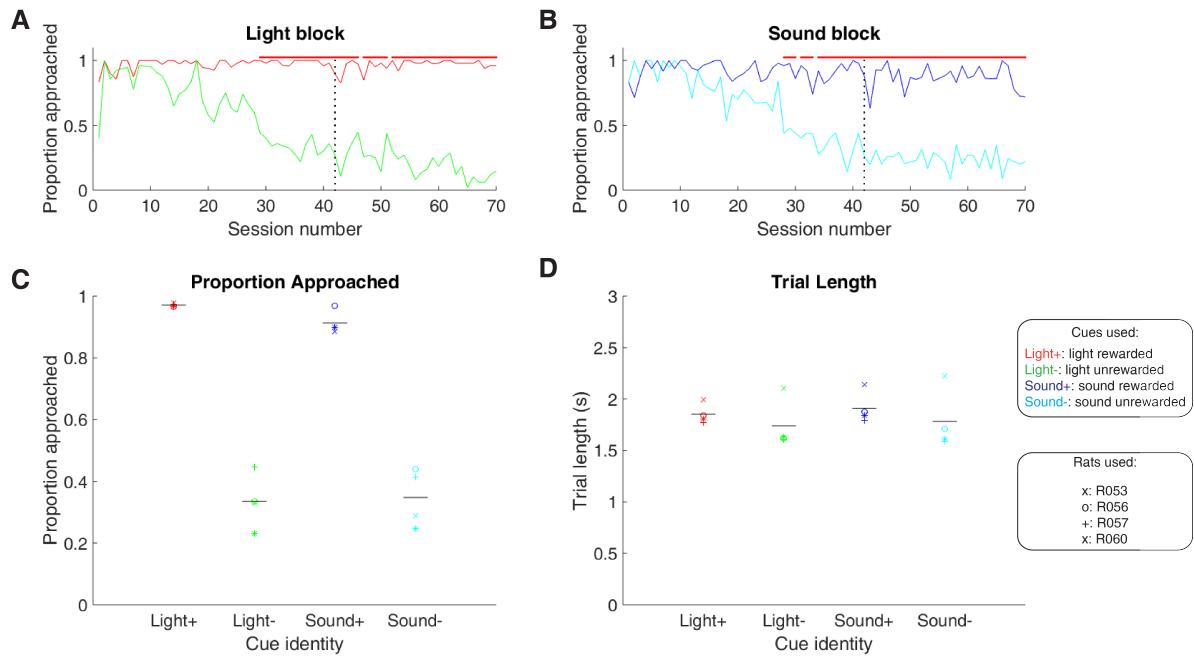


Figure 4: Performance on the behavioral task. A-B. Example learning curves across sessions from a single subject (R060) showing the proportion approached for rewarded (red line for light block, navy blue line for sound block) and unrewarded trials (green line for light block, light blue line for sound block). Fully correct performance corresponds to approach proportion of 1 for rewarded trials and 0 for unrewarded trials. Note that this subject starts out approaching on both rewarded and unrewarded trials, and learns with experience to skip non-rewarded trials. 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 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 (+) and unrewarded (-) light cues, rewarded and unrewarded sound cues). Different symbols correspond to individual subjects; black line shows the mean. All rats learned to discriminate between rewarded and unrewarded cues, as indicated by the clear difference of proportion approached between rewarded (0.9x approached) and unrewarded cues (0.3x 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

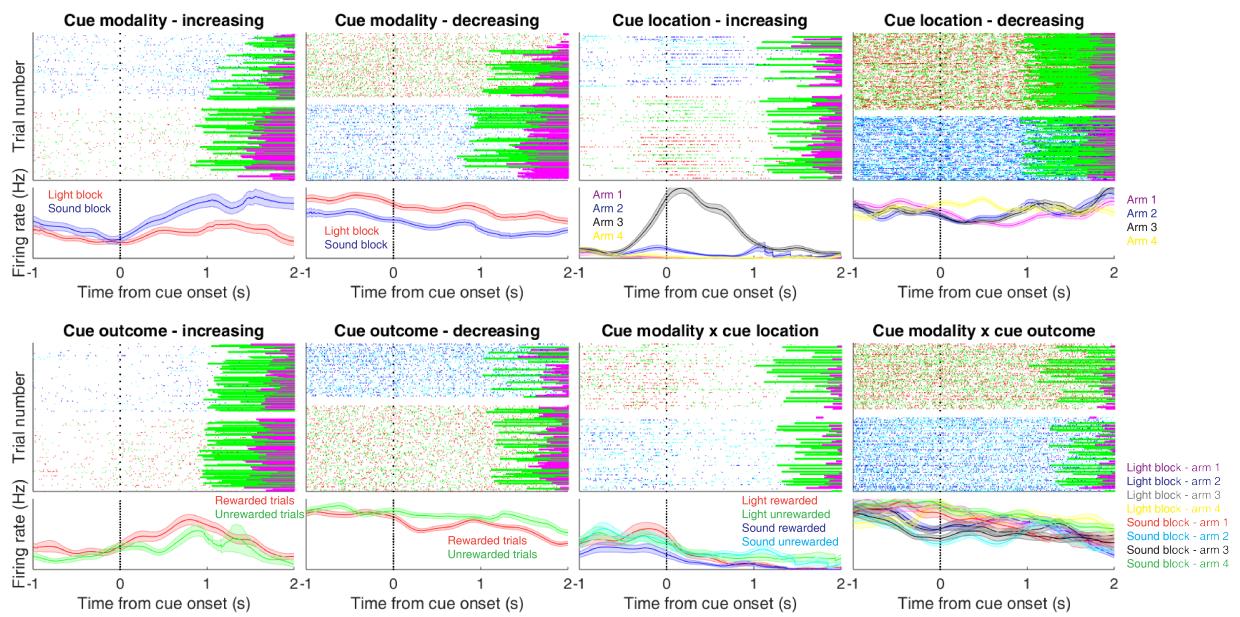


Figure 5: Examples of different cue-modulated NAc units influenced by various task parameters.

A. Example of a cue-modulated NAc unit that showed an increase in firing in response to the cue, and encoded cue modality. 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 light; green: unrewarded light; navy blue: rewarded sound; light blue: unrewarded 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 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.

E. This firing rate of this unit only changed on arm 3 of the task.

F. Firing decreased for this unit on all arms but arm 4.

G-H. Cue-modulated units that encoded cue outcome, with the PETHs comparing rewarded (red) and unrewarded (green) trials.

E. This unit showed a slightly higher response during presentation of rewarded cues.

F. This unit showed a dip in firing when presented with rewarded cues.

G-H. Examples of cue-modulated units that encoded multiple cue features.

G. This unit integrated cue modality and outcome.

H. An example of a unit that integrated cue modality and location.

6 - GLM.png

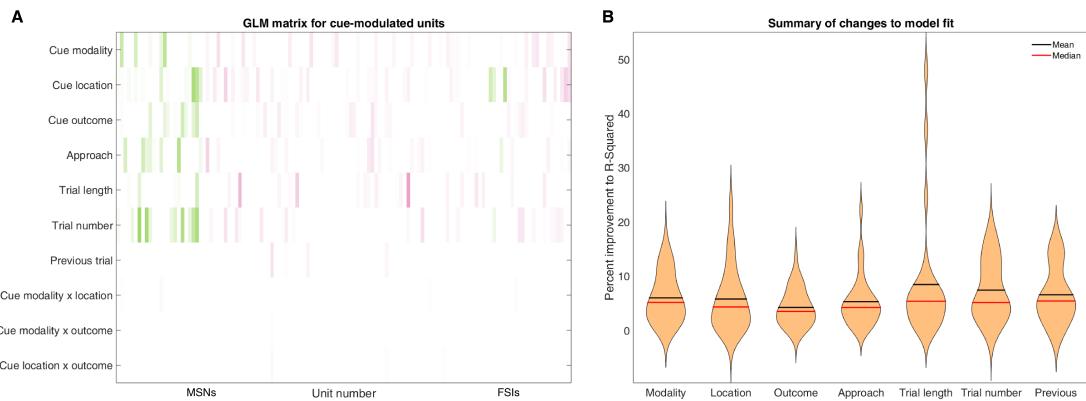


Figure 6: Summary of influence of various task parameters on cue-modulated NAc units after cue-onset. A. GLM matrix demonstrating impact of various task parameters on NAc firing rates. A stepwise GLM was fit to each unit that showed evidence of cue modulation by a Wilcoxon signed-rank test. Each row 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), MSNs with a decreasing response (red, left), FSIs with an increasing response (green, right), FSIs with a decreasing response (red, right). Darker shades indicate more firing rate variance explained by a given predictor. 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

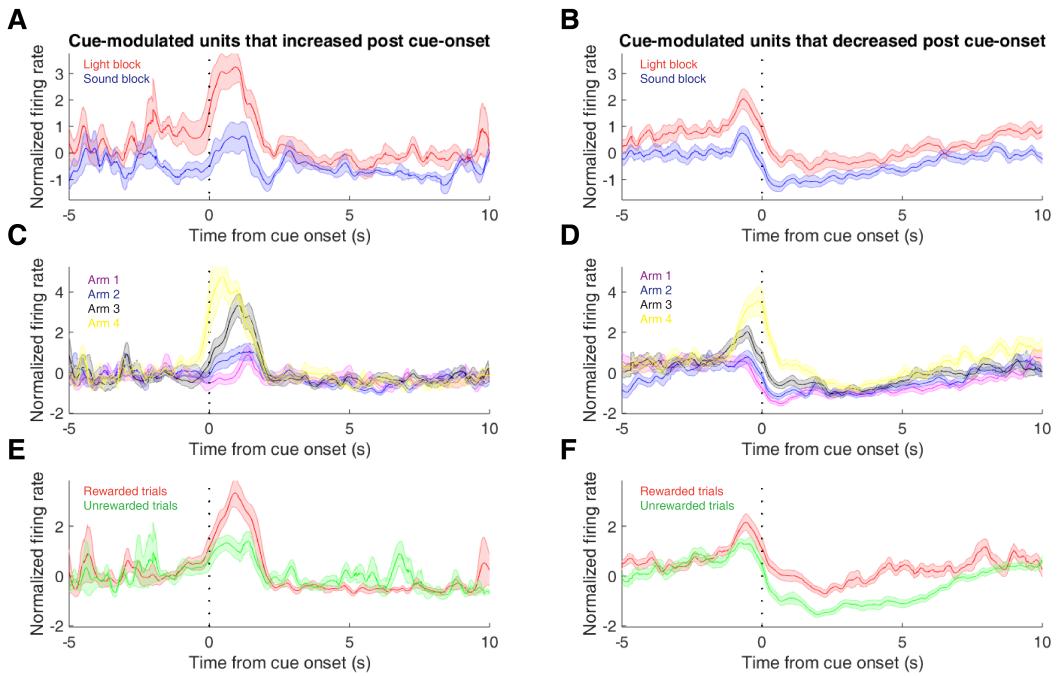
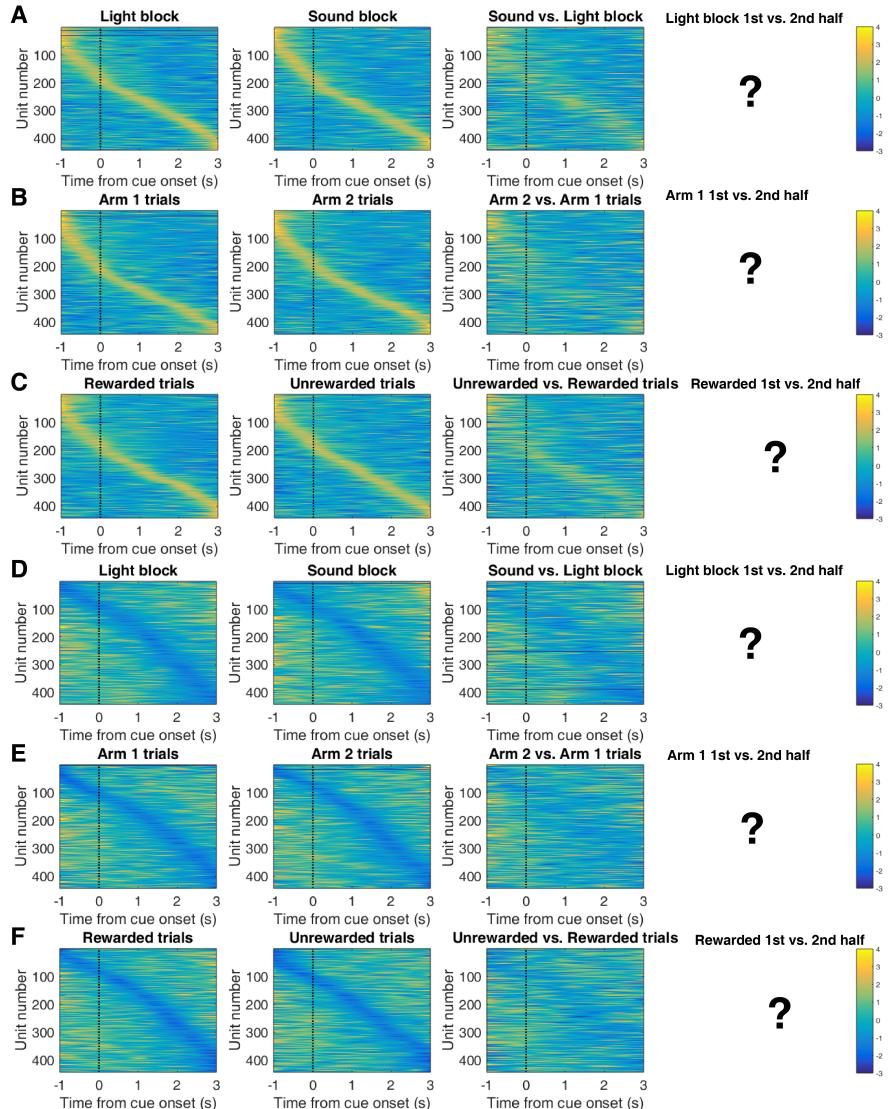


Figure 7: Population-level averages of cue feature sensitive NAc units. A. Average smoothed normalized (zscored) activity for cue-modulated units where cue modality was a significant predictor in the GLM, aligned to cue-onset. Activity is plotted for preferred stimulus block (red) and non-preferred stimulus 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. 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) 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, 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: 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, 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. 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 and unrewarded 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 different stimulus blocks as in A, but with units ordered according to the time of their minimum firing rate. F. Responses during different stimulus blocks as in A, but with units ordered according to the time of their minimum firing rate.

9 - NP GLM.png

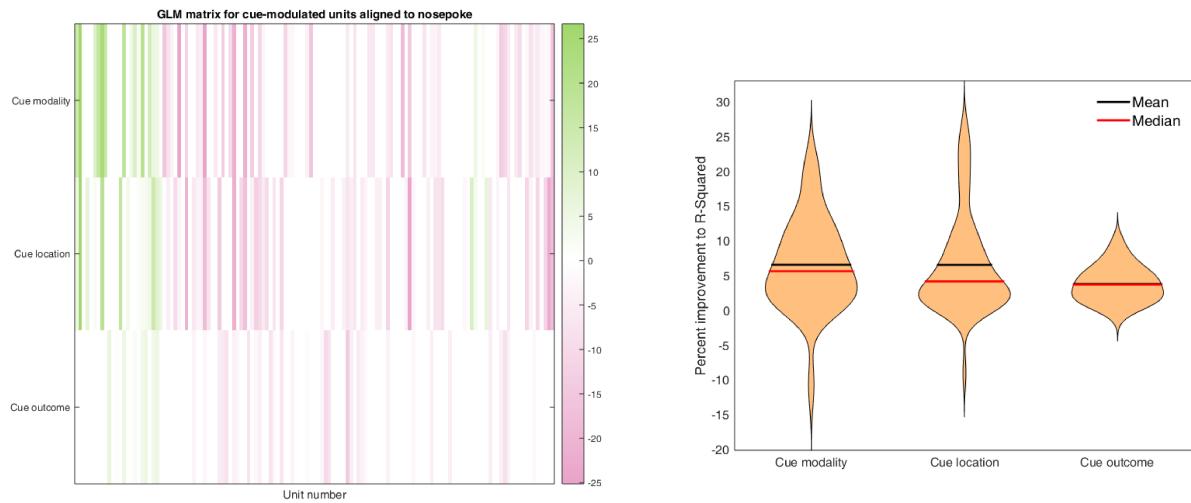


Figure 9: Summary of influence of various task parameters of cue-modulated NAc units during nosepoke. A. GLM matrix demonstrating impact of various task parameters on NAc firing rates. A stepwise GLM was fit to each unit that showed evidence of cue modulation by a Wilcoxon signed-rank test. Each row 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), MSNs with a decreasing response (red, left), FSIs with an increasing response (green, right), FSIs with a decreasing response (red, right). Darker shades indicate more firing rate variance explained by a given predictor. 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.

10 - NP population averages.png

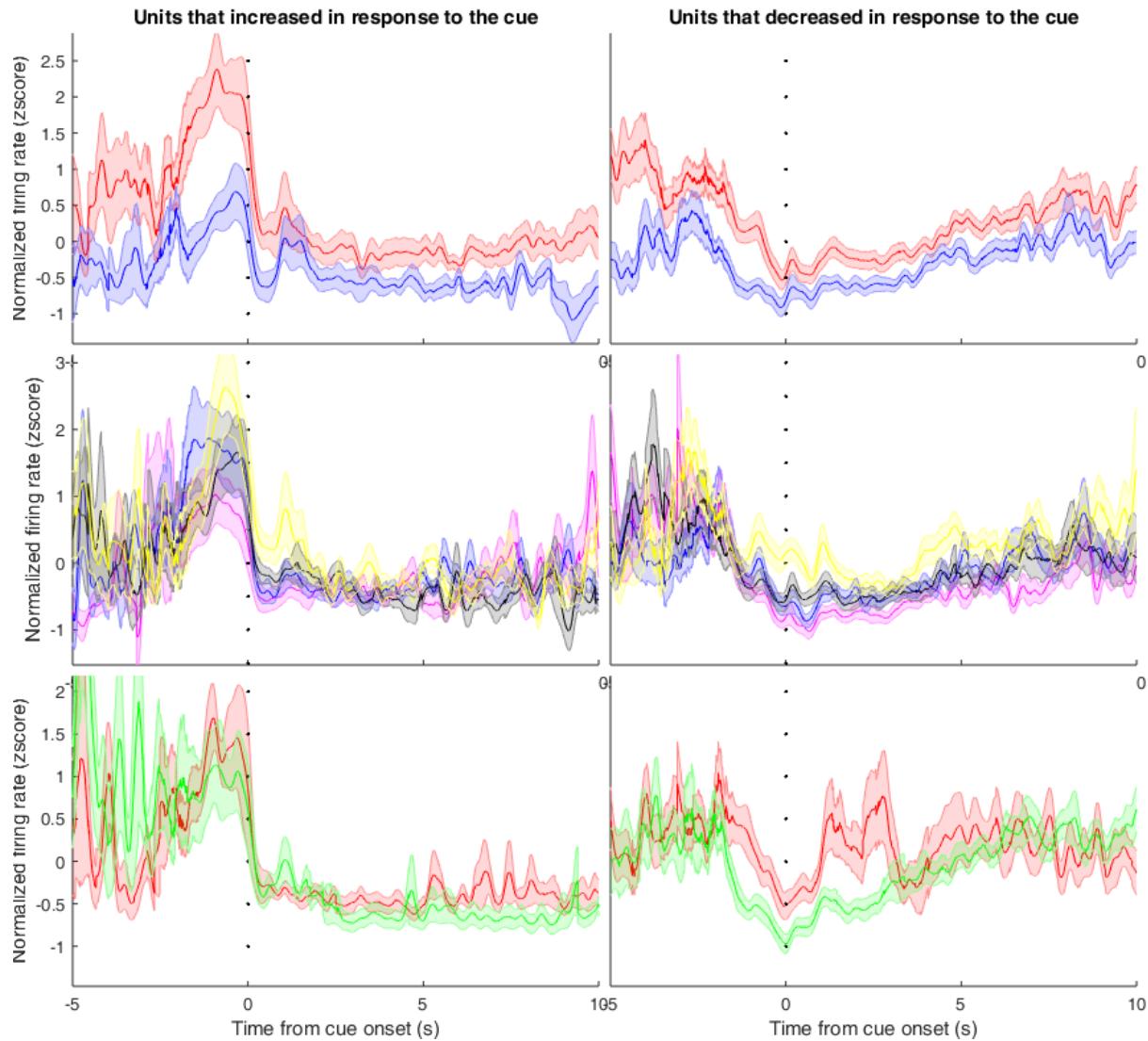


Figure 10: Population-level averages of cue feature sensitive NAc units during a nosepoke. A. Average smoothed normalized activity for cue-modulated units where cue modality was a significant predictor in the GLM, aligned to nosepoke with reward delivery occurring 1 s after nosepoke. Activity is plotted for preferred stimulus block (red) and nonpreferred stimulus block (blue). Dashed line indicates nosepoke. 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).

11 - NP task tiling.png

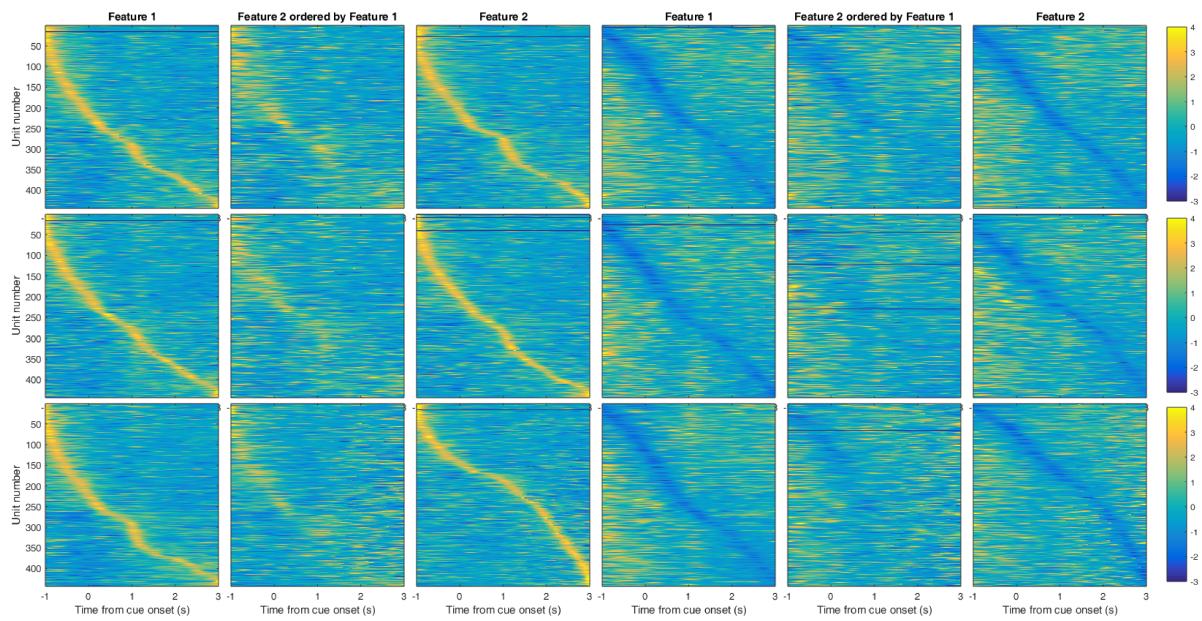


Figure 11: Distribution of NAc firing rates across task space during approach trials. Each panel shows normalized firing rates for all recorded NAc units (each row corresponds to one unit) as a function of time (time 0 indicates nosepoke), averaged across all approach trials for a specific cue type, indicated by text labels. A, 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 nosepoke with reward delivery occurring 1 s after nosepoke for rewarded 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: 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, 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. 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. E: Same layout as in A, except that panels now compare rewarded and unrewarded trials. Notice the absence of a cluster of tiling around reward receipt for unrewarded 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 (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