

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

Authors: Jimmie M. Gmaz¹, James E. Carmichael¹, Matthijs A. A. van der Meer^{1*}

¹Department of Psychological and Brain Sciences, Dartmouth College, Hanover NH 03755

*Correspondence should be addressed to MvdM, Department of Psychological and Brain Sciences, Dartmouth College, 3 Maynard St, Hanover, NH 03755. E-mail: mvdm@dartmouth.edu.

Number of Figures: 11

Number of Tables: 1

Total Word Count: ?

Abstract Word Count: ?

Introduction Word Count: ?

Discussion Word Count: ?

Acknowledgments: We thank Nancy Gibson, Martin Ryan and Jean Flanagan for animal care, and Min-Ching Kuo and Alyssa Carey for technical assistance. This work was supported by Dartmouth College (Dartmouth Fellowship to JMG and JEC, and start-up funds to MvdM) and the Natural Sciences and Engineering Research Council (NSERC) of Canada (Discovery Grant award to MvdM, Canada Graduate Scholarship to JMG).

Conflict of Interest: The authors declare no competing financial interests.

1 Abstract

2 to do

3 Significance Statement (120 words)

4 to do

5 Introduction

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

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

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

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

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

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

44 [Figure 1 about here.]

45 **Methods**

46 **Subjects:**

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

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

53 **Overall timeline:**

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

62 **Behavioral task and training:**

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

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

92 [Figure 2 about here.]

93 **Surgery:**

94 Surgical procedures were as described previously (Malhotra et al., 2015). Briefly, animals were anesthetized

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

102 **Data acquisition and preprocessing:**

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

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

113 **Data analysis:**

114 To investigate the contribution of various cue features on NAc firing rates we first determined whether firing
115 rates for a unit were modulated by the onset of a cue by collapsing across all cues and comparing the firing
116 rates for the 1 s preceding cue-onset with the 1 s following cue-onset. Single units were considered to be

cue-responsive if a Wilcoxon signed-rank test comparing pre- and post-cue firing had a $p < .01$. (Excluded:
the mean firing rate difference between pre- and post-cue onset was within the lower or upper 2.5% of a
shuffled distribution, as there is redundancy using both, and I did both because I was paranoid about just
using one.). Cue-modulated units were then classified as either increasing or decreasing in response to the
cue if the post-cue activity was higher or lower than the pre-cue activity, respectively.

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

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

To visualize NAc representation of task space within cue conditions, normalized spike trains for all units
were ordered by the location of their maximum or minimum firing rate for a specified cue condition (Figure
8). To compare representation of task space across cue conditions for a cue feature, the ordering of units

¹⁴¹ derived for one condition (e.g. light block) was then applied to the normalized spike trains for the other
¹⁴² condition (e.g. sound block).

¹⁴³ To identify the responsivity of units to different cue features at the time of a nosepoke into a reward recepta-
¹⁴⁴ cle, the same cue-responsive units from the cue-onset analyses were analyzed at the time of nosepoke using
¹⁴⁵ identical analysis techniques (Figures 9,10,11).

¹⁴⁶ Given that some of our analyses compare firing rates across time, particularly comparisons across blocks,
¹⁴⁷ we sought to exclude units with unstable firing rates that would generate spurious results reflecting a drift
¹⁴⁸ in firing rate over time unrelated to our task. To do this we ran a Mann-Whitney U test comparing the cue-
¹⁴⁹ evoked firing rates for the first and second half of trials within a block, and excluded units from analysis that
¹⁵⁰ showed a significant change for either block. All analyses were completed in MATLAB R2015a, and the
¹⁵¹ code is available on GitHub.

¹⁵² **Histology:**

¹⁵³ Upon completion of the experiment, rats were anesthetized with 5% isoflurane, then asphyxiated with carbon
¹⁵⁴ dioxide. Transcardial perfusions were performed, and brains were fixed and removed. Brains were sliced
¹⁵⁵ in 50 um coronal sections and stained with thionin. Slices were visualized under light microscopy, tetrode
¹⁵⁶ placement was determined, and electrodes with recording locations in the NAc were analyzed (Figure 3).

¹⁵⁷ [Figure 3 about here.]

158 **Results**

159 **Behavior**

160 Rats were trained to discriminate between cues signaling the availability and absence of reward on a square
161 track with four identical arms for two distinct sets of cues. During each session, rats were presented with
162 two behavioral blocks containing different sensory cues in sequential order, a light and a sound block, with
163 each block containing a cue that predicted reward, and a cue that signalled the absence of reward. An
164 example learning curve is seen in Figure 4A,B. All four rats learned to discriminate between the rewarded
165 and unrewarded cue for both the light and sound blocks as determined by reaching significance ($p < .05$)
166 on a daily chi-square test comparing approach behavior for rewarded and unrewarded cues for each block,
167 for at least three consecutive days (range for time to criteria: 22 - 57 days). Maintenance of behavioral
168 performance during recording sessions was assessed using linear mixed effects models for both proportion
169 of trials where the rat approached the receptacle, and trial length. Analyses revealed that cue type had an
170 influence on the likelihood of a rat to make an approach ($p < .001$), but not for the length of time taken to
171 complete a trial ($p = .13$)(Figure 4C,D).

172 [Figure 4 about here.]

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

174 **General responses to cue:**

175 We sought to address which parameters of our task were encoded by NAc activity, specifically whether the
176 NAc encodes aspects of motivationally relevant cues not directly tied to reward, such as the identity and

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

195 [Table 1 about here.]

196 [Table 2 about here.]

197 [Figure 5 about here.]

198 [Figure 6 about here.]

199 **Population level responses:**

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

216 [Figure 7 about here.]

217 **NAc units segment the task:**

218 Another method to get a sense of how the NAc is behaving at the population level is to analyze NAc unit
219 activity across time, and NAc neurons have been shown to have correlates across an entire task space. To
220 look at the distribution of responses throughout our task space and see if this distribution is modulated by cue
221 features, we z-scored the firing rate of each unit and plotted the normalized firing rates of all units aligned

222 to cue-onset and according to peak firing rate (Figure 8). We did this separately for both the light and sound
223 blocks, and found a nearly uniform distribution of firing fields in task space that was not limited to alignment
224 to the cue (Figure 8A). Furthermore, to see if this population level activity was similar across blocks, we
225 also organized firing during the sound blocks according to the ordering derived from the light blocks. This
226 revealed that while there was some preservation of order, the overall firing was qualitatively different across
227 the two blocks. Additionally, given that the majority of our units showed an inhibitory response to the cue,
228 we also plotted the firing rates according to the lowest time in firing, and again found some maintenance
229 of order, but largely different ordering across the two blocks (Figure 8B). This process was repeated for
230 cue location and cue outcomes, showing that NAc segmentation of the task is qualitatively different across
231 various cue conditions (Figure 8C-F).

232 [Figure 8 about here.]

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

234 In order to be useful for learning, a trace of the cue must be maintained until the outcome. Fitting a GLM
235 to the firing rates of cue-modulated units at the time of a nosepoke response showed that a variety of units
236 still discriminated firing according to various cue features, but not other task parameters, showing that NAc
237 activity discriminates various cue conditions well into a trial (Table 3, Figure 9). Fitting a GLM to all
238 recorded units...*Jimmie finish this* (data not shown). Population level averages for units that increased
239 to cue-onset showed a ramping up of activity that peaked upon nosepoke, whereas units that decreased to
240 cue-onset showed a gradual reduction of firing activity that reached a minimum upon nosepoke (Figure
241 10). Additionally, a peak is seen for preferred cue outcome in decreasing units at 1 second post cue-onset
242 when reward was received, demonstrating an integration of expected and received reward (Figure 10F).
243 Furthermore, aligning normalized peak firing rates to nosepoke onset, revealed a clustering of responses
244 around reward receipt for all cue conditions where the rat would have received reward (Figure 11). Alongside
245 this, fitting a GLM to the firing rates of cue-modulated units at the time of reward receipt, revealed 10 units

246 (8%) where cue outcome accounted for an average of 32% of firing rate variance (data not shown).

247 [Table 3 about here.]

248 [Figure 9 about here.]

249 [Figure 10 about here.]

250 [Figure 11 about here.]

251 **Discussion**

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

265 vigor, to detangle this from our results we included the trial length as a predictor in our GLMs, and found
266 cells with correlates independent of trial length.

267 **Cue modality:**

268 Our finding that ventral striatal units can discriminate between cues from different sensory modalities ex-
269 pands upon an extensive literature examining neural correlates of conditioned stimuli. Perhaps the most
270 comparable work in rodents comes from a study that found distinct coding for an odor when it predicted
271 separate but equally valued rewards (Cooch). The present work is complementary to this as it shows that
272 ventral striatal cells have representations of identifiable aspects of the cue itself, in addition to the reward
273 it predicts. Another study paired separate cues with appetitive or aversive outcomes, and found separate
274 populations of cells that encode each cue, with many switching selectivity after reversal of the associations
275 between the cues and outcomes, providing evidence that the NAc encodes the biological significance of
276 stimuli. Once again, our study was different as we recorded neural responses to distinct cues encoding the
277 same anticipated outcome, suggesting that even when the biological relevance of these stimuli is similar, the
278 NAc dissociates their representations at the level of the single-unit (Setlow). Another possibility is that these
279 modality specific cells were encoding the context, rule, or sequence within a session as some cells responded
280 similar for both rewarded and unrewarded cues within a block. This interpretation is in alignment with a
281 recent paper from the primate realm that recorded ventral striatal responses during the Wisconsin Card Sort-
282 ing Task (WCST), a common set-shifting task used in both the laboratory and clinic, and found cells that
283 preferred firing to stimuli when a certain rule, or rule category was currently active (Sleeker). Indeed, an
284 encoding of the current strategy could be an explanation as to why differentially tiling of task structure was
285 observed across blocks in the current study. Further support for a modulation of NAc responses by strategy
286 comes from an fMRI study that examined BOLD levels during a set-shifting task (FitzGerald et al., 2014). In
287 this task, participants learned two sets of stimuli-reward contingencies, a visual set and auditory set. During
288 testing they were presented with both simultaneously, and the stimulus dimension that was relevant was peri-
289 odically shifted between the two. Here, they found that bilateral NAc activity reflected value representations

290 of whatever the currently relevant stimulus dimension was, and not the irrelevant stimulus. The current find-
291 ing of separate, but overlapping, populations of cells encoding cue modality and expected value, suggests
292 that the fMRI finding is generated by the combined activity of several different functional cell types.

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

303 **Encoding of position:**

304 Our finding that cue-evoked activity was modulated by cue location sides with some of the literature (Lavoie,
305 1994; Tabuchi, 2000; Strait, 2016). An alternative explanation for a pure spatial representation, is that
306 these are task segmentation correlates, keeping track of where in the task the rat is. A previous non-human
307 primate paper has shown that when reward is contingent upon completion of a series of trials, separate
308 populations of NAc neurons signal the start of a schedule, subsequent trials in the schedule, and the first
309 trial in extended schedules (Shidara et al., 1998). This signalling of position within a sequence has been
310 observed in subsequent studies, and it is possible that the our rats were keeping track of which specific arm
311 they were in as part of a sequence of arms, and not just strictly a spatial representation (Mulder, 2004 and
312 2005; Khamassi et al., 2008; Berke, 2009). Also, given that our task is pseudo-random, it is possible that the
313 rats learned which cue to anticipate, and the neural activity could reflect this. However, this is unlikely as

314 including a previous trial variable in the analysis did not explain a significant amount of firing rate variance
315 in response to the cue for the vast majority of cells..

316 **Mixed selectivity:**

317 Several other papers have reported unit profiles that integrate different task-related variables. These papers
318 report integrated coding between expected value and subsequent motor responses, expected value and iden-
319 tity of a reward, and a combination of spatial-, movement-, and reward-related features (Roesch, Lavoie,
320 Cooch). However, our study is the first to show mixed selectivity among identifying features of a cue and
321 expected outcome or behavior. The presence of mixed selectivity responses confers a larger number of input-
322 output relationships that are available to a given neuron. A possible functional consequence of this attribute
323 of NAc units, is the combination and transformation of various motivationally relevant features into a sig-
324 nal informing downstream decoders such as the ventral pallidum about appropriate behaviors in obtaining
325 motivationally relevant goals and biasing action selection towards these behaviors. Mixed selectivity in the
326 NAc could be a consequence of synaptic integration from a variety of anatomically distinct inputs, as seen
327 in experiments examining the convergence of various NAc afferents at the level of synaptic transmission and
328 stimulation-induced firing (Goto and Grace 2008). In one such experiment it was shown that NAc cells that
329 responded to stimulation of either the fornix, amygdala, or PFC, typically responded to stimulation from all
330 inputs (ODonnell and Grace, 1995). Furthermore, an interaction between these inputs was observed such
331 that PFC stimulation failed to elicit spiking in the NAc neurons unless they were in a depolarized UP-state, a
332 state induced by hippocampal stimulation and was dependent on an intact fornix. Hippocampal-induced sup-
333 pression of other inputs has also been observed for the BLA (Mulder et al., 1998). Recently, it has also been
334 shown that train stimulation of PFC afferents reduces hippocampal-evoked NAc responses, suggesting that
335 there is competition between various inputs (Calhoon and ODonnell, 2013). These studies suggest that the
336 integration of the variables we saw could be the result of this gating observed in behaviorally-independent
337 preparations. However, given that we did not systematically manipulate these various limbic and cortical
338 afferents, comments on the anatomical origins of the observed mixed selectivity responses are speculative at

339 this point.

340 Integrating cue identity and value, as seen in the present study, could be one neural instantiation of how
341 value is associated with the appropriate predictive stimuli (credit assignment), keeping in mind that value
342 encoding is distributed, redundantly in some aspects, across various structures (Hayden Nat Neuro opinion).
343 Indeed, lesions of the NAc impair the ability to learn changes in reward value or identity in an unblocking
344 experiment, as well as disrupting dopamine RPEs generated by modification of timing of reward (McDannald
345 2011, Takahashi 2016). Would be interesting to see if uncoupling the integrated coding of stimulus features
346 and predictive properties of a cue has an effect on the ability of a rat to use reward-predictive cues to pursue
347 the associated reward.

348 **Tiling of task structure:**

349 Additionally, we found that the population of recorded units had a relatively uniform distribution of firing
350 fields within our task space, similar to what has been reported previously (Shidara, 1998; Berke, 2009;
351 Lansink, 2012). Uniquely, we found that this representation differed according to whether the rat was cur-
352 rently engaged in the light or sound block, suggesting that this could be a possible neural correlate for
353 encoding the currently relevant strategy in the NAc. During progress through a predictable trial series, neu-
354 rons represented state value of cue (Shidara 1998). Single-unit responses allowed the monkey to know how
355 it was progressing throughout the task. Likewise, the tiling we saw could be a consequence of upstream cor-
356 tical or limbic inputs informing the striatum of the current task rules. Another possibility is that the NAc not
357 only pays attention to progress throughout a task within a trial, but also higher-order task information, like
358 blocks. Cue location was a behaviorally irrelevant variable in the current experiment, but it is possible that
359 if this tiling is dependent on hippocampal input, or related to a state value representation, that making cue
360 location a relevant variable by adding positional contingencies such as only alternating arms are rewarded
361 in one block, would result in a further separation of the mapping within a block between the rewarded and
362 unrewarded arms. Furthermore, dopamine levels in the NAc fluctuate through a trial, and it is possible that

363 the observed tiling could be a NAc-representation of state value related to this temporally evolving dopamine
364 signal. Future experiments should monitor this mapping of task structure during the application of dopamine
365 antagonists. Finally, the presence of functional correlates not evident when looking at single-unit responses
366 time-locked to salient task events emphasizes the need to employ ensemble level analyses across all aspects
367 of a task.

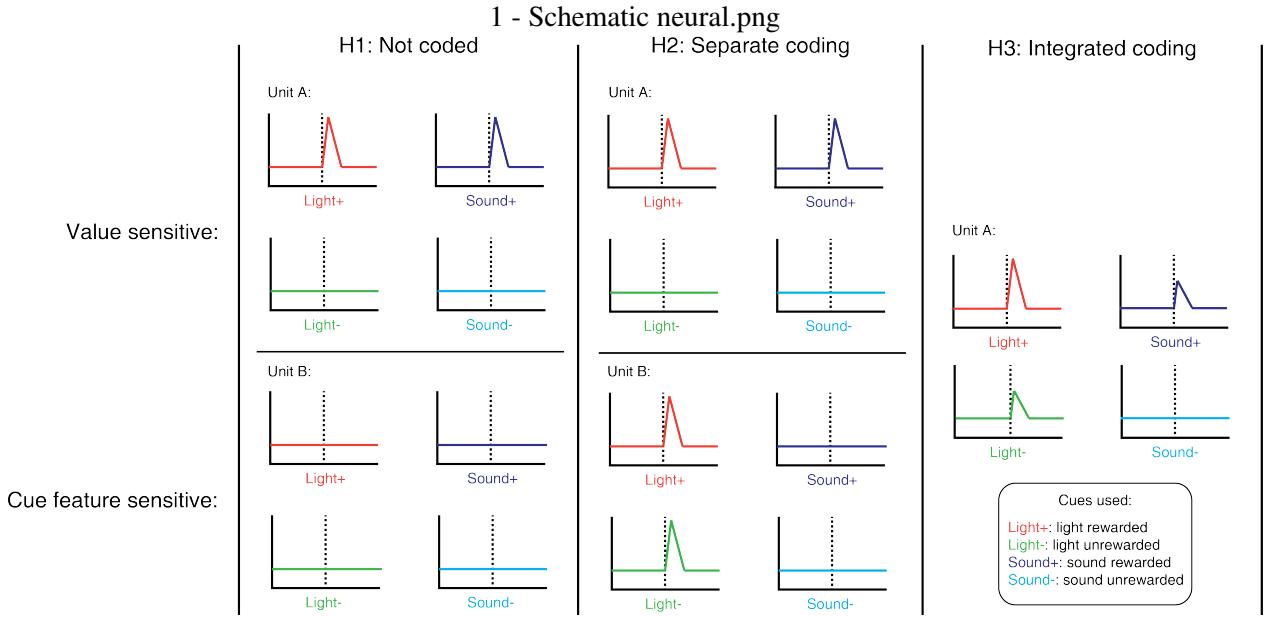


Figure 1: Schematic of potential coding strategies employed by single units in the NAc. Displayed are schematic PETHs illustrating putative responses to different cues under different hypotheses of how cue identity and value are coded. H1 (left panel): Coding of identifying cue features is absent in 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.

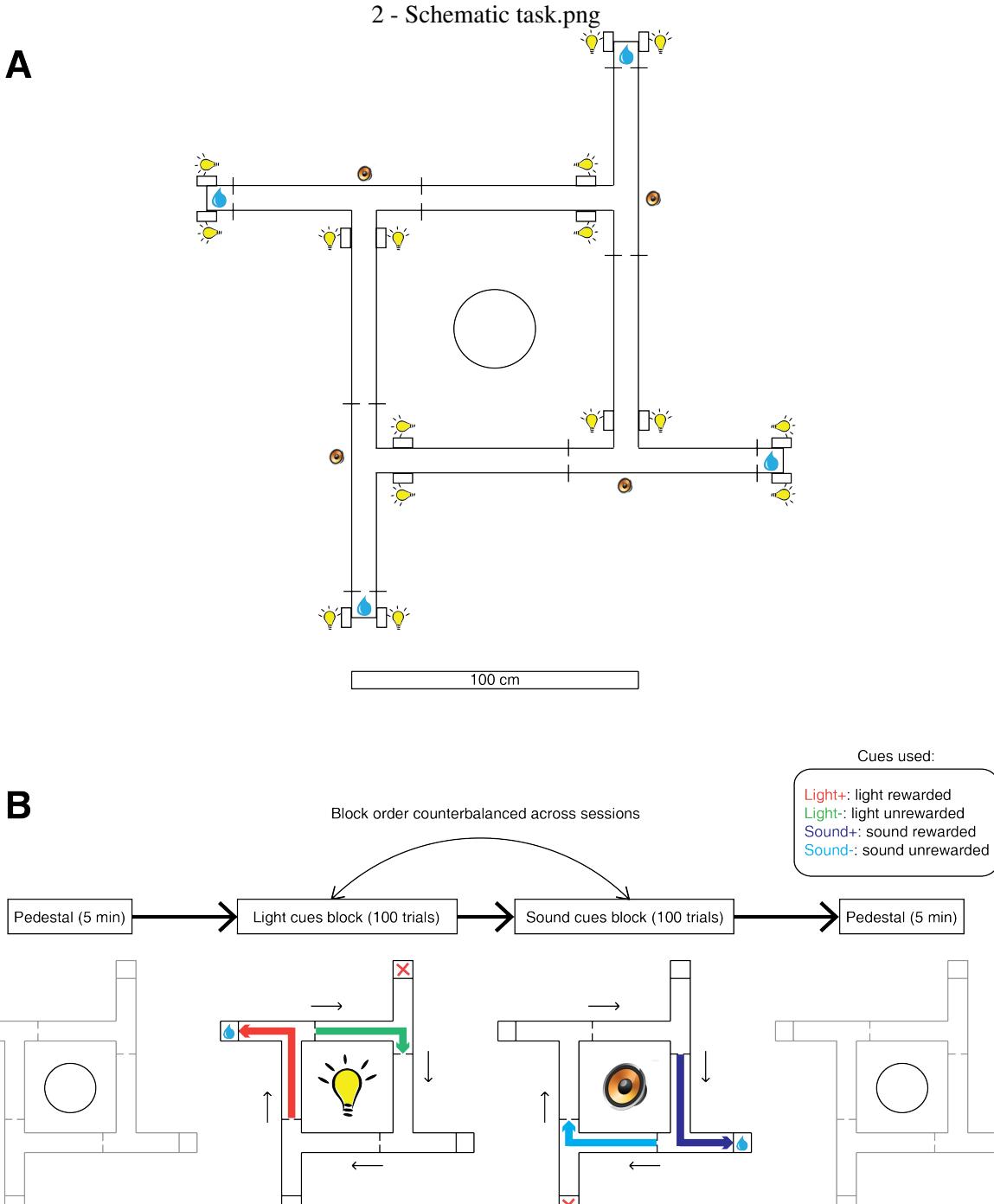
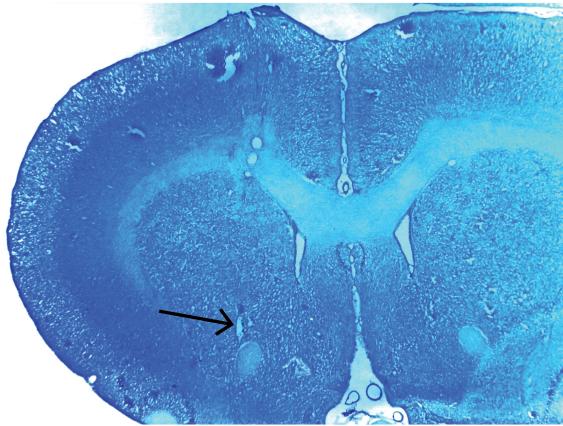


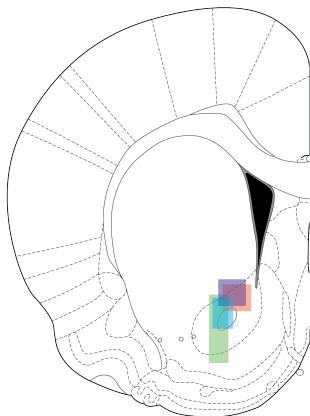
Figure 2: Schematic of behavioral task. A. To scale depiction of square track consisting of multiple identical T-choice points. Dashed lines indicate location of photobeams. Photobeams on the center, square portion of the track triggered the start of a trial and onset of a cue, whereas photobeams at 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

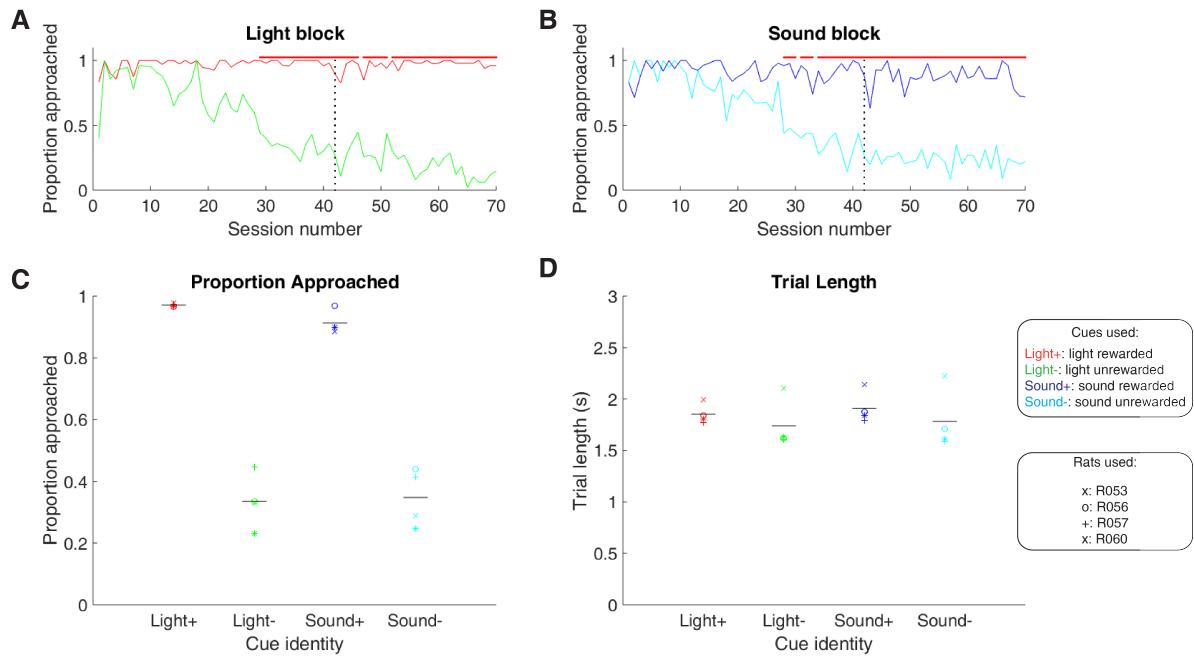


Figure 4: Performance on the behavioral task. A-B. Example learning curves across sessions from a single subject (R060) showing the proportion approached for rewarded (red line for light block, navy blue line for sound block) and unrewarded trials (green line for light block, light blue line for sound 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

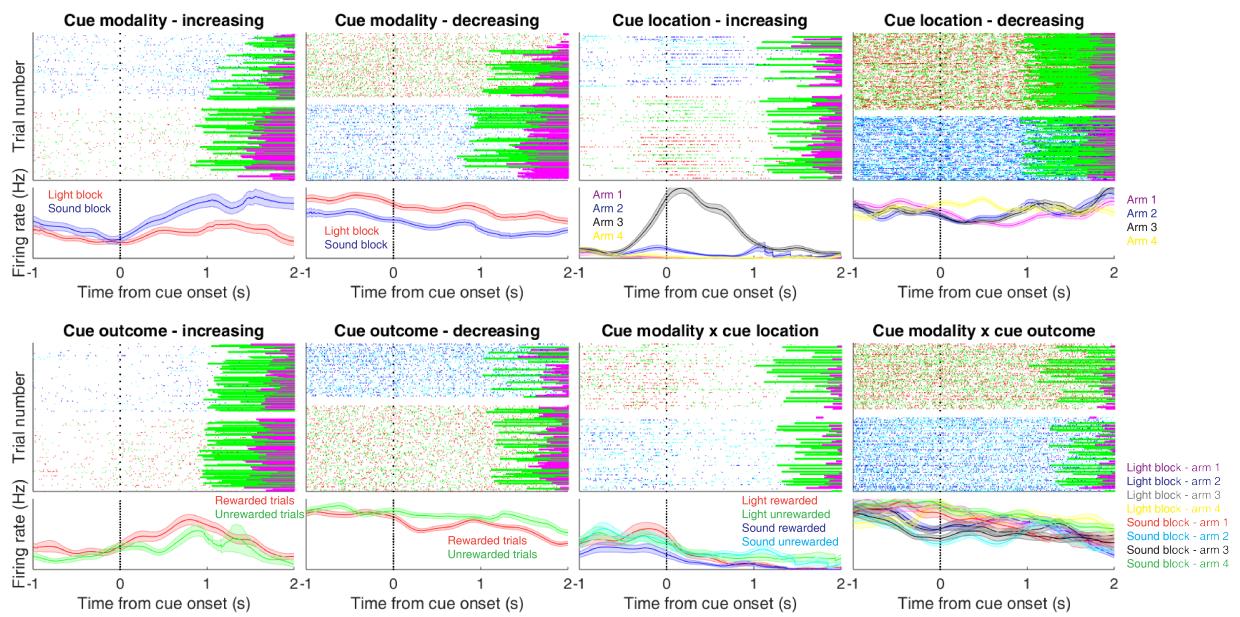


Figure 5: Examples of different cue-modulated NAc units influenced by various task parameters.

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

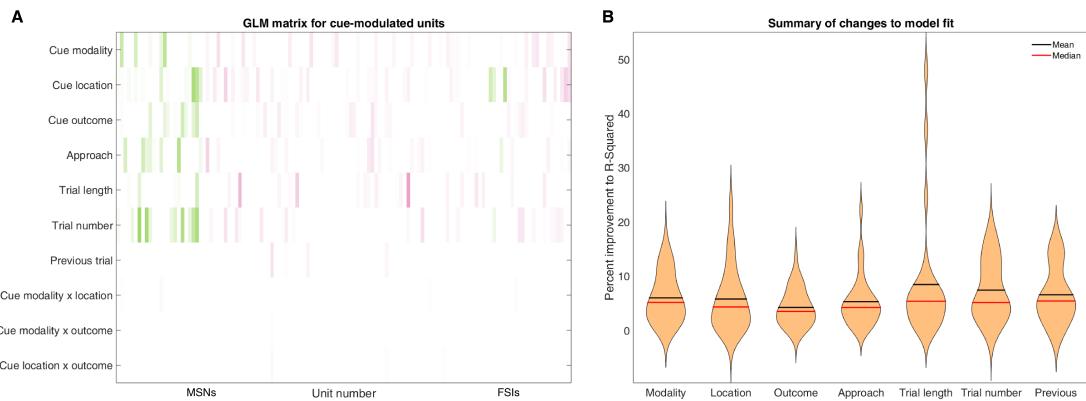


Figure 6: Summary of influence of various task parameters on cue-modulated NAc units after cue-onset. A. GLM matrix demonstrating impact of various task parameters on NAc firing rates. A stepwise GLM was fit to each unit that showed evidence of cue modulation by a Wilcoxon signed-rank test. Each row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

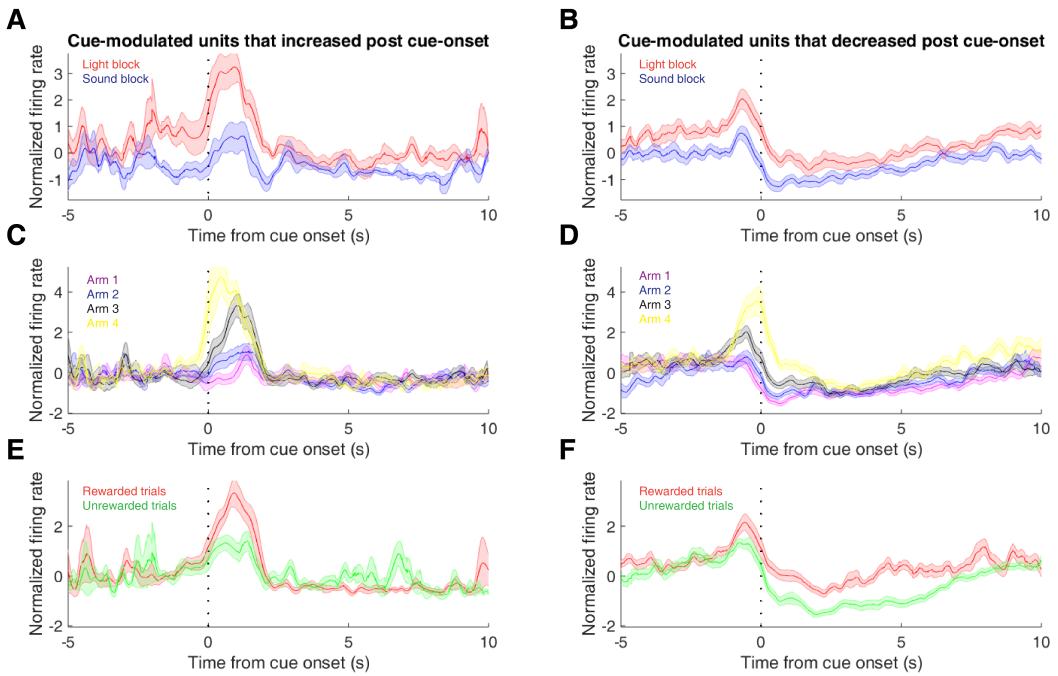
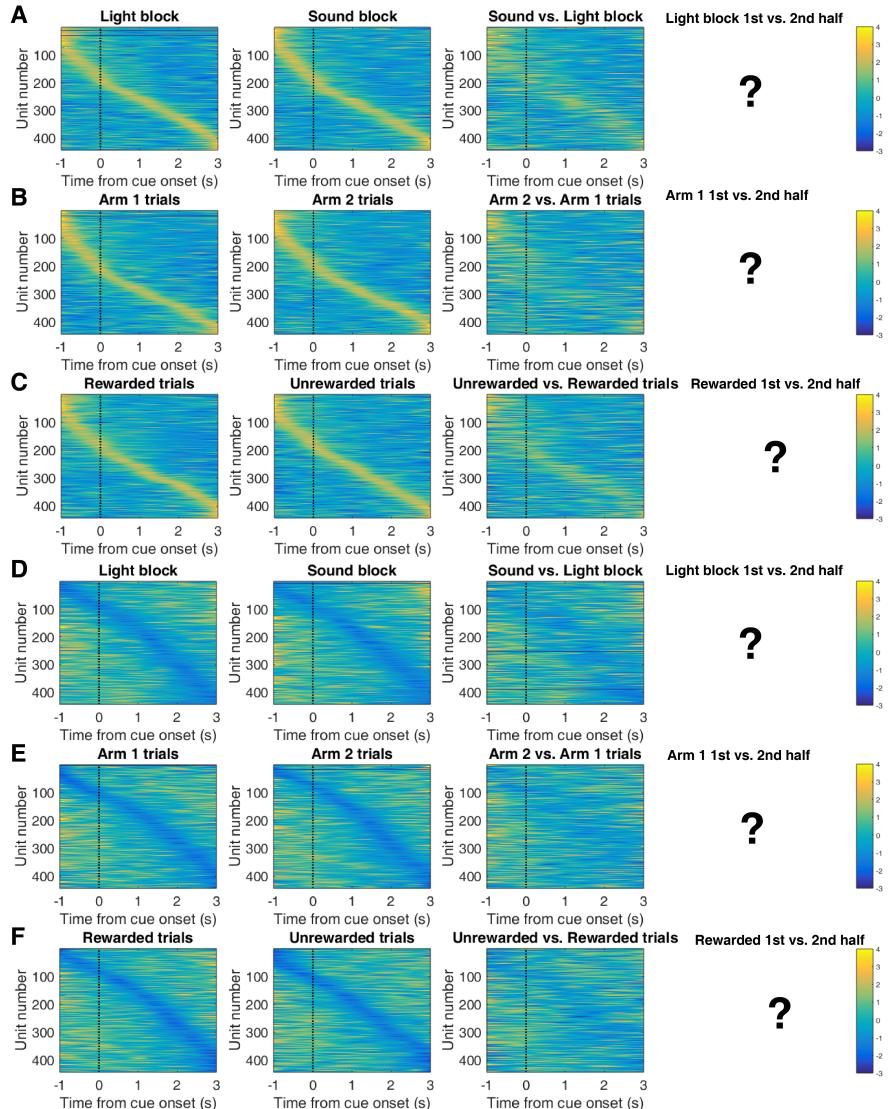


Figure 7: Population-level averages of cue feature sensitive NAc units. A. Average smoothed normalized (zscored) activity for cue-modulated units where cue modality was a significant predictor in the GLM, aligned to cue-onset. Activity is plotted for preferred stimulus block (red) and non-preferred stimulus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

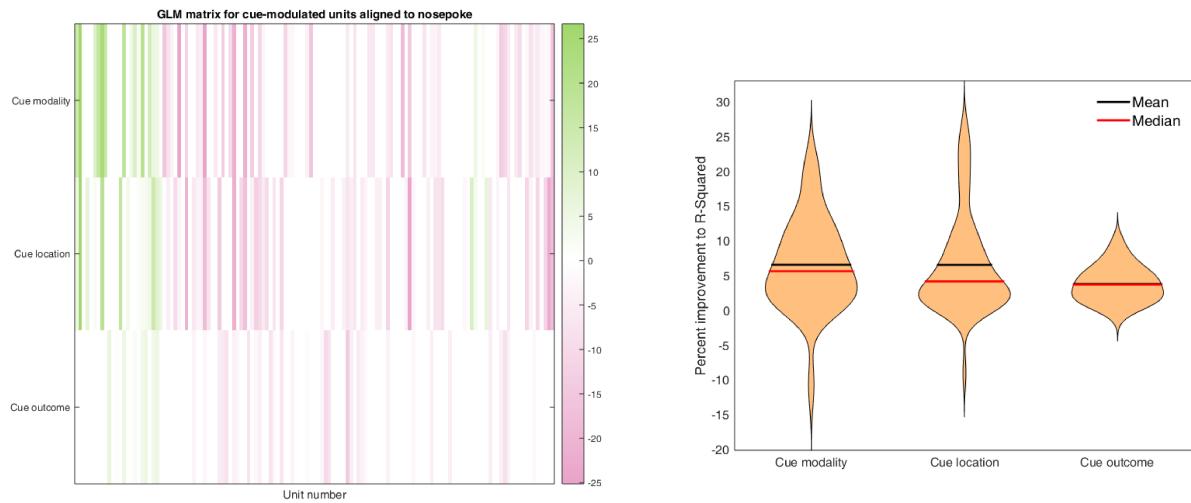


Figure 9: Summary of influence of various task parameters of cue-modulated NAc units during nosepoke. A. GLM matrix demonstrating impact of various task parameters on NAc firing rates. A stepwise GLM was fit to each unit that showed evidence of cue modulation by a Wilcoxon signed-rank test. Each row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

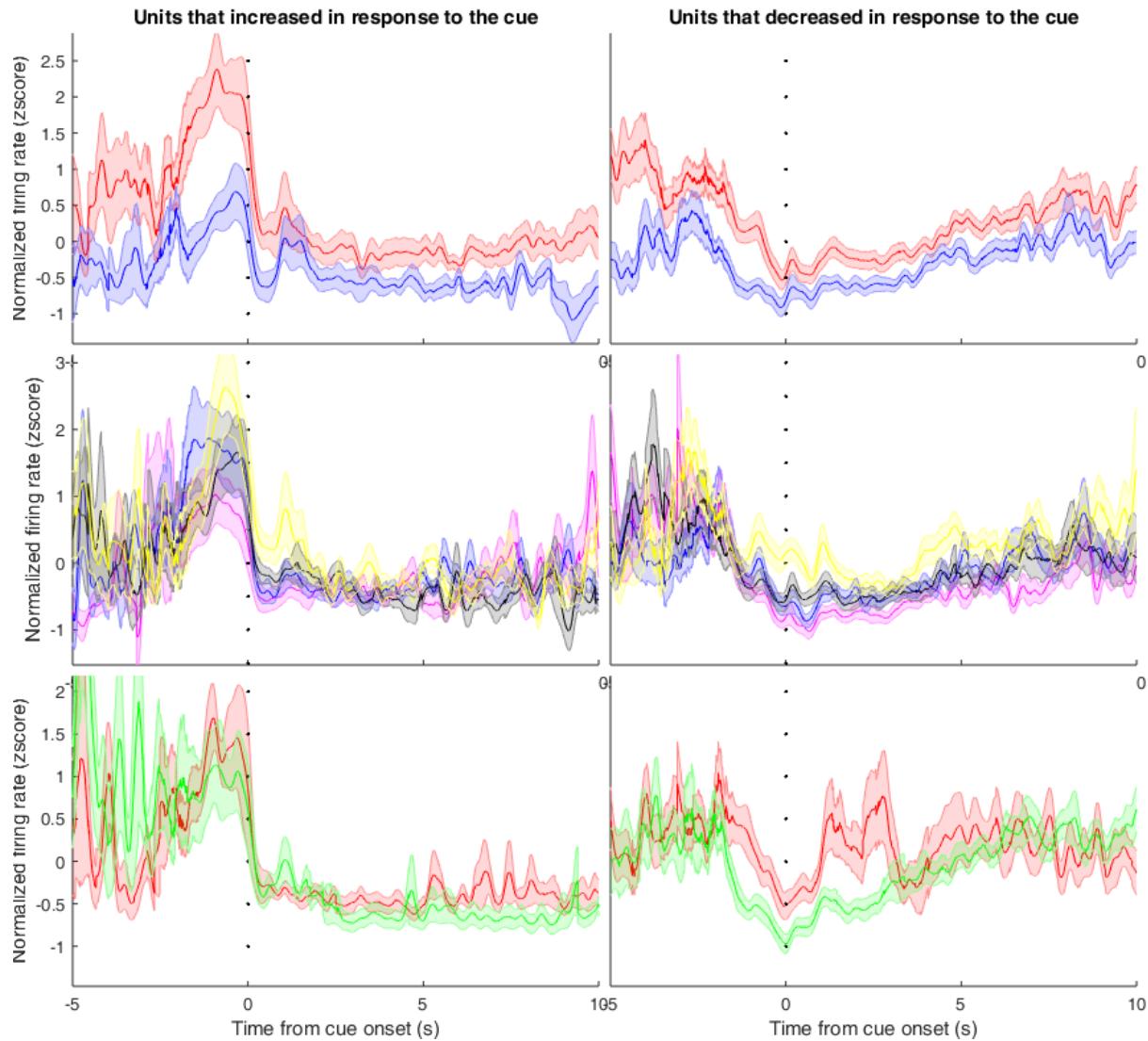


Figure 10: Population-level averages of cue feature sensitive NAc units during a nosepoke. A. Average smoothed normalized activity for cue-modulated units where cue modality was a significant predictor in the GLM, aligned to nosepoke with reward delivery occurring 1 s after nosepoke. Activity is plotted 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

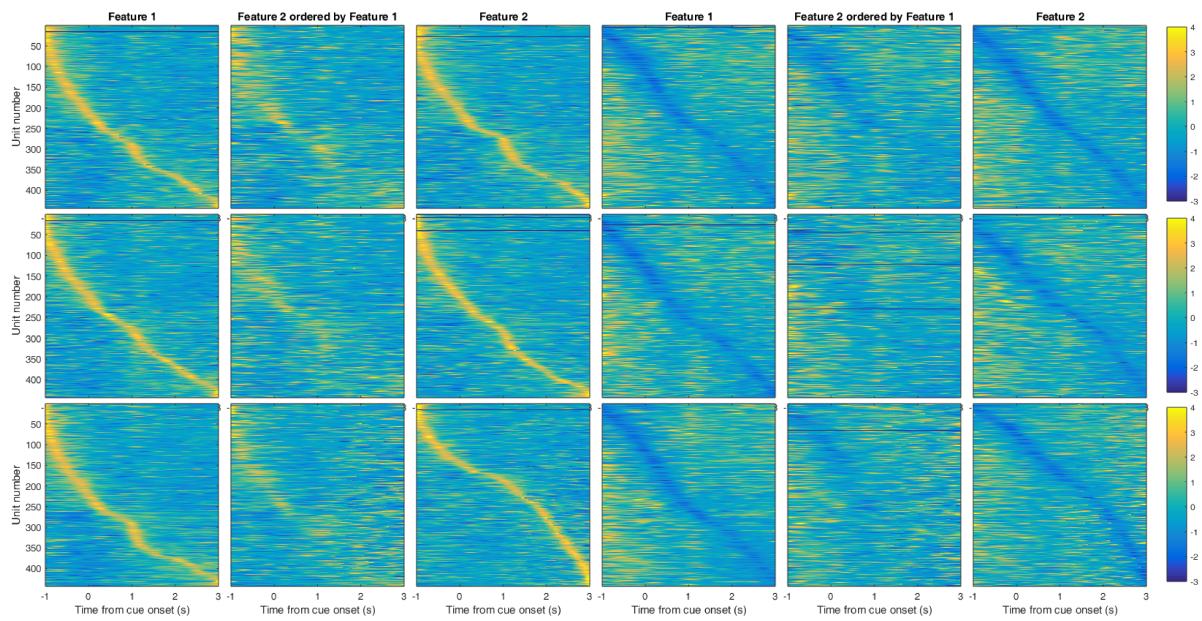


Figure 11: Distribution of NAc firing rates across task space during approach trials. Each panel shows normalized firing rates for all recorded NAc units (each row corresponds to one unit) as a function of time (time 0 indicates nosepoke), averaged across all approach trials for a specific cue type, 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