

A hippocampus-accumbens code guides goal-directed appetitive behavior

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1 **Neurons in dorsal hippocampus (dHPC) encode a rich repertoire of task-relevant environmental features, while downstream regions such as the nucleus accumbens (NAc) translate this information into task-adaptive behaviors. Yet, the contents of this information stream and their acute role in behavior remain largely unknown. Here, we used *in vivo* dual-color two-photon imaging to simultaneously record from both large numbers of hippocampal neurons and an identified subpopulation of NAc-projecting neurons during goal-directed navigation towards a hidden reward zone. We found that NAc-projecting neurons contain enriched spatial information and enhanced representations of non-spatial task-relevant behaviors such as deceleration and appetitive licking, both of which could be elicited by optogenetic activation of dHPC terminals in NAc. A generalized linear model revealed enhanced conjunctive coding in NAc-projecting dHPC neurons, improving the identification of the reward zone. We propose that dHPC routes specific reward-related spatial and behavioral state information to guide NAc action selection.**

19 hippocampus | nucleus accumbens | spatial memory | place cell | reward
20 learning | conjunctive coding | appetitive behavior | calcium imaging

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23 Introduction

24 Memories allow an organism to use past experience to optimize current and future behaviors (1). The hippocampus (HPC) is widely recognized as one of the main sites of memory-related plasticity (2–4). Yet, while our understanding of memory processing within the HPC has greatly advanced, surprisingly little is known about the translation of this information into behavioral action: which hippocampal output pathways send which types of information to guide memory-driven behavior? This lack of knowledge corresponds with the scarcity of data available on the activity profile of large numbers of identified hippocampal projection neurons while animals perform memory-dependent behaviors.

37 The HPC is a major part of the brain’s limbic system, processing various kinds of memory by receiving highly processed sensory information from the entorhinal cortex, and sending behaviorally relevant outputs to diverse brain regions via hippocampal Cornu Ammonis field 1 (CA1) and subiculum (5). Its elevated role for spatial memory is highlighted by the presence of spatially tuned “place cells” that form a “cognitive map” to support goal-directed navigation (6, 7).

45 This cognitive map is further supported by neuronal coding of navigationally relevant features such as borders, speed, reward/goal locations (8–11), but also non-spatial information such as that about future decisions or behavioral tasks (12, 13). The conjunction of this variety of encoded features is suggested to provide a “scaffold” that supports the formation of episodic memories (14).

52 Previous models of hippocampal memory processing assumed largely homogeneous cell populations (15, 16). However, hippocampal principal neurons are increasingly recognized as structurally and functionally diverse in terms of their morphology, electrophysiology, transcriptomic cell types, anatomical differences across its three axes, and projection patterns (17, 18). Such heterogeneity may provide the hippocampus with the intrinsic flexibility to meet the various demands of diverse environments (17), and to route task-relevant information to specific output targets (19, 20).

62 One target receiving strong projections from both dorsal and ventral CA1 and subiculum is the nucleus accumbens (NAc) (21, 22), a basal ganglia brain structure crucial for value-based action selection (23, 24). Described as the “interface between limbic and motor circuitry” (25), it has been suggested as the main site of transformation from a hippocampal spatial code into a motivation-driven motor code (26). In line with this, NAc neurons are spatially tuned, required for spatial memory acquisition and consolidation, and display task-dependent synchrony with dHPC neurons (27–30). Indeed, disabling HPC→NAc projections diminished conditioned place preference (CPP) (31, 32), and optogenetic stimulation was sufficient to artificially induce CPP (33, 34). While these findings cement the NAc’s role as an indispensable hippocampus output node, surprisingly little is known about which spatial, contextual and behavioral patterns of hippocampal information the NAc receives. Here, we set out to understand the specific contents of this information stream during goal-directed navigation and its acute role in behavior. We hypothesized that the HPC may selectively route both spatial as well as other task-relevant information to aid the NAc in action selection.

84 To test this, we employed dual-color two-photon imaging, capturing *in vivo* calcium signals of large populations of neurons in dHPC, while using a projection-specific red fluorophore to allow identification of NAc-projecting neurons (dHPC→NAc). Directly comparing dHPC→NAc activity with

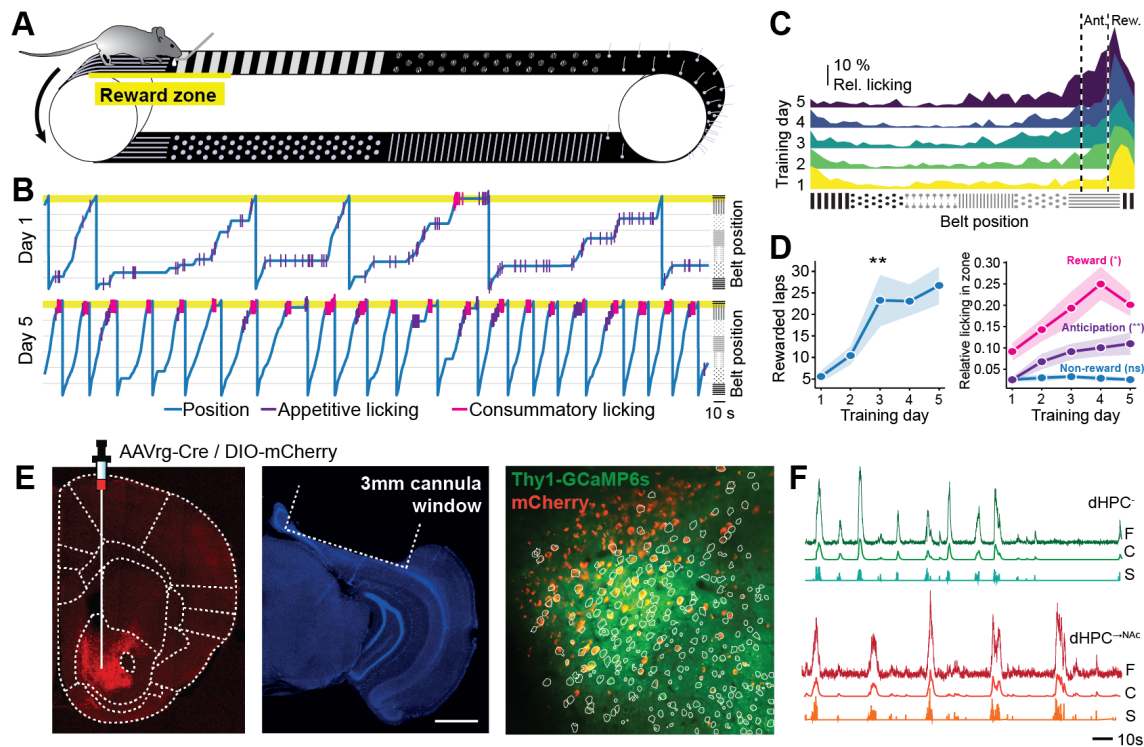


Figure 1. Dual-color two-photon calcium imaging of dHPC allows projection-specific activity monitoring during goal-directed navigation. (A-D) Training mice on a spatial reward learning task. (A) Schematic of behavioral task. Mice moved on a cue-enriched self-propelled treadmill belt of 360 cm length and obtained a liquid reward when licking at a spout in a hidden reward zone of 30 cm length (yellow). (B) Representative traces of one mouse's behavior on training day 1 and day 5. (C) Licking in anticipation (Ant.) and reward (Rew.) zone increases over the course of training. (D) Number of rewarded laps per training session ($F(4) = 6.344$, GG-correction), anticipatory ($F(4) = 3.803$) and reward licking ($F(4) = 4.276$, GG-correction) significantly increased over the course of five training days ($n = 18$ mice, repeated-measures ANOVA). See also Figure S1. (E) Schematic of dual-color projection neuron imaging method. Thy1-GCaMP6s mice were injected with AAVrg-Cre in the medial NAc and DIO-mCherry in dHPC. Representative coronal brain slice showing axonal mCherry expression in NAc (AP -1.3; left). Representative coronal brain slice stained with DAPI (blue) of dHPC, showing the outlines of the 3 mm cannula window used for imaging; scale bar represents 1 mm (second left). Field of view of one sample experiment showing Thy1-GCaMP6s expression in green and mCherry expression of putative NAc-projecting neurons in red; outlines show detected components used for analysis (right). (F) Two representative neurons' raw ("F"), denoised and deconvolved ("C") and event ("S") traces; red traces indicate mCherry co-expression (dHPC \rightarrow NAc). See also Figure S2. All data are presented as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$.

89 the rest of the dHPC population (dHPC $^{-}$), we found enhanced
 90 spatial tuning in dHPC \rightarrow NAc neurons, with strong modulation
 91 by local cue boundaries and a reward zone. We also found
 92 elevated coding of low velocities and appetitive licking, both
 93 of which could be elicited by optogenetic stimulation. Lastly,
 94 we show stronger conjunctive coding of space, velocity, and
 95 appetitive licking, which improves classification of the re-
 96 ward zone with a linear decoder. We thus propose that dHPC
 97 routes reward context-enriched spatial and behavioral state
 98 information to bias NAc action selection.

99 Results

100 **Dual-color calcium imaging during goal-directed navigation.** To investigate coding of spatial information and
 101 goal-directed behavior in dHPC \rightarrow NAc neurons, we trained
 102 food-restricted mice on a head-fixed spatial reward learning
 103 task. Mice had to run on a self-propelled treadmill, traversing
 104 a 360 cm long textile belt with six differently textured zones,
 105 including one otherwise unmarked 30 cm long fixed reward
 106 zone (35, 36). Licking a spout in this zone causes a liquid
 107 reward to be dispensed once per lap (Figure 1A and Figure
 108 S1A-E). Mice underwent five days of training in which they
 109 learned to obtain more rewards by progressively increasing
 110

their licking in both reward zone and a 30 cm “anticipation
 111 zone” preceding the reward zone (Figure 1B-D and Figure
 112 S1F-N).
 113

To understand the neural coding properties of large numbers
 114 of dHPC \rightarrow NAc neurons in behaving animals, we turned to
 115 dual-color two-photon imaging in mice pan-neuronally ex-
 116 pressing the calcium indicator GCaMP6s, as well as the static
 117 red marker mCherry in defined NAc-projecting neurons. For
 118 this, we injected AAVrg-Cre in NAc and DIO-mCherry in
 119 dHPC (CA1/subiculum border region) of Thy1-GCaMP6s
 120 mice (37). This approach allowed us to obtain dynamic cal-
 121 cium signals both in a large majority of mCherry-negative
 122 hippocampal neurons (dHPC $^{-}$) and specifically mCherry co-
 123 expressing NAc-projecting neurons (dHPC \rightarrow NAc), simultane-
 124 ously within the same field of view using the same calcium
 125 indicator (Figure 1E and Figure S2). It allowed us to over-
 126 come constraints of electrophysiological studies such as rela-
 127 tively low sample sizes (19, 20) or indirect connectivity mea-
 128 surements (38, 39). Optical access to dorsal CA1 and pro-
 129 subiculum (also known as proximal subiculum (40, 41)) was
 130 established by implanting a chronic hippocampal window af-
 131 ter virus injections (Figure S1C) (42, 43). Imaging data was
 132 acquired after 5 days of behavioral training, was motion-
 133 corrected using NormCorre, and spatio-temporal components
 134

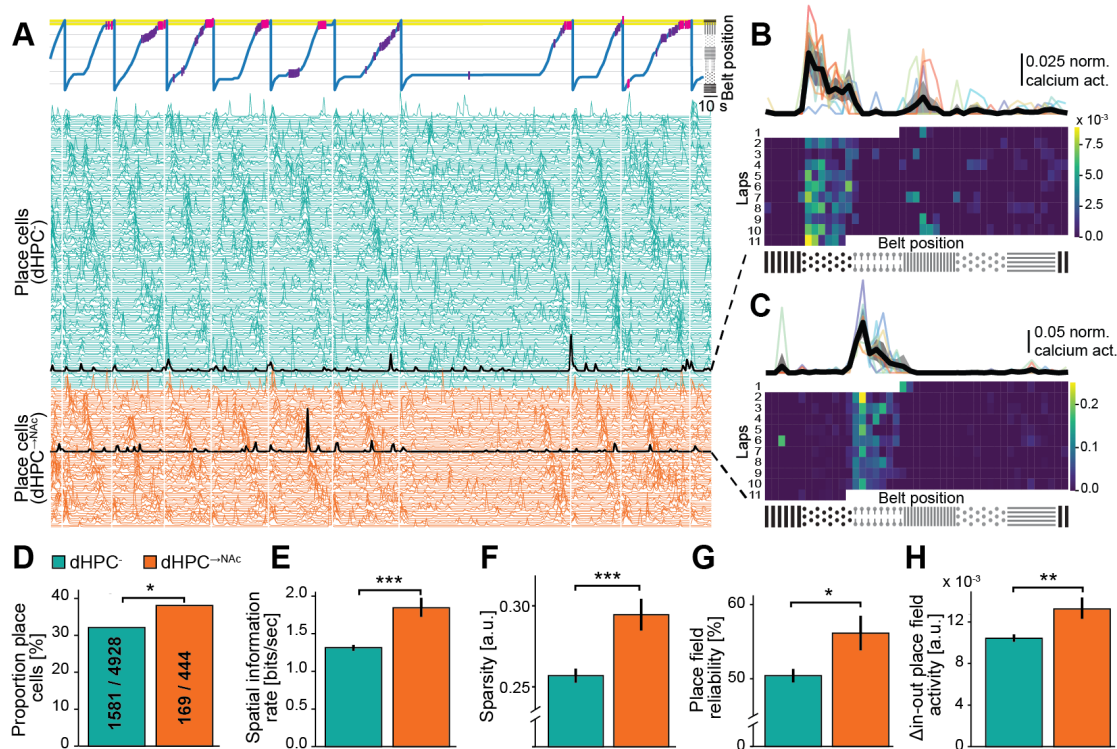


Figure 2. dHPC^{→NAc} neurons carry enhanced and more reliable spatial information. (A) Excerpt from one representative recording session showing behavioral activity (top) and denoised neural activity of identified place cells (bottom) over the course of several minutes from one mouse. White vertical lines mark new laps. Traces are ordered according to whether neurons co-expressed mCherry (red; dHPC^{→NAc}) or not (green; dHPC⁻) and their respective place fields. Black traces are example neurons shown in (B and C). (B and C) Spatial activity of one neuron without (B) and with (C) mCherry co-expression over one session. Calcium events are binned by position on the belt (x axis) and laps (y axis) and show consistent activity at one position on the belt. (D-H) Comparisons of spatial tuning characteristics between dHPC⁻ (green) and dHPC^{→NAc} (red) neurons. NAc-projecting neurons contain a higher proportion of place cells (D, $\chi^2(1, 5372) = 6.364$); these place cells contain more spatial information per second (E, Welch's $t(186.55) = 4.770$), show increased sparsity (F, Welch's $t(218.79) = 3.657$), higher reliability (G, probability of firing maximally within their place field per lap; Welch's $t(203.46) = 2.479$), and higher in-place field activity (H, Welch's $t(190.32) = 2.884$). $n = 6$ mice, 19 imaging sessions, 5,372 (inc. 444 mCherry-coexpressing) neurons, 1,750 (inc. 169 mCherry-coexpressing) place cells. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. See also Figure S3.

135 were extracted using constrained non-negative matrix factorization (CNMF) (44). We thus obtained calcium signals for further analysis from a total of 5,372 GCaMP-expressing neurons including 444 putative dHPC^{→NAc} neurons in 6 mice across 19 imaging sessions (Figure 1F and Figures S2 and S3). These numbers approximate previously established proportions of NAc-projecting neurons in dorsal presubiculum and distal CA1 (20, 45, 46).

143 **Enhanced spatial coding by dHPC^{→NAc} neurons.** Given the dHPC's well-established role in representing spatial information and the role of dHPC^{→NAc} projections in spatial memory tasks (31, 32), we wondered if and how spatial information may be encoded by dHPC^{→NAc} neurons compared to dHPC⁻ neurons. For this, we extracted calcium events for each neuron and compared this activity across the linear spatial environment of the treadmill belt as mice traversed it lap by lap. Indeed, we found large numbers of both dHPC⁻ and dHPC^{→NAc} neurons with repeatedly elevated calcium levels on the same positions of the belt (Figure 2A-C and Figure S3). Comparing each neuron's spatial information content (47) with that of a randomly shuffled distribution, we identified a significantly higher proportion of such place cells in dHPC^{→NAc} neurons (169/444 neurons; 38 %) compared to dHPC⁻ neurons (1,581/4,928 neurons; 32 %; $\chi^2, p = 0.012$;

Figure 2D). Within this population of place cells, we further analyzed how specifically space is encoded, and found that dHPC^{→NAc} place cells had a higher spatial information rate (47) ($p < 0.001$, Welch's t -test; Figure 2E). They also had significantly higher levels of sparsity ($p < 0.001$, Welch's t -test; Figure 2F), a measure for how diffuse a neuron is firing in the spatial domain (48). Furthermore, both the relative calcium activity (activity inside the place field – activity outside the place field; $p = 0.0044$, Welch's t -test) and reliability of in-place field activity per lap ($p = 0.014$, Welch's t -test) were significantly higher for dHPC^{→NAc} place cells compared to dHPC⁻ neurons (Figure 2G-H). These results suggest that the dHPC routes enhanced and more reliable spatial information to NAc compared to the general dHPC population, in line with previous results pointing towards the necessity of dHPC^{→NAc} projections for spatial memory expression (31, 32).

Place fields are modulated by local cue boundaries and are overrepresented near the reward zone. Previous studies found that place fields are often not homogeneously distributed across the environment but can be modulated by salient environmental features such as textures, borders, or reward/goal zones (11, 49, 50). We hypothesized that information about such spatial features may be preferen-

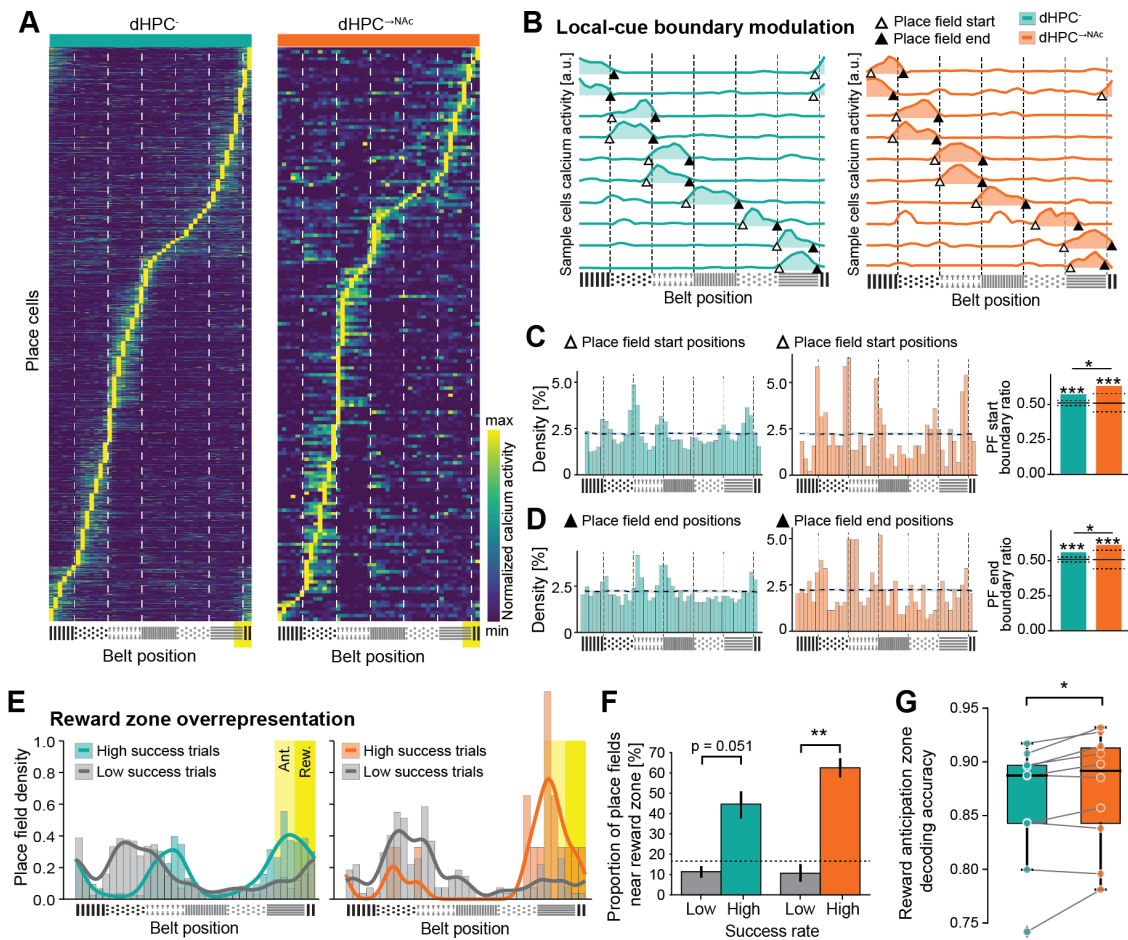


Figure 3. dHPC[→]NAc place fields are modulated by local cues and reward zone. (A) Heat maps of dHPC⁻ (left) and dHPC[→]NAc (right) place cells' normalized position-binned average calcium events, ordered by place field location. Texture boundaries are indicated as white dashed lines. Yellow rectangle represents reward zone. (B) Example place fields with edges near belt texture boundaries. Primary place fields are indicated by color fill. Triangles mark start (no fill) and end (fill) points. Dashed black lines represent texture boundaries. (C and D) Place field edges accumulate near texture boundary areas. Histograms of dHPC⁻ (green) and dHPC[→]NAc (red) neurons' place field start and end positions are significantly overrepresented at the 99.9th percentile (dotted black lines) compared to a randomly shuffled distribution. Start ($\chi^2(1, 5134) = 5.735$) and end positions ($\chi^2(1, 5217) = 4.397$) of dHPC[→]NAc place fields are furthermore significantly overrepresented compared to the dHPC⁻ population. (E and F) Place cells are overrepresented near reward zone in high success trials. (E) Histograms (bars) and kernel density estimations (KDEs; lines) of place field centers for dHPC⁻ (left) and dHPC[→]NAc (right) neurons, split into high success trials (green/red) and low success trials (gray). Reward zone (Rew.; yellow) and anticipation zone (Ant.; bright yellow) are indicated as rectangles. (F) Proportion of place fields in reward and vicinity zone is significantly higher in high-success trials (colored bars) compared to low-success trials (gray bars) in NAc-projecting neurons (red) but not in dHPC⁻ neurons (green). 2-way ANOVA, $F_{\text{success}}(1,1) = 54.918$, $p < 0.001$, $F_{\text{projection}}(1,1) = 0.958$, $p = 0.338$, $F_{\text{interaction}}(1,1) = 2.969$, $p = 0.098$. Post-hoc Welch's *t*-tests with Bonferroni correction: $t_{\text{dHPC}^-}(3.561) = 3.698$; $t_{\text{dHPC}^{\rightarrow\text{NAc}}}(3.479) = 8.671$; $t_{\text{low success}}(13.629) = 0.093$, $p = 1$; $t_{\text{high success}}(3.952) = 2.075$, $p = 0.215$. Dashed line represents an even distribution of reward and anticipation zone. (G) A linear classifier shows significantly increased decoding accuracy of reward anticipation zone based on dHPC[→]NAc neural activity compared to that of sample size-matched dHPC⁻ neurons. Wilcoxon's *t*-test, $W(9) = 5.0$, $n = 10$ imaging sessions. All data are presented as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. See also Figure S4.

183 tially routed to NAc, given the relevance of NAc for spatial
184 memory (29, 30) and particularly for reward-related behav-
185 iors (51).

186 Upon inspection of place cells' spatially binned calcium pro-
187 files (Figure 3A-B and Figures S3, S4A), we noticed an over-
188 abundance of place fields that seemed to cover entire texture
189 zones, starting at the beginning of a texture zone and end-
190 ing before the next texture zone began. To quantify this ob-
191 servation, we plotted the densities of place field start and
192 end positions across the belt and compared the observed
193 densities with those of randomly shuffled place fields. We
194 found a significant overrepresentation of place field start and
195 end positions (but not centers) near texture boundaries for
196 both dHPC⁻ and dHPC[→]NAc populations (ratios >99.9th per-

centile, permutation test). This effect was more pronounced
197 in dHPC[→]NAc neurons (PF start: $p = 0.033$, PF end: $p =$
198 0.036 , χ^2 ; Figure 3C-D and Figure S4A-C). This suggests
199 that dHPC[→]NAc neurons are more strongly modulated by local
200 cue boundaries.
201

202 Mice were trained to lick for reward in a hidden reward
203 zone. Previous studies have shown that such zones and their
204 preceding vicinity are often over-represented by place cells
205 (35, 52, 53) – we hypothesized that this effect might involve
206 dHPC[→]NAc neurons, given the NAc's role in reward-related
207 behaviors (51). When pooled across all sessions, we found
208 little evidence of such an overrepresentation (Figure S4D-
209 E). Mouse behavior generally shows great variability though,
210 and previous work demonstrated the dependence of such an

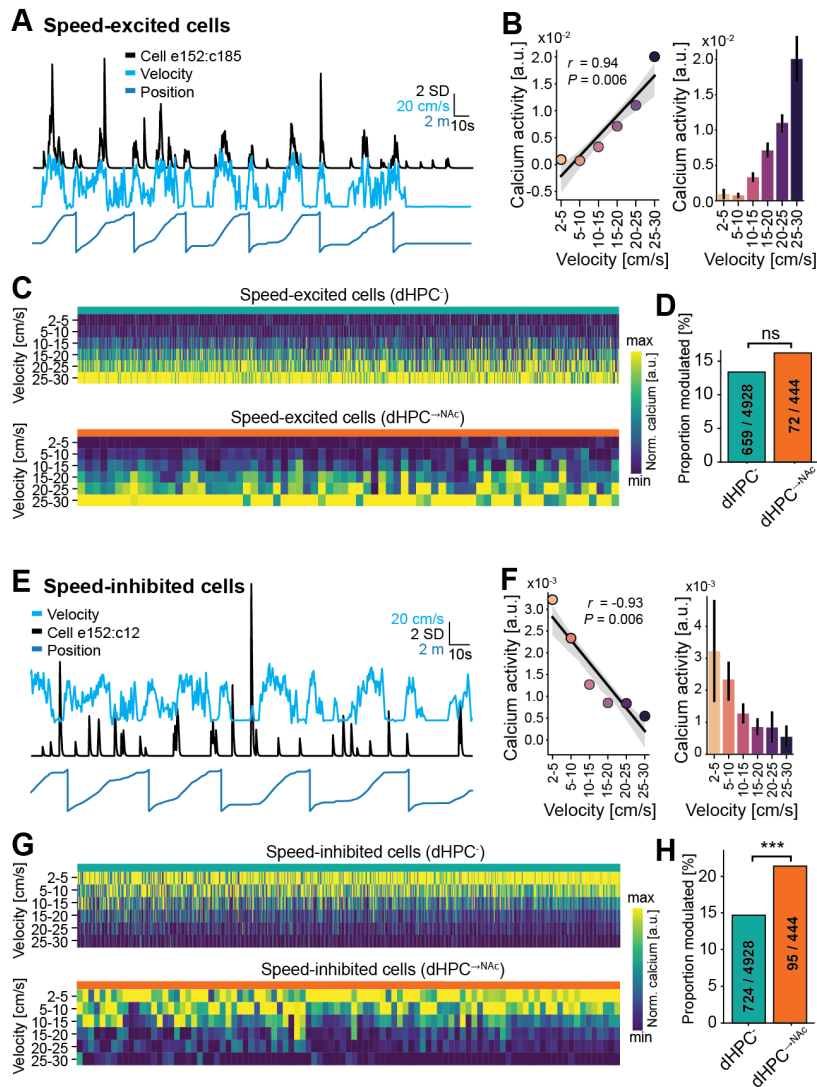


Figure 4. Speed-inhibited cells are overrepresented in dHPC^{→NAc} neurons. (A-D) Speed-excited dHPC neurons. (A and B) Representative example of one speed-excited neuron. (A) Sample traces of velocity, position and one neuron's denoised calcium activity. Note the increased calcium activity in times of high velocity, irrespective of position. (B) Linear regression on average calcium events per velocity bin shows a significant positive relationship (slope = 7.43×10^{-4} , intercept = -2.097×10^{-3} , $r = 0.937$, $p = 0.0058$). (C) Heatmaps of speed-binned normalized calcium activity of all significantly positively speed-modulated dHPC⁻ (top) and dHPC^{→NAc} (bottom) neurons. (D) Proportions of speed-excited neurons are comparable between dHPC⁻ and dHPC^{→NAc} populations ($\chi^2(1, 5372) = 2.565$, $p = 0.109$). (E-H) Speed-inhibited dHPC neurons. (E and F) Representative example of one speed-inhibited neuron. (E) Sample traces of velocity, position and one neuron's denoised calcium activity. Note the increased calcium activity in times of low velocity, irrespective of position. (F) Linear regression on average calcium events per velocity bin shows a significant negative relationship (slope = -1.0437×10^{-4} , intercept = 2.814×10^{-3} , $r = -0.933$, $p = 0.0065$). (G) Heatmaps of speed-binned normalized calcium activity of all significantly negatively speed-modulated dHPC⁻ (top) and dHPC^{→NAc} (bottom) neurons. (H) Negatively tuned neurons are overrepresented in the NAc-projecting population ($\chi^2(1, 5372) = 13.66$, $p = 0.00022$). Speed-modulated neurons were classified as showing a significant linear regression at $p < 0.05$ after Benjamini/Hochberg FDR correction. All data are presented as mean \pm SEM. ns: not significant, *** $p < 0.001$.

211 overrepresentation on individual task success (35, 52). We
 212 thus divided sessions into high- and low-success based on
 213 lick performance (lick precision and reward dispensation,
 214 see Methods) and found significantly more dHPC^{→NAc} place
 215 cells near the reward zone (reward and anticipation zones) in
 216 high-success sessions compared to low-success sessions (p
 217 = 0.0036, Welch's t -test). In contrast, dHPC⁻ neurons only
 218 showed a trend towards significance ($p = 0.051$, Welch's t -
 219 test; Figure 3E-F). In line with previous studies, both neu-
 220 ron populations also showed a strong correlation between
 221 the success rate (percentage of rewarded laps) and the pro-
 222 portion of place fields near the reward zone across sessions (r
 223 = 0.62; Figure S4F).

224 If reward zone information is preferentially encoded in
 225 dHPC^{→NAc} populations and NAc neurons play an integral
 226 part in reward-related behaviors, we would expect linear
 227 decoders to identify reward or anticipation zones more accu-
 228 rately based on dHPC^{→NAc} activity compared to that of
 229 dHPC⁻. To test this, we trained a linear classifier based on
 230 a support vector machine (SVM) with calcium activity and
 231 reward zone information on odd/even laps and tested decod-

ing accuracy on even/odd laps, using either dHPC⁻ or sam-
 232 ple size-matched dHPC^{→NAc} populations (see Methods). We
 233 found that, within individual sessions, decoding accuracy of
 234 the reward anticipation zone was significantly enhanced for
 235 dHPC^{→NAc} populations ($p = 0.0195$, Wilcoxon's test; Fig-
 236 ure 3G). These findings demonstrate significant modulation
 237 of dHPC^{→NAc} neurons by local cue boundaries and enhanced
 238 reward zone coding.
 239

Enhanced coding of low velocities in dHPC^{→NAc} neu-
 240 **rons.** As correct performance in the spatial reward learning
 241 task goes hand in hand with a reduction in velocity and an
 242 increase of licking near the reward zone (see Figure S1K-
 243 N), we wondered if such non-spatial task-relevant behavioral
 244 features were encoded by dHPC neurons and its projections
 245 to NAc (9, 54, 55). We hypothesized that NAc may have
 246 privileged access to information on low velocities as mice
 247 generally slow down near the reward zone, presumably to al-
 248 low for better discrimination and to engage in anticipatory
 249 licking. In line with previous analyses of speed coding in
 250 hippocampal and parahippocampal regions (8, 56), we av-
 251 eraged each neuron's calcium activity per velocity bin from
 252

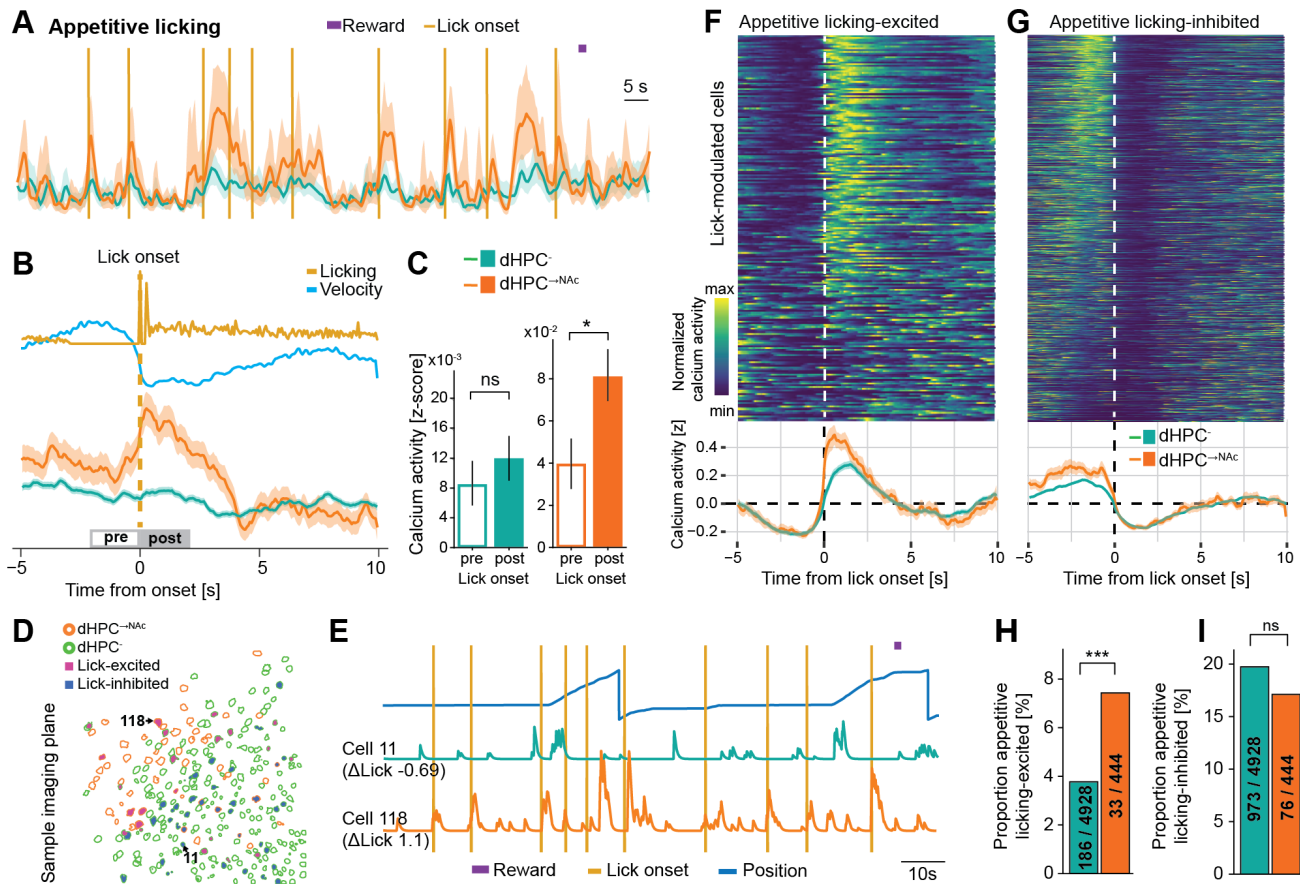


Figure 5. dHPC[→]NAc neurons are excited by appetitive licking and are over-represented in lick-excited neurons. (A-C) Appetitive licking is accompanied by increased neural activity in dHPC[→]NAc neurons but not dHPC⁻ neurons. (A) Representative example showing reward dispensation (purple bar) and appetitive licking onsets (golden vertical lines) and population calcium activity from dHPC⁻ (green) and dHPC[→]NAc (red) neurons. Note the robust increase of neural activity around lick onsets in the dHPC[→]NAc population. (B) Event-triggered average traces around appetitive licking onset, including licking and speed as well as population average calcium activity of dHPC⁻ (green) and dHPC[→]NAc (red) neurons. Gray rectangles indicate time windows for comparing calcium activity shown in (C). (C) Calcium activity is differentially modulated by appetitive licking onset only in dHPC[→]NAc neurons; two-way mixed ANOVA; $F_{licktiming}(1, 5370) = 2.843, p = 0.0918, F_{projection}(1, 5370) = 43.779, p < 0.001, F_{interaction}(1, 5370) = 7.073, p = 0.0079$. Post-hoc t -tests with Bonferroni correction: $t_{dHPC^-}(4927) = 0.871, p = 0.768; t_{dHPC \rightarrow NAc}(443) = 2.470, p = 0.0277$. (D and E) Example imaging session and traces showing lick-excited and lick-inhibited neurons. (D) Field of view showing spatial profiles of dHPC⁻ (green outlines) and dHPC[→]NAc (red outlines), some of which are classified as lick-excited (violet fill) or lick-inhibited (dark blue fill); neurons #11 and #118 are highlighted. (E) Behavioral traces and calcium activity of sample neurons #11 (lick-inhibited) and #118 (lick-excited). (F and G) Heatmaps (top) and event-triggered calcium activity averages (bottom) of neurons classified as appetitive licking-excited (F) and licking-inhibited (G). (H) Proportion of appetitive licking-excited neurons is higher in NAc-projecting neurons; $\chi^2(1, 5372) = 13.018, p = 0.00031$. (I) Proportion of appetitive licking-inhibited neurons is not different between populations; $\chi^2(1, 5372) = 1.626, p = 0.202$. All data are presented as mean \pm SEM. ns: not significant, * $p < 0.05$, *** $p < 0.001$. See also Figure S5.

253 2 to 30 cm/s and regressed this activity against velocity.
 254 Neurons with a significant regression model (after correct-
 255 ing for false discovery rate) and positive slope were class-
 256 ified as speed-excited (Figure 4A-B). We found around 15 %
 257 of neurons were positively speed-modulated, with compar-
 258 able proportions between dHPC⁻ and dHPC[→]NAc neurons (13
 259 % vs. 16 %, $p = 0.109, \chi^2$; Figure 4C-D). We also identified
 260 neurons with a significant velocity regression but a negative
 261 slope (Figure 4E-F). We found approximately 15 % of such
 262 speed-inhibited neurons, with a significantly larger propor-
 263 tion among the dHPC[→]NAc population (15 % vs. 21 %, $p =$
 264 0.00022, χ^2 ; Figure 4G-H). These results suggest widespread
 265 modulation of dHPC neuronal activity by non-spatial features
 266 such as velocity, with dHPC[→]NAc neurons specifically over-
 267 representing low speeds.

268 **Overrepresentation of appetitive licking-excited**
 269 **dHPC[→]NAc neurons.** Besides a decrease in velocity when

approaching the reward zone, mice also increasingly engaged
 in licking behavior (see Figure 1B-D). Given the NAc's dual
 role in appetitive and consummatory behaviors (57), we
 wondered if licking behaviors might be reflected in the neural
 activity of dHPC[→]NAc neurons. For this, we distinguished
 between consummatory licking which occurs after a reward
 is dispensed and allows the mouse to consume the reward
 provided, and appetitive licking which is an operant behavior
 that will lead to reward dispensation when performed at
 the correct location on the belt. We found a significant
 decrease of calcium activity during reward consumption in
 both dHPC⁻ and dHPC[→]NAc populations (Figure S5A-C).
 Appetitive licking, on the other hand, had no apparent effect
 on neural activity in dHPC⁻ neurons but coincided with a
 significant increase in calcium activity in the dHPC[→]NAc
 population that began about one second before lick onset
 and then fell below baseline 4 seconds after licking (Fig-
 ure 5A-C). We investigated if this population-averaged data

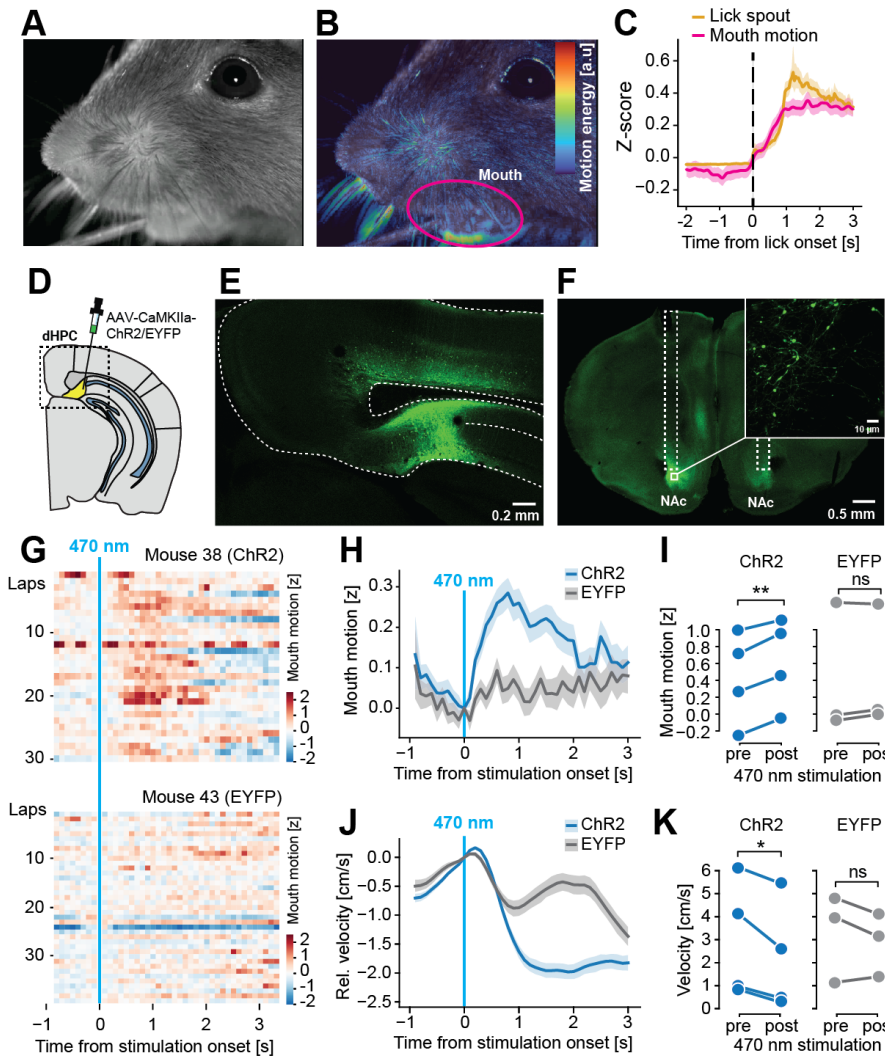


Figure 6. Optogenetic stimulation of dHPC axons in NAc induces mouth movement and deceleration. (A-C) Tracking of orofacial movements via infrared camera recordings. (A) Example still image from near-infrared camera, sampled at 75 Hz. (B) False-color coded motion energy (pixel-by-pixel intensity difference to previous image) overlaid over sample image from (A). Automatically segmented mouth region is indicated by a purple line. (C) Average motion energy in mouth region increases with licking. Golden line shows analog lick spout signal. (D-F) Experimental approach for optogenetic dHPC \rightarrow NAc stimulation. (D) Injection schematic. (E) Somatic expression of ChR2-EYFP in dorsal pre-subiculum. (F) Axonal expression of ChR2-EYFP in the NAc, where light fibers are placed (tracts indicated by white dotted lines). (G) Representative examples of mouth motion around onset of optogenetic stimulation in an animal expressing ChR2 (top) or EYFP control (bottom). (H) Trial-averaged mouth motion activity around time of optogenetic stimulation in ChR2 (blue) and EYFP (gray)-expressing mice. (I) Mouth motion is significantly increased with optogenetic stimulation in ChR2 animals ($t(3) = 7.485$; $p = 0.00494$) but not EYFP animals ($t(2) = 1.353$; $p = 0.309$). Paired t -tests; $n = 4$ mice (ChR2), $n = 3$ mice (EYFP). (J) Trial-averaged relative velocity around time of optogenetic stimulation in ChR2 (blue) and EYFP (gray)-expressing mice. (K) Velocity is significantly decreased with optogenetic stimulation in ChR2 animals ($t(3) = -3.551$; $p = 0.0381$) but not EYFP animals ($t(2) = -1.263$; $p = 0.334$). Paired t -tests; $n = 4$ mice (ChR2), $n = 3$ mice (EYFP). All data are presented as mean \pm SEM. ns: not significant, * $p < 0.05$, ** $p < 0.01$.

is reflected on the single-cell level and if there are individual cells that are reliably modulated by appetitive licking (Figure 5D-E and Figure S5D-E). Comparing the pre- and post-lick neural activity for each neuron for each appetitive lick event, we identified a total of 1,268 neurons (24 %) that were significantly (negatively or positively) modulated by appetitive licking (Figure 5F-I). Interestingly, while the proportion of lick-inhibited neurons was comparable between dHPC \rightarrow and dHPC \rightarrow NAc populations (19.7 % vs 17.1 %, $p = 0.20$, χ^2 ; Figure 5I), we found a significantly larger proportion of lick-excited neurons in dHPC \rightarrow NAc populations (3.8 % vs 7.4 %, $p < 0.001$, χ^2 ; Figure 5H). These findings suggest that dHPC does not route reward information per se to NAc, but rather information on appetitive behaviors required to obtain such rewards.

Optogenetic activation of dHPC terminals in NAc induces mouth movement and deceleration. Given the NAc's hypothesized role as an interface between limbic and motor systems (25), we investigated if the observed increase of calcium activity in dHPC \rightarrow NAc neurons may be a corollary signal of motor action or indeed serve a causal role in appetitive behaviors (58). To enable high-resolution behavioral

tracking, we monitored mouse orofacial movements using a high-speed near-infrared camera (Figure 6A-C). To test for a causal role of excitatory dHPC \rightarrow NAc projections in appetitive behaviors, we injected animals with either CaMKIIa-driven ChR2 or EYFP into dHPC and implanted light fibers in the NAc ($n = 4/3$ mice; Figure 6D-F). After habituating mice to run on the treadmill and receive rewards upon licking on the lick spout, mice were given 5 mW of 473 nm 20 Hz (5 ms duration) pulsed laser light for up to 10 seconds upon entry into a hidden light stimulation zone.

We found that, shortly after stimulation onset, ChR2-expressing mice reliably showed increased mouth movement for up to two seconds after stimulation, while we observed no effects in mice expressing EYFP ($p_{\text{ChR2}} = 0.0099$, $p_{\text{EYFP}} = 0.617$, paired t -tests; Figure 6G-H). In line with this, we also found a significant deceleration of running on the treadmill upon light delivery in ChR2 animals but not EYFP animals ($p_{\text{ChR2}} = 0.0381$, $p_{\text{EYFP}} = 0.334$, paired t -tests; Figure 6J-K). These findings support the idea that dHPC \rightarrow NAc projections may not simply represent task-related non-spatial behavioral features but may in fact represent a driving force in the generation of task-relevant appetitive behavior.

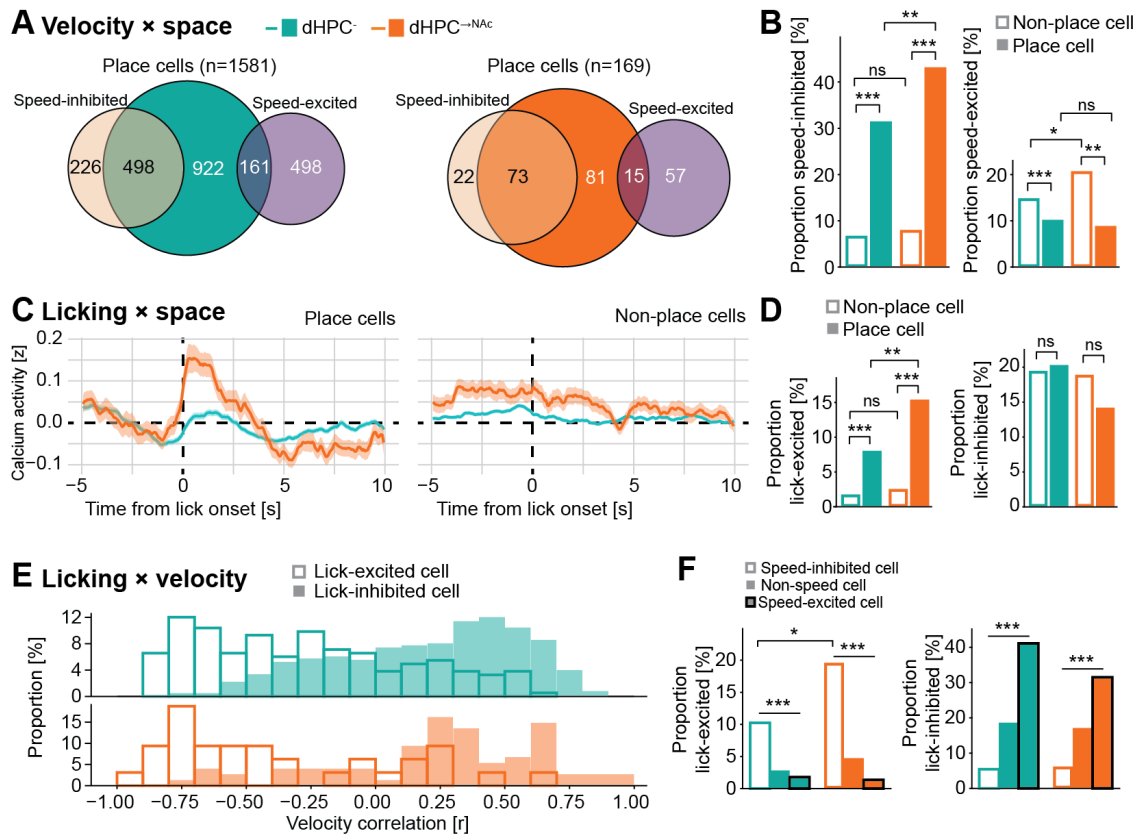


Figure 7. Enhanced conjunctive coding of space, velocity and licking in NAc-projecting neurons. (A and B) Conjunctive coding of space and velocity. (A) Venn diagrams of dHPC⁻ (left) and dHPC^{→NAc} (right) place cells and their overlaps with negatively (ocher) and positively (purple) tuned speed cells. Numbers shown refer to absolute numbers of neurons classified. (B) Proportions of dHPC⁻ (green) and dHPC^{→NAc} (red) place cells (fill) and non-place cells (no fill) that are also significantly speed-modulated. A larger proportion of place cells is speed-inhibited compared to non-place cells (dHPC⁻: $\chi^2(1, 4928) = 522.71$; dHPC^{→NAc}: $\chi^2(1, 444) = 75.02$). dHPC^{→NAc} place cells also have a higher proportion of speed-inhibited cells than dHPC⁻ place cells ($\chi^2(1, 1750) = 8.977$). Speed-excited neurons are overrepresented in non-place cells compared to place cells (dHPC⁻: $\chi^2(1, 4928) = 20.034$; dHPC^{→NAc}: $\chi^2(1, 444) = 9.966$). dHPC^{→NAc} non-place cells also have a higher proportion of speed-excited cells than dHPC⁻ non-place cells ($\chi^2(1, 3622) = 6.255$). (C and D) Conjunctive coding of space and licking. (C) Event-triggered average calcium traces for dHPC⁻ (green) and dHPC^{→NAc} (red) place cells (left) and non-place cells (right). (D) Proportions of lick-excited neurons are significantly enriched in dHPC⁻ ($\chi^2(1, 4928) = 114.515$) and dHPC^{→NAc} ($\chi^2(1, 444) = 23.248$) place cells compared to non-place cells. The proportion of dHPC^{→NAc} lick-excited place cells is also higher than the proportion of dHPC⁻ lick-excited place cells ($\chi^2(1, 1750) = 9.442$); there is no difference between non-place cells ($\chi^2(1, 3622) = 0.488$, $p = 0.485$). There is no difference in the proportions of lick-inhibited place and non-place dHPC⁻ and dHPC^{→NAc} neurons (χ^2 s, all $p > 0.05$). (E and F) Conjunctive coding of velocity and licking. (E) Histogram of lick-excited (no fill) and lick-inhibited (fill) cells' velocity correlations (green: dHPC⁻; red: dHPC^{→NAc}). Note the negative velocity correlations of lick-excited cells and positive velocity correlations for lick-inhibited cells. (F) Proportions of lick-excited (left) and lick-inhibited (right) cells among speed-inhibited (no fill), non-speed-modulated (fill, black stroke) dHPC⁻ (green) and dHPC^{→NAc} (red) cells. Proportions of lick-excited cells are overrepresented in speed-inhibited cells (dHPC⁻: $\chi^2(2, 4928) = 100.484$; dHPC^{→NAc}: $\chi^2(2, 444) = 27.608$). Lick-excited neurons are further enriched in speed-inhibited dHPC^{→NAc} neurons compared to dHPC⁻ neurons ($\chi^2(1, 830) = 6.564$). Proportions of lick-inhibited cells are overrepresented in speed-excited cells (dHPC⁻: $\chi^2(2, 4928) = 290.832$; dHPC^{→NAc}: $\chi^2(2, 444) = 18.825$). All data are presented as mean \pm SEM. ns: not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

332 **Enhanced conjunctive coding of space, velocity, and**
 333 **appetitive behaviors in dHPC^{→NAc} neurons.** We identi-
 334 fied cells modulated by space, velocity, and appetitive lick-
 335 ing. Previous studies suggested that individual hippocampal
 336 neurons do not necessarily exclusively code for one single
 337 feature but are instead able to conjunctively encode various
 338 environmental properties (9, 55, 59, 60). Such conjunctive
 339 coding may be particularly relevant for downstream linear
 340 decoders to select task-appropriate actions (61, 62). We thus
 341 investigated speed and lick modulation of projection-specific
 342 place cells and interactions between velocity and lick modu-
 343 lation.

344 We first analyzed speed coding in dHPC^{→NAc} place cells
 345 and compared it to the dHPC⁻ population (Figure 7A-B). We
 346 found about one third of hippocampal place cells were also
 347 speed-inhibited, in contrast to only about 7 % of non-place
 348 cells. This effect was particularly pronounced in dHPC^{→NAc}

349 place cells (43 % vs. 31 %, $p = 0.0027$, χ^2). Conversely,
 350 place cells were significantly less likely to be speed-excited
 351 than non-place cells (10 % vs. 15 %, $p < 0.001$, χ^2), an ef-
 352 fect that was again more pronounced in dHPC^{→NAc} neurons.
 353 This shows that dHPC place cells, and in particular those pro-
 354 jecting to NAc, are more likely to be speed-inhibited, and less
 355 likely to be speed-excited.

356 We next analyzed lick modulation of place cells and, sur-
 357 prisingly, found that the previously observed lick-related in-
 358 crease in calcium activity (Figure 5B) was largely carried by
 359 place cells and not by non-place cells (Figure 7C). This ef-
 360 fect seems to be mostly carried by lick-excited neurons that
 361 are significantly overrepresented in place cells compared to
 362 non-place cells (8 % vs. 2 %, $p < 0.001$, χ^2), particularly
 363 in dHPC^{→NAc} neurons (15 % vs. 3 %, $p < 0.001$, χ^2 ; Fig-
 364 ure 7D). Lick-inhibited neurons, on the other hand, were
 365 distributed equally between place and non-place dHPC⁻ and

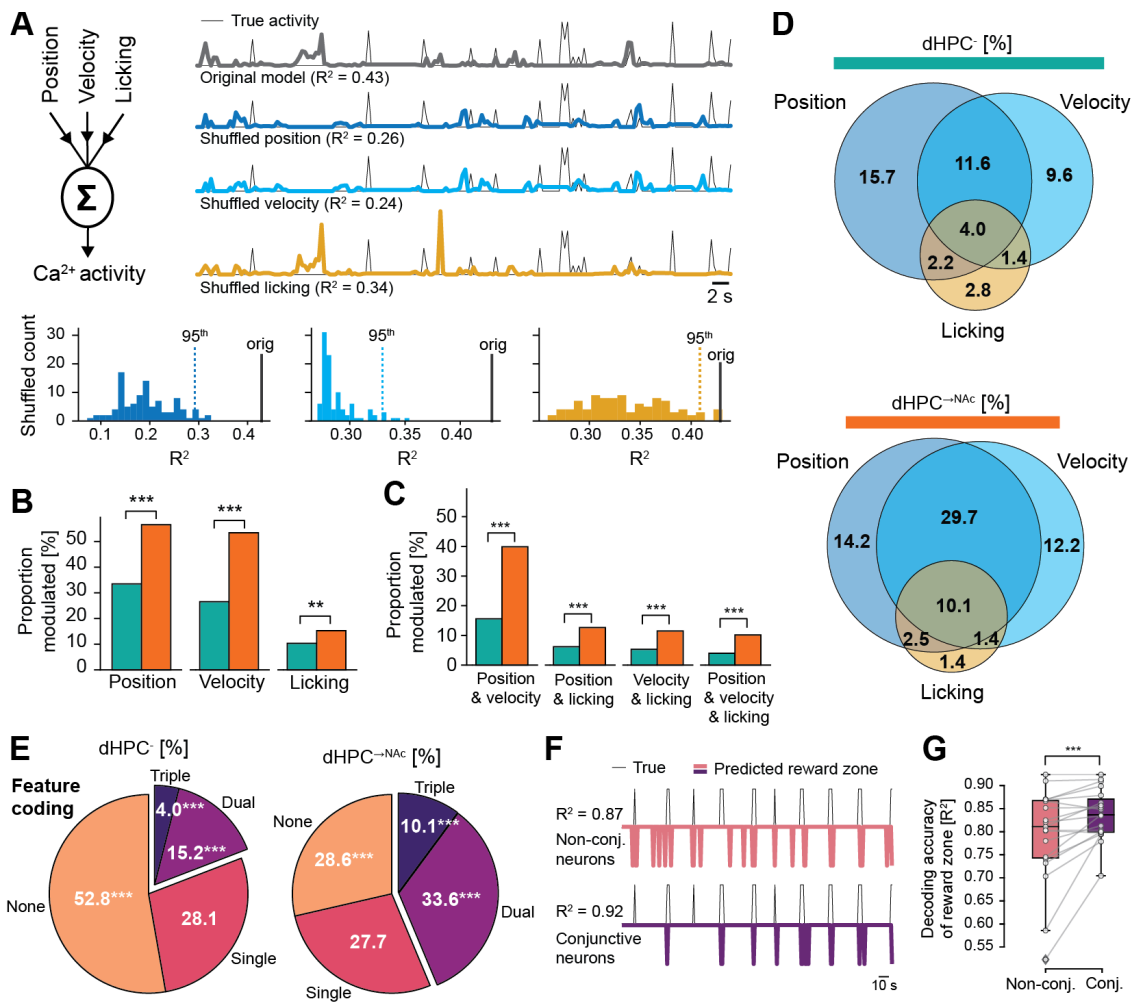


Figure 8. A generalized linear model confirms enhanced conjunctive coding in NAc-projecting neurons. (A) Schematic of the generalized linear model (GLM) using position, velocity, and appetitive licking data as predictors for each neuron's calcium activity (left). Example modelling approach for a triple conjunctive neuron (right). Upper traces show true calcium activity (downsampled and normalized) of test dataset (thin black line) as well as predictions of original model (thick gray) and one example each for each shuffled feature. Note the reductions in prediction quality when features are shuffled. Bottom histograms show R^2 distributions for 100 shuffled models for each feature. Dotted line represents value of 95th percentile, thick black line represents original model's R^2 . (B) Increased proportions of dHPC⁻ neurons modulated by position ($\chi^2(1, 5372) = 93.634$), velocity ($\chi^2(1, 5372) = 141.86$), and licking ($\chi^2(1, 5372) = 10.050$). (C) Increased proportions of conjunctive coding in dHPC⁻ neurons for position & velocity ($\chi^2(1, 5372) = 163.97$), position & licking ($\chi^2(1, 5372) = 26.029$), velocity & licking ($\chi^2(1, 5372) = 27.145$), and position & velocity & licking ($\chi^2(1, 5372) = 34.993$). (D) Venn diagrams showing overlap of neurons GLM-classified as modulated by position, velocity and licking in dHPC⁻ (top) and dHPC^{→NAc} (bottom) neurons. (E) Proportions of n -feature coding neurons in dHPC⁻ (left) and dHPC^{→NAc} (right) populations. Non-coding neurons are overrepresented in dHPC⁻ neurons ($\chi^2(1, 5372) = 35.382$); single-coding neurons are comparably distributed ($\chi^2(1, 5372) = 0.0057$); dual-coding ($\chi^2(1, 5372) = 61.336$) and triple-coding ($\chi^2(1, 5372) = 30.447$) neurons are overrepresented in dHPC^{→NAc} neurons. (F and G) A linear classifier to decode presence of reward zone. (F) Example true presence of reward zone (thin line) and decoder predictions based on non-conjunctive neurons (pink; top) and conjunctive-coding neurons (dark purple; bottom). (G) Conjunctive-coding neurons allow a linear decoder to classify the presence of reward zone more accurately than non-conjunctive coding neurons (Wilcoxon's $W(18) = 14.0$). $**p < 0.01$, $***p < 0.001$. See also Figure S6.

dHPC^{→NAc} neurons. These findings show that a large majority of lick-excited neurons, especially in the dHPC^{→NAc} population, also code for spatial information. Finally, we investigated interactions between lick and velocity modulation. Comparing velocity correlations of lick-excited and lick-inhibited neurons, we observed a clear skew of lick-excited cells to have more negative velocity correlations and lick-inhibited cells to have more positive velocity correlations, visible in both dHPC⁻ and dHPC^{→NAc} populations (Figure 7E). This results in significantly more speed-inhibited cells to be lick-excited compared to speed-excited cells (11 % vs. 2 %, $p < 0.001$, χ^2), an effect that was even more pronounced in dHPC^{→NAc} neurons (20 % vs. 1 %, $p < 0.001$, χ^2 ; Figure 7F). Conversely, speed-excited neurons were much more likely to be lick-inhibited than speed-inhibited neurons (40

% vs. 6 %, $p < 0.001$, χ^2). These results demonstrate that there is a strong inverse relationship between lick and velocity modulation.

One caveat of such conjunctive coding analyses is that in our behavioral task, trained mice often show highly stereotypical behavior, such that mice would mostly lick at one location where they would also slow down (see Figure 1C and Figure S1K-N). In light of this, conjunctive coding could be an epiphenomenon of collinear behavioral features. To account for this collinearity, we modelled the influence of three key behavioral features (space, velocity, and appetitive licking) on the activity of each neuron by building a generalized linear model for each neuron (GLM; Figure 8A). On average, we found that our models could explain close to 40% of the variance observed in our test datasets (Figure S6A),

396 with dHPC \rightarrow NAc neurons showing increased feature impor-
397 tance for position and licking (Figure S6B). To determine
398 significant contributions of the three behavioral features, we
399 next built 3×100 models in which one of the behavioral fea-
400 tures was randomly shuffled against time, and compared the
401 variance explained to the original model (Figure 8A; see also
402 ref (63)). Thus, if neural activity spuriously coincided with
403 the activity of one behavioral feature that could be simi-
404 larly explained by a largely collinear behavioral feature, the
405 model's predictive performance should be mostly unaffected
406 by this shuffling. The average drop in variance explained
407 to the full model was significantly stronger in dHPC \rightarrow NAc
408 neurons compared to dHPC $^-$ for position and velocity but
409 not licking (Figure S6C). We classified significant modula-
410 tion as behavioral features whose shuffling led to a reduction
411 in variance explained in more than 95% of shuffled models.
412 We found that cells thus encoding space, velocity, and lick-
413 ing were overrepresented in dHPC \rightarrow NAc compared to dHPC $^-$
414 neurons (space: $p < 0.001$; velocity: $p < 0.001$; licking: p
415 = 0.0015; χ^2 ; Figure 8B). Importantly, we also found sig-
416 nificantly increased proportions of conjunctive coding for all
417 three feature combinations as well as triple-conjunctive neu-
418 rons in dHPC \rightarrow NAc neurons compared to dHPC $^-$ (all com-
419 binations $p < 0.001$, χ^2 ; Figure 8C-D). This results in a sig-
420 nificantly higher proportion of conjunctive coding neurons in
421 dHPC \rightarrow NAc neurons compared to dHPC $^-$ (44 % vs. 19 %, p
422 < 0.001, χ^2 ; Figure 8E).

423 As conjunctive coding has been suggested to aid downstream
424 linear decoders to select task-appropriate actions (55, 61),
425 we wondered if this increased conjunctive code might al-
426 low linear decoders to identify the presence of the reward
427 zone more correctly in our task. We thus trained an SVM-
428 based linear classifier on each trial's odd/even laps' reward
429 zones and tested the decoding accuracy on even/odd laps,
430 based on conjunctive or randomly sample size-matched non-
431 conjunctive coding neurons (Figure 8F). We found enhanced
432 reward zone decoding accuracy for conjunctive compared to
433 non-conjunctive coding neurons ($p < 0.001$, Wilcoxon's test;
434 Figure 8G). Thus, our data suggest that the enhanced con-
435 junctive coding observed in dHPC \rightarrow NAc neurons allows NAc
436 neurons to better identify the reward zone and guide task-
437 appropriate appetitive behavior.

438 Discussion

439 While our understanding of the principal mechanisms by
440 which internal and environmental features are processed by
441 hippocampal circuits has greatly advanced (5, 6), the infor-
442 mation content of its various output streams and their spe-
443 cific contributions to behavior remain largely unresolved.
444 The NAc, with its proposed role as an integrator between
445 limbic and motor systems (25) seems an ideal candidate to
446 transform hippocampal mnemonic and contextual informa-
447 tion into task-relevant behaviors (26), but evidence for such a
448 role has remained sparse.

449 One main finding was the identification of a larger proportion
450 of place cells among the dHPC \rightarrow NAc population that also en-
451 coded more spatial information and showed both enhanced

452 trial-to-trial reliability and in-place-field activity. Our data
453 confirm previous suggestions that the NAc receives spatial in-
454 formation from dHPC (26) and likely explains previously de-
455 scribed spatial tuning of NAc neurons (29). The dHPC \rightarrow NAc
456 population's observed enhanced spatial tuning and stronger
457 modulation of local cues parallels previously described dif-
458 ferences between deep and superficial dHPC neurons (18).
459 Indeed, vHPC \rightarrow NAc projection neurons have been shown
460 to accumulate mostly in deep layers (64), raising the possi-
461 bility that previously observed anatomical-functional differ-
462 ences could at least partly be explained by differences in pro-
463 jection targets, thus adding another important layer of com-
464 plexity to pyramidal cell heterogeneity (17).

465 Place cells in CA1 and subiculum have long been appreci-
466 ated to overrepresent reward/goal zones (53, 65, 66) in a way
467 that correlates with behavioral performance (35, 52). This
468 effect likely depends on the interaction of inputs from en-
469 torhinal cortex layer 3, dopaminergic inputs from VTA, and
470 hippocampal NMDA receptors (36, 52, 67). While previ-
471 ous studies suggested a role for a largely undefined dedi-
472 cated population of dHPC cells in this overrepresentation (65) and
473 differences have been observed along the radial dCA1 axis
474 (35), evidence for projection-specific reward zone represen-
475 tations has remained sparse. Although synchronous activity
476 has been observed between dHPC and NAc during spatial re-
477 ward learning tasks (38, 68, 69), in line with a central role of
478 NAc in reward learning (reviewed in ref (70)), our findings
479 present direct evidence of increased reward zone overrepre-
480 sentation by dHPC \rightarrow NAc neurons. Interestingly, a previous
481 study (19) showed projection-specific ambiguity of reward
482 zone activity depending on vCA1 \rightarrow NAc collaterals. Future
483 studies should investigate potential functional differences in
484 collaterals of dHPC projections (41).

485 Successful behavioral performance in our spatial reward
486 learning task depended on a decrease in velocity and in-
487 creased appetitive lick activity as mice approached the hidden
488 reward zone. In line with previous work (8, 9, 12, 20), we
489 found a considerable proportion of speed-modulated dHPC
490 neurons. Interestingly, NAc-projecting neurons were more
491 likely to be speed-inhibited, suggesting an elevated role in
492 reward approach behaviors (see also ref (12)). Similarly,
493 we observed significant modulation of hippocampal neurons
494 during appetitive and consummatory licking (see also refs
495 (12, 54)). This modulation was dichotomous: consumma-
496 tory licking resulted in widespread suppression of dHPC ac-
497 tivity, while appetitive licking led to enhanced activity in
498 dHPC \rightarrow NAc neurons. Activity of dHPC neurons, and espe-
499 cially those projecting to NAc, thus seems to mirror the activ-
500 ity within NAc: Inhibition of NAc DIR medium spiny neu-
501 rons (MSNs) has been observed during lick behavior (71–73)
502 and was shown to be both necessary (71, 74) and sufficient
503 (71, 75) for consummatory licking. We found that the origin
504 of these signals may be localized upstream, similar to recent
505 findings of an inhibitory permissive drive for licking in vHPC
506 projections to the NAc (72, 76) and that inhibiting these pro-
507 jections facilitates licking (72). This suggests a similar in-
508 volvement of dHPC $^-$ and vHPC $^-$ accumbens projections for

509 consummatory licking.
510 Contrasting this consumption-related inhibition, we found
511 appetitive lick-related dHPC \rightarrow NAc excitation and optogenetic
512 induction of lick-related behaviors. This dichotomy likely re-
513 flects previously described differences in the NAc's role for
514 consummatory and appetitive behaviors (57): vHPC \rightarrow NAc
515 activity was shown to increase during active food investiga-
516 tion (76) and around the time of lever pressing to obtain a
517 reward (Iyer E, Muir J, Namuhoranye B, Bagot, R. Gluta-
518 matergic afferents to the nucleus accumbens integrate out-
519 comes in reward-learning. Poster S01-181, FENS Forum
520 2022), while optogenetic stimulation of this projection facili-
521 tated nose poke and lever pressing behaviors (33, 77). Given
522 that optogenetic stimulation of D2R MSNs in the NAc shell
523 was also found to produce repetitive jaw movements (78), it
524 is likely that D1R and D2R MSNs, both of which are tar-
525 geted by HPC axons (34, 79, 80), might play dichotomous
526 roles in appetitive and consummatory behaviors (see also refs
527 (34, 81)). Future studies should aim to resolve the behavioral
528 roles of specific NAc cell types targeted by HPC neurons.

529 In our analysis of dHPC \rightarrow NAc coding properties, we un-
530 covered more neurons individually coding for two or three
531 aspects of space, velocity, and lick activity specifically in
532 identified projection neurons. Such conjunctive coding, or
533 mixed selectivity, has been previously found in hippocam-
534 pal neurons to combine coding of space with direction, ve-
535 locity, appetitive behaviors, behavioral tasks, future paths
536 ("splitter cells"), context, or objects (9, 12, 13, 55, 66, 82).
537 These conjunctions are hypothesized to combine "where"
538 and "what" information to disambiguate different experi-
539 ences occurring in the same location, thus building a "scaf-
540 fold" for episodic memories that allows unambiguous later
541 retrieval (14, 83, 84). Relatedly, theoretical and experimen-
542 tal studies demonstrated that such high-dimensional coding
543 facilitates action selection by putative downstream linear de-
544 coders tasked with action selection and generating behavior
545 (61, 62, 85), and was found to scale with task demands and
546 performance (55, 62, 85–87). Indeed, our data show that con-
547 junctive coding improved reward zone detection by a linear
548 decoder, similar to a recent study in retrosplenial cortex that
549 demonstrated enhanced decoding near a reward zone by con-
550 junctive coding neurons (87). In line with previous findings
551 of increased conjunctive coding in prefrontal cortex \rightarrow NAc
552 projection neurons (88) and elevated proportions of splitter
553 cells in dSub \rightarrow NAc neurons (20), we thus propose that dHPC
554 routes strongly conjunctive task-relevant information to facil-
555 itate NAc action selection.

556 The NAc has been described as a key node transforming moti-
557 vational information from the limbic system into motor be-
558 haviors (25), but the information flow of specific projection
559 neurons has remained elusive. Our data demonstrate that
560 dHPC routes enhanced spatial information to the NAc that
561 is conjunctively enriched by further spatial and non-spatial
562 task-relevant features. We show that this conjunctive code
563 improves linear decoding to guide downstream action selec-
564 tion, and that dHPC can drive the execution of appetitive mo-
565 tor behaviors via the basal ganglia, as early studies suggested

(89). Thus, our findings identify a direct role for the hip-
566 pocampus in the generation of motor behaviors and build an
567 important bridge in our understanding of how sensory and
568 mnemonic processes guide behavioral action.
569

570 Materials and Methods

571 Animals. Experiments were performed in adult male and fe-
572 male mice. C57Bl/6 ($n = 16$) and Thy1-GCaMP6s ($n = 6$)
573 mice (GP4.3; The Jackson Laboratory, Bar Harbor, USA)
574 were bred under specific pathogen-free conditions. Heterozy-
575 gous mice were group-housed with 12 hours reversed dark
576 light cycle at 21°C and ad libitum food/water access until
577 mice had recovered from surgery. Experiments were per-
578 formed during the dark phase. All experiments were per-
579 formed according to the Directive of the European Communi-
580 ties Parliament and Council on the protection of animals used
581 for scientific purposes (2010/63/EU) and were approved by
582 the animal care committee of North Rhine-Westphalia, Ger-
583 many.

584 Viral vectors. To co-express the static red fluorophore
585 mCherry in Thy1-GCaMP6s animals, we injected retrograde
586 (rg) serotype (90) adeno-associated virus (AAV) under con-
587 trol of the polyglycokinase (pgk) promoter (AAVrg-pgk-Cre,
588 Catalog #24593-AAVrg, titer: 1×10^{13} viral genomes (vg)
589 ml^{-1} , Addgene, Watertown, USA) in the NAc to enter axons
590 and induce Cre expression in NAc-projecting neurons. Then,
591 we used double-floxed inverse open reading frame (DIO)
592 Cre-dependent mCherry under the human synapsin pro-
593 moter in dHPC (AAV5-hSyn-DIO-mCherry, titer: 1.1×10^{13}
594 vg/ml , Catalog #50459-AAV5, Addgene, Watertown, USA).
595 For optogenetic experiments, we used unfloxed ChR2 (91)
596 or control EYFP in excitatory neurons under the CaMKII
597 promoter in the dHPC of C57Bl/6 mice (AAV2-CaMKII-
598 hChR2(H134R)-EYFP-WPRE, titer: 4×10^{12} transducing
599 units (TU), UNC #AV4381E, Vector Core at the University of
600 North Carolina, Chapel Hill, USA; rAAV2-CaMKII-EYFP,
601 titer: 4.3×10^{12} TU, UNC #AV6650, Vector Core at the Uni-
602 versity of North Carolina, Chapel Hill, USA).

603 Stereotactic virus injections. For stereotactic injection of
604 AAVs, mice were anesthetized with an intraperitoneal (i.p.)
605 injection of a mixture of ketamine (0.13 mg/g) and xylazine
606 (0.01 mg/g). Mice were head-fixed using a head holder
607 (MA-6N, Narishige, Tokyo, Japan), placed into a motorized
608 stereotactic frame (LuigsNeumann, Ratingen, Germany) and
609 warmed by a self-regulating heat pad (Fine Science Tools,
610 Heidelberg, Germany). After skin incision (5 mm) and re-
611 moval of the periosteum, placement of the injection was de-
612 termined in relation to bregma. A 0.5 mm wide hole was
613 drilled through the skull (Ideal micro drill, World Preci-
614 sion Instruments, Berlin, Germany). Stereotactic coordinates
615 were taken from Franklin and Paxinos, 2008 (The Mouse
616 Brain in Stereotaxic Coordinates, Third Edition, Academic
617 Press). To induce retrograde labelling of NAc-projecting
618 neurons in dHPC, 2×500 nl of AAVrg-pgk-Cre were in-
619 jected into the ipsilateral (right) nucleus accumbens (-1.3 mm

620 anterior-posterior, -1.0 mm lateral, 5.0 and 4.3 mm ventral,
621 relative to Bregma) at 100 nl/min, using a UltraMicroPump,
622 34G cannula and Hamilton syringe (World Precision Instru-
623 ments, Berlin, Germany). To label projection neurons in red,
624 200 nl of AAV5-hSyn-DIO-mCherry were injected into the
625 ipsilateral dorsal hippocampus (3.38 mm anterior-posterior,
626 -2.5 mm lateral, 1.8 mm ventral, relative to Bregma, at a 10°
627 angle). For optogenetic experiments, 200 nl of either AAV2-
628 CaMKII-hChR2(H134R)-EYFP-WPRE or rAAV2-CaMKII-
629 EYFP were injected each bilaterally into dorsal hippocam-
630 pus (3.38 mm anterior-posterior, ± 2.5 mm lateral, 1.8 mm
631 ventral, relative to Bregma, at a 10° angle). After surgery,
632 buprenorphine (0.05 mg/kg) was administered thrice daily for
633 3 consecutive days. Implant surgery followed two weeks af-
634 ter AAV injection.

635 **Cranial window surgery.** For awake hippocampal Ca2+
636 imaging, a window was surgically implanted over the right
637 dorsal hippocampus, one week after virus injections. The
638 hippocampal window was assembled from a 1.7 mm long
639 stainless-steel cannula (3 mm outer diameter) and a round
640 cover slip (3 mm diameter). The coverslip was glued to
641 the end of the cannula using UV curable adhesive (NOA81,
642 Thorlabs, Dachau/Munich, Germany). Mice were anes-
643 thetized and prepared for surgery as described above. The
644 skin over the parietal skull and the periosteum were removed
645 and wound edges sealed with Vetbond tissue adhesive (3M
646 Animal Care Products, St Paul, USA). Skull surface was
647 roughened by briefly applying gel etchant phosphoric acid
648 (37.5 %; Kerr Dental, Scafati, Italy), carefully washing the
649 skull surface and applying two-component dental adhesive
650 (OptiBond FL, Kerr Dental, Scafati, Italy). A circular piece
651 of the skull (3 mm in diameter) centered over the injection
652 was carefully cut out using a sharp drill (Ideal micro drill,
653 World Precision Instruments, Berlin, Germany). The dura
654 was removed with forceps and mild vacuum suction was used
655 to slowly remove the cortex within the craniotomy. Blood
656 and aspirated tissue were washed out using a constant flow of
657 artificial cerebrospinal fluid (ACSF). Intracranial aspiration
658 was continued until the external capsule was exposed. The
659 external capsule remained intact. The hippocampal window
660 was manually inserted. During continuous perfusion with
661 ACSF, the hippocampal window was lowered until sealing
662 onto the external capsule. The hippocampal window was fix-
663 ated, and the craniotomy sealed using UV curable dental ce-
664 ment (Gradia Direct Flo, GC Corporation, Tokyo, Japan). An
665 angular metal bar (Luigs & Neumann, Ratingen, Germany)
666 for head fixation was placed paramedian on the skull. After
667 surgery, buprenorphine (0.05 mg/kg) was administered thrice
668 daily for 3 days.

669 **Behavioral task.** 3-4 days after recovery from surgery, mice
670 were provided with spinning wheels in their cages and were
671 placed under a reverse light/dark cycle. One week after
672 surgery, food restriction and two-week habituation schedules
673 were initiated. Mice were food-restricted by providing about
674 80 % of their measured daily food pellets every 24 h. In
675 the course of this, mice lost about 10-20 % of their origi-

676 nal weight before the start of training. Habituation consisted
677 of progressive exposure of mice to manual handling by the
678 experimenter, obtaining milk rewards through a metal can-
679 nula, gentle manual head fixation, the treadmill apparatus,
680 and, finally, head-fixed running on an unmarked treadmill
681 belt with random rewards provided through a metal cannula
682 lick spout after licking on it. The self-propelled treadmill
683 (Luigs & Neumann) consisted of three rotating cylinders cov-
684 ered by a 7 cm wide and 360 cm long textile belt (Luigs &
685 Neumann) including six differently textured zones: horizon-
686 tal and vertical glue stripes, glue dots, Velcro dots, vertical
687 tape stripes and upright nylon spikes. The reward zone for
688 imaging experiments was 30 cm long and was placed be-
689 tween the end of the horizontal glue stripes and the begin-
690 ning of the vertical tape stripes. The position of the mouse
691 was recorded via an optical sensor (Luigs & Neumann) mea-
692 suring the rotation of the treadmill cylinder underneath the
693 mouse. Lick signals were measured by an analog piezo sen-
694 sor; one full belt rotation was measured by an optical infrared
695 sensor. All signals were collected at 10 kHz by an I/O board
696 (USB-6212 BNC, National Instruments, Austin, USA) and
697 recorded using custom-written Python software. After two
698 weeks of habituation, mice were placed daily for 15 minutes
699 on a cued (see above) treadmill belt on which they needed to
700 lick on the metal spout in this hidden reward zone to obtain
701 a liquid reward in the form of condensed milk. Milk was
702 released by a miniature peristaltic pump (RP-Q1-S-P45A-
703 DC3V, Takasago Fluidic Systems, Nagoya, Japan) that was
704 triggered by custom-written Python software via an I/O board
705 (USB-6212 BNC, National Instruments, Austin, USA). Af-
706 ter five days of training, calcium activity was recorded while
707 mice performed the learned task.

708 **Infrared camera behavioral tracking.** Headfixed mouse
709 behavior was continuously monitored by simultaneously us-
710 ing two monochrome CCD cameras (Basler acA 780-75gm)
711 positioned at approximately 15 cm from the mouse. To cap-
712 ture face dynamics, we used a high-resolution zoom lens (50
713 mm FL, Thorlabs MVL50TM23); for body dynamics, we
714 used a wide-angle lens (12 mm FL, Edmund Optics #33-
715 303). Infrared illumination was provided via two 850 nm
716 LED arrays (Thorlabs LIU850A), and cameras were outfitted
717 with 850/40 nm bandpass filters (Thorlabs FB850-40). Both
718 cameras' positions were aligned for each mouse before the
719 start of recordings. Camera images were acquired at 25 or
720 75 Hz with 782×582 pixels using pylon Camera Software
721 Suite (Basler), each frame triggered by TTL pulses from the
722 recording software. Files were saved in compressed MP4 for-
723 mat before further processing.

724 **Two-photon calcium imaging.** Two-photon imaging was
725 performed using an upright LaVision BioTec (Bielefeld, Ger-
726 many) TrimScope II resonant scanning microscope, equipped
727 with a Ti:sapphire excitation laser (Chameleon Ultra II,
728 Coherent, Santa Clara, USA) operated at 920 nm for
729 GCaMP6s fluorescence excitation, a second 1045 nm fixed-
730 wavelength laser (Spectra Physics HighQ-2-IR, Newport
731 Corp., Irvine, USA) for mCherry fluorescence excitation,

732 and a 16x objective (N16XLWD-PF, Nikon, Düsseldorf, Ger- 788
733 many). GCaMP6s fluorescence emission was isolated using 789
734 a band-pass filter (525/40, Semrock, Rochester, USA) 790
735 and detected using a GaAsP PMT (H7422-40, Hamamatsu, 791
736 Herrsching am Ammersee, Germany). mCherry fluorescence 792
737 emission was isolated using a band-pass filter (590/40, Sem- 793
738 rock, Rochester, USA) and detected using a GaAsP PMT 794
739 (H7422-40, Hamamatsu, Herrsching am Ammersee, Ger- 795
740 many). Both lasers were aligned such that they excited the 796
741 same focal plane. Inspector software (LaVision BioTec) was 797
742 used for microscope control and image acquisition. Image se- 798
743 ries (2 channels, 1024×1024 pixels or 512×512 pixels, 350– 799
744 850 μm square field of view) were acquired at 15.2 Hz or 30.5 800
745 Hz, respectively. Frame capture signals were recorded by an 801
746 I/O board (USB-6212 BNC, National Instruments, Austin, 802
747 USA) that allowed subsequent data synchronization. 803

748 **Optogenetic stimulation.** For optogenetic experiments, di- 804
749 rectly following virus injections, the skin over the parietal 805
750 skull and the periosteum were removed and wound edges 806
751 sealed with Vetbond tissue adhesive (3M Animal Care Prod- 807
752 ucts, St Paul, USA). Skull surface was roughened by briefly 808
753 applying gel etchant phosphoric acid (37.5 %; Kerr Dental, 809
754 Scafati, Italy), carefully washing the skull surface and ap- 810
755 plying two-component dental adhesive (OptiBond FL, Kerr 811
756 Dental, Scafati, Italy). Two small 0.5 mm wide holes 812
757 were drilled through the skull (Ideal micro drill, World Pre- 813
758 cision Instruments, Berlin, Germany) and a two-ferrules 814
759 fiber-optic cannula (TFC_200/230-0.37_5.5mm_TS2.0_FLT, 815
760 Doric Lenses, Quebec, Canada) was implanted bilaterally on 816
761 top of NAc (coordinates: +1.3 mm anterior-posterior, +/- 1 817
762 mm lateral, -4.9 mm ventral from brain surface, relative to 818
763 bregma). The craniotomy was then sealed using UV curable 819
764 dental cement (Gradia Direct Flo, GC Corporation, Tokyo, 820
765 Japan). An angular metal bar (Luigs & Neumann, Ratin- 821
766 gen, Germany) for head fixation was placed paramedian on 822
767 the skull. After surgery, buprenorphine (0.05 mg/kg) was 823
768 administered thrice daily for 3 days. After one week of 824
769 recovery, the food restriction and a two-week habituation 825
770 schedules were started. After successful habituation, head- 826
771 fixed mice received light stimulation via a fiber-coupled 473 827
772 nm diode laser (LuxX 473-80, Omicron-Laserage) through 828
773 a branching fiberoptic patchcord (BFP(2)_200/220/900- 829
774 0.37_2m_FCM*-2xZF1.25; Doric Lenses, Quebec, Canada), 830
775 at 5 mW light intensity, measured at the fiber output. Custom- 831
776 written Python scripts were used to send a trigger signal to 832
777 an analog isolated pulse generator (Model 2100, A-M Sys- 833
778 tems, Sequim, USA) once animals entered a hidden optoge- 834
779 netic stimulation zone. The pulse generator produced 5 ms 835
780 long pulses at 20 Hz for a maximum duration of 10 seconds 836
781 or until the mouse passed beyond the stimulation zone. 837

782 **Behavioral analysis.** Analog signals for position, licking, 838
783 reward pump, full rotation of belt, and digital signals from 839
784 infrared camera triggers, two-photon scanning triggers, and 840
785 optogenetic stimulation triggers were collected at 10 kHz 841
786 and saved as TDMS files. Data processing and analysis were 842
787 performed in Python. Position signals in cm were reconstructed

using the optical sensor detecting a full rotation of the tread-
mill belt. Velocity was calculated using a Kalman filter ap-
plied to the position signal. Behavioral data were then down-
sampled to either match the sampling rate of camera track-
ing (25/75 Hz) for training data or to match the sampling
rate of two-photon imaging (15/30 Hz), by using each time
window's arithmetic mean (velocity), median (position, lap
number, camera/scanner trigger), maximum (reward pump,
optogenetic trigger), or standard deviation (licking). Discrete
lick events were detected using Scipy's *find_peaks* function
with a minimum temporal distance of 0.33 s and a dynamic
minimum height threshold that was individually determined
by inspecting the synchronized infrared camera video. Lick
bouts were classified as lick events that were <2 seconds apart
from one another. Appetitive lick onsets were defined as lick
bout onsets that were preceded by at least 3 seconds absence
of lick events. Consummatory licking onset was defined as
the first lick event between 0.5 seconds up to 5 seconds after
reward pump trigger. Rewarded laps were defined as laps in
which the reward pump was triggered by the animal's lick-
ing. Relative licking refers to the cumulative analog lick sig-
nal per position bin or reward zone. Successful laps were
defined as laps in which mice showed at least 50 % of rela-
tive licking in reward and anticipation zones and received a
reward. High success trials were defined as trials consisting
of at least 50 % of successful laps.

814 **Calcium signal processing.** Two-photon imaging data was 814
815 processed using custom-written software in Python, largely 815
816 based on CaImAn (v1.6.2; (44)). Green and red chan- 816
817 nel 16-bit TIFF stack files (512×512 or 1024×1024 pix- 817
818 els times 9,000 or 18,000 frames) were first resampled to 818
819 1 px/μm before motion-correcting the red static channel us- 819
820 ing NormCorre piecewise rigid (parameters: *max_shifts* = 820
821 40, *num_frames_split* = 2000, *overlaps* = 46, *splits_els* = 821
822 4, *strides* = 255). The motion-corrected red channel image 822
823 was then averaged over t and used for later identification 823
824 of mCherry-positive components. Motion correction vectors 824
825 were then applied on the dynamic green channel before us- 825
826 ing constrained non-negative matrix factorization (CNMF) 826
827 for cell segmentation. Resulting traces were detrended and 827
828 deconvolved before filtering spatiotemporal components us- 828
829 ing quality criteria followed by identity- and behavior-blind 829
830 manual curation based on visual inspection of spatial and 830
831 temporal footprints and the quality of deconvolution. To 831
832 identify mCherry-positive components, resulting spatial foot- 832
833 prints were overlaid over the red channel average and a dy- 833
834 namic threshold applied to visually match the optimal signal 834
835 discovery. 835

836 **Place field analysis.** Continuous belt positions were binned 836
837 into 45 bins of 8 cm length. For calcium signals, de- 837
838 convolved events were used to avoid differential effects of 838
839 GCaMP6s calcium signal tails at different animal velocities 839
840 across space. Only time points with a velocity >2 cm/s were 840
841 considered for spatial tuning analysis. Spatial information 841

842 (SI) was calculated as follows (47):

$$843 \quad SI = \sum_i o_i a_i \log_2(a_i/\bar{a}) \quad (1)$$

843 where i denotes the i -th spatial bin, o_i is the animal's occu-
844 pancy at spatial bin i , a_i is the mean of deconvolved events at
845 spatial bin i , and \bar{a} is the overall mean calcium activity. Place
846 cells were defined as cells whose SI was higher than the 95th
847 percentile of 1000x randomly position-shuffled SI values (see
848 ref (65)). Each shuffle consisted of randomly circularly shift-
849 ing the activity time course by at least 500 frames, segment-
850 ing the activity in n blocks that were randomly permuted. n
851 was automatically chosen for each session to match approx-
852 imately twice the number of laps run to account for running
853 differences between animals. The resulting shuffled trace was
854 then used to calculate SI as above. Spatial information rate
855 in bits per second was calculated by multiplying SI with the
856 average firing rate during times of movement (>2 cm/s). Fir-
857 ing rate was defined as the number of deconvolved calcium
858 events >0 per second. Sparsity was calculated according to
859 ref (48), using the same denotation as above:

$$860 \quad \text{Sparsity} = \frac{(\sum o_i a_i)^2}{(\sum o_i a_i^2)} \quad (2)$$

860 Place field reliability was calculated as the fraction of laps in
861 which the maximum spatially binned deconvolved calcium
862 activity occurred within the cell's place field. Δ in-out place
863 field activity was defined as the average deconvolved calcium
864 event activity within a cell's place field subtracted by that
865 activity outside the place field. Place fields were obtained
866 by first averaging deconvolved calcium event activity at ve-
867 locities >2 cm/s per 45 spatial bins, replicating the result-
868 ing trace by a factor of two to account for circularity of the
869 belt, and applying a Savitzky-Golay filter (using Scipy's *sav-*
870 *gol* function with a window of 5 frames at the second order
871 polynomial) to account for skewed place field activity. The
872 resulting filtered spatial calcium activity was then searched
873 for peaks using Scipy's *find_peaks* function (*width*: 1 frame;
874 *prominence*: 1.5 standard deviations; *relative height*: 0.8),
875 with the most prominent peak used as primary place field and
876 left/right interpolated intersection points as place field begin-
877 ning/end. Place fields that would stretch into the replicated
878 positions were translated back to the original 45 spatial bins.
879 Place field boundary ratio was calculated as segmenting po-
880 sition bins into texture transition or middle zones, depending
881 on their proximity to a texture transition, and calculating the
882 ratio of place field beginnings/ends in a transition zone over
883 those in a middle zone. To compare this ratio with a shuf-
884 fled distribution, all place fields underwent 1000 iterations of
885 randomly circularly shifting both beginnings and ends simul-
886 taneously and saving the resulting ratio both cell populations
887 after each iteration. Place field centers were defined as the
888 center of mass (COM) of significantly spatially modulated
889 neurons (see above). For this, calcium activity at velocities
890 >2 cm/s per 45 spatial bins was transformed to polar coor-
891 dinates with q as position bin and r as average deconvolved
892 calcium event activity at that position (see also ref (65)). The

two-dimensional centroid was calculated, and the resulting 893
angle transformed back to belt position to yield the place field 894
center. "Place fields near reward zone" refers to place cells 895
with COMs in either the anticipation zone (starting 30 cm 896
before reward zone) or reward zone. 897

Decoding of reward and anticipation zones. For decod- 898
ing of reward and anticipation zones, all non-zero decon- 899
volved calcium event activity was normalized into quantiles. 900
Position and calcium activity were only considered from time 901
points with velocity >2 cm/s. Data were downsampled to 902
1 Hz by accumulating calcium activity and averaging posi- 903
tion data. Positions in the 30 cm reward zone or the preced- 904
ing 30 cm anticipation zone were binarized (1 inside zone, 905
0 outside zone). Models were trained using calcium data 906
of either (non-)projecting or (non-)conjunctive neurons and 907
100 randomly sample size-matched neurons of the respec- 908
tively larger population, ultimately using the average R^2 of 909
the random sampling procedure. Neurons with fewer than 10 910
time bins of non-zero calcium activity in train/test datasets 911
were excluded from training/testing of the model. A linear 912
support vector machine (SVM) classifier (Sklearn's *linear_* 913
model.SGDClassifier function; *loss*: hinge, *penalty*: L2) 914
was cross-validated by training on even laps and testing on 915
odd laps, and vice versa, using the average of the resulting 916
 R^2 . For models comparing (non-)projection neurons only 917
imaging sessions with >10 projection neurons were included. 918

Velocity and lick coding. Velocity modulation was as- 919
sessed by linear regression of each neuron's average decon- 920
volved calcium event activity against 1 cm/s velocity bins 921
from 2 up to 30 cm/s using Scipy's *stats.linregress* func- 922
tion (see also ref (56)). Cells were considered to be signif- 923
icantly velocity modulated at $P_{\text{adjusted}} < 0.05$ after correct- 924
ing for false discovery rate by using Benjamini/Hochberg cor- 925
rection. Significantly velocity modulated cells with positive 926
slopes were termed "speed-excited" and those with negative 927
slopes "speed-inhibited". To assess population-level appet- 928
itive/consummatory lick modulation, average deconvolved 929
calcium activity before and after each lick event (see above 930
for definition) were compared within the respectively de- 931
scribed time windows. For single-cell level appetitive lick 932
modulation analysis, each neuron's average pre/post decon- 933
volved calcium activity was compared across events using 934
Wilcoxon's non-parametric paired test. Cells were classified 935
as "licking-excited" if they were significantly modulated and 936
showed a positive difference, or "licking-inhibited" if they 937
showed a significant negative difference. 938

Generalized linear model. To determine cellular coding of 939
space, velocity, and licking, we used a generalized linear 940
model to predict each neuron's calcium activity. We nor- 941
malized calcium activity by dividing each neuron's decon- 942
volved calcium events by their signal-to-noise ratio and the 943
resulting standard deviation. Normalized calcium activity 944
was then smoothed using a Gaussian window of 0.5 sec- 945
onds ($SD = 2$) and temporally downsampled by averaging 946
to 3 Hz. Finally, downsampled non-zero calcium data were 947

948 normalized into quintiles. The feature matrix consisted of
949 normalized position, velocity, and lick data. Position data
950 were binned into 45 equally sized spatial bins (8 cm each)
951 and median-averaged to 3 Hz, used as factors. Velocity data
952 were normalized by dividing data by its mean and averaging
953 to 3 Hz. Lick bouts were calculated as described above.
954 Appetitive lick bouts were defined as all lick bouts occur-
955 ring up to 5 seconds before and at least 5 seconds after re-
956 ward dispensation. Appetitive lick bouts were downsampled
957 by median-averaging to 3 Hz. Both downsampled velocity
958 and downsampled licking (appetitive lick bouts) were repli-
959 cated with time shifts spanning 3 seconds before to 3 sec-
960 onds after original timing at 3 Hz to account for anticipatory
961 or delayed calcium activity. Generalized linear Poisson mod-
962 els were trained, validated and tested using Python's H2O
963 library (*H2OGeneralizedLinearEstimator*; λ : 0) with
964 a train/validation/test ratio of 0.8/0.1/0.1. The resulting R^2
965 score based on the test dataset was saved and compared to
966 that of randomly shuffled feature models. For each predic-
967 tor group (position, velocity, licking), 100 shuffled models
968 were generated by circularly shifting the respective group's
969 data randomly between 200 and 700 time points, saving each
970 model's R^2 score. Significant predictors for each neuron's
971 calcium activity were defined as those whose random shuf-
972 fling procedure resulted in reductions in R^2 for more than 95
973 % of cases. This means that at least 96 of the 100 models with
974 one randomly shuffled predictor group had to have decreased
975 R^2 values compared to the full model, in order for this predic-
976 tor to be considered significant. Conjunctive-coding neurons
977 were defined as those with at least two significant predictor
978 groups.

979 **Infrared camera recordings analysis.** Markerless pose
980 estimation (Deep Graph Pose (92) and DeepLabCut (93)) was
981 used to detect facial and body movements for both types of
982 videos. For this, a deep neural network was trained to au-
983 tomatically discriminate 15 markers for videos of the body
984 (paw, tail and head segments) and 13 markers for videos of
985 the face (6 for pupil, eye, nose, mouth, etc). The network was
986 trained on a large variety of lighting conditions and angles
987 until it reached satisfactory performance. Mouth facial re-
988 gions of interest (ROIs) were automatically segmented using
989 video-averaged marker points of nose tip, the eye's tear duct
990 and the mouth as stable landmarks. Mouth motion energy
991 was calculated as the ROI's average pixel-by-pixel intensity
992 differences from t_{-1} to t_0 , and z -scoring the resulting activ-
993 ity.

994 **Statistical analysis.** Statistical analysis was performed us-
995 ing Python's pingouin library. Statistical tests are indicated
996 in the figure legends and text. To evaluate statistical signif-
997 icance, data from Figures 1D and 3F was subjected to one-
998 or two-way ANOVAs. Data in Figures 2D, 4D, 4H, 5H-I,
999 7B, 7D, 7F, 8B-C, and 8E was subjected to chi-square tests.
1000 Data in Figures 2E-H was subjected to Welch's t -tests. Data
1001 in Figures 3C-D was subjected to bootstrapping analysis (see
1002 above) and chi-square tests. Data in Figure 3G and 8G was
1003 subjected to Wilcoxon's test for nonparametric data. Data in

Figure 5C was subjected to a repeated-measures ANOVA fol-
lowed by post-hoc t -tests with Bonferroni correction. Data
in Figures 6I and 6K was subjected Student's paired t -test.
For all analyses data are presented as mean \pm SEM, and the
threshold for significance was at $p < 0.05$.

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AUTHOR CONTRIBUTIONS

O.B. and S.R. conceived and designed experiments. O.B. performed and analyzed imaging experiments. O.B. and P.M. performed optogenetic experiments, O.B. analyzed these. O.B. wrote the manuscript with help from S.R. and P.M..

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

CODE AND DATA AVAILABILITY

Code and data will be made available upon final publication.

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1370 **Supplementary videos**

1371 **Supplemental Video 1. *In vivo* dual-color two-photon**
1372 **calcium imaging of a mouse during goal-directed nav-**
1373 **igation.** Representative example of dual-color two-photon
1374 calcium imaging combined with goal-directed navigation and
1375 lick behavior. Infrared camera images show face (top left)
1376 and body (top right) tracking. Calcium activity is shown
1377 by overlaying denoised GCaMP6s activity (green) onto av-
1378 erage mCherry signal (red; bottom left); traces represent de-
1379 noised GCaMP6s activity of mCherry-negative (green) and
1380 mCherry-positive (red; NAc-projecting) hippocampal neu-
1381 rons (bottom right). Playback speed is 2× original speed.

1382 **Supplemental Video 2. Optogenetic stimulation in-**
1383 **duces mouth movement.** Five representative optogenetic
1384 stimulation trials from one experimental mouse expressing
1385 ChR2 and one control mouse expressing EYFP only. A light
1386 fiber in NAc stimulated excitatory hippocampal projections
1387 by shining 473 nm laser light at times indicated by blue
1388 squares (top) and blue traces (bottom). Optogenetic stimu-
1389 lation repeatedly induces lick behavior and a decrease in ve-
1390 locity in ChR2-expressing animals but not those expressing
1391 EYFP. Playback speed equals original speed.

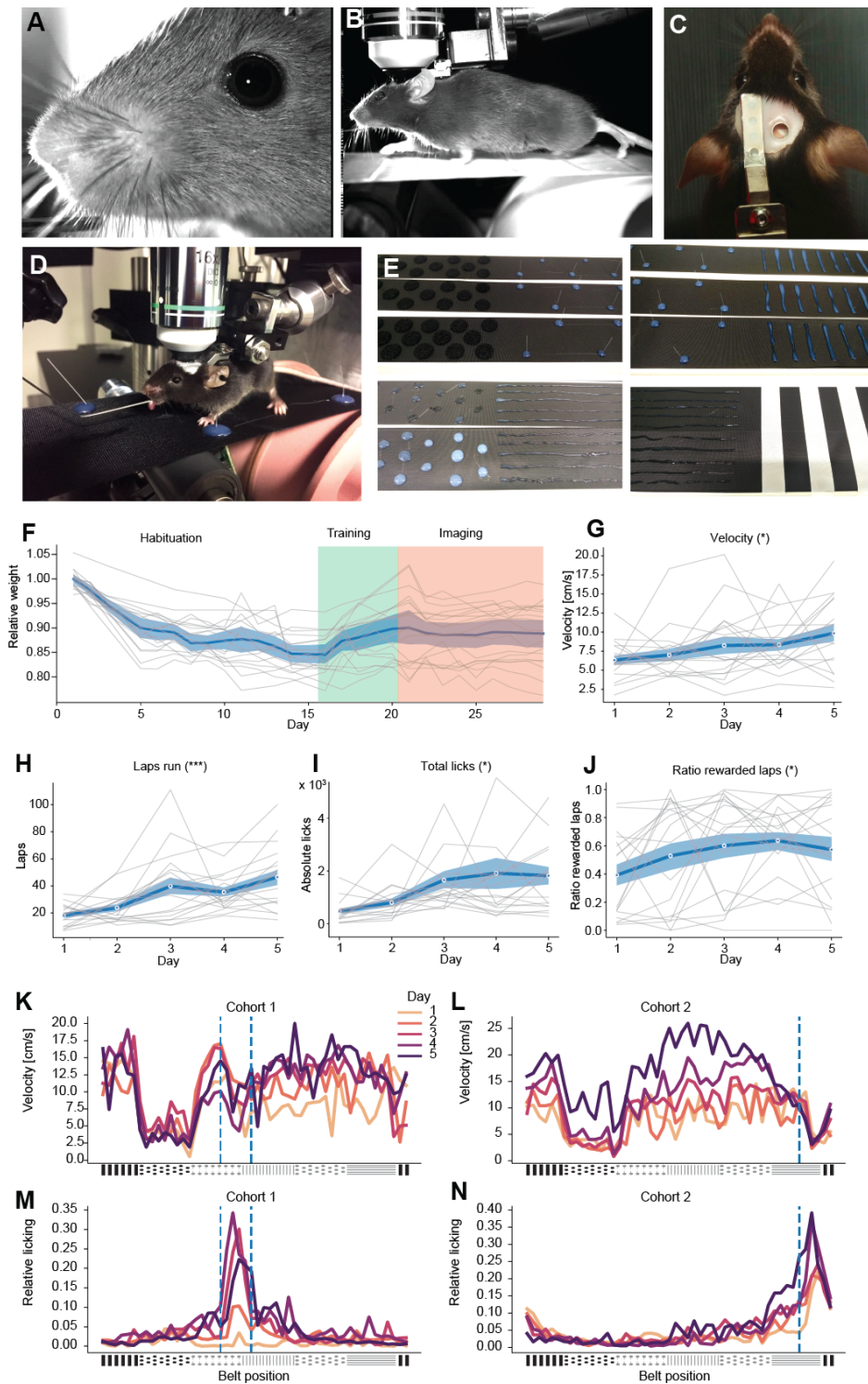


Figure S1. Spatial reward learning task. (A and B) Sample infrared images of face (A) and body (B) that were continuously captured at 25/75 Hz. (C) Image of one head-fixed experimental mouse from top, illustrating craniotomy and head-fixation. (D) Sample image of two-photon imaging of a head-fixed mouse on treadmill belt, licking at the lick spout. (E) Sample images of the six different belt texture zones, including Velcro dots, nylon spikes, vertical glue stripes, glue dots, horizontal glue stripes, white tape stripes (from left top to right bottom). (F) Weight changes after introduction of food restriction and during training and imaging. (G-J) Average velocity (G, $F(4) = 2.631$, $p = 0.0430$), number of laps run (H, $F(4) = 8.771$, $p < 0.001$, GG-corrected), number of licks (I, $F(4) = 3.883$, $p = 0.0326$, GG-corrected), and ratio of rewarded laps (J, $F(4) = 3.331$, $p = 0.0157$) increase over the course of five training days (all repeated-measures ANOVAs). Gray lines indicate data points of individual animals, blue shade represents SEM, blue line represents mean. (K-N) Average velocity (K and L) and average licking (M and N) changes across belt position and days of mice tested with reward zone in “center” of belt (K and M) and “end” of belt (L and N). Blue dashed line represents presence of reward zone. $n = 9$ (cohort 1), $n = 9$ (cohort 2). All data are presented as mean \pm SEM. * $p < 0.05$, *** $p < 0.001$.

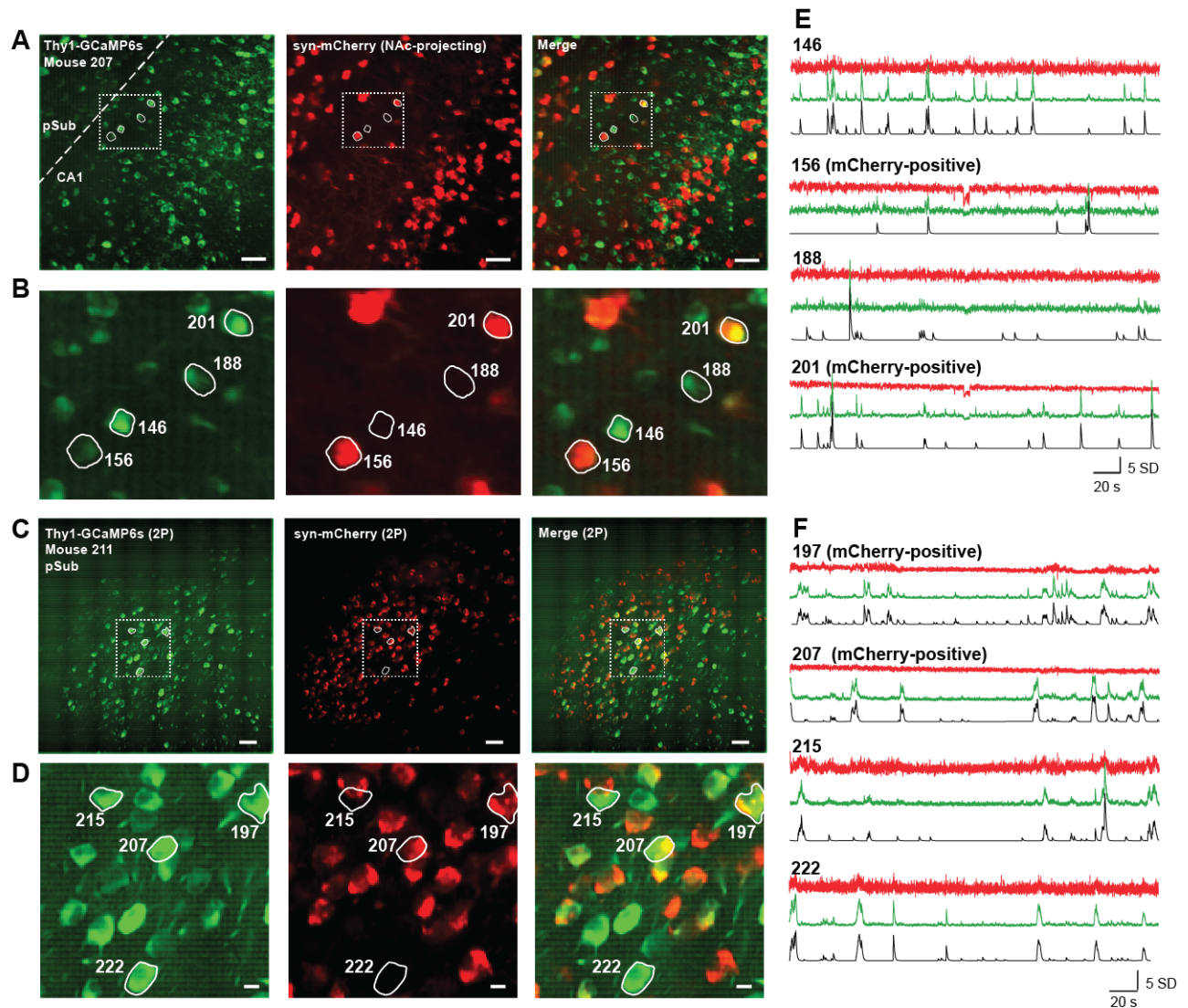
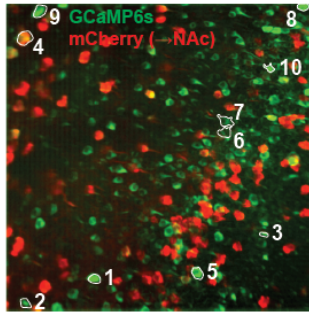
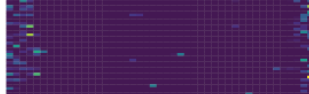


Figure S2. *In vivo* dual-color two-photon imaging of dynamic GCaMP and static mCherry signals. (A) Sample field of view (FOV) of one imaged region crossing putative CA1 and prosubiculum (pSub). Green channel shows GCaMP6s local correlations image, red channel shows average motion-corrected mCherry signal. (B) Detail of region in (A) clearly outlining spectrally separate channels. (C and D) Sample FOV of another region in putative prosubiculum (C) with detailed view (D). Scale bars represent 50 μ m (A and C) or 10 μ m (B and D). (E and F) Z-scored fluorescent temporal sample traces of regions of interest (ROIs) shown in (A-D). Red traces show raw fluorescence dynamics from imaging red channel, green traces show raw fluorescence dynamic from green channel, and black traces show denoised calcium signal.

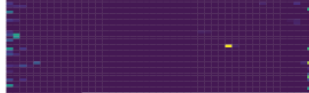
Mouse: 207, session: 103,
cells: 37 / 200



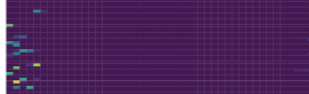
1 - SI: 1.65***, Sp: 0.18, Rel: 0.82



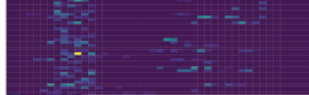
2 - SI: 1.06**, Sp: 0.10, Rel: 0.88



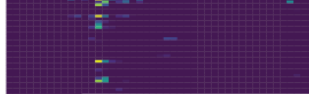
3 - SI: 0.55, Sp: 0.08, Rel: 0.91



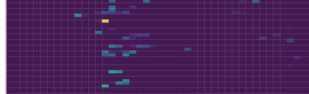
4 - SI: 0.87*, Sp: 0.27, Rel: 0.74



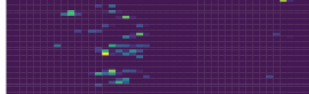
5 - SI: 1.08*, Sp: 0.08, Rel: 0.38



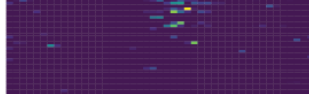
6 - SI: 0.79, Sp: 0.16, Rel: 0.44



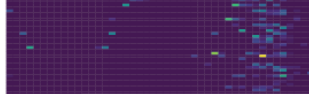
7 - SI: 0.97*, Sp: 0.24, Rel: 0.47



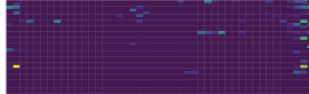
8 - SI: 0.43, Sp: 0.20, Rel: 0.32



9 - SI: 3.40***, Sp: 0.42, Rel: 0.65

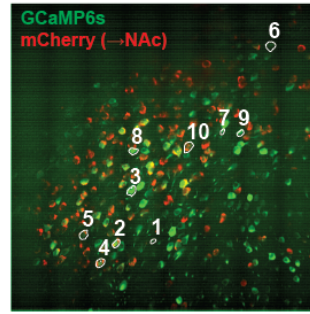


10 - SI: 1.59**, Sp: 0.24, Rel: 0.85



Rewarded laps: 33/35
Avg. velocity: 20.98 cm/s

Mouse: 211, session: 152,
cells: 79 / 156



1 - SI: 1.57*, Sp: 0.15, Rel: 0.89



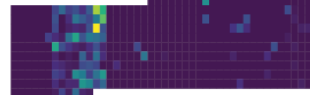
2 - SI: 4.79***, Sp: 0.17, Rel: 0.89



3 - SI: 6.29***, Sp: 0.31, Rel: 0.78



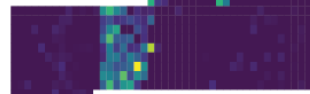
4 - SI: 2.98***, Sp: 0.27, Rel: 0.89



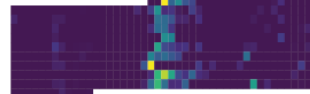
5 - SI: 2.73***, Sp: 0.14, Rel: 0.89



6 - SI: 1.71**, Sp: 0.19, Rel: 0.78



7 - SI: 1.05, Sp: 0.17, Rel: 1.00



8 - SI: 1.37, Sp: 0.27, Rel: 0.89



9 - SI: 1.75*, Sp: 0.32, Rel: 0.78

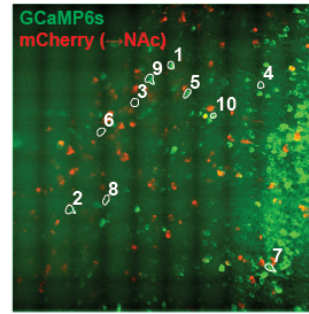


10 - SI: 1.27, Sp: 0.31, Rel: 0.78

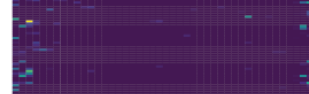


Rewarded laps: 7/10
Avg. velocity: 5.93 cm/s

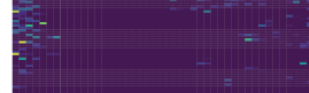
Mouse: 210, session: 137,
cells: 18 / 255



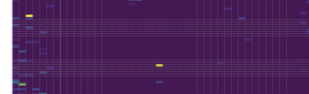
1 - SI: 2.24***, Sp: 0.23, Rel: 0.83



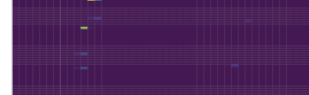
2 - SI: 1.54***, Sp: 0.33, Rel: 0.68



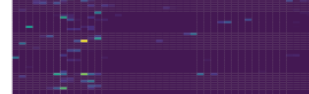
3 - SI: 0.64***, Sp: 0.22, Rel: 0.78



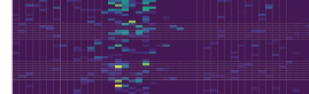
4 - SI: 0.24, Sp: 0.07, Rel: 0.10



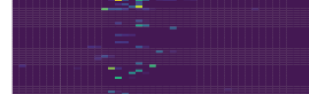
5 - SI: 0.74, Sp: 0.23, Rel: 0.46



6 - SI: 1.15***, Sp: 0.23, Rel: 0.83



7 - SI: 0.62, Sp: 0.11, Rel: 0.49



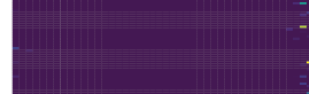
8 - SI: 0.45, Sp: 0.16, Rel: 0.29



9 - SI: 2.63***, Sp: 0.34, Rel: 0.80



10 - SI: 1.91***, Sp: 0.14, Rel: 0.61



Rewarded laps: 36/42
Avg. velocity: 25.68 cm/s

Figure S3. Spatial tuning of different neurons in sample FOVs.

Three representative fields of view (FOVs) from three different mice are shown, with single-lap spatial calcium activity of each 10 representative neurons, some putatively NAc-projecting. FOV information is shown on top, including numbers of identified dHPC⁻ (green) and dHPC^{→NAc} (red) neurons. FOVs shown are composites of CalmAn maximum local correlation images (*caiman.summary_images.max_correlation_image*) of GCaMP channel (green) and the averaged motion-corrected red channel with NAc-projecting mCherry fluorescence. Contours of ten representative neurons from each FOV are indicated with white outlines and numbers that refer to spatially averaged calcium activity below. Each neuron's normalized average calcium activity across 45 spatial bins per lap (y axis) is shown with key spatial information values above (SI: spatial information (47), Sp: Sparsity (48), Rel: reliability of each neuron's per-lap maximum activity to occur within the place field). Orange numbers refer to NAc-projecting neurons.

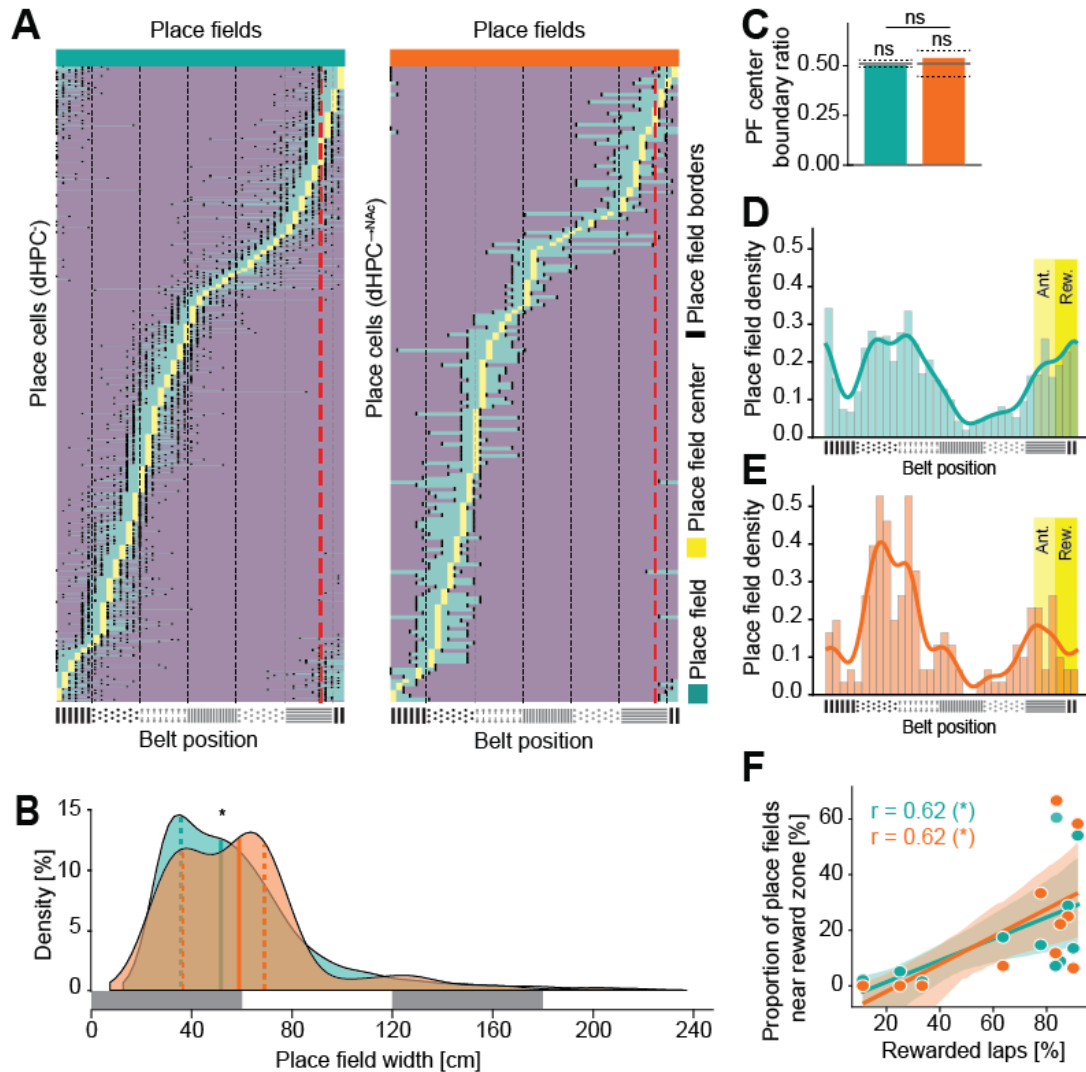


Figure S4. Place field distribution across the belt and behavioral performance. (A) Extent of place fields of dHPC⁻ (left) and dHPC^{→NAc} (right) place cells, sorted by each place cell's center of mass (COM). Turquoise represents place field, yellow represents COM, black lines represent left and right borders of place fields. Red dashed line represents beginning of reward zone, black dashed lines represent belt texture borders. (B) Width of place fields is unevenly distributed between dHPC⁻ (green; quartiles 35.6 cm / 51.7 cm / 69.0 cm) and dHPC^{→NAc} (red; quartiles 36.5 cm / 58.9 cm / 69.0 cm) place cells (Kolmogorov-Smirnov test, $D = 0.116$, $p = 0.0299$). Gray bars represent belt texture zones (60 cm). (C) Place field centers are not biased with respect to texture boundaries (percentile < 95th, permutation test) and the ratio is not different between neuronal populations ($\chi^2(1, 5372) = 1.646$, $p = 0.1995$). (D and E) Place field density between reward and anticipation zones compared to the rest of the belt for dHPC⁻ (D) and dHPC^{→NAc} (E) populations. (F) Reward and anticipation zone overrepresentation correlates with behavioral success (percentage of rewarded laps per session) for both dHPC⁻ (green) and dHPC^{→NAc} (red) populations (Pearson correlation; $r_{\text{dHPC}^-} (15) = 0.622$, $p = 0.0107$; $r_{\text{dHPC}^{\rightarrow\text{NAc}}} (10) = 0.619$, $p = 0.0421$). ns: not significant, * $p < 0.05$.

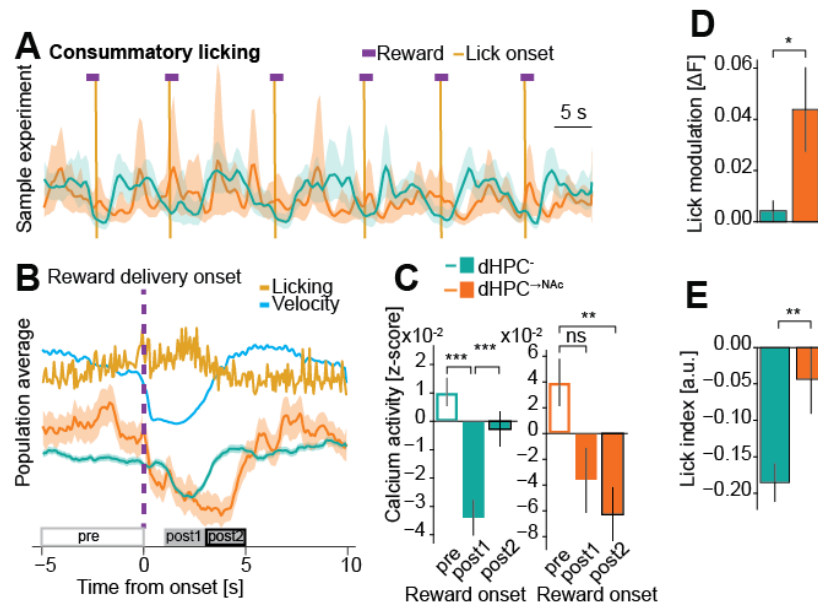


Figure S5. Lick modulation during consumption and general licking. (A-C) Consummatory licking leads to a depression in neural activity. (A) Sample experiment showing reward dispensation (purple bars) and consummatory licking onsets (golden vertical lines) and population calcium activity from dHPC⁻ (green) and dHPC^{→NAc} (red) neurons. Note the robust depression of neural activity during times of reward consumption. (B) Event-triggered average traces around reward delivery onset, including licking and speed as well as population average calcium activity of dHPC⁻ (green) and dHPC^{→NAc} (red) neurons. Gray rectangles indicate time windows for comparing calcium activity shown in (C). (C) Calcium activity is differentially modulated by reward onset (two-way mixed ANOVA; $F_{\text{reward_timing}}(2, 10740) = 19.380$, $p(\text{GG-corrected}) < 0.001$, $F_{\text{projection}}(1, 5370) = 0.798$, $p = 0.372$, $F_{\text{interaction}}(2, 10740) = 5.559$, $p = 0.0039$). Post-hoc pairwise t -tests with Bonferroni correction were performed for each interaction and the results indicated with asterisks. (D) Lick modulation (average of each trial's pre-post difference) is significantly increased in dHPC^{→NAc} neurons (Welch's $t(496.11) = 2.379$, $p = 0.0177$). (E) Lick index (average calcium activity difference during licking) is significantly increased in dHPC^{→NAc} neurons (Welch's $t(454.19) = 2.729$, $p = 0.0066$). All data are presented as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

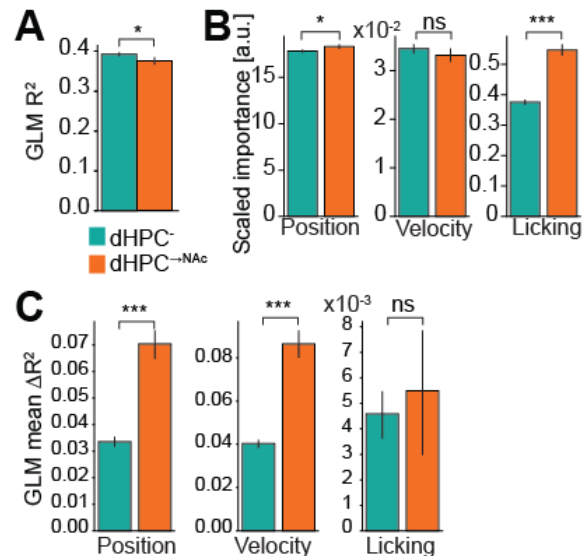


Figure S6. Generalized linear model reveals coding differences for dHPC^{→NAc} neurons. (A) GLMs' explained variance R^2 is significantly lower for dHPC^{→NAc} neurons (Welch's $t(615.15) = 2.147$, $p = 0.032$). (B) Scaled feature importance (standardized GLM coefficients retrieved by H2O's varimp() method) is higher in dHPC^{→NAc} neurons for position (Welch's $t(2508.09) = 2.162$, $p = 0.031$) and licking (Welch's $t(1879.75) = 9.328$, $p < 0.001$), but not for velocity (Welch's $t(2609.15) = 0.881$, $p = 0.378$). (C) Mean R^2 differences between full and feature-shuffled models are significantly greater in dHPC^{→NAc} neurons for position (Welch's $t(523.23) = 5.984$, $p < 0.001$), velocity (Welch's $t(497.69) = 7.704$, $p < 0.001$), but not for licking (Welch's $t(552.63) = 0.3626$, $p = 0.717$). All data are presented as mean \pm SEM. ns: not significant, * $p < 0.05$, *** $p < 0.001$.