The nucleus accumbens shell regulates hedonic feeding via a rostral hotspot

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KEY WORDS

anterior-posterior, aversion, consumption, fiber photometry, food intake, molecular diversity, neural circuits, neuroanatomy, nucleus accumbens, nucleus accumbens shell, optogenetics, reward, rostral-caudal, RNA sequencing, topographical gradients.

ABSTRACT

The medial nucleus accumbens shell (medNAcSh) is increasingly recognized as a key node in the regulation of hedonic feeding, exerting strong control over reward consumption through its projections to downstream structures. Recent studies showed that one of the primary cellular mediators of these effects are dopamine 1 receptor positive striatal projection neurons (D1-SPNs). Specifically, population D1-SPN activity gets inhibited during reward consumption and such inhibition is necessary and sufficient to authorize reward consumption, independent of metabolic need. Anatomically, the medNAcSh spans a 1 to 1.5 mm distance along the rostro-caudal axis in mice and previous studies have reported functional gradients along this axis in behavioral control. For instance, classical pharmacological studies have suggested that rostral rather than caudal medNAcSh regulates appetitive behavior. However, the cellular, circuit, and molecular mechanisms underlying this topographical gradient remain unknown. Here we hypothesized that D1-SPNs contribute to this gradient by regulating hedonic feeding via a specific hotspot in the rostral medNAcSh. Using population level calcium monitoring with fiber photometry in mice, we show that rostral medNAcSh D1-SPNs demonstrate inhibitory responses during reward consumption, while caudal D1-SPNs do not. Consistently, optogenetic stimulation of rostral medNAcSh D1-SPNs inhibits consumption, while stimulation of caudal D1-SPNs had minimal effects, confirming the existence of a functional rostro-caudal gradient. Importantly, we observed no differences between rostral and caudal D1-SPNs in their behavioral or activity responses to aversive stimuli, suggesting that the D1-SPN gradient is specific to appetitive contexts. To investigate potential molecular correlates of this functional gradient, we leveraged open-source anatomy datasets and performed fluorescent in situ hybridization, identifying Stard5 and Peg10 as markers enriched in the rostral and caudal medNAcSh, respectively. Finally, we developed a novel Stard5-Flp driver mouse line, which allowed us to target the rostral hotspot selectively. We demonstrated that Stard5+ neurons recapitulate activity patterns of rostral medNAcSh D1-SPNs. Together, these findings establish a spatially confined rostral subregion of the medNAcSh as a critical regulator of reward consumption and introduce Stard5 as a molecular tool for its manipulation - offering new opportunities for intervention in dysregulated eating beyond classical homeostatic circuits.

INTRODUCTION

Feeding behavior is a multifaceted physiological process governed by interconnected homeostatic and hedonic feeding brain circuits (Marinescu and Labouesse, 2024; Stuber et al., 2025; Zhu et al., 2025). While homeostatic pathways in the hypothalamus and brainstem regulate energy balance according to metabolic signals, non-homeostatic hedonic circuits are distributed more broadly throughout the brain to adjust feeding behavior according to sensory signals, motivation, associative learning or pleasure (Stuber et al., 2025). Among key brain regions involved in the latter, the nucleus accumbens shell (NAcSh) has emerged as a significant player, in particular its most medial section, the medial NAcSh (medNAcSh) (reviewed in (Marinescu and Labouesse, 2024)). Pharmacological studies in the 1990s and 2000s in rodents showed that inactivation of the medNAcSh dramatically increases the consumption of food rewards, even in fed animals (Maldonado-Irizarry et al., 1995; Stratford and Kelley, 1997). The medNAcSh consists of a mixed population of neurons of which a considerable proportion pause during reward consumption, an inhibitory signal that is necessary and sufficient to authorize food consumption, i.e. excitation suppresses, and pauses permit feeding (Domingues et al., 2025; Krause et al., 2010; O'Connor et al., 2015; Pedersen et al., 2022; Roitman et al., 2005; Taha and Fields, 2006, 2005). These pauses are driven primarily by cortical and limbic incoming circuits into the medNAcSh (Reed et al., 2018; Vachez et al., 2021), can be found in individual dopamine 1 receptor-positive striatal projection neurons (D1-SPNs) and D2-SPNs (Domingues et al., 2025; O'Connor et al., 2015; Pedersen et al., 2022), and are thought to allow rapid control over food consumption in response to motivational or sensory signals (Kelley et al., 2005; O'Connor et al., 2015). Importantly, recent studies showed that one of the primary cellular mediators of the eating-inhibitory effects in the medNAcSh are D1-SPNs, one of the two main output populations of the NAc (O'Connor et al., 2015; Thoeni et al., 2020; Bond et al., 2020).

Topographically, the NAcSh spans a 1 to 1.5 mm distance along the rostro-caudal (anterior-posterior) axis in mice with studies demonstrating strong polarity gradients along this axis in the control of various behaviors (Castro et al., 2016; Castro and Berridge, 2014; Krause et al., 2010; Reed et al., 2018; Reynolds and Berridge, 2002, 2008; Richard and Berridge, 2011). For instance, microinjection of the glutamate antagonist DNQX or the GABA agonist muscimol into the rostral medNAcSh promotes appetitive responses like feeding, while the same manipulations in the caudal medNAcSh trigger aversive behaviors (Reynolds and Berridge, 2001; Richard and Berridge, 2011). D1, but not D2 antagonists were able to block the glutamate-mediated feeding responses in the rostral medNAcSh, suggesting a more important role for the D1-SPN pathway (Richard and Berridge, 2011). Altogether, these findings suggest the existence of a hotspot for feeding located in the rostral sections of the medNAcSh possibly involving D1-SPNs. However, the cellular, circuit, and molecular mechanisms underlying this rostral hotspot remain unknown.

Against this background, we hypothesized that D1-SPNs regulate feeding behavior according to a rostro-caudal gradient within the medNAcSh. We use fiber photometry recordings and optogenetic activation studies, finding a functional dichotomy between rostral vs. caudal medNAcSh D1-SPNs in reward consumption but not in aversive behavioral assays. We perform neuroanatomical investigations in the rostral vs. caudal medNAcSh and identify various circuit and molecular signatures of these two subregions, including a molecular marker, Stard5, densely expressed in 80% of rostral medNAcSh D1-SPNs and D2-SPNs. Finally, we generate a new Flp driver line which targets the Stard5 rostral medNAcSh subpopulation and use it in proof of concept experiments, finding that Stard5 cells replicate the population-level activity signatures of rostral medNAcSh D1-SPNs during reward consumption and aversion. Our work outlines the importance of functional and molecular gradients in the medNAcSh, demonstrating the existence of a D1 rostral hotspot for feeding in the medNAcSh that is partly recapitulated by Stard5+ cells, a newly described molecular marker to access this feeding hub. These findings offer

new potential access points for targeting dysregulated eating through a spatially confined medNAcSh region, outside traditional homeostatic feeding circuits.

RESULTS

Rostral medNAcSh D1-SPNs regulates feeding

We first set to identify points of divergence in the feeding-related properties of D1-SPNs along the rostro-caudal medNAcSh axis. It is well established that, although D1-SPNs show both excitatory and inhibitory responses during feeding, a considerable proportion of cells are inhibited, in the form of prolonged pauses (Domingues et al., 2025; Krause et al., 2010; O'Connor et al., 2015; Pedersen et al., 2022; Roitman et al., 2005; Taha and Fields, 2006, 2005) which can be reflected at population level activity (Domingues et al., 2025). These pauses are thought to authorize eating by disinhibiting downstream circuits (O'Connor et al., 2015; Thoeni et al., 2020; Bond et al., 2020). To determine whether such pause responses change along the rostro-caudal axis, we used fiber photometry to measure population level D1-SPN activity in both the rostral and caudal medNAcSh while sated mice were fed a palatable food source (condensed milk). Drd1-cre mice received an adeno-associated virus (AAV) expressing a cre-dependent calcium indicator (jGCaMP8m (Y. Zhang et al., 2023)) and an optic fiber above the rostral or caudal medNAcSh, targeting specifically the dorsal region given its strong role in feeding (O'Connor et al., 2015) (**Figure 1a-b;** see also **Supplementary Figure S1**). Mice were allowed to consume up to 30 food rewards per session delivered at random intervals (**Figure 1c**). As expected, population D1-SPN activity in the rostral medNAcSh showed a prolonged pause upon reward consumption, with average activity decreasing at eating onset, and returning to baseline after the 10 s consumption period (**Figure 1d**). This was reflected by a significant decrease in the ΔF/F0 minima during the consumption as compared to pre-consumption period (**Figure 1e**). The inhibition of D1-SPN activity during reward consumption in rostral medNAcSh aligns well with previous findings (Bond et al., 2020; Krause et al., 2010; O'Connor et al., 2015; Thoeni et al., 2020). On the other hand, D1-SPNs in the caudal medNAcSh showed an acute excitatory response at consumption onset that returned to baseline after ~2.5 s, followed by a minor prolonged inhibition (**Figure 1f**). This was reflected by a significant increase in the ΔF/F0 maxima in the consumption as compared to pre-consumption period (**Figure 1g**). To confirm regional specificity of the recordings, histological validation in a subset of animals showed correct placement of the optic fibers at the intended stereotaxic coordinates. Additionally, injections targeting the rostral medNAcSh exhibited minimal to no viral expression at caudal coordinates, while injections targeting the caudal medNAcSh showed minimal to no viral expression at rostral coordinates (**Supplementary Figure S10a-d**). These data indicate a strong divergence in the responses of D1-SPNs to reward consumption depending on their topographical location, highlighting the existence of a rostro-caudal gradient. It further demonstrates that the rostral, but not caudal, medNAcSh represents the primary locus for the expression of pause responses by D1-SPNs at a population level during reward consumption.

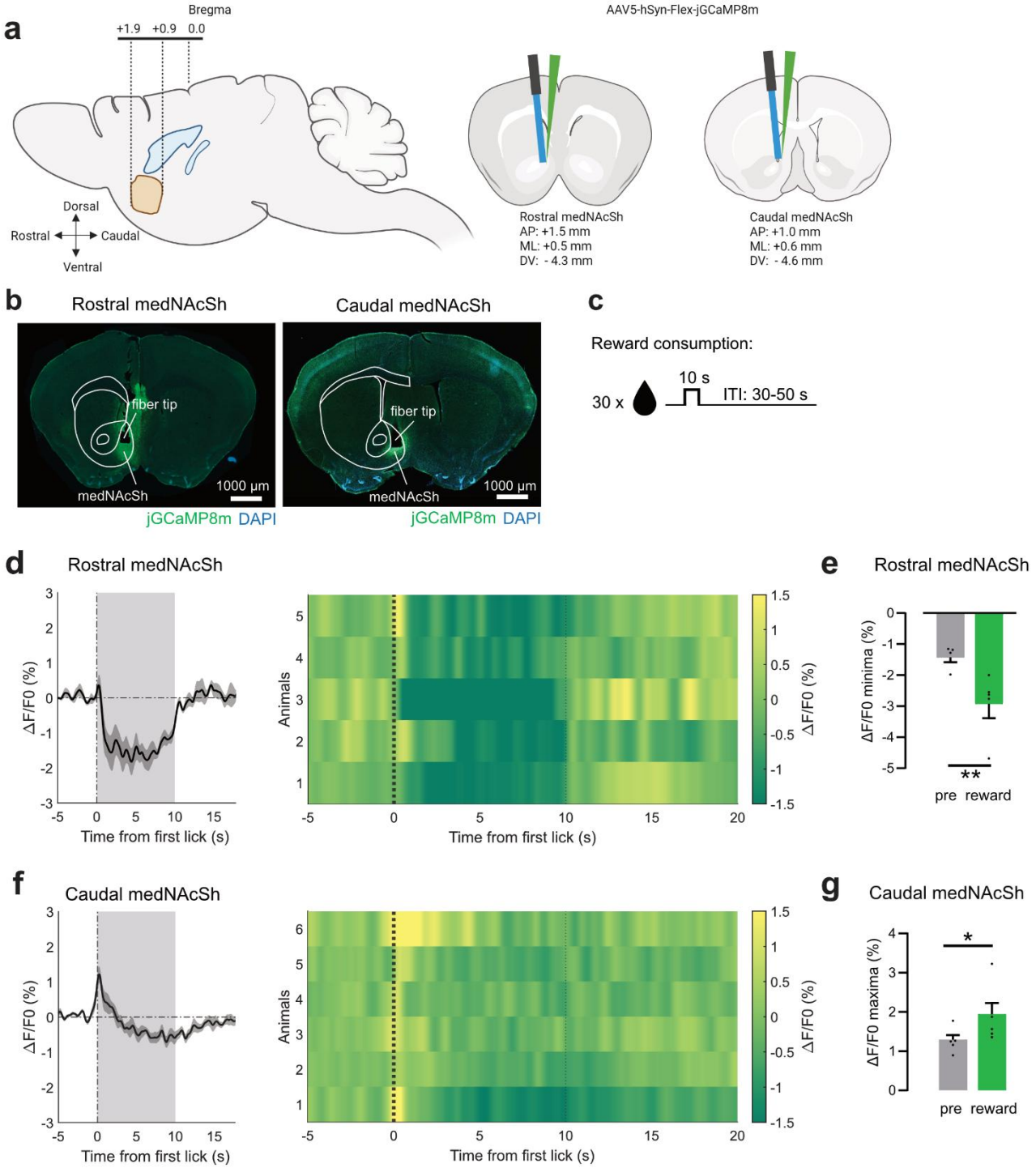

Figure 1. D1-SPNs in the rostral vs. caudal medNAcSh respond differentially to reward consumption. a. Surgery schematics of Drd1-cre mice injected with a cre-dependent calcium indicator (jGCaMP8m) and implanted with unilateral optic fibers in the rostral or caudal medNAcSh to image calcium photometry signals. Stereotaxic coordinates for injection and optic fiber implantation in the rostral and caudal are shown on the right. **b.** Representative coronal images of rostral (left) vs. caudal (right) medNAcSh mice. **c.** Protocol for the unpredicted reward consumption behavioral task: 30 rewards were available for 10 s at random intervals. **d.** Left: Average calcium activity in D1-SPNs (all trials) shown as normalized fluorescence (ΔF/F0, %) in the rostral medNAcSh over

time aligned to the onset of reward consumption, i.e. first lick onset (0 s). Grey shading represents the 10 s epoch for reward consumption. Right: Heatmap showing average calcium activity across individual trials (each row represents an animal) aligned to reward consumption i.e. lick onset (0 s, thick dotted line). Thin dotted line: end of reward access. On average, mice completed 16.2 of 30 trials successfully (54%).**e.** Quantification of the data in (d.) depicts a significant decrease in rostral medNAcSh activity upon reward consumption, as shown by a significant decrease in ΔF/F0 minima in the 0 to 5 s reward epoch vs. -6 to -1 s pre-reward epoch. Paired t-test, $t(4) = 4.610$, $p = 0.0100$. N =5 mice, 1 female, 4 male. **f.** same as (d.) for the caudal medNAcSh. Animals completed an average of 17.67 successful trials out of 30 (59%). **g.** Quantification of the data in (f.) depicts a significant increase in caudal medNAcSh activity upon reward consumption, as shown by a significant increase in ΔF/F0 maxima in the 0 to 5 s reward epoch vs. -6 to -1s pre-reward epoch. Paired t-test, $t(5) = 3.059$, $p = 0.0281$. N = 6 mice, 4 female, 2 male. Data is mean ± SEM. *p<0.05; **p<0.01; ***p<0.001. See also **Supplementary Figure S1**. Subfigure a. was created in BioRender.

Previous work showed that medNAcSh D1-SPNs can also causally impact hedonic eating, where optogenetic activation of D1-SPNs (using rostral-centric medNAcSh coordinates) inhibits reward consumption while optogenetic inhibition promotes consumption independent of metabolic needs (Bond et al., 2020; O'Connor et al., 2015). This is similar to what is found with global, cell-unspecific, medNAcSh pharmacological inhibition (Maldonado-Irizarry et al., 1995; Stratford and Kelley, 1997). Based on this extensive literature and our photometry results, we asked whether reward consumption effects were confined to the rostral side of the medNAcSh or whether similar functions extended to the caudal medNAcSh. Drd1-cre mice received bilateral injections of an AAV expressing a cre-dependent excitatory opsin, ChrimsonR, and optic fibers above the rostral or caudal medNAcSh (**Figure 2a-b** and **Supplementary Figure S10e-h**). We first conducted a proof-of-concept experiment in a small cohort to validate previously published optogenetic protocols. Mice were exposed to palatable food rewards with alternating blocks of 8 min: blocks 1 and 3 without laser stimulation (laser off) and block 2 with stimulation (laser on) (**Figure 2c**). In ChrimsonR Rostral medNAcSh mice, lick count was initially high with laser off, then decreased during laser on, and rebounded again with laser off. This consumption pattern is very similar to what is seen in the literature (Bond et al., 2020; O'Connor et al., 2015). When averaging lick counts per epoch type, we could detect a significant reduction in reward consumption with optogenetic stimulation of rostral medNAcSh D1-SPNs. No significant change in consumption was seen in mCherry controls. Although these mice began with a slightly lower (non-significant) baseline compared to ChrimsonR mice, there was no evidence of a floor effect (**Figure 2d-e**). Altogether, this feasibility experiment confirmed published findings (Bond et al., 2020; O'Connor et al., 2015), namely that activation of rostral medNAcSh D1-SPNs inhibit reward consumption. We then went on to test a full experimental cohort to evaluate the differential contribution of rostral vs. caudal medNAcSh D1-SPNs in reward consumption. Here we modified the protocol to enhance the sensitivity of the assay, minimize potential confounds such as increasing satiety over time and improve our ability to detect even minor differences in reward consumption between the three groups. We started with a laser on epoch and expanded the number of epochs from 3 to 5 (3 laser on, 2 laser off); we also reduced epoch duration from 8 to 5 min (**Figure 2f**). As before, and despite the modified protocol, we again found that optogenetic stimulation of D1-SPNs in the rostral medNAcSh significantly inhibited reward consumption with no effects in mCherry controls (**Figure 2g-i**). A similar (as seen in **Figure 2d**) rebound in consumption was detected after laser on epochs in rostral medNAcSh mice but not mCherry controls (**Figure 2g**). Importantly, although there was a non-significant trend for an opto-mediated eating-inhibitory effect in caudal medNAcSh mice (see **Figure 2g**), and the number of caudal medNAcSh animals demonstrating opto-mediated inhibition of

consumption was 64% (vs. 100% of rostral medNAcSh mice and 30% mCherry) (**Figure 2i**), the mean effect was too small to reach statistical significance (p = 0.5244) (**Figure 2h**). To determine whether the reduced reward consumption observed in Rostral ChrimsonR mice could be explained by changes in locomotion, we quantified the total distance traveled during this task. Optogenetic stimulation led to an increase in locomotion in the small cohort of Rostral ChrimsonR mice in the reward consumption experiment shown in **Figure 2d-e** (**Supplementary Figure S9a**), while no change in locomotion was observed across epochs in mCherry controls, ChrimsonR Rostral and Caudal mice (**Supplementary Figure S9b, related to Figure 2g-i**). This indicates that the reduced feeding in Rostral ChrimsonR mice is not due to impaired locomotion. In sum, rostral, but not caudal, medNAcSh D1-SPNs inhibit reward consumption. Altogether this indicates the existence of a rostro-caudal gradient in the regulation of hedonic eating by medNAcSh D1-SPNs, supporting the existence of a D1 rostral medNAcSh appetitive hotspot.

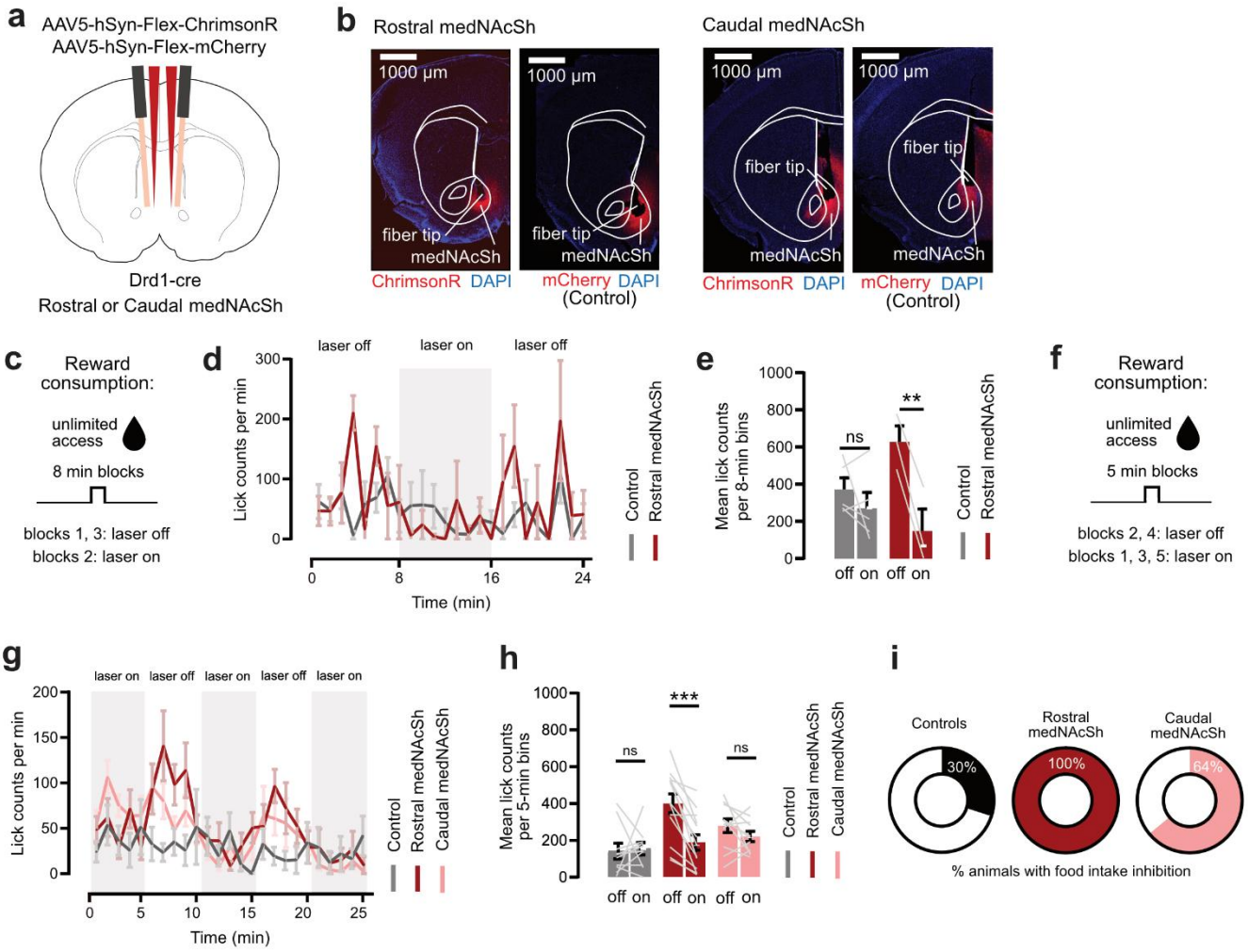

Figure 2. In vivo optogenetic stimulation of rostral medNAcSh inhibits reward consumption. a. Surgery schematics of Drd1-cre mice injected with a cre-dependent activating opsin (ChrimsonR) or mCherry control AAV and implanted with bilateral optic fibers in the rostral or caudal medNAcSh to stimulate local D1-SPNs. **b.** Representative coronal images of rostral (left) vs. caudal (right) medNAcSh mice expressing relevant AAVs. **c.** Protocol for the proof-of-concept reward consumption experiment: mice had unlimited access to the reward spout. Blocks of 8 min without (laser off, 'non-opto') or with opto-stimulation (laser on, 'opto') were alternated (off/on/off) for a total of 3 blocks. **d.** Quantification of the lick counts per min in mCherry mice vs. ChrimsonR (rostral medNAcSh) mice, showing a lower lick count per min in rostral medNAcSh mice during opto stimulation vs. 'non-

opto' epochs; lick counts unchanged across epochs in mCherry mice. **e.** Quantification of mean licks per session in the opto-stimulation vs. non-opto-stimulation epochs shows a significant decrease in lick counts following stimulation of rostral medNAcSh D1-SPNs (and not in mCherry controls) confirming published studies. 2-way RM-ANOVA (group x epoch). Main effects: group $F(1,6) = 0.4089$, $p = 0.5461$; epoch $F(1,6) = 18.44$, $p = 0.0051$; group x epoch $F(1,6) = 7.846$, $p = 0.0311$. Sidak post-hoc opto-stimulation vs. non opto-stimulation: Controls $t(6) = 1.219$, $p = 0.4648$; ChrimsonR Rostral medNAcSh $t(6) = 4.488$, $p = 0.0083$; N = 5 Controls, 3 female 2 male; N = 3 ChrimsonR Rostral medNAcSh, 1 female, 2 male. **f.** Protocol for the full reward consumption experiment: mice had unlimited access to the reward spout. Blocks of 5 min with or without opto-stimulation were alternated (on/off/on/off/on) for a total of 5 blocks. **g.** Quantification of the lick counts per min, showing that ChrimsonR rostral medNAcSh mice eat less during on vs. off epochs as in **d.**; there is a trend for a similar observation for ChrimsonR caudal medNAcSh mice. No change across epochs for mCherry control mice, as in (**d.**). **h.** Quantification of mean licks per session in the opto-stimulation vs. non-opto-stimulation epochs shows a significant decrease in lick counts following stimulation of rostral, but not caudal medNAcSh D1-SPNs (and no change in mCherry controls) indicating that activation of rostral but not caudal medNAcSh D1-SPNs significantly decrease reward consumption. 2-way RM-ANOVA (group x epoch). Main effects: group $F(2,31) = 4.548$, $p = 0.0185$; epoch $F(1,31) = 10.58$, $p = 0.0028$; group x epoch $F(2,31) = 6.651$, $p = 0.0039$. Sidak post-hoc opto-stimulation vs. non opto-stimulation: Controls $t(31) = 0.2669$, $p = 0.9909$; Rostral medNAcSh $t(31) = 5.010$, $p < 0.0001$; Caudal medNAcSh $t(31) = 1.253$, $p = 0.5244$. N = 10 Controls, 5 female, 5 male; N = 13 Rostral medNAcSh, 6 female, 7 male; N = 11 Caudal medNAcSh, 4 female, 7 male. **i.** Pie charts showing % of mice showing food intake inhibition (mean Δlick counts non-opto/opto>0) in each group: 100% of ChrimsonR rostral medNAcSh mice, 30% of controls; and although caudal medNAcSh opto-stimulation does not lead to significant reward consumption inhibition as shown in **h.**, 64% of mice show an inhibition, indicating the effect is present but the effect size is too small to get captured statistically. Data is mean ± SEM. *p<0.05; **p<0.01; ***p<0.001. See also **Supplementary Figure S7**.

Rostral and caudal medNAcSh D1-SPNs show similar function in aversive assays

Previous work has shown that the medNAcSh plays strong roles in not only appetitive but also aversive behaviors (Al-Hasani et al., 2015; de Jong et al., 2019; Domingues et al., 2025; Liu et al., 2022; Yang et al., 2018; Zhou et al., 2022). For instance, Liu et al. showed that medNAcSh D1 projections to the ventral pallidum (VP) (but not D1 projections to the ventral tegmental area, VTA) are acutely activated by aversive stimuli and in turn optogenetic stimulation of these projections promotes aversion in a real time place preference vs. avoidance assay (RTTPA) (Liu et al., 2022). We therefore asked whether regulation of aversive behaviors by medNAcSh D1-SPNs differed along the rostro-caudal axis, as was the case for the reward consumption task (**Figure 1,2**). Using fiber photometry, we first recorded D1-SPN population activity in the rostral and caudal medNAcSh while mice were exposed to aversive stimuli in the form of 10 unpredicted shocks delivered at random intervals (**Figure 3a-b**). We found that both rostral and caudal D1-SPNs showed similar responses (albeit not fully overlapping in the period post-shock), with both groups depicting strong acute excitatory responses to the shocks (**Figure 3c,e**). This was reflected by a significant increase in the ΔF/F0 in the shock vs. pre-shock period in both groups (**Figure 3d,f**). Given prior reports of cell-type differences in NAcSh responses to aversive stimuli (Liu et al., 2022) and the absence of differences detected in our assay, our findings likely reflect a common activation of rostral and caudal D1-SPNs during acute aversive events. To further probe for potential regional differences, confirm the sensitivity of our approach and to ensure that this effect is not specific to a single stressor, we also assessed responses to a distinct aversive stimulus: tail lift, a recognized ethologically relevant stressor (**Figure 3g**). Here again, we identified similar acute excitatory responses in rostral and caudal D1-SPNs to the stimuli, reflected by significant increases in ΔF/F0 in the lift vs. pre-

241 lift period (**Figure 3h-k**). Hence, at the population level, rostral and caudal medNAcSh D1-SPNs similarly respond
242 to aversive stimuli. Finally, we determined whether direct optogenetic manipulation of these neurons would trigger
243 aversive responses in a well established RTPPA assay (Al-Hasani et al., 2015; Liu et al., 2022; Zhou et al., 2022)
244 (**Figure 4a-d**). We found that optogenetic stimulation of both rostral and caudal medNAcSh D1-SPNs promoted
245 avoidance of the opto-paired chamber, with no effects in controls, suggesting that stimulation of these neurons is
246 aversive. Quantification of locomotion showed no reduction in distance traveled in the light-paired chamber
247 (**Supplementary Figure S9c**), indicating that the avoidance was not driven by impaired locomotion. These data
248 indicate that medNAcSh D1-SPNs generally promote aversion without affecting locomotion and without major
249 differences along the rostro-caudal axis. This lack of functional differences between the rostral and caudal
250 medNAcSh contrasts with other studies showing divergent responses between, for example, medial and lateral
251 NAcSh (Chen et al., 2023) or medNAcSh D1-SPN projections to different downstream regions (Liu et al., 2022).
252 Altogether our data highlight the existence of a rostral D1 medNAcSh hotspot that is primarily restricted to an
253 appetitive feeding context.
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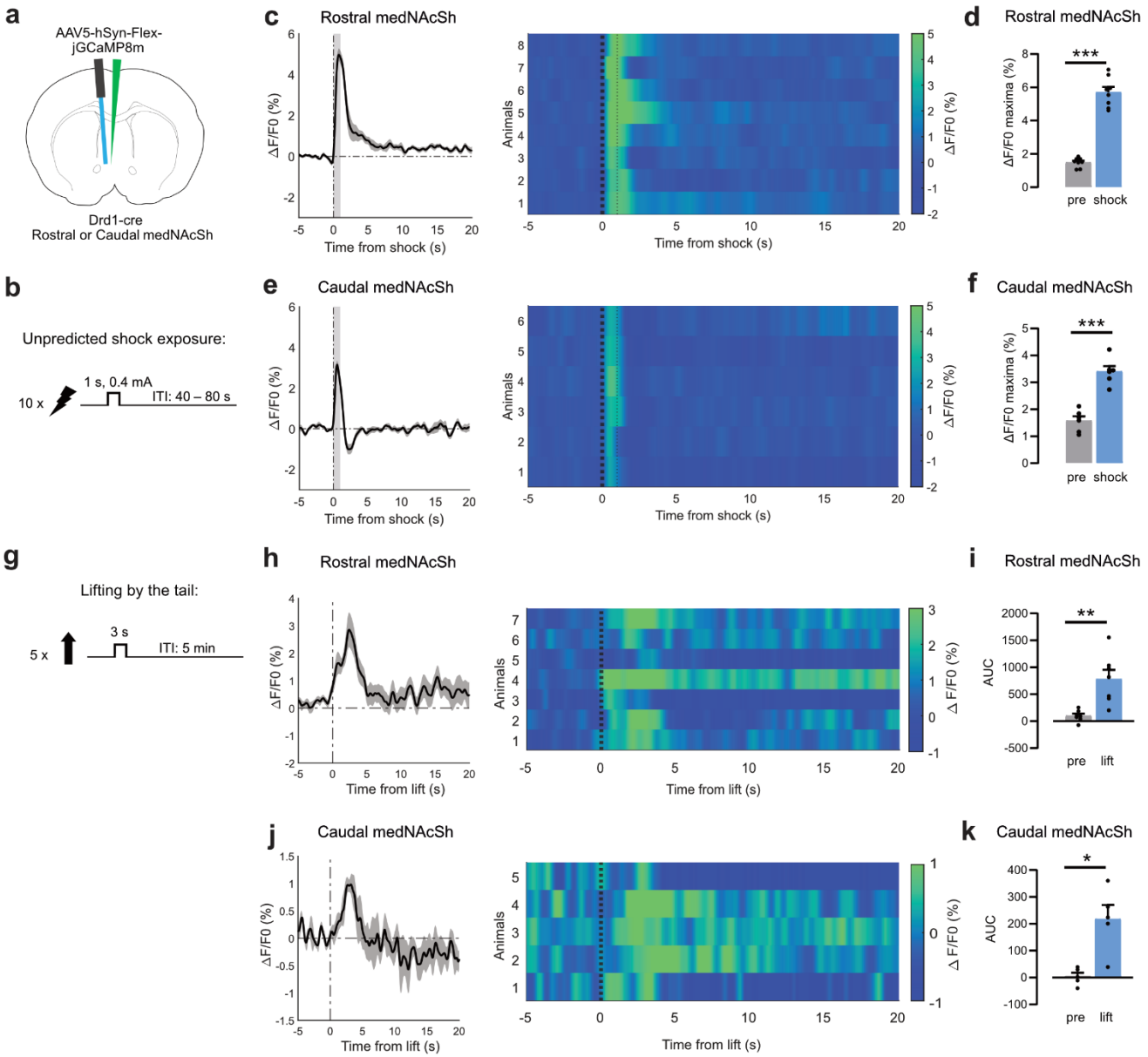

Figure 3. D1-SPNs in the rostral vs. caudal medNAcSh respond similarly to aversion. a. Surgery schematics of Drd1-cre mice injected with a cre-dependent calcium indicator (jGCaMP8m) and implanted with unilateral optic fibers in the rostral or caudal medNAcSh to image calcium photometry signals. **b.** Protocol for the unpredicted shock behavioral task: 10 shocks (0.4 mA) were available for 1 s at random intervals. **c.** Left: Average calcium activity in D1-SPNs (all trials) shown as normalized fluorescence (ΔF/F0, %) in the rostral medNAcSh over time aligned to shock onset (0 s), showing acute excitation at shock onset, followed by a slow return to baseline. Grey shading: shock epoch. Right: Heatmap showing calcium activity across individual trials (each row represents a trial) aligned to shock onset (0 s, thick dotted line). Thin dotted line: end of shock. **d.** Quantification of the data in (**c.**) depicts a significant increase in rostral medNAcSh activity upon shock onset, as shown by a significant increase in ΔF/F0 maxima in the 0 to 5 s shock epoch vs. -6 to -1s pre-shock epoch. Paired t-test, $t(7) = 14.91$, $p = 0.0001$. N = 8 mice, 2 female, 6 male. **e.** same as (**d.**) for the caudal medNAcSh showing acute excitation at shock onset, followed by a rapid return below zero before returning to baseline; the significance of this is unknown. **f.** same as (**d.**) for the caudal medNAcSh. Paired t-test, $t(5) = 8.312$, $p = 0.0004$. N = 6 mice, 4 female, 2 male. **g.** Protocol for the tail lift task: 5 tail lifts (3 s to lift the mouse, followed by 55 s stabilized in the air then back to the cage. Only the first 20 s are shown), at 5 min intervals. **h.** Left: Average calcium activity in D1-SPNs (all trials) shown as normalized fluorescence (ΔF/F0, %) in the rostral medNAcSh over time aligned to lift onset (0 s), showing acute excitation at

lift onset, followed by return to baseline. Right: Heatmap showing calcium activity across individual trials (each row represents a trial) aligned to lift onset (0 s, thick dotted line). **i.** Quantification of the data in (**h.**) depicts a significant increase in rostral medNAcSh activity upon lift onset, as shown by a significant increase in ΔF/F0 maxima in the 0 to 4 s lift epoch vs. -4 to 0 s pre-lift epoch. Paired t-test, t(6) = 4.214, p = 0.0056. N = 7 mice, 2 female, 5 male. **j.** same as (**h.**) for the caudal medNAcSh showing acute excitation at lift onset (starts at t = 0 s), followed by a rapid return below zero before returning to baseline; the significance of this is unknown. **k.** same as (**i.**) for the caudal medNAcSh. Paired t-test, t(4) = 4.435, p = 0.0114. N = 5 mice, 3 female, 2 male. Data is mean ± SEM. ns non-significant; *p<0.05; **p<0.01; ***p<0.001.

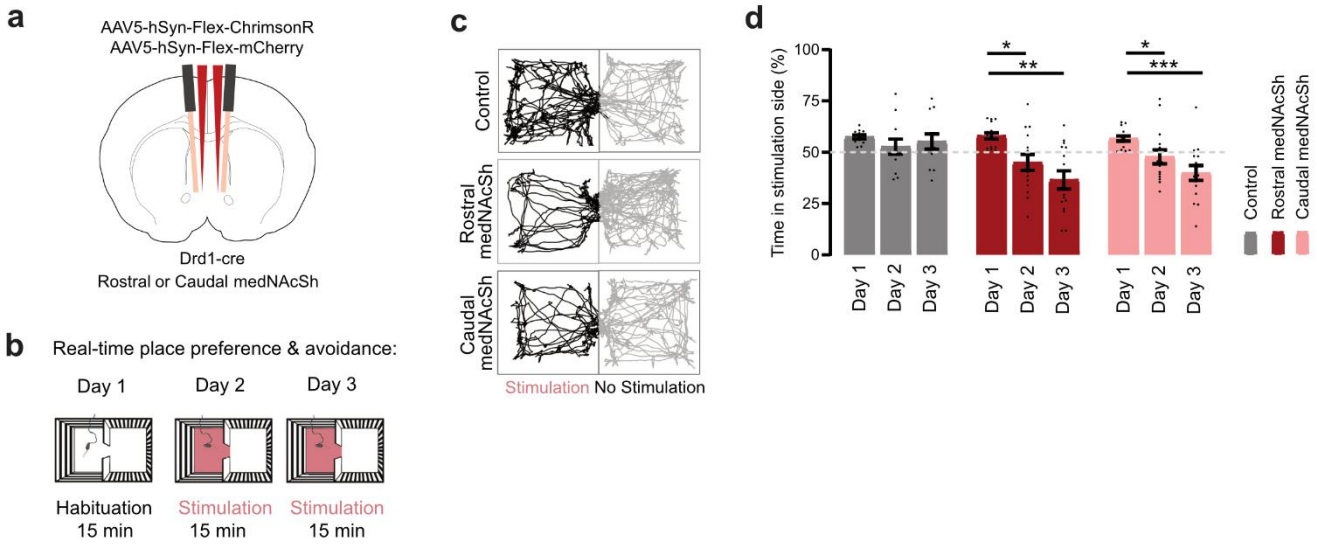

Figure 4. Optogenetic stimulation of D1-SPNs in the rostral and caudal medNAcSh lead to aversion. a. Surgery schematics of Drd1-cre mice injected with a cre-dependent activating opsin (ChrimsonR) or mCherry control AAV and implanted with bilateral optic fibers in the rostral or caudal medNAcSh to stimulate local D1-SPNs. **b**. Protocol for the real-time place preference or avoidance (RTPPA) behavioral task: After 1 habituation (unstimulated) day, mice were tested on 2 days where opto-stimulation occurred as soon as mice entered the laser-paired chamber (session duration 15 min). **c.** Representative locomotor patterns of animals in the RTPPA chamber on day 3 in all groups. **d.** Quantification of the percent (%) time spent in the stimulation side vs. non-stimulated side. Opto-stimulation led to a decrease in the time spent in the stimulation chamber in rostral medNAcSh and caudal medNAcSh mice but not in mCherry mice, indicating that activation of rostral and caudal medNAcSh D1-SPNs is aversive. 2-way RM-ANOVA (group x day). Main effects: group F(2,39) = 3.539, p = 0.0387; day F(1.822,71.05) = 19.08, p = 0.0001; group x day F(4,78) = 3.302, p = 0.0149. Sidak post-hoc day 2 vs. day 1: Controls t(11) = 1.232, p = 0.4277; Rostral medNAcSh t(14) = 2.951, p = 0.0209; Caudal medNAcSh t(14) = 2.700, p = 0.0342. Sidak post-hoc day 3 vs. day 1: Controls t(11) = 0.5776, p = 0.8195; Rostral medNAcSh t(14) = 4.250, p = 0.0016; Caudal medNAcSh t(14) = 4.661, p = 0.0007. N = 12 Controls, 7 female, 5 male; N = 15 Rostral medNAcSh, 7 female, 8 male; N = 15 Caudal medNAcSh, 7 female, 8 male. Data is mean ± SEM. ns non-significant; *p<0.05; **p<0.01; ***p<0.001.

Molecular rostro-caudal gradients across the medNAcSh highlight Stard5 and Peg10 as unique markers

305 Rostro-caudal functional differences in feeding could reflect distinct molecular compositions across the medNAcSh.
306 Indeed, recent work using fluorescent in-situ hybridization (FISH) or single-cell RNA sequencing (scRNAseq) has
307 delineated the strong molecular and spatial diversity within the NAc (Chen et al., 2021; Stanley et al., 2020),
308 identifying cellular clusters within D1-SPNs and/or D2-SPNs that express combinations of marker genes and are
309 often enriched in spatial subregions of the NAc. Hence it is possible that unique molecular markers are enriched in
310 the rostral vs. caudal medNAcSh and contribute to functional differences. We used published in-situ hybridization
311 datasets from the Allen Mouse Brain Atlas to identify novel molecular markers enriched in one of these two
312 subregions. Screening 48 markers flagged by the Allen Brain Atlas as expressing in the NAc, we found that most
313 genes showed relatively stable expression across the rostro-caudal axis (e.g. Rasal1, LOC381076, Stra6, Lypd1;
314 which are representative examples shown in **Supplementary Figure S2**). However, we detected 5 genes depicting
315 differential rostro-caudal gradient expression: Stard5, Smug1 and Cartpt were enriched in the rostral medNAcSh
316 while Peg10 and Sgsm1 were enriched in the caudal medNAcSh (**Figure 5a**), which was also confirmed by optical
317 density analysis (**Figure 5b**). Among all markers, Stard5 and Peg10 showed particular strong enrichment in the
318 rostral and caudal medNAcSh, respectively, as opposed to neighboring regions (**Figure 5b**). These data
319 demonstrate the existence of molecular gradients across the rostro-caudal axis of the medNAcSh and highlight
320 Stard5 and Peg10 as markers enriched in rostral vs. caudal medNAcSh, respectively.
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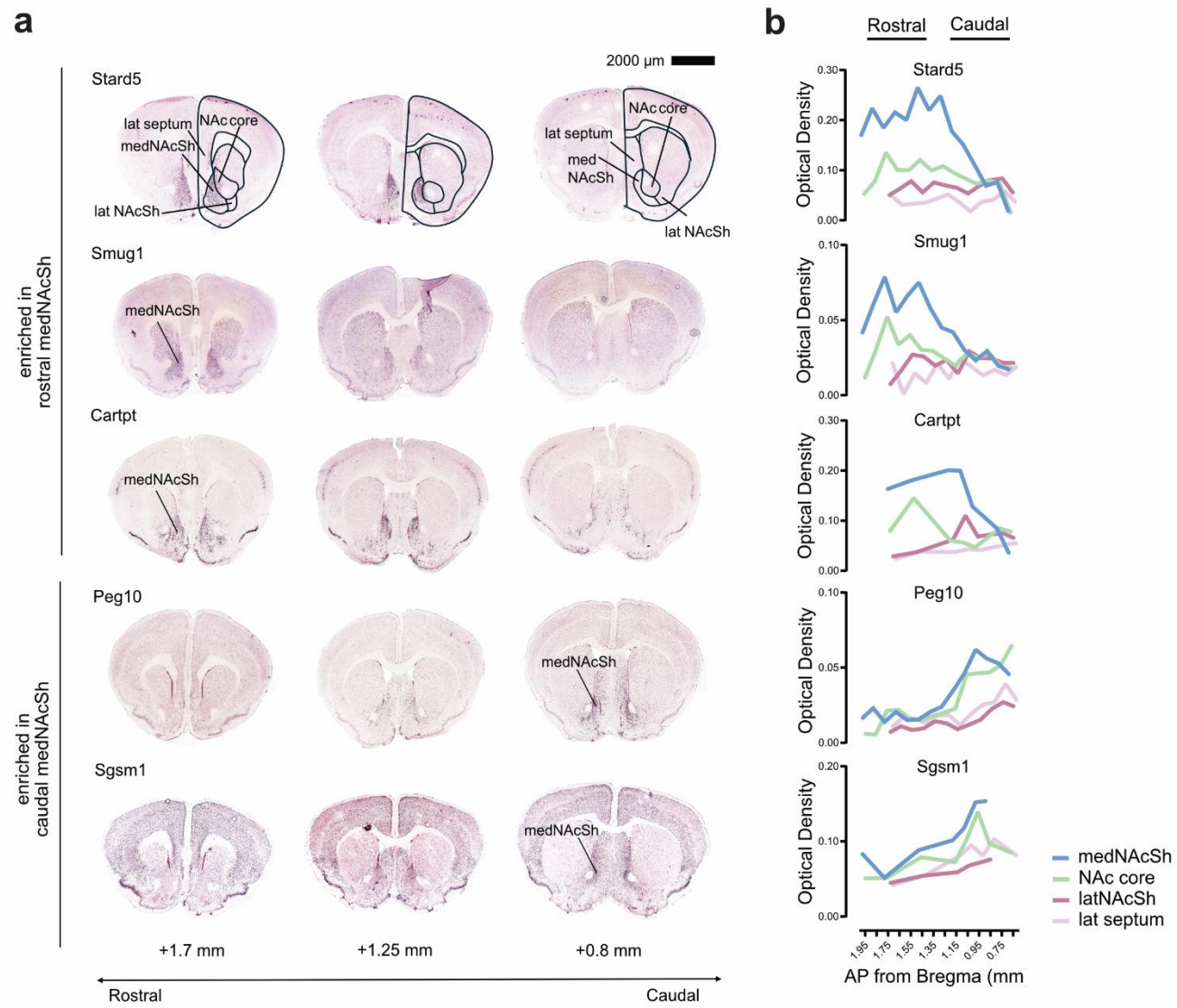

Figure 5. Stard5 is enriched in the rostral region and Peg10 in the caudal region of the medNAcSh, respectively. a. Representative in-situ hybridization images obtained from the Allen Brain Atlas (https://mouse.brain-map.org/) for 5 markers showing enriched expression in the rostral medNAcSh (Stard5, Smug1, Cartpt) or the caudal medNAcSh (Peg10, Sgsm1). Data for the NAc core, lateral NAcSh (latNAcSh) and lateral septum (lat septum) are also shown. All 48 markers available in the Allen Brain Atlas labeling the nucleus accumbens were screened. Images on the left are more rostral (bregma +1.65 mm); images on the right are more caudal (bregma +0.85 mm). **b.** Quantification of expression levels of markers from a. in the medNAcSh along the rostral-caudal axis (bregma +1.95 mm to +0.75 mm) using optical density measurements (n = 1 mouse, 7-14 sections total, data shown is the mean from 2 measures/section). Stard5, Smug1 and Cartpt show enrichment in the rostral medNAcSh with Stard5 showing the strongest total expression and strongest relative expression in medNAcSh as compared to other regions. Peg10 and Sgsm1 show enrichment in the caudal medNAcSh with Peg10 showing the strongest relative expression in medNAcSh as compared to latNAcSh and lateral (lat) septum (but not NAc core). See also **Supplementary Figure S2**.

We analyzed a published scRNAseq dataset of the entire NAc (core and shell) (Chen et al., 2021) to further characterize the cellular expression patterns of Stard5 and Peg10 (**Figure 6a-c**, see also below). We found that, in the NAc, Stard5 is expressed predominantly in D1-SPNs (34.91% of Stard5+ cells) and D2-SPNs (30.41%) with

340 lesser expression in other cell types, mostly interneurons (17,60%) (**Supplementary Figure S3a**). A similar pattern
341 was observed for Peg10 with strongest expression in D1-SPNs (36.52% of Peg10+ cells), D2-SPNs (26.69%) and
342 interneurons (33.13%) (**Supplementary Figure S3b**).
343 Since Stard5 and Peg10 are expressed in both D1- and D2-SPNs, we performed triple FISH to further
344 characterize their expression profiles within these two main cell populations (D1- and D2-SPNs) across subregions
345 of the NAc (**Figure 6d,f**). First, we confirmed that Stard5 is strongly expressed within the D1-SPN (30.62% of Drd1+
346 cells) and D2-SPN (32.47% of Drd2+ cells) populations amongst the entire NAc, similar to what we see with
347 scRNAseq 'entire NAc' data (29.55% of Drd1+ cells and 38.19% of Drd2+ cells) (**Figure 6b**). Importantly, subregion
348 analyses revealed that Stard5 is predominantly expressed in the rostral and intermediate sections of the
349 medNAcSh, in particular its dorsal part, precisely the rostral feeding hotspot identified in this study and others (Reed
350 et al., 2018; Reynolds and Berridge, 2001; Richard and Berridge, 2011) (**Figure 6e**). Stard5 was expressed in a
351 large majority of D1-SPNs (79% of Drd1+ cells) and D2-SPNs (76% of Drd2+ cells) in the dorsal rostral medNAcSh
352 (**Figure 6e**), hence representing an almost ubiquitous marker for SPNs in this region. Stard5 expression in D1-
353 SPNs and D2-SPNs gradually decreased when moving more caudally, ventrally and laterally into the NAc core and
354 lateral shell (**Figure 6e**), confirming that its expression follows a molecular gradient rather than a binary on/off
355 expression. We performed the same FISH analysis for Peg10, and found a similar gradient pattern with strongest
356 expression in the caudal medNAcSh and gradually decreasing expression moving more rostrally, ventrally and
357 laterally (**Figure 6f,g**). In the caudal medNAcSh, Peg10 was expressed in 16.45% of D1-SPNs (Drd1+) and 13.34%
358 of D2-SPNs (Drd2+), similar to what we see with scRNAseq 'entire NAc' data (16.72% of Drd1+ cells and 16.09%
359 of Drd2+ cells) (**Figure 6c**). Moreover, in caudal sections, Peg10 was enriched in dorsal and ventral medNAcSh
360 D1-SPNs (33% dorsal and 31% ventral, of Drd1+ cells) similar to Stard5; while it showed relatively stable expression
361 across NAc subregions for D2-SPNs (i.e. no medNAcSh enrichment) (**Figure 6g**). In summary, we confirm the
362 existence of molecular gradients across the rostro-caudal medNAcSh axis, revealing Stard5 as a strong marker for
363 both types of SPNs in the rostral medNAcSh feeding hotspot, in particular its dorsal part.
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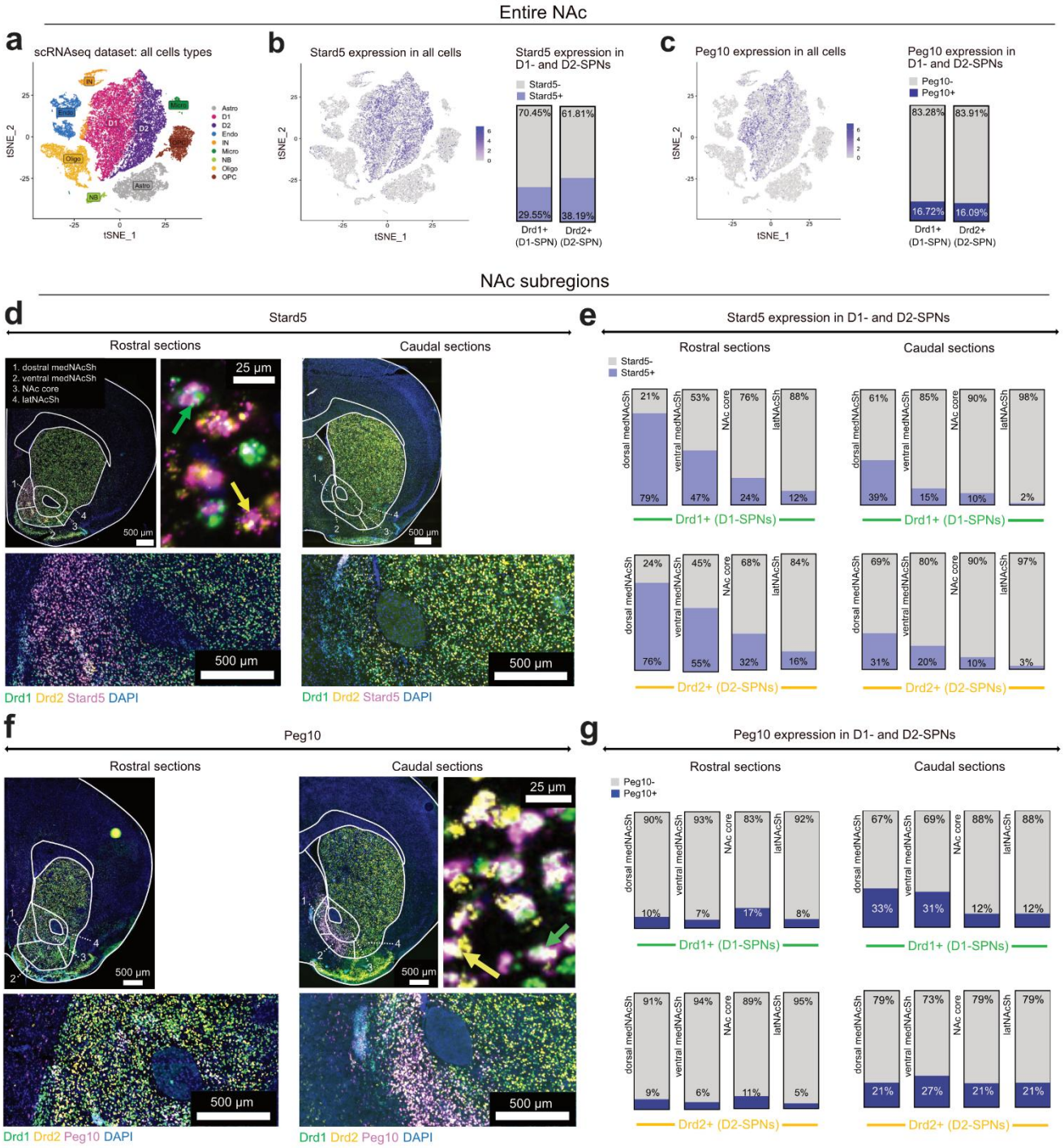

Figure 6. Stard5 is enriched in D1- and D2-SPNs of the rostral medNAcSh. a. t-SNE plot showing cell types in the entire NAc based on re-analysis of a published scRNAseq dataset, GSE118020 (Chen et al., 2021). Data is pooled from 11 mice, 36670 cells. Different cell clusters are color-coded. Clusters mark cell types identified by canonical markers, as used in (Chen et al., 2021). Astro: astrocytes, D1: D1-SPN, D2: D2-SPN, Endo: endothelial cells, IN: interneurons, Micro: microglia, NB: neuroblasts, Oligo: oligodendrocytes, OPC: oligodendrocyte progenitor cells. **b.** Left: t-SNE plots showing expression of Stard5 across NAc cells; the densest expression is found in the D1- and D2-SPNs clusters. Stard5 expression level is color-coded from 0 (low) to 6 (high). Right: Quantification of cells positive or negative for Stard5 within D1- and D2-SPNs (identified by Drd1 and Drd2 expression). About 1/3 of D1- and 1/3 of D2-SPNs express Stard5. **c.** Left: t-SNE plots showing expression of Peg10 across NAc cells; the

379 densest expression is found in the D1-SPN, D2-SPN and interneuron clusters. Peg10 expression level is color-
380 coded from 0 (low) to 6 (high). Right: Quantification of cells positive or negative for Peg10 within D1- and D2-SPNs
381 (identified by Drd1 and Drd2 expression). A small proportion (16 to 17%) of D1- and of D2-SPNs express Peg10.
382 **d.** Left: Representative image of a fluorescent in-situ hybridization (RNAscope) assay for Drd1, Drd2 (labelling D1-
383 and D2-SPNs) and Stard5 in subregions of the rostral and caudal sections of the NAc (dorsal and ventral
384 medNAcSh, NAc core and latNAcSh). Lower panel: zoom-in and Right panel: high resolution magnification showing
385 Stard5+,Drd1+ (green arrow) or Stard5+,Drd2+ (yellow arrow) neurons. **e.** Quantification of expression of Stard5 in
386 D1- and D2-SPNs in subsections of the NAc (dorsal and ventral medial [med] NAcSh, NAc core and lateral [lat]
387 NAcSh) in the rostral and caudal regions of the NAc. An average of 6-8 sections in total, including 2-3 for most
388 rostral sections and 2-3 for most caudal sections; and 5 mice in total were analyzed. Data shows strong enrichment
389 (75-80%) of Stard5 in D1- and D2-SPNs of the rostral, dorsal medNAcSh (and much less in the caudal medNAcSh
390 or other NAc regions). See also **Supplementary Figure S3** showing minor Stard5 expression in other cell types. **f.**
391 Same as (d.) for Peg10. **g.** Same as (e.) for Peg10 (n= 5 mice). Data shows enrichment of Peg10 in D1- and D2-
392 SPNs of the dorsal and ventral caudal medNAcSh (very little expression in the rostral medNAcSh). See also
393 **Supplementary Figure S3** showing Peg10 expression in interneurons (in addition to D1- and D2-SPNs).
394
395 **Medial NAcSh Stard5 cells recapitulate phenotypes of rostral medNAcSh D1-SPNs**
396 Stard5 could represent an interesting molecular marker to label or functionally target cellular populations in the
397 rostral medNAcSh feeding hotspot. To evaluate this possibility, we used CRISPR/Cas9 genetic engineering to
398 generate a new driver line, Stard5-Flp, expressing the Flp recombinase in Stard5+ cells (**Figure 7a,**
399 **Supplementary Figure S4**), i.e. primarily in Stard5+ D1-, D2-SPNs and interneurons. Stard5-Flp-positive mice
400 bred well, were viable and showed no behavioral or metabolic abnormalities as compared to Stard5-Flp-negative
401 mice indicating absence of obvious genomic off-target expression (**Supplementary Figure S5**). Stard5-Flp mice
402 were injected with an AAV expressing a Flp-dependent fluorophore (jGCaMP8m) into the rostral medNAcSh or in
403 neighboring regions (**Figure 7b**). We found strong fluorophore expression in the rostral medNAcSh but very low or
404 no expression when injecting in the neighboring NAc core and dorsal striatum, respectively (**Figure 7c**), confirming
405 that Stard5 expression is enriched in the medNAcSh as opposed to neighboring regions (**Figure 6**). Very low ectopic
406 expression was found in Stard5-Flp negative mice (a ratio 1:16 vs Stard5-Flp positive mice, **Figure 7c**), confirming
407 minimal (but not entirely absent) Flp leakage of the AAV construct, a very common yet often ignored issue, which
408 could be mitigated in the future using modern AAV design strategies (Fischer et al., 2019).
409 Finally, we used the Stard5-Flp mouse to determine whether Stard5 cells recapitulate some of the feeding-
410 related phenotypes of rostral medNAcSh D1-SPNs. Stard5-Flp mice were injected in the rostral medNAcSh with an
411 AAV expressing a synaptophysin tagged Flp-dependent EGFP to evaluate the projection sites of rostral medNAcSh
412 Stard5+ cells. We found strong expression in the VP, VTA and lateral hypothalamus (LH) (**Supplementary Figure**
413 **S6**), confirming that Stard5+ cells project to the three classical projection targets of medNAcSh D1-SPNs (see
414 **Supplementary Figure S1**) and D2-SPNs. Stard5-Flp mice were then injected with an AAV expressing a Flp-
415 dependent calcium indicator (jGCaMP8m) and optic fibers implanted above the rostral medNAcSh to record the
416 population activity of Stard5+ cells (**Figure 7d,e**). Similarly to rostral medNAcSh D1-SPNs, we found that Stard5+
417 cells pause during reward consumption (**Figure 7f-h**), and get acutely excited upon exposure to aversive stimuli
418 (**Figure 7f,i-j**). This confirms that Stard5+ cells recapitulate some of the behavioral phenotypes of rostral
419 medNAcSh D1-SPNs. It further suggests that Stard5 is a molecular marker of the rostral medNAcSh feeding
420 hotspot.
421

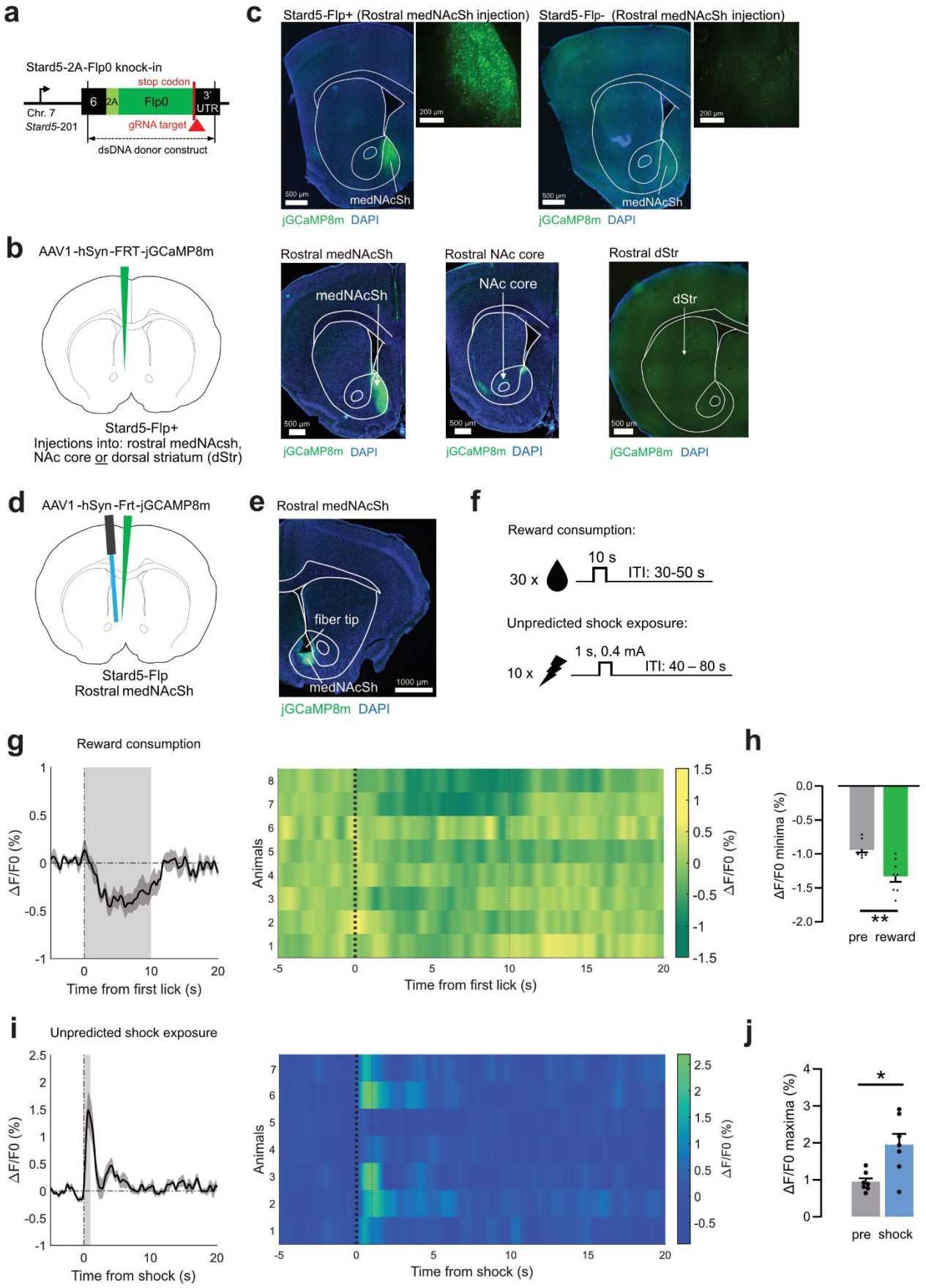

Figure 7. Stard5 is a molecular marker for the rostral medNAcSh feeding hotspot. a. CRISPR/Cas9-based strategy to generate a Stard5-2A-Flp0 knock-in mouse (abbreviated Stard5-Flp). A double-stranded (ds) DNA knock-in template expressing the Flp0 recombinase (dark green bands), the RAKR and T2A self-cleaving peptide

426 (light green bands) and Stard5 homology arms spanning exon 6 and the 3'UTR of the Stard5 gene (black bands)
427 was used to create the model. The guide (g)RNA (red triangle) targets the stop codon (blue) of the Stard5 gene
428 (Chromosome 7) at its most expressed transcript in the NAc (Stard5-201; see **Supplementary Figure S4**). Knock-
429 in was achieved by homologous recombination. **b.** Left: Surgery schematic for the validation of the Stard5-Flp
430 mouse. An AAV expressing a flp-dependent vector (jGCaMP8m) was injected into the rostral or caudal medNAcSh,
431 dorsal striatum or NAc core of Stard5-Flp mice. **c**. Top: Representative coronal images and zoom-in showing
432 successful expression of a Flp-dependent fluorophore in the rostral medNAchSh of Stard5-Flp mice, with very
433 minimal expression in Stard5-Flp negative mice (few cells) indicating a minor Flp leak of the AAV. Close to no
434 expression could be detected in the caudal medNAcSh, NAc core or dorsal striatum (dStr) after injection directly
435 into those regions, confirming that, within the striatal complex, Stard5 expression is primarily confined to the
436 medNAcSh. **d.** Surgery schematics of Stard5-Flp mice injected with a flp-dependent calcium indicator (FRT-
437 jGCaMP8m) and implanted with unilateral optic fibers in the rostral medNAcSh to image calcium photometry
438 signals. **e.** Representative coronal image of rostral medNAcSh Stard5-Flp mice expressing FRT-jGCaMP8m and
439 implanted with an optic fiber. **f.** Top: Protocol for the unpredicted reward behavioral task: 30 rewards were available
440 for 10 s at random intervals. Bottom: Protocol for the unpredicted shock behavioral task: 10 shocks (0.4 mA) were
441 available for 1 s at random intervals. **g.** Left: Average calcium activity in Stard5+ neurons (all trials) shown as
442 normalized fluorescence (ΔF/F0, %) in the rostral medNAcSh over time aligned to the onset of reward consumption,
443 i.e. first lick onset (0 s). Grey shading represents the ~10 s epoch for reward consumption. Right: Heatmap showing
444 calcium activity across individual trials (each row represents an animal) aligned to reward consumption. Thin dotted
445 line: end of reward access. The average number of successful lick trials was 18.5 out of 30 (62%). **h.** Quantification
446 of the data in (g.) depicts a significant decrease in rostral medNAcSh Stard5 cell activity upon reward consumption,
447 as shown by a significant decrease in ΔF/F0 minima in the 0 to 5 s reward epoch vs. -6 to -1 s pre-reward epoch.
448 Paired t-test, $t(7) = 4.630$, $p = 0.0024$. N = 8 mice, 4 female, 4 male. **i.** Left: Average calcium activity in Stard5+
449 neurons (all trials) shown as normalized fluorescence (ΔF/F0, %) in the rostral medNAcSh over time aligned to
450 shock onset (0 s). Grey shading: shock epoch. Right: Heatmap showing calcium activity across individual trials
451 (each row represents a trial) aligned to shock onset. Thin dotted line: end of reward access. **j.** Quantification of the
452 data in (i.) depicts a significant increase in rostral medNAcSh Stard5 cell activity upon shock exposure, as shown
453 by a significant increase in ΔF/F0 maxima in the 0 to 5 s shock epoch vs. -6 to -1 s pre-shock epoch. Paired t-test,
454 $t(6) = 3.305$, $p = 0.0163$. N = 7 mice, 1 female, 6 male. Data is mean ± SEM. *p<0.05; **p<0.01; ***p<0.001. See
455 also **Supplementary Figures S4, S5, S6**.
456
457
458 **DISCUSSION**
459 In recent years, the medNAcSh has gained increasing appreciation as a key brain region that controls hedonic
460 feeding (reviewed in (Marinescu and Labouesse, 2024). At the cellular level, studies have shown that D1-SPNs,
461 are one of the primary cellular mediators of these effects as they can rapidly and powerfully regulate the amount of
462 food consumed in rodents independent of metabolic need (O'Connor et al., 2015; Thoeni et al., 2020; Bond et al.,
463 2020; Maldonado-Irizarry et al., 1995; Stratford and Kelley, 1997). At the topographical level, in situ pharmacological
464 studies have indicated the existence of a rostro-caudal gradient in the medNAcSh, suggesting that its rostral but
465 not caudal subregion is the primary hotspot for feeding regulation (Castro et al., 2016; Castro and Berridge, 2014;
466 Krause et al., 2010; Reed et al., 2018; Reynolds and Berridge, 2008, 2002; Richard and Berridge, 2011). However,
467 the cellular, circuit and molecular mechanisms of this gradient were, this far, unclear. Here, we combined fiber
468 photometry, optogenetic and neuroanatomical experiments, identifying a strong functional dichotomy between

469 rostral and caudal medNAcSh D1-SPNs in feeding behavior, but not in aversive assays, thus demonstrating the
470 existence of a rostral medNAcSh D1-SPN feeding hotspot. Using open-source ISH, scRNAseq datasets and in-
471 house FISH experiments, we show that functional gradients in the medNAcSh are paralleled by molecular gradients
472 along the rostro-caudal axis, delineating the existence of Stard5 and Peg10 as markers of the rostral and caudal
473 medNAcSh, respectively. Finally, we use our newly developed Stard5-Flp driver mouse line, finding that Stard5
474 cells replicate functional phenotypes of the rostral D1 feeding hotspot. This work provides insights into the functional
475 and molecular organization of the medNAcSh in the context of feeding and aversion behavior and delineates a
476 novel marker, Stard5, to access the rostral medNAcSh feeding hub. Importantly, our results indicate that spatial
477 organization also defines functional specialization in the medNAcSh, and that molecular markers such as Stard5
478 provide access to these spatially defined subterritories rather than labeling a single, homogenous neuronal subtype.
479
480 Our work extends previous research delineating a behavioral and anatomical dichotomy between rostral and caudal
481 sections of the medNAcSh (Baumgartner et al., 2020; Castro et al., 2016; Castro and Berridge, 2014; Faure et al.,
482 2010; Krause et al., 2010; Pedersen et al., 2022; Reed et al., 2018; Reynolds and Berridge, 2008, 2002; Richard
483 and Berridge, 2011) and align with similar concepts demonstrated in the dorsal striatum (Hintiryan et al., 2016;
484 Valjent and Gangarossa, 2021). In our photometry work, we show that at the population level, rostral medNAcSh
485 D1-SPNs pause, while caudal medNAcSh are acutely excited upon reward consumption. This finding corroborates
486 past work showing that forebrain excitatory inputs into the medNAcSh pause during consumption at rostral but not
487 caudal medNAcSh sites (Reed et al., 2018) and that individual D1- and D2-SPNs show rostro-caudal gradients in
488 their responses to feeding (Pedersen et al., 2022). We also find that optogenetic stimulation of D1-SPNs in the
489 rostral medNAcSh suppress reward consumption, while there was a trend for an eating-inhibitory effect that was
490 not significant in the caudal medNAcSh, suggesting the existence of a gradient rather than a binary on/off
491 phenomenon. In addition, comparison of licking behavior during the laser-off blocks revealed an interesting effect:
492 following cessation of opto-stimulation, Rostral ChrimsonR mice licked more than Caudal ChrimsonR and Control
493 mice, suggesting a possible compensatory overconsumption. One possible interpretation is that the optogenetic
494 parameters used suppressed consummatory behavior without reducing the motivation to obtain the reward.
495 Furthermore, consistent with the RTPPA results, activation of rostral D1-SPNs may be experienced as aversive
496 and termination of the optogenetic stimulation could produce relief, which in turn reinforces the licking behavior.
497 Further investigations are required to test these possibilities. Importantly, our behavioral effects could not be
498 explained by reduced locomotor activity; indeed locomotion was either unchanged or increased in a small cohort of
499 Rostral ChrimsonR mice following optogenetic stimulation. The increased locomotion likely reflected appetitive
500 behavior and is consistent with past chemogenetic studies (Zhu et al., 2016).
501 Of note, in this paper we decided to use the term “rostro-caudal gradient”, motivated by converging evidence
502 from prior pharmacological studies (see below) and scRNA sequencing data (Chen et al., 2021; Stanley et al.,
503 2020), which show continuous molecular and functional changes along the rostro-caudal axis of the medNAcSh
504 rather than sharply defined boundaries. Our use of the term ‘gradient’ therefore reflects this established molecular
505 organization, even though our functional experiments sampled only two representative positions along this
506 continuum. Our findings align with previous studies identifying rostro-caudal gradients in the medNAcSh, which
507 found that *in situ* infusion of the glutamate antagonist DNQX or the GABA agonist muscimol in the rostral
508 medNAcSh promotes appetitive responses like feeding. The DNQX effects were also suppressed by a D1 but not
509 D2 antagonist (Reynolds and Berridge, 2001; Richard and Berridge, 2011).
510 Interestingly, studies also found that DNQX or muscimol infusions into the caudal, but not rostral, medNAcSh
511 promote aversive behaviors indicating the existence of rostro-caudal gradients also for aversion (Reynolds and

512 Berridge, 2001; Richard and Berridge, 2011). Here, we further tested the existence of rostro-caudal gradients for
513 aversion, asking whether D1-SPNs in the rostral vs. caudal medNAcSh respond differently to aversive stimuli. To
514 ensure that any observed effects were not specific to a single stressor, we tested two distinct aversive stimuli (foot
515 shock and tail lift). In both cases, we found no rostro-caudal differences, as D1-SPNs in both subregions responded
516 with excitation. We also asked whether optogenetic activation of these neurons is perceived as aversive. Stimulation
517 of D1-SPNs in both rostral and caudal medNAcSh promoted aversive behavioral responses in the RTPPA
518 experiment. Hence, in contrast to the pharmacological inhibitions mentioned above, we did not detect differences
519 in aversive behaviors according to the rostro-caudal medNAcSh site. Here again, our behavioral effects were not
520 explained by reduced locomotor activity as no locomotion differences were detected in the RTTPA.

521 The lack of divergent responses within subregions of the medNAcSh in aversion contrasts with published
522 work identifying differences in either the photometry activity patterns or the RTTPA functional responses when
523 comparing medial to lateral NAcSh (Chen et al., 2023) or medNAcSh D1-SPN projections to VP vs. VTA (Liu et al.,
524 2022). In our study, the lack of subregional differences could be due to the fact that our injection sites in rostral and
525 caudal medNAcSh were too close to each other to distinguish region-specific effects. However, in a subset of
526 animals we validated and confirmed accurate injection and optic fiber placements within the intended medNAcSh
527 subregions. Moreover, since we did detect significant differences between rostral and caudal medNAcSh in the
528 reward consumption photometry and optogenetic assays, this suggests that injection site proximity was not a
529 limiting factor. Future studies using minimal AAV injection volumes and/or tapered optic fibers (Pisanello et al.,
530 2017) would help to more precisely restrict the manipulation, thereby enabling refined dissection of spatially
531 confined subregions. Another interesting possibility is that D2-SPNs mediate such rostro-caudal aversive gradient,
532 a cell type we did not manipulate in the present study, which represents a limitation of this work. This possibility
533 would align with previous work showing that the modulation of aversive behaviors by the caudal medNAcSh requires
534 both D1Rs and D2Rs (Richard and Berridge, 2011).

535 Intriguingly, past studies found that rostro-caudal gradients are plastic, i.e. they can be retuned by the valence
536 of the environment. While in a standard test chamber, rostral DNQX promotes feeding and caudal DNQX promotes
537 aversion, the feeding zone expands caudally in a calm, home-like condition, while the aversion zone expands
538 rostrally in a highly stressful condition (Baumgartner et al., 2020; Reynolds and Berridge, 2008; Richard et al., 2013;
539 Richard and Berridge, 2011). Future work could address whether the same environmental retuning impacts the
540 behavioral effects of medNAcSh D1-SPNs, and in turn what are the possible molecular or circuit mediators of these
541 effects. Moreover, it would be interesting to determine if the food consumption effects of D1-SPN optogenetic
542 manipulation are related to the avoidance effects in the RTPPA assay, i.e. is the inhibition of food consumption due
543 to an internal state of aversion. Since we show that optogenetic stimulation of the caudal medNAcSh induces
544 aversion without suppressing food consumption, this suggests that aversive signaling and feeding behavior can be
545 dissociated. Instead, our findings support the idea that D1-SPNs in the rostral medNAcSh function as a neural hub
546 at the intersection of reward processing and motor (consumption) output. Future studies should dissect this further.

547 We here determined the projection patterns of medNAcSh subregions and find that the rostral and caudal
548 medNAcSh both project to the LH (**Supplementary Figure S1**), an important feeding center (Rossi, 2023). Although
549 in this study we did not quantify the density of projections, previous data identified a stronger medNAcSh to LH
550 projection arising from rostral as compared to caudal medNAcSh sections (Thoeni et al., 2020). Such differential
551 projections to the LH could explain behavioral differences between these two brain subregions as multiple studies
552 have demonstrated a dominant role for the medNAcSh to LH projection in the D1-SPN eating effects (O'Connor et
553 al., 2015; Stratford and Kelley, 1999; Thoeni et al., 2020). Since both LH GABA and LH glutamate neurons receive
554 D1-SPN input (Liu et al., 2024; O'Connor et al., 2015; Thoeni et al., 2020; Zhou et al., 2022), future studies could

555 investigate the relative contribution of these two cell types in the feeding effects of rostral medNAcSh D1-SPNs.
556 The contribution of rostral medNAcSh D1-SPN projections to the VP and VTA (Bond et al., 2020; Pedersen et al.,
557 2022) should also be addressed in future work. Moreover, whether D2-SPNs also regulate behavior differentially
558 according to a rostro-caudal axis remains to be determined. Optogenetic activation of D2-SPNs in the medNAcSh
559 did not affect feeding (O'Connor et al., 2015), but it is possible that rostro-caudal differences emerge when
560 restricting manipulations to either subregion. Moreover, a recent preprint indicated that medNAcSh D2-SPNs, in
561 particular its projections to the VP, regulate reward preference (Pedersen et al., 2022), while other studies have
562 shown that the medNAcSh regulates not only food consumption but also food liking, food wanting (Castro and
563 Berridge, 2014) and extinction in a Pavlovian task (Domingues et al., 2025). Additionally, a new study showed that
564 manipulation of D2-SPN cell bodies in the medNAcSh modulates reward preference, self-stimulation, and palatable
565 food intake in a frequency- and context-dependent manner (Requejo-Mendoza et al., 2025). Together, these
566 findings suggest that D1- and D2-SPNs within the medNAcSh play complementary rather than opposing roles in
567 reward processing. Hence, the potential role of rostral and caudal medNAcSh D1- and D2-SPNs in food-related
568 behaviors beyond the act of consumption could be addressed in future work.
569 Other possible circuit mechanisms could explain the rostro-caudal functional differences identified here, in
570 particular the differential circuit inputs into the rostral vs. caudal medNAcSh. It is well established that the
571 medNAcSh receives strong excitatory input from the ventral hippocampus (vHipp), amygdala (Amy), paraventricular
572 nucleus of the thalamus (PVT) and prefrontal cortex (PFC) (reviewed in (Marinescu and Labouesse, 2024)).
573 Interestingly, previous work found that these forebrain regions innervate the rostral and caudal medNAcSh via
574 distinct cellular subpopulations (Reed et al., 2018). vHipp inputs to the rostral medNAcSh were also found to be
575 stronger as compared to the caudal medNAcSh (Trouche et al., 2019). Moreover, optogenetic inhibition of excitatory
576 inputs into the rostral medNAcSh promotes feeding, while similar phenotypes are not detected in the caudal
577 medNAcSh (Reed et al., 2018). Hence, it is very likely that differential innervation patterns into the rostral vs. caudal
578 medNAcSh explain the emergence of a rostral feeding hotspot. Previous work demonstrated that, in addition to
579 excitatory forebrain inputs, the VP also sends strong inhibitory inputs into the medNAcSh and directly regulates
580 feeding (Vachez et al., 2021); future work could address the rostro-caudal innervation patterns of these VP
581 arkypallidal cells and their contributions to functional differences.
582
583 Having established a robust functional dichotomy of D1-SPNs along the rostro-caudal axis in reward consumption,
584 we next asked whether this functional organization is mirrored by differences in molecular composition across the
585 medNAcSh. Using multiple anatomical techniques, we find strong differences in the molecular composition of the
586 rostral vs. caudal medNAcSh, which in turn could explain behavioral differences between these brain subregions.
587 Indeed, we detected multiple markers showing enriched expression in the rostral vs. caudal medNAcSh. In
588 particular, we identified and later validated Stard5 as a molecular marker labeling the rostral medNAcSh and Peg10
589 the caudal medNAcSh, respectively. Future work using spatial transcriptomics could extend these findings to
590 identify further relevant rostro-caudal markers at the transcriptome-wide level (Piwecka et al., 2023). Importantly,
591 we find that molecular markers do not exhibit a binary on/off expression pattern but rather demonstrate a gradient
592 like pattern, being enriched vs. depleted in specific subregions of the NAc. This aligns with previous scRNAseq and
593 MERFISH studies, highlighting the existence of spatial gradients of molecularly defined cells within the NAc or
594 dorsal striatum (Chen et al., 2021; Stanley et al., 2020; M. Zhang et al., 2023). Authors outlined the existence of
595 two types of markers: 1) cluster markers that selectively mark rare and unique cellular subtypes and 2) spatial
596 markers that are broadly expressed in a spatially restricted manner within NAc subregions. One example of the
597 former are Serpinb2 and Tac2, which labeled small and separate clusters of D1-SPNs in the medial NAc (Chen et

al., 2021) and regulate behavior in a non-canonical manner (Liu et al., 2024; Zhao et al., 2022a). Here we build on past findings (Chen et al., 2021; Stanley et al., 2020), showing that Stard5 is one of the latter, namely a spatial marker for a spatially defined subterritory, the dorsal part of the rostral medNAcSh. Rather than labeling a unique rare cell type, Stard5 expression is almost ubiquitous within D1- and D2-SPNs in that subregion (up to 80% of SPNs), making it a noteworthy marker to broadly target the projections of this functionally unique NAc subregion, the rostral medNAcSh feeding hotspot. This makes Stard5 a spatial molecular landmark that captures the cellular ensemble of the rostral feeding hotspot, rather than a marker defining a single functional cell class. It is interesting that Stard5, a START-domain protein implicated in cholesterol metabolism and cellular stress responses (Alpy and Tomasetto, 2005; Rodriguez-Agudo et al., 2012; Calderon-Dominguez et al., 2014), and Peg10, an imprinted gene with roles in embryonic development and cancer (Mou et al. 2025), mark distinct rostro-caudal domains of the medNAcSh. Whether these genes themselves causally contribute to appetitive and consummatory behaviors, or aversive processing in this region remains an important question for future studies. Using our new Stard5-Flp mouse driver line, we confirm that rostral medNAcSh Stard5 cells recapitulate feeding-related phenotypes of rostral but not caudal medNAcSh D1-SPNs, confirming that molecular markers and spatial location intersect to define cellular function. One important caveat of the Stard5-Flp mouse is that it targets not only D1- but also D2-SPNs as well as other cell types (primarily interneurons) in the rostral medNAcSh. This heterogeneity does not undermine the utility of the Stard5-Flp line as a targeting strategy, but it reflects the broader principle that the functional specialization arises also from spatially organized ensembles rather than strictly cell-type-restricted populations. However, the Stard5-Flp line was designed using a Flp recombinase so that it could be crossed with selected cre lines to label more restricted cell types. This strategy could be used in future work to dissect the contribution of D1+ vs. non-D1+ Stard5 cells in the phenotypes we describe herein, something we could not address in the current study, which represents a limitation.

Together with multiple other studies (Chen et al., 2023; Liu et al., 2024; Zhao et al., 2022), our findings suggest that the molecular composition of NAc cellular clusters or NAc subregions strongly dictate their function. This aligns with recent work showing that other cellular properties such as spatial location, circuit inputs or circuit outputs also regulate function (Al-Hasani et al., 2015; Chen et al., 2023; Liu et al., 2024; Zhao et al., 2022; Zhou et al., 2022). For instance, recent work finds that medNAcSh cells receiving PVT input drive aversion via LH GABA neurons, while those receiving BLA input drive reinforcement via VTA GABA and LH glutamate cells (Zhou et al., 2022). Thus, the NAcSh is composed of intermingled neural circuits that can dictate behavioral outcome via multiple and sometimes opposing sub-circuits. The finding that NAc molecular composition strongly regulates function represents an important piece of information with critical implications for future therapeutic strategies. Indeed, if druggable targets such as receptors, transporters or enzymes can be identified as molecular markers labeling unique NAc subregions or unique NAc cell clusters, this could allow to develop targeted therapies to increase or decrease reward consumption in a specific manner by up or downregulating the activity of unique NAc cells.

Our study has potential applications in applied and therapeutic research. First, it could inform future studies investigating the neuronal regulation of body weight and energy balance by the NAc. In this study we show that the rostral medNAcSh acutely regulates food intake: it would be interesting to determine if these effects are persistent over several days and in turn if chronic modulation is sufficient to trigger changes in body weight or body composition. Although these experiments have not been conducted, previous work showed that synaptic plasticity at medNAcSh D1-SPN terminals in the LH regulates overeating over more than 24 hours (Thoeni et al., 2020). Moreover, we and others have used broader manipulations of the NAc or dorsal striatum showing that these brain

640 regions as a whole can powerfully impact body weight, energy metabolism and obesity risk (Labouesse et al., 2018;
641 Matikainen-Ankney et al., 2023; Walle et al., 2024). Hence, future work could determine whether the rostral
642 medNAcSh plays particularly important roles in these effects. In addition, the NAc is implicated not only in food
643 intake regulation but also in neuropsychiatric diseases like depression or addiction (Lüscher and Janak, 2021).
644 Dissecting which subregions of the NAc regulates which types of behavior could have important implications for the
645 understanding of the pathophysiology of these brain disorders. For instance, it would be interesting to determine
646 whether the addiction-related impact of NAcSh D1-SPNs or incoming inputs also follow a rostro-caudal gradient
647 (Corre et al., 2018; Gibson et al., 2018). The NAc is the target of multiple therapeutic agents including anti-
648 depressants (Xu et al., 2020) or anti-obesity drugs, including glucagon-like peptide 1 (GLP-1) mimetics (Adan,
649 2013; Müller et al., 2022). Better delineating the cellular targets of these compounds could support the further
650 refinement of these therapeutic agents into drugs with lesser side effects or stronger impact, e.g. by inhibiting
651 hedonic eating to support weight loss or weight maintenance in the context of obesity. Although there are GLP-1
652 receptors in the NAc (shell and core), peripheral GLP-1 mimetics do not reach these brain regions due to limited
653 blood-brain-barrier permeability (reviewed in (Marinescu and Labouesse, 2024)). Since the medNAcSh can get
654 activated by GLP-1 agonists, (see e.g. (Labouesse et al., 2012)) these effects are likely occurring via indirect circuit
655 effects. Moreover, the NAc is an important candidate for deep brain stimulation (DBS) treatment, in particular for
656 obsessive compulsive disorder or depression (Bewernick et al., 2010; Creed et al., 2015; Nho et al., 2024) but also
657 disorders of dysregulated eating (Hounchonou et al., 2023; Shivacharan et al., 2022; Zhang et al., 2015). Describing
658 a topographical map of how NAc subregions control various types of behavior could support the refinement of
659 electrode targeting strategies in the context of DBS-based therapy.

660 In summary, our study provides clues into the cellular, circuit and molecular mediators of a rostro-caudal feeding
661 gradient, identifying a D1 rostral hotspot in the medNAcSh with critical roles in feeding behavior. We describe a
662 molecular marker, Stard5, that allows labeling the rostral medNAcSh feeding hotspot using a new Flp driver line.
663 Our work also provides insights into the functional organization of the medNAcSh, demonstrating the importance
664 of functional and molecular gradients. This could inform more localized targeting of future therapeutic interventions
665 aiming at treating disorders of dysregulated eating.
666
667
668 **METHODS**
669 **Mice**
670 Adult (>8 weeks old) male and female C57Bl6/J mice, Drd1-cre (B6.FVB(Cg)-Tg(Drd1-cre)EY262Gsat/Mmucd) and
671 Stard5-2A-Flp0 (C57BL/6J-Stard5(2A-FLPo)Em1Ethz/Mlab) (Stard5-Flp, generated for this paper; see below)
672 backcrossed onto C57BL/6 J background were used for experiments. All animals were housed at 22-25˚C under a
673 reversed 12-hour light-dark cycle (dark 8 am to 8 pm) fed with a regular chow diet (18.5% proteins, 4.5% fat, 4.5%
674 fibers, 54.2% digestible carbohydrates; 3.85 kcal/g gross energy, #3437, Kliba-Nafag, Switzerland). Behavioral
675 testing was done in the dark phase unless otherwise indicated. Animals for behavior were handled and if necessary
676 habituated to patch cord tethering before testing. All animal procedures were performed in accordance to the Animal
677 Welfare Ordinance (TSchV 455.1) of the Swiss Federal Food Safety and Veterinary Office and were approved by
678 the Zurich Cantonal Veterinary Office or other relevant National and Institutional regulatory bodies.
679
680 **Generation of the Stard5-2A-Flp-mice via Crispr/Cas9 genetic engineering**

681 *Analysis of Stard5 transcript expression in the striatum.* We set out to design a Stard5-2A-Flp0 mouse line by
682 inserting a 2A-Flp0 sequence to the 3'UTR of the Stard5 gene (prior to the stop codon). The Stard5 gene has 2
683 differential splicing variants that contain open reading frames (protein coding): Stard5-201 and Stard-202 which
684 have different 3'UTR regions and terminal exons. To ensure 2A-Flp0 is targeted to a variant that is expressed in
685 the NAc, we analyzed a bulk RNAseq dataset (n=2 samples) of actively translating transcripts in Stard5-expressing
686 cells of the NAc generated using a Stard5-TRAP mouse line (BioProject PRJNA575143 (Heintz, 2019)). Paired-
687 end FASTQ files were downloaded from the SRA using the `fastq-dump` function of the sratoolkit. Read quality was
688 inspected using FastQC (Andrews, 2024). Quality-based and length-based filtering and adapter removal were
689 performed using fastp (Chen et al., 2018). Alignment was performed using the STAR aligner (Dobin et al., 2013)
690 using the GENCODE (Frankish et al., 2021) mouse genome build GRCm39 (release M28). The output BAM files
691 were filtered to the Stard5 region, and track plots generated in R using the package Gviz (Hahne and Ivanek, 2016).
692 Sashimi plots were generated to visualize the total counts of transcripts that align to specific exons (histograms)
693 and predicted splice junctions (curved lines). The sequencing library preparation was unstranded, preventing us
694 from determining whether reads come from the sense or antisense strand. Hence, some of the Stard5 reads on the
695 sense strand in the last exon of the Stard5-202 variant overlap with the IL16 gene on the antisense strand, making
696 it impossible to deconvolute reads that map to Stard5 or IL16 in those overlapping regions, leading to artificial splice
697 junctions across Stard5 and IL16. Still, despite these limitations, the results were unambiguous, showing a large
698 proportion of the reads (total splice junction counts) mapping to the last exon of the Stard5-201 isoform (5742 reads
699 mapped across both samples) rather than to the last two exons of the Stard5-202 isoform (246 reads)
700 (**Supplementary Figure S4**). Hence the Stard5-201 transcript and its 3'UTR region are an adequate genomic
701 region to be targeted. *Generation of the Stard5-2A-Flp0 transgenic mouse line.* The Stard5-2A-FlpO transgenic
702 mouse line (official strain name: C57BL/6J-Stard5(2A-FLPo)Em1Ethz/Mlab) was created at the Transgenic Core of
703 the ETH Phenomics Center. In this model, the FlpO gene, a mouse codon-optimized version of the FLP gene, was
704 inserted at the C-terminus of the Stard5 gene. SV40 nuclear localization signal was included at its N-terminus.
705 Gene expression was controlled via a 2A self-cleaving peptide (T2A) sequence, preceded by a Furin cleavage site
706 (RA^KA), and terminated by the Stard5 polyA signal. To generate this transgenic line, gRNAs were designed using
707 the CRISPOR algorithm (http://crispor.gi.ucsc.edu/) to target the region near the stop codon of the mouse Stard5
708 gene. The selected gRNA sequence was CAATAGGCGACTGTCAGTGA. A targeting template, 1962 bp in length,
709 was used to facilitate homologous recombination (detailed sequence in Annex). This template was dialyzed against
710 OmniPur® Water (Merck Millipore) and IDTE buffer (Integrated DNA Technologies) using a hydrophilic MCE filter
711 membrane (0.22 µm pore size, Millipore Merck). The Stard5 sequence modification was achieved using
712 CRISPR/Cas9 in 2-cell stage mouse embryos. Female C57BL/6J donors were primed for ovulation through
713 intraperitoneal injections of 5 IU Pregnant Mare Serum Gonadotropin (PMSG, Novatein Bioscience), followed 48
714 hours later by 5 IU Human Chorionic Gonadotrophin (hCG) (Chorulon®, MSD Animal Health). Following hCG
715 administration, females were mated with males of the same strain, and zygotes were collected from oviducts.
716 Remaining cumulus cells were removed using a 5 min incubation in 0.3% Hyaluronidase (Sigma-Aldrich) in M2
717 medium (Sigma-Aldrich). Embryos were cultured overnight at 37°C and 5% CO2 in Embryomax KSOM medium
718 (Sigma-Aldrich). Microinjections were performed on a microinjection system that included an inverted microscope
719 with Nomarski optics (Leica), micromanipulators (Eppendorf), and a FemtoJet microinjection unit (Eppendorf). The
720 injection mix contained IDTE buffer pH 7.5, annealed Alt-R™ CRISPR-Cas9 crRNA and tracrRNA (50 ng/µl), Alt-R
721 S.p.Hifi-Cas9 Nuclease V3 (50 ng/µl), Alt-R™ HDR Enhancer V2 (1uM), and Alt-R™ HDR Donor Block double-
722 stranded template (20 ng/µl) from IDT. Injection mix was filtered using Ultrafree®-MC column (Merck Millipore).
723 After injection, surviving embryos were transferred into the oviducts of 8–20-week-old 0.5 days post coitus

724 pseudopregnant CD-1 (RjOrl:SWISS) females mated with vasectomized males. Offspring genomic DNA was
725 extracted from toe clip biopsies using the NucleoSpin Tissue kit (Macherey-Nagel) and analyzed for correct knock-
726 in insertion via PCR using Phusion™ Plus DNA Polymerase (Thermo Fisher Scientific) and Sanger sequencing
727 (Microsynth AG). The following primers were used for PCR: 5' GCACCTAGCTGTCCTACCAG 3' and 5'
728 GATGTCGGTGATGTCGCTCT 3' (842 bp amplicon spanning the 5' homology arm) and 5'
729 TCAGCAACAGCCTGAGCTTC 3' and 5' TACAGCAACCAGCACACAGG 3' (1403 bp amplicon spanning the 3'
730 homology arm). Animals from the F2 generation onwards were used in experiments.
731
732 **Metabolic cages and body composition of Stard5-Flp mice**
733 Body composition was assessed using a quantitative nuclear magnetic resonance (NMR) scanner (Echo MRI-100H
734 Body Composition Analyzer, Echo Medical Systems, Houston, USA) as previously described (Labouesse et al.,
735 2018). Scans were performed by placing animals into a plastic cylinder, where they were kept immobile (no
736 anesthesia) for the duration of the scan (20 s). Scans computed estimations of fat mass (all fat molecules in the
737 body) and lean mass (all body parts containing water, excluding fat, bone minerals, free water and hair or cartilage).
738 Indirect calorimetry measurements were performed with the Promethion metabolic cages (Sable Systems
739 International) according to the manufacturer's guidelines and protocols. Energy expenditure analysis was
740 performed by the Promethion software version using the Weir equation (Weir, 1949): EE = 3.9 x VO2 + 1.1 x VCO2
741 (kg/cal) where V02 and VC02 are the volume of oxygen and carbon dioxide (L) consumed and produced,
742 respectively. The respiratory quotient was estimated by calculating the ratio of CO2 production to O2 consumption.
743 Locomotor activity (infrared photobeam based monitoring), food and water intake were monitored throughout the
744 whole measurement. Recordings were done at 22°C. Animals were single-caged. Data were collected and
745 averaged from 3 consecutive days after an initial 1-3 days acclimatization period to the cages and the single-caging.
746 Data was analyzed using the CalR application (Mina et al., 2018).
747
748 **Surgical Procedures**
749 Stereotaxic surgery was performed on a mouse stereotaxic apparatus (Stoelting) using glass pipettes pulled on a
750 Universal Puller (DMZ Zeitz), a Nanoject II (Drummond) and a stereomicroscope (Kaps Optik SOM 82). Fibers were
751 secured in place with miniscrews (FST), iBond (Demadent), dental cement (Charisma A2, Kulzer and Tetric
752 EvoFlow, Ivoclar Vivadent; Demadent) cured with UV light (Woodpecker), and the skin closed with Vetbond (3M).
753 Photometry fibers (2.5mm, 400 µm, 0.5 NA; R-FOC-BF400C-50NA) and optogenetic fibers (1.25mm, 200 µm, 0.22
754 NA; R-FOC-L200C-22NA) were purchased from RWD. Drd1-cre mice were injected unilaterally with 230 nL of
755 AAV9-CAG-Flex-Synaptophysin-jGCaMP8s for tracing (self-production via ETH VVPP, order 037, titer 2.5 x10^12,
756 originally generated in (Labouesse et al., 2023)) or AAV5-hSyn-Flex-jGCaMP8m for photometry (VVF Zurich v628-
757 5, titer 8.1 x 10E12 vg/mL, diluted 2x in PBS), at AP +1.5, ML +0.5, DV -4.6, 4.4, 4.2 mm from bregma for the rostral
758 medNAcSh and AP +1.0, ML +0.6, DV -4.8, -4.6, -4.5 for the caudal NAcSh. Photometry fibers were implanted at
759 DV -4.3 (rostral medNAcSh) or DV -4.6 (caudal medNAcSh). For optogenetics, bilateral injections of 300 nL AAV5-
760 hSyn-Flex-ChrimsonR (VVF Zurich v298-5, titer 5.2 x 10E12 vg/ml, undiluted) or AAV5-hSyn-Flex-mCherry (VVF
761 v116-5, titer 7.5 x 10E12, undiluted) were done at AP +1.5, ML +0.5, DV -4.6, -4.4, -4.2 (rostral medNAcSh) or AP
762 +1.0, ML +0.5, DV -4.6, -4.4, -4.2 (caudal medNAcSh) on both sides. Optogenetic fibers were implanted at the
763 same AP, ML location at DV -4.2 [straight] and at AP +1.5, ML 0.5, DV- 4.2 with an angle of 13.4° [angled] (rostral
764 medNAcSh) and DV -4.2 [straight] and AP +1.0, ML 1.5, DV -4.2 with an angle of 13.4°[angled] (caudal medNAcSh).
765 Stard5-Flp were injected with 300 nL of AAV2-CAG-dFRT-ratSynaptophysin-eGFP (VVF 780-2, titer 4.5 x 10E12

766 vg/mL, undiluted) and AAV1-hSyn-FRT-jGCaMP (VVF 740-1, titer 6.4 x 10E12 vg/ml, 2x diluted in PBS) for tracing
767 the projections (**Supplementary Fig S6**) and for validation experiments/photometry, respectively, at the same
768 coordinates as for the rostral medNAcSh above; or into the NAc core (AP 1.3, ML 1.35, DV -4.4) or dorsal striatum
769 (AP +0.9, ML 1.5, DV -3.5). Behavioral experiments began 4-6 weeks after surgery. Animals for neuroanatomy
770 were prepared >4 weeks after surgery. AAV and fiber locations were validated post-hoc.
771
772 **Immunohistochemistry and neuroanatomical tracing**
773 Mice were transcardially perfused with ice-cold 4% paraformaldehyde in PBS under deep anesthesia. For animals
774 with fibers, skulls were immersed into PFA for 2-4 days. Brains were then extracted from PFA-fixed skulls and
775 washed in PBS. Alternatively, brains were extracted after perfusion. Free-floating 50 µm coronal sections were
776 immunostained using the following primary antibodies: chicken GFP (Abcam # ab13970, RRID AB_300798, used
777 1:1000), rabbit DsRed (Clontech, #632496, RRID AB_10013483, used 1:500-1:1000) and secondary antibodies
778 from Thermofisher used 1:1000: 488 goat anti-chicken (A11039, RRID AB_2534096), 568 goat anti-rabbit (A-
779 11036, RRID AB_10563566). Digital images were acquired using a Nikon Eclipse T2 epifluorescence microscope
780 or an Olympus/Evident Slideview™ VS200 Slide Scanner, then processed with ImageJ.
781
782 **Analysis of Allen Brain Atlas in-situ hybridization data**
783 We used the open access resource Allen Brain Atlas (https://mouse.brain-map.org/) to identify molecular markers
784 enriched in either the rostral or caudal medNAcSh. Available datasets were filtered for flagged expression in the
785 NAc using the Fine Structure Search "nucleus accumbens". The markers included: 4932418E24Rik, Atp6v1c2,
786 Calb1, Carhsp1, Cartpt, Ddit4l, Dlk1, Fxyd7, Gabrg3, Gpr12, Hpcal4, Hs6st2, Ids, Itgb1bp1, Kcng3, Kcnq5, Kctd4,
787 Kctd8, Krt9, Lin7a, Lmo3, LOC381076, Lrrtm1, Lypd1, Man1a, MglI, Nrsn1, Pbx3, Peg10, Prkch, Prkg1, Rab27b,
788 Rab3c, Rasal1, Rgs10, Sdc2, Sgsm1, Slc8a1, Smug1, Sorcs2, Stard5, Stra6, Sv2b, Tac2, Th, Zcchc12, Zdbf2,
789 Zdhhc1. All 48 markers available were screened manually for a visibly strongly enriched expression in the rostral
790 or caudal medNAcSh, also with good expression differences between the medNAcSh and neighboring regions (e.g.
791 NAc core, NAc lateral shell and lateral septum). Quantification of marker expression in the medNAcSh was
792 performed using optical density (OD) calculations in ImageJ. High-resolution coronal images (7-14 images spanning
793 the rostral-caudal axis from AP +1.95 mm to +0.65 mm from bregma) from the Allen Brain Atlas (Allen Institute for
794 Brain Science, 2004) for 1 dataset (1 animal) were downloaded and OD assessed within the regions of interest
795 (ROI, medNAcSh, NAc core, lateral NAcSh, lateral septum) using 100 x 100 pixel squares on the raw unedited
796 images. The data from both hemispheres (2 measures) were averaged for each ROI. OD values from the ROIs
797 were subtracted from the average OD of the background (taken in the anterior commissure). For the purpose of the
798 figure, images were edited in Microsoft PowerPoint, using contrast and brightness filters applied the same way to
799 all images of the same staining, so as to improve staining visibility.
800
801 **Analysis of scRNAseq data**
802 To determine expression patterns of Stard5 and Peg10 in the NAc, we utilized a published scRNAseq dataset from
803 the NAc (Chen et al., 2021). Gene expression matrix and metadata were downloaded from GEO
804 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE118020) and imported into Seurat version 5.1.0 (Butler
805 et al., 2018), using the 'min.cells' and 'min.features' parameters set to 3 and 1,250 respectively. Then the following
806 functions were used: 'NormalizeData()' using the "LogNormalize" method with a scale factor of 1e6; 'ScaleData()'
807 using 'nUMI', 'percent.mito', and 'Sample' as regression variables; 'FindVariableFeatures()' using the
808 'mean.var.plot' option, and mean and dispersion cutoffs from 1 to infinite. Then, PCA, UMAP, and tSNEs were

809 computed, with UMAP and tSNE using the PCs 1:15. Separate objects for Drd1a+ (D1-SPN) and Drd2+ (D2-SPN)
810 cells were generated, with the 'FindVariableFeatures()' and dimension reductions recomputed for each. Due to their
811 relatively low expression in the dataset, cells were labelled in the Seurat metadata as either Stard5+, Drd1a+,
812 Drd2+, or Peg10+, simply by the presence of a single copy of the respective gene in the main data assay. Feature
813 plots of gene expression were generated using tSNE and the main data assay, with the 'FeaturePlot()' Seurat
814 function. Proportions of the cells that were labelled as both Stard5 (or Peg10), and either Drd1a or Drd2 positive,
815 were calculated by taking the number of cells that expressed both genes against the total number of cells.
816 Proportions of the Stard5+ (or Peg10+) cells that are also either Drd1a+ or Drd2+ were calculated by taking the
817 number of Stard5+ (or Peg10+) cells divided by the number of those Stard5+ (or Peg10+) cells that were non-zero
818 for the other gene. Data is pooled from 11 mice, 36670 cells.
819
820 **Fluorescent in-situ hybridization assay**
821 Stard5 and Peg10 expression patterns in NAc D1- and D2-SPNs was determined by fluorescent multiplex in-situ
822 hybridization (RNAscope, Advanced Cell Diagnostics, CA, USA). Following rapid decapitation of wild-type mice,
823 brains were rapidly frozen in powdered dry ice and stored at -80°C. Coronal sections of OCT-embedded brains
824 were cut at 20 µm at -20°C and thaw-mounted onto Super Frost Plus slides (Fisher). Slides were stored at -80°C
825 until further processing. Fluorescent in situ multiplex hybridization was performed according to the RNAscope
826 Multiplex Fluorescent Reagent Kit v2 and the User Manual for fresh frozen tissue (Advanced Cell Diagnostics, Inc.,
827 CA, USA). Briefly, sections were fixed in 4% PFA, dehydrated with increasing percentage of EtOH, incubated with
828 Hydrogen Peroxide and treated with protease III solution (Advanced Cell Diagnostics, Inc., CA, USA). Sections
829 were then incubated with target probes for mouse Drd1 to label D1-SPNs (Drd1-C1, Cat No. 406491), mouse Drd2
830 to label D2-SPNs (Drd2-C2, Cat No. 406501-C2), mouse Stard5 to label Stard5+ cells (Stard5-C3, Cat No. 880931-
831 C3) or Peg10 to label Peg10+ cells (Peg10-C3, Cat No. 512921-C3). Following probe hybridization, sections
832 underwent a series of probe signal amplification steps followed by incubation of fluorescently labeled probes (TSA
833 fluorophore detection kit, PerkinElmer, MA, USA) designed to target the specified channel associated with Stard5
834 or Peg10 (TSA Vivid Fluorophore 690), Drd1 (TSA Vivid Fluorophore 520) and Drd2 (TSA Vivid Fluorophore 570)
835 as a triplex. Slides were counterstained with DAPI, and coverslipped with Fluoromount-G mounting medium
836 (SouthernBiotech, Birmingham, AL, USA). High-resolution images (20X) were obtained on a fluorescent
837 microscope (Olympus/Evident Slideview™ VS200 Slide Scanner), exported and analyzed in Qupath-0.5.1
838 (Bankhead et al., 2017). Annotations were created for both hemispheres for the medNAcSh, lateral NAcSh and
839 core. Cell detection was performed for each annotation using QuPath's built-in "Cell Detection" algorithm, with DAPI
840 as the detection channel. The following nucleus parameters were used: background radius 8 µm, median filter
841 radius 0 µm, sigma 1.2 µm, intensity threshold of 1000–3000, and cell expansion of 5 µm. Single measurement
842 classifiers were created for all channels (FITC, Cy3, Cy5) to count the number of cells that were FITC+ (Drd1+
843 only), Cy3+ (Drd2+ only), Cy5+ (Stard5+ or Peg10+), or double/triple-positive. For each brain, 6-8 sections of the
844 medNAcSh were included (covering rostral to caudal bregma levels +1.95 to +0.65 mm; on average 2-3 sections
845 for most rostral and 2-3 sections for most caudal sections). Percent labeling was averaged for n=5 mice for Stard5
846 and n=5 for Peg10.
847
848 **Fiber photometry**
849 Fiber photometry was conducted using a RZ10X LUX-I/O Processor with integrated LEDs (Tucker-Davis
850 Technologies) combined with a filter/mirror minicube set (FMC6_IE(400-410)_E1(467-497)_F1(507-554)_E2(566-
851 586)_F2(600-680)_S) (Doric Lenses). The 405 nm LED was passed through a 400–410 nm bandpass filter, the

852 465 nm LED through a 467–497 nm GFP excitation filter, then both were coupled to a dichroic mirror to split
853 excitation and emission lights. Low-autofluorescence patch cords (400 µm/0.57 NA, Doric) were attached to the
854 optic fibers (400 µm/0.5 NA, RWD) on the mouse's head to collect fluorescence emissions. Signals were filtered
855 through 507-554 nm GFP emission filters coupled to a photodetector on the RZ10X. Signals were sinusoidally
856 modulated, using the TDT Synapse software at 210 and 330 Hz (405 and 465 nm, respectively) via a lock-in
857 amplification detector, then demodulated and low-passed filtered at 6 Hz on-line. Data were sampled at 1017.3 Hz.
858 465 nm power at the patch cord was set to 30-35 µW.
859
860 **Photometry recordings during reward consumption or aversive stimuli**
861 For behavioral experiments combined with medNAcSh photometry, an iCon behavior control interface (Tucker-
862 Davis Technologies) was used to control an operant box (length 22 cm x width 20 cm x height 38 cm; ENV-307W-
863 CT, Med Associates). The behavioral interface was directly connected to the RZ10X processor and controlled by
864 custom-written codes written in Pynapse (version 96), a Python-based coding interface integrated into the TDT
865 Synapse software. The iCon consisted of two modules – an iM10 multi-function interface and an iH16 28V control
866 interface. The iM10 control interface contained the touch detector and received input from the lickometer. The iH16
867 controlled the retractable lickometer (ENV-352-2W Retractable Sipper Tube, Med Associates) and the aversive
868 stimulator (ENV-414S, MedAssociates), house light and house fan. Mouse behavior was monitored using a camera
869 (C270 HD webcam, Logitech) controlled by Synapse. The reward port access were custom-modified using a 3D
870 printer to facilitate reward collection. For unpredicted reward delivery, mice were first trained across 7-10 days. On
871 the first 2 days mice were given unrestricted access to the reward for 10 min followed by 10 min of restricted access
872 with 10 s rewards being accessible at random intertrial intervals (ITIs) of 20-40 s. On days 3-5, mice were trained
873 over 30 trials with the 10 s rewards accessible at random ITIs of 20-40 s. If the animal collected the reward within
874 the specified reward windows, the trial was called a successful trial. If the mouse tried to collect the reward outside
875 of the reward windows, a timeout period of 60 s was introduced during the training sessions. Once the mouse
876 achieved a minimum of 10 successful trials, it was tethered for 2 days and trained similarly to days 3-5. After 10
877 successful trials, training was repeated for 3 days, with ITIs set at 30-50 s. Upon 12 successful trials, animals were
878 tested in combination with in-vivo fiber photometry recordings under the same conditions. In case of >1 day break
879 just before recordings (e.g. weekends), mice underwent an additional day of training before recording. The learning
880 rate from Day 1 until testing is shown in **Supplementary Figure S8.** For Drd1-cre mice, the experiment was
881 repeated 3 times (all gave the same results; no learning effects), and the session on the last day was analyzed. For
882 Stard5-Flp mice the experiment was conducted only once. For unpredicted shock (aversive stimuli #1), mice were
883 exposed (without training) to 10 aversive shocks (0.4 mA, 1 s) with intertrial intervals of 40 to 80 s. Mice were placed
884 inside a black-and-white striped cylinder in the operant box to create a context distinct from that of the reward
885 experiment. For tail lift (aversive stimuli #2), mice were lifted by the tail 5 times with intertrial intervals of 5 min, in a
886 cage similar to their home cage. The initial lift took an average of 3 s, followed by a period of stabilization in the air
887 for a total of 60 s then mice were returned on the cage floor promptly. The clearest response was found at the acute
888 tail lift, hence that data is shown and analyzed. A 3 min period at the beginning of all sessions with no stimuli were
889 observed, and the data for these 3 min removed before analysis. MedNAcSh responses to reward consumption or
890 to aversive stimuli were quantified using custom in-house Matlab scripts as done previously (Labouesse et al.,
891 2024, 2023). Events were identified using TTLs within the software. Data was 10x downsampled to 100 Hz and
892 low-pass filtered (1 Hz). The first 3 min were trimmed to remove fast bleaching artefacts. Change in fluorescence,
893 ΔF/F0 (%), was defined as (F-F0)/F0 x 100, where F represents the fluorescent signal (465 nm) at each time point.
894 F0 was calculated by applying a least-squares linear fit (polyfit) to the 405 nm signal to align with the 465 nm signal,

done on fluorescent data obtained in the -10 to +20 s or 30 s around events. Event-related ΔF/F0 was baseline corrected against the ΔF/F0 average in the -10 to -1 s timewindow before event onset. Data was aligned to events of interest, namely the first lick during the 10 s window of reward availability or the shock onset. Average peri-stimulus time histogram (PSTH) curves generated by averaging the data from all mice within a session and heatmaps showing all trials in a session were computed showing the ΔF/F0 values (%) in windows of -10 or -5 to +20 s around events. Two epochs were used for analysis: the baseline epoch spanning -6 to -1 s before event start and event epoch spanning 0 to 5 s from event start. Area under the curve (AUC) was calculated for each epoch using the trapezoidal method. Peak maxima and minima ΔF/F0 were obtained by calculating the maxima or minima value for each epoch.

Optogenetic manipulations in a reward consumption task

Optogenetic stimulation was provided by a amber-light emitting laser (635 nm, Laserglow) and operant boxes and laser were driven using the iCon behavioral interface and custom-written Python code in Pynapse/Synapse software (TDT). Lasers were connected via optical patch cords (200 µm, 0.22 NA) and a rotary joint to the animals' optic fibers. Rewards were available ad libitum via a lickometer (ENV-350CW, Med Associates). Initial training consisted of exposing mice to the lickometer during 3-5 sessions of 15-25 min. In the first two sessions mice were not tethered to the patch cord to facilitate training. Once the variability in lick counts across two consecutive sessions was less than 30 %, animals were moved to the test session. Two mice did not reach criterion and were excluded from the experiment. The proof-of-concept experiment consisted of three 8-min blocks, where laser stimulation occurred from minute 8 to minute 16. The stimulation was delivered continuously at 20 Hz (20 ms pulses, 10 mW power at fiber tip). The full optogenetic experiment consisted of five 5-min blocks, with laser stimulation during the first, third, and fifth blocks. Optogenetic stimulation was delivered continuously at 20 Hz (20 ms pulses, 300 µW power at fiber tip) throughout blocks 1, 3 and 5. All licks were detected with the Synapse software. Data was analyzed using custom-written Matlab codes to generate lick counts and lick counts/min. Additionally, to assess general locomotor activity during the reward consumption task, mice were tracked in the operant box. A small subset of frames (136 frames from 10 video recordings) was manually annotated to define the animal's body center and nose, as well as the four corners of the operant box. These annotations were used to train a YOLO- based pose estimation model (Redmon et al., 2015). Locomotion metrics, such as total distance moved, were subsequently derived from the temporal integration of positional data and aligned to opto-on and opto-off epochs of the feeding task.

Optogenetic manipulations in a real-time place preference and avoidance (RTPPA)

Optical stimulation was provided by an amber-light emitting laser (635 nm, Laserglow), which was activated using Anymaze version 7.37 (Stoelting). Lasers were connected via optical patch cords (200 µm, 0.22 NA) and a rotary joint to the animals' optic fibers. To activate ChrimsonR, we used 20 ms light pulses at 20 Hz with a power of 10 mW (or 300 µW, data not shown, results consistent) at the fiber tip. The RTPPA chamber consisted of 2 chambers of same dimension (30 x 30 x 30 cm, width, length, height) with different cues in each chamber. For the main experiment with 10 mW optogenetic laser power (shown in **Figure 4**), one chamber had thick vertical black stripes, while the other had horizontal ones. The experiment was repeated with lower laser power (300 µW) where we found the same results (data not shown): here, one chamber featured a black-and-white checkerboard pattern, and the other had diagonal black-and-white lines; experiment performed 5 months after the 10 mW one. Visual cues were also positioned outside the chamber. Testing consisted of 3 trials of 15 min each on 3 consecutive days. On day 1, animals were exposed to both sides of the chamber, and their preference was determined. Laser stimulation was

assigned to the preferred chamber. On days 2 and 3, the laser was stimulated as soon as animals entered the laser-assigned chamber and for the entire duration of their visit. This was triggered in closed loop using a custom-written protocol in the Anymaze software. Time spent and total distance moved in each chamber was computed using Anymaze. Some animals were euthanized early to validate optic fiber surgery implantation; hence not all mice were used in both RTTPA and reward consumption tasks.

Statistics

Statistical analyses were performed using GraphPad Prism 9 or 10. Data are expressed as mean ± standard error of the mean (SEM). 2-group data were compared with paired or unpaired two-tailed Student's t-tests. Multiple comparisons were evaluated by ANOVA (no Greenhouse correction) and in the case of significant interactions, Sidak post-hoc tests were used. A p-value of <0.05 was considered statistically significant.

SUPPLEMENTARY INFORMATION

Supplementary Information includes 3 Datasets and 10 Figures, which can be found with this article online.

MATERIAL AVAILABILITY

The Stard5-Flp mouse is deposited at the ETH Phenomics Center (administration@epic.ethz.ch) in the form of cryopreserved straws (line 3072) under the group of Prof. Ferdinand von Meyenn (ETH). Its CRISPR sequence is available in **Supplementary Data S1**.

CODE AVAILABILITY

Original Python code to drive Pynapse is provided in **Supplementary Data S2**. Please cite this paper upon usage of the code. No new MATLAB code was developed but the original code can be found in (Labouesse et al., 2024, 2023).

DATA AVAILABILITY

Source data is provided as **Supplementary Data S3**.

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AUTHOR CONTRIBUTIONS

AMM designed and conducted the experiments, performed the surgeries, wrote Python scripts for Pynapse, edited MATLAB scripts, analyzed data, supervised junior students, wrote the paper and conducted the revision process. EK wrote Python scripts for Pynapse and helped with in vivo photometry experiments, pilot anatomy experiments and data analysis. PL analyzed TRAPseq and scRNAseq data. KNB and CLF helped with proof-of-concept in vivo optogenetic experiments. MK helped with metabolic cage experiments and EchoMRI. NS designed and generated the Stard5-Flp mouse line. MAL acquired funding, established lab equipment, designed experiments, helped with pilot experiments, provided MATLAB scripts, analyzed data, wrote the paper, helped with the revision process and supervised the overall work. All authors contributed to the final version of the paper.

COMPETING INTERESTS

The authors have no competing interests to declare. With no relation to the present manuscript, MAL declares having been recently employed by Novartis.

MATERIALS AND CORRESPONDENCE

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dr. Marie A. Labouesse (marie.labouesse@hest.ethz.ch).

997 **REFERENCES**

998 Adan, R.A.H., 2013. Mechanisms underlying current and future anti-obesity drugs. Trends Neurosci 36, 133–140.
999 https://doi.org/10.1016/j.tins.2012.12.001
1000 Al-Hasani, R., McCall, J.G., Shin, G., Gomez, A.M., Schmitz, G.P., Bernardi, J.M., Pyo, C.-O., Park, S.I.,
1001 Marcinkiewcz, C.M., Crowley, N.A., Krashes, M.J., Lowell, B.B., Kash, T.L., Rogers, J.A., Bruchas, M.R.,
1002 2015. Distinct Subpopulations of Nucleus Accumbens Dynorphin Neurons Drive Aversion and Reward.
1003 Neuron 87, 1063–1077. https://doi.org/10.1016/j.neuron.2015.08.019
1004 Alpy, F., Tomasetto, C., 2005. Give lipids a START: the StAR-related lipid transfer (START) domain in mammals.
1005 Journal of Cell Science 118, 2791–2801. https://doi.org/10.1242/jcs.02485
1006 Andrews, S., 2024. FastQC: A Quality Control Tool for High Throughput Sequence Data.
1007 Bankhead, P., Loughrey, M.B., Fernández, J.A., Dombrowski, Y., McArt, D.G., Dunne, P.D., McQuaid, S., Gray,
1008 R.T., Murray, L.J., Coleman, H.G., James, J.A., Salto-Tellez, M., Hamilton, P.W., 2017. QuPath: Open
1009 source software for digital pathology image analysis. Sci Rep 7, 16878. https://doi.org/10.1038/s41598-
1010 017-17204-5
1011 Baumgartner, H.M., Cole, S.L., Olney, J.J., Berridge, K.C., 2020. Desire or Dread from Nucleus Accumbens
1012 Inhibitions: Reversed by Same-Site Optogenetic Excitations. J. Neurosci. 40, 2737–2752.
1013 https://doi.org/10.1523/JNEUROSCI.2902-19.2020
1014 Bewernick, B.H., Hurlemann, R., Matusch, A., Kayser, S., Grubert, C., Hadrysiewicz, B., Axmacher, N., Lemke,
1015 M., Cooper-Mahkorn, D., Cohen, M.X., Brockmann, H., Lenartz, D., Sturm, V., Schlaepfer, T.E., 2010.
1016 Nucleus accumbens deep brain stimulation decreases ratings of depression and anxiety in treatment-
1017 resistant depression. Biol Psychiatry 67, 110–116. https://doi.org/10.1016/j.biopsych.2009.09.013
1018 Bond, C.W., Trinko, R., Foscue, E., Furman, K., Groman, S.M., Taylor, J.R., DiLeone, R.J., 2020. Medial Nucleus
1019 Accumbens Projections to the Ventral Tegmental Area Control Food Consumption. J. Neurosci. 40,
1020 4727–4738. https://doi.org/10.1523/JNEUROSCI.3054-18.2020
1021 Butler, A., Hoffman, P., Smibert, P., Papalexi, E., Satija, R., 2018. Integrating single-cell transcriptomic data
1022 across different conditions, technologies, and species. Nat Biotechnol 36, 411–420.
1023 https://doi.org/10.1038/nbt.4096
1024 Calderon-Dominguez, M., Gil, G., Medina, M.A., Pandak, W.M., Rodríguez-Agudo, D., 2014. The StarD4
1025 subfamily of steroidogenic acute regulatory-related lipid transfer (START) domain proteins: New players
1026 in cholesterol metabolism. The International Journal of Biochemistry & Cell Biology 49, 64–68.
1027 https://doi.org/10.1016/j.biocel.2014.01.002
1028 Castro, Daniel C., Berridge, K.C., 2014. Opioid Hedonic Hotspot in Nucleus Accumbens Shell: Mu, Delta, and
1029 Kappa Maps for Enhancement of Sweetness “Liking” and “Wanting.” J. Neurosci. 34, 4239–4250.
1030 https://doi.org/10.1523/JNEUROSCI.4458-13.2014
1031 Castro, D.C., Berridge, K.C., 2014. Advances in the neurobiological bases for food ‘liking’ versus ‘wanting.’
1032 Physiol Behav 0, 22–30. https://doi.org/10.1016/j.physbeh.2014.05.022
1033 Castro, D.C., Terry, R.A., Berridge, K.C., 2016. Orexin in Rostral Hotspot of Nucleus Accumbens Enhances
1034 Sucrose ‘Liking’ and Intake but Scopolamine in Caudal Shell Shifts ‘Liking’ Toward ‘Disgust’ and ‘Fear.’
1035 Neuropsychopharmacol 41, 2101–2111. https://doi.org/10.1038/npp.2016.10
1036 Chen, G., Lai, S., Bao, G., Ke, J., Meng, X., Lu, S., Wu, X., Xu, H., Wu, F., Xu, Y., Xu, F., Bi, G.-Q., Peng, G.,
1037 Zhou, K., Zhu, Y., 2023. Distinct reward processing by subregions of the nucleus accumbens. Cell
1038 Reports 42, 112069. https://doi.org/10.1016/j.celrep.2023.112069
1039 Chen, R., Blosser, T.R., Djekidel, M.N., Hao, J., Bhattacherjee, A., Chen, W., Tuesta, L.M., Zhuang, X., Zhang,
1040 Y., 2021. Decoding molecular and cellular heterogeneity of mouse nucleus accumbens. Nat Neurosci 1–
1041 15. https://doi.org/10.1038/s41593-021-00938-x
1042 Chen, S., Zhou, Y., Chen, Y., Gu, J., 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34,
1043 i884–i890. https://doi.org/10.1093/bioinformatics/bty560
1044 Corre, J., van Zessen, R., Loureiro, M., Patriarchi, T., Tian, L., Pascoli, V., Lüscher, C., 2018. Dopamine neurons
1045 projecting to medial shell of the nucleus accumbens drive heroin reinforcement. Elife 7, e39945.
1046 https://doi.org/10.7554/eLife.39945
1047 Creed, M., Pascoli, V.J., Lüscher, C., 2015. Refining deep brain stimulation to emulate optogenetic treatment of
1048 synaptic pathology. Science 347, 659–664. https://doi.org/10.1126/science.1260776
1049 de Jong, J.W., Afjei, S.A., Pollak Dorocic, I., Peck, J.R., Liu, C., Kim, C.K., Tian, L., Deisseroth, K., Lammel, S.,
1050 2019. A Neural Circuit Mechanism for Encoding Aversive Stimuli in the Mesolimbic Dopamine System.
1051 Neuron 101, 133-151.e7. https://doi.org/10.1016/j.neuron.2018.11.005
1052 Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., Gingeras, T.R.,
1053 2013. STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29, 15–21.
1054 https://doi.org/10.1093/bioinformatics/bts635
1055 Domingues, A.V., Carvalho, T.T.A., Martins, G.J., Correia, R., Coimbra, B., Bastos-Gonçalves, R., Wezik, M.,
1056 Gaspar, R., Pinto, L., Sousa, N., Costa, R.M., Soares-Cunha, C., Rodrigues, A.J., 2025. Dynamic
1057 representation of appetitive and aversive stimuli in nucleus accumbens shell D1- and D2-medium spiny
1058 neurons. Nat Commun 16, 59. https://doi.org/10.1038/s41467-024-55269-9

Faure, A., Richard, J.M., Berridge, K.C., 2010. Desire and Dread from the Nucleus Accumbens: Cortical Glutamate and Subcortical GABA Differentially Generate Motivation and Hedonic Impact in the Rat. PLOS ONE 5, e11223. https://doi.org/10.1371/journal.pone.0011223

Fischer, K.B., Collins, H.K., Callaway, E.M., 2019. Sources of off-target expression from recombinase-dependent AAV vectors and mitigation with cross-over insensitive ATG-out vectors. Proc Natl Acad Sci U S A 116, 27001–27010. https://doi.org/10.1073/pnas.1915974116

Frankish, A., Diekhans, M., Jungreis, I., Lagarde, J., Loveland, J.E., Mudge, J.M., Sisu, C., Wright, J.C., Armstrong, J., Barnes, I., Berry, A., Bignell, A., Boix, C., Carbonell Sala, S., Cunningham, F., Di Domenico, T., Donaldson, S., Fiddes, I.T., García Girón, C., Gonzalez, J.M., Grego, T., Hardy, M., Hourlier, T., Howe, K.L., Hunt, T., Izuogu, O.G., Johnson, R., Martin, F.J., Martínez, L., Mohanan, S., Muir, P., Navarro, F.C.P., Parker, A., Pei, B., Pozo, F., Riera, F.C., Ruffier, M., Schmitt, B.M., Stapleton, E., Suner, M.-M., Sycheva, I., Uszczynska-Ratajczak, B., Wolf, M.Y., Xu, J., Yang, Y.T., Yates, A., Zerbino, D., Zhang, Y., Choudhary, J.S., Gerstein, M., Guigó, R., Hubbard, T.J.P., Kellis, M., Paten, B., Tress, M.L., Flicek, P., 2021. GENCODE 2021. Nucleic Acids Res 49, D916–D923. https://doi.org/10.1093/nar/gkaa1087

Gibson, G.D., Prasad, A.A., Jean-Richard-dit-Bressel, P., Yau, J.O.Y., Millan, E.Z., Liu, Y., Campbell, E.J., Lim, J., Marchant, N.J., Power, J.M., Killcross, S., Lawrence, A.J., McNally, G.P., 2018. Distinct Accumbens Shell Output Pathways Promote versus Prevent Relapse to Alcohol Seeking. Neuron 98, 512-520.e6. https://doi.org/10.1016/j.neuron.2018.03.033

Hahne, F., Ivanek, R., 2016. Visualizing Genomic Data Using Gviz and Bioconductor. Methods Mol Biol 1418, 335–351. https://doi.org/10.1007/978-1-4939-3578-9_16

Heintz, N., 2019. Transcriptional profiling of neurons in the ventra... (ID 575143) - BioProject - NCBI.

Hintiryan, H., Foster, N.N., Bowman, I., Bay, M., Song, M.Y., Gou, L., Yamashita, S., Bienkowski, M.S., Zingg, B., Zhu, M., Yang, X.W., Shih, J.C., Toga, A.W., Dong, H.-W., 2016. The mouse cortico-striatal projectome. Nat Neurosci 19, 1100–1114. https://doi.org/10.1038/nn.4332

Hounchonou, H.F., Tang, H., Paulat, R., Kühn, A., Spranger, J., van Riesen, C., Maurer, L., 2023. Continuous deep brain stimulation of the nucleus accumbens reduces food intake but does not affect body weight in mice fed a high-fat diet. Sci Rep 13, 18952. https://doi.org/10.1038/s41598-023-45511-7

Kelley, A.E., Baldo, B.A., Pratt, W.E., 2005. A proposed hypothalamic-thalamic-striatal axis for the integration of energy balance, arousal, and food reward. J Comp Neurol 493, 72–85. https://doi.org/10.1002/cne.20769

Krause, M., German, P.W., Taha, S.A., Fields, H.L., 2010. A Pause in Nucleus Accumbens Neuron Firing Is Required to Initiate and Maintain Feeding. J. Neurosci. 30, 4746–4756. https://doi.org/10.1523/JNEUROSCI.0197-10.2010

Labouesse, M.A., Sartori, A.M., Weinmann, O., Simpson, E.H., Kellendonk, C., Weber-Stadlbauer, U., 2018. Striatal dopamine 2 receptor upregulation during development predisposes to diet-induced obesity by reducing energy output in mice. Proc. Natl. Acad. Sci. U.S.A. 115, 10493–10498. https://doi.org/10.1073/pnas.1800171115

Labouesse, M.A., Stadlbauer, U., Weber, E., Arnold, M., Langhans, W., Pacheco-López, G., 2012. Vagal afferents mediate early satiation and prevent flavour avoidance learning in response to intraperitoneally infused exendin-4. J Neuroendocrinol 24, 1505–1516. https://doi.org/10.1111/j.1365-2826.2012.02364.x

Labouesse, M.A., Torres-Herraez, A., Chohan, M.O., Villarin, J.M., Greenwald, J., Sun, X., Zahran, M., Tang, A., Lam, S., Veenstra-VanderWeele, J., Lacefield, C.O., Bonaventura, J., Michaelides, M., Chan, C.S., Yizhar, O., Kellendonk, C., 2023. A non-canonical striatopallidal Go pathway that supports motor control. Nat Commun 14, 6712. https://doi.org/10.1038/s41467-023-42288-1

Labouesse, M.A., Wilhelm, M., Kagiampaki, Z., Yee, A.G., Denis, R., Harada, M., Gresch, A., Marinescu, A.-M., Otomo, K., Curreli, S., Serratosa Capdevila, L., Zhou, Xuehan, Cola, R.B., Ravotto, L., Glück, C., Cherepanov, S., Weber, B., Zhou, Xin, Katner, J., Svensson, K.A., Fellin, T., Trudeau, L.-E., Ford, C.P., Sych, Y., Patriarchi, T., 2024. A chemogenetic approach for dopamine imaging with tunable sensitivity. Nat Commun 15, 5551. https://doi.org/10.1038/s41467-024-49442-3

Liu, Y., Wang, Y., Zhao, Z., Xie, G., Zhang, C., Chen, R., Zhang, Y., 2024. A subset of dopamine receptor-expressing neurons in the nucleus accumbens controls feeding and energy homeostasis. Nat Metab 6, 1616–1631. https://doi.org/10.1038/s42255-024-01100-0

Liu, Z., Le, Q., Lv, Y., Chen, X., Cui, J., Zhou, Y., Cheng, D., Ma, C., Su, X., Xiao, L., Yang, R., Zhang, J., Ma, L., Liu, X., 2022. A distinct D1-MSN subpopulation down-regulates dopamine to promote negative emotional state. Cell Res 32, 139–156. https://doi.org/10.1038/s41422-021-00588-5

Lüscher, C., Janak, P.H., 2021. Consolidating the Circuit Model for Addiction. Annu Rev Neurosci 44, 173–195. https://doi.org/10.1146/annurev-neuro-092920-123905

Maldonado-Irizarry, C., Swanson, C., Kelley, A., 1995. Glutamate receptors in the nucleus accumbens shell control feeding behavior via the lateral hypothalamus. J. Neurosci. 15, 6779–6788. https://doi.org/10.1523/JNEUROSCI.15-10-06779.1995

Marinescu, A.-M., Labouesse, M.A., 2024. The nucleus accumbens shell: a neural hub at the interface of homeostatic and hedonic feeding. Front. Neurosci. 18. https://doi.org/10.3389/fnins.2024.1437210

Matikainen-Ankney, B.A., Legaria, A.A., Pan, Y., Vachez, Y.M., Murphy, C.A., Schaefer, R.F., McGrath, Q.J., Wang, J.G., Bluitt, M.N., Ankney, K.C., Norris, A.J., Creed, M.C., Kravitz, A.V., 2023. Nucleus

Accumbens D1 Receptor-Expressing Spiny Projection Neurons Control Food Motivation and Obesity. Biol Psychiatry 93, 512–523. https://doi.org/10.1016/j.biopsych.2022.10.003

Mina, A.I., LeClair, R.A., LeClair, K.B., Cohen, D.E., Lantier, L., Banks, A.S., 2018. *CalR*: A Web-Based Analysis Tool for Indirect Calorimetry Experiments. Cell Metabolism 28, 656-666.e1. https://doi.org/10.1016/j.cmet.2018.06.019

Mou, D., Wu, S., Chen, Y., Wang, Y., Dai, Y., Tang, M., Teng, X., Bai, S., Bai, X., 2025. Roles of PEG10 in cancer and neurodegenerative disorder (Review). Oncol Rep 53, 1–9. https://doi.org/10.3892/or.2025.8893

Müller, T.D., Blüher, M., Tschöp, M.H., DiMarchi, R.D., 2022. Anti-obesity drug discovery: advances and challenges. Nat Rev Drug Discov 21, 201–223. https://doi.org/10.1038/s41573-021-00337-8

Nho, Y.-H., Rolle, C.E., Topalovic, U., Shivacharan, R.S., Cunningham, T.N., Hiller, S., Batista, D., Feng, A., Espil, F.M., Kratter, I.H., Bhati, M.T., Kellogg, M., Raslan, A.M., Williams, N.R., Garnett, J., Pesaran, B., Oathes, D.J., Suthana, N., Barbosa, D.A.N., Halpern, C.H., 2024. Responsive deep brain stimulation guided by ventral striatal electrophysiology of obsession durably ameliorates compulsion. Neuron 112, 73-83.e4. https://doi.org/10.1016/j.neuron.2023.09.034

O'Connor, E.C., Kremer, Y., Lefort, S., Harada, M., Pascoli, V., Rohner, C., Lüscher, C., 2015. Accumbal D1R Neurons Projecting to Lateral Hypothalamus Authorize Feeding. Neuron 88, 553–564. https://doi.org/10.1016/j.neuron.2015.09.038

Pedersen, C.E., Castro, D.C., Gray, M.M., Zhou, Z.C., Piantadosi, S.C., Gowrishankar, R., Kan, S.A., Murphy, P.J., O'Neill, P.R., Bruchas, M.R., 2022. Medial accumbens shell spiny projection neurons encode relative reward preference. https://doi.org/10.1101/2022.09.18.508426

Pisanello, F., Mandelbaum, G., Pisanello, M., Oldenburg, I.A., Sileo, L., Markowitz, J.E., Peterson, R.E., Della Patria, A., Haynes, T.M., Emara, M.S., Spagnolo, B., Datta, S.R., De Vittorio, M., Sabatini, B.L., 2017. Dynamic illumination of spatially restricted or large brain volumes via a single tapered optical fiber. Nat Neurosci 20, 1180–1188. https://doi.org/10.1038/nn.4591

Piwecka, M., Rajewsky, N., Rybak-Wolf, A., 2023. Single-cell and spatial transcriptomics: deciphering brain complexity in health and disease. Nat Rev Neurol 19, 346–362. https://doi.org/10.1038/s41582-023-00809-y

Redmon, J., Divvala, S., Girshick, R., Farhadi, A., 2015. You Only Look Once: Unified, Real-Time Object Detection. https://doi.org/10.48550/ARXIV.1506.02640

Reed, S.J., Lafferty, C.K., Mendoza, J.A., Yang, A.K., Davidson, T.J., Grosenick, L., Deisseroth, K., Britt, J.P., 2018. Coordinated Reductions in Excitatory Input to the Nucleus Accumbens Underlie Food Consumption. Neuron 99, 1260-1273.e4. https://doi.org/10.1016/j.neuron.2018.07.051

Requejo-Mendoza, N., Arias-Montaño, J.-A., Gutierrez, R., 2025. Nucleus accumbens D2-expressing neurons: Balancing reward and licking disruption through rhythmic optogenetic stimulation. PLoS ONE 20, e0317605. https://doi.org/10.1371/journal.pone.0317605

Reynolds, S.M., Berridge, K.C., 2008. Emotional environments retune the valence of appetitive versus fearful functions in nucleus accumbens. Nat Neurosci 11, 423–425. https://doi.org/10.1038/nn2061

Reynolds, S.M., Berridge, K.C., 2002. Positive and Negative Motivation in Nucleus Accumbens Shell: Bivalent Rostrocaudal Gradients for GABA-Elicited Eating, Taste "Liking"/"Disliking" Reactions, Place Preference/Avoidance, and Fear. J. Neurosci. 22, 7308–7320. https://doi.org/10.1523/JNEUROSCI.22-16-07308.2002

Reynolds, S.M., Berridge, K.C., 2001. Fear and Feeding in the Nucleus Accumbens Shell: Rostrocaudal Segregation of GABA-Elicited Defensive Behavior Versus Eating Behavior. J. Neurosci. 21, 3261–3270. https://doi.org/10.1523/JNEUROSCI.21-09-03261.2001

Richard, J.M., Berridge, K.C., 2011. Nucleus Accumbens Dopamine/Glutamate Interaction Switches Modes to Generate Desire versus Dread: D1 Alone for Appetitive Eating But D1 and D2 Together for Fear. J. Neurosci. 31, 12866–12879. https://doi.org/10.1523/JNEUROSCI.1339-11.2011

Richard, J.M., Plawecki, A.M., Berridge, K.C., 2013. Nucleus accumbens GABAergic inhibition generates intense eating and fear that resists environmental retuning and needs no local dopamine. European Journal of Neuroscience 37, 1789–1802. https://doi.org/10.1111/ejn.12194

Rodriguez-Agudo, D., Calderon-Dominguez, M., Medina, M.A., Ren, S., Gil, G., Pandak, W.M., 2012. ER stress increases StarD5 expression by stabilizing its mRNA and leads to relocalization of its protein from the nucleus to the membranes. Journal of Lipid Research 53, 2708–2715. https://doi.org/10.1194/jlr.M031997

Roitman, M.F., Wheeler, R.A., Carelli, R.M., 2005. Nucleus Accumbens Neurons Are Innately Tuned for Rewarding and Aversive Taste Stimuli, Encode Their Predictors, and Are Linked to Motor Output. Neuron 45, 587–597. https://doi.org/10.1016/j.neuron.2004.12.055

Rossi, M.A., 2023. Control of energy homeostasis by the lateral hypothalamic area. Trends Neurosci 46, 738–749. https://doi.org/10.1016/j.tins.2023.05.010

Shivacharan, R.S., Rolle, C.E., Barbosa, D.A.N., Cunningham, T.N., Feng, A., Johnson, N.D., Safer, D.L., Bohon, C., Keller, C., Buch, V.P., Parker, J.J., Azagury, D.E., Tass, P.A., Bhati, M.T., Malenka, R.C., Lock, J.D., Halpern, C.H., 2022. Pilot study of responsive nucleus accumbens deep brain stimulation for loss-of-control eating. Nat Med 28, 1791–1796. https://doi.org/10.1038/s41591-022-01941-w

Stanley, G., Gokce, O., Malenka, R.C., Südhof, T.C., Quake, S.R., 2020. Continuous and Discrete Neuron Types of the Adult Murine Striatum. Neuron 105, 688-699.e8. https://doi.org/10.1016/j.neuron.2019.11.004

Stratford, T.R., Kelley, A.E., 1999. Evidence of a Functional Relationship between the Nucleus Accumbens Shell and Lateral Hypothalamus Subserving the Control of Feeding Behavior. J. Neurosci. 19, 11040–11048. https://doi.org/10.1523/JNEUROSCI.19-24-11040.1999

Stratford, T.R., Kelley, A.E., 1997. GABA in the Nucleus Accumbens Shell Participates in the Central Regulation of Feeding Behavior. J. Neurosci. 17, 4434–4440. https://doi.org/10.1523/JNEUROSCI.17-11-04434.1997

Stuber, G.D., Schwitzgebel, V.M., Lüscher, C., 2025. The neurobiology of overeating. Neuron. https://doi.org/10.1016/j.neuron.2025.03.010

Taha, S.A., Fields, H.L., 2006. Inhibitions of nucleus accumbens neurons encode a gating signal for reward-directed behavior. J Neurosci 26, 217–222. https://doi.org/10.1523/JNEUROSCI.3227-05.2006

Taha, S.A., Fields, H.L., 2005. Encoding of Palatability and Appetitive Behaviors by Distinct Neuronal Populations in the Nucleus Accumbens. J. Neurosci. 25, 1193–1202. https://doi.org/10.1523/JNEUROSCI.3975-04.2005

Thoeni, S., Loureiro, M., O'Connor, E.C., Lüscher, C., 2020. Depression of Accumbal to Lateral Hypothalamic Synapses Gates Overeating. Neuron 107, 158-172.e4. https://doi.org/10.1016/j.neuron.2020.03.029

Trouche, S., Koren, V., Doig, N.M., Ellender, T.J., El-Gaby, M., Lopes-dos-Santos, V., Reeve, H.M., Perestenko, P.V., Garas, F.N., Magill, P.J., Sharott, A., Dupret, D., 2019. A Hippocampus-Accumbens Tripartite Neuronal Motif Guides Appetitive Memory in Space. Cell 176, 1393-1406.e16. https://doi.org/10.1016/j.cell.2018.12.037

Vachez, Y.M., Tooley, J.R., Abiraman, K., Matikainen-Ankney, B., Casey, E., Earnest, T., Ramos, L.M., Silberberg, H., Godynyuk, E., Uddin, O., Marconi, L., Le Pichon, C.E., Creed, M.C., 2021. Ventral arkypallidal neurons inhibit accumbal firing to promote reward consumption. Nature Neuroscience 24, 379–390. https://doi.org/10.1038/s41593-020-00772-7

Valjent, E., Gangarossa, G., 2021. The Tail of the Striatum: From Anatomy to Connectivity and Function. Trends in Neurosciences 44, 203–214. https://doi.org/10.1016/j.tins.2020.10.016

Walle, R., Petitbon, A., Fois, G.R., Varin, C., Montalban, E., Hardt, L., Contini, A., Angelo, M.F., Potier, M., Ortole, R., Oummadi, A., De Smedt-Peyrusse, V., Adan, R.A., Giros, B., Chaouloff, F., Ferreira, G., de Kerchove d'Exaerde, A., Ducrocq, F., Georges, F., Trifilieff, P., 2024. Nucleus accumbens D1- and D2-expressing neurons control the balance between feeding and activity-mediated energy expenditure. Nat Commun 15, 2543. https://doi.org/10.1038/s41467-024-46874-9

Weir, J.B. de V., 1949. New methods for calculating metabolic rate with special reference to protein metabolism. The Journal of Physiology 109, 1–9. https://doi.org/10.1113/jphysiol.1949.sp004363

Xu, L., Nan, J., Lan, Y., 2020. The Nucleus Accumbens: A Common Target in the Comorbidity of Depression and Addiction. Front. Neural Circuits 14. https://doi.org/10.3389/fncir.2020.00037

Yang, H., de Jong, J.W., Tak, Y., Peck, J., Bateup, H.S., Lammel, S., 2018. Nucleus Accumbens Subnuclei Regulate Motivated Behavior via Direct Inhibition and Disinhibition of VTA Dopamine Subpopulations. Neuron 97, 434-449.e4. https://doi.org/10.1016/j.neuron.2017.12.022

Zhang, C., Wei, N.-L., Wang, Y., Wang, X., Zhang, J.-G., Zhang, K., 2015. Deep brain stimulation of the nucleus accumbens shell induces anti-obesity effects in obese rats with alteration of dopamine neurotransmission. Neuroscience Letters 589, 1–6. https://doi.org/10.1016/j.neulet.2015.01.019

Zhang, M., Pan, X., Jung, W., Halpern, A.R., Eichhorn, S.W., Lei, Z., Cohen, L., Smith, K.A., Tasic, B., Yao, Z., Zeng, H., Zhuang, X., 2023. Molecularly defined and spatially resolved cell atlas of the whole mouse brain. Nature 624, 343–354. https://doi.org/10.1038/s41586-023-06808-9

Zhang, Y., Rózsa, M., Liang, Y., Bushey, D., Wei, Z., Zheng, J., Reep, D., Broussard, G.J., Tsang, A., Tsegaye, G., Narayan, S., Obara, C.J., Lim, J.-X., Patel, R., Zhang, R., Ahrens, M.B., Turner, G.C., Wang, S.S.-H., Korff, W.L., Schreiter, E.R., Svoboda, K., Hasseman, J.P., Kolb, I., Looger, L.L., 2023. Fast and sensitive GCaMP calcium indicators for imaging neural populations. Nature 615, 884–891. https://doi.org/10.1038/s41586-023-05828-9

Zhao, Z., Han, X., Chen, R., Liu, Y., Bhattacherjee, A., Chen, W., Zhang, Y., 2022a. A molecularly defined D1 medium spiny neuron subtype negatively regulates cocaine addiction. Science Advances 8, eabn3552. https://doi.org/10.1126/sciadv.abn3552

Zhao, Z., Han, X., Chen, R., Liu, Y., Bhattacherjee, A., Chen, W., Zhang, Y., 2022b. A molecularly defined D1 medium spiny neuron subtype negatively regulates cocaine addiction. Science Advances 8, eabn3552. https://doi.org/10.1126/sciadv.abn3552

Zhou, K., Xu, H., Lu, S., Jiang, S., Hou, G., Deng, X., He, M., Zhu, Y., 2022. Reward and aversion processing by input-defined parallel nucleus accumbens circuits in mice. Nat Commun 13, 6244. https://doi.org/10.1038/s41467-022-33843-3

Zhu, X., Ottenheimer, D., DiLeone, R.J., 2016. Activity of D1/2 Receptor Expressing Neurons in the Nucleus Accumbens Regulates Running, Locomotion, and Food Intake. Front. Behav. Neurosci. 10. https://doi.org/10.3389/fnbeh.2016.00066

Zhu, Z., Gong, R., Rodriguez, V., Quach, K.T., Chen, X., Sternson, S.M., 2025. Hedonic eating is controlled by dopamine neurons that oppose GLP-1R satiety. Science 387, eadt0773. https://doi.org/10.1126/science.adt0773

1253 **SUPPLEMENTARY FIGURES**

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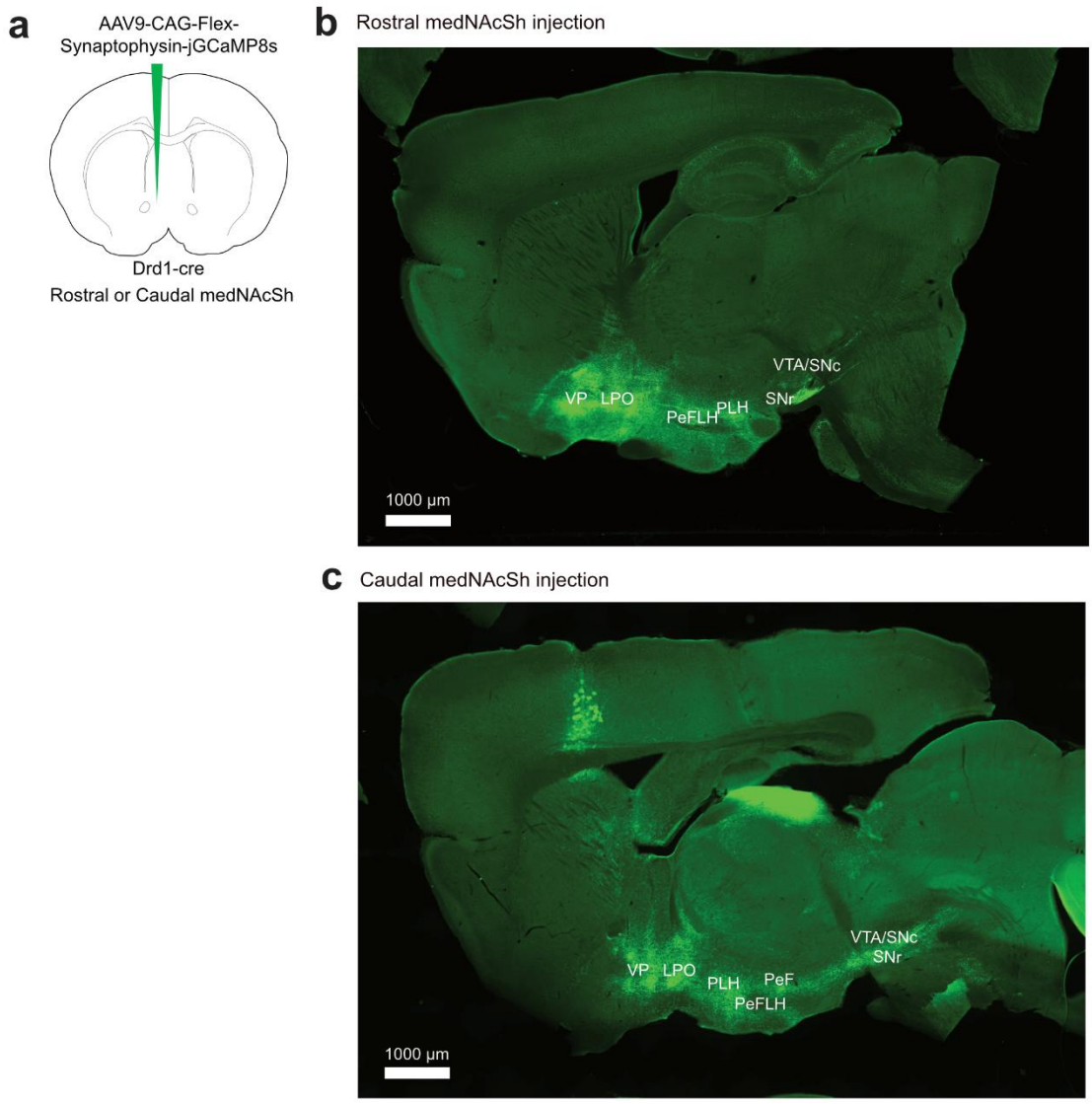

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1257 **Supplementary Figure S1. Projections sites of D1-SPNs from the rostral and caudal medNAcSh. a.** Surgery
1258 schematics of Drd1-cre mice injected with a cre-dependent green anterograde tracer (Synaptophysin-jGCaMP8s)
1259 in the rostral or caudal medNAcSh to trace projections **b.** Representative sagittal images of rostral (upper panel)
1260 vs. caudal (lower) medNAcSh mice injected with the tracer. Axons arising from rostral vs. caudal medNAcSh D1-
1261 SPNs both similarly reach the canonical target regions of D1-SPNs, namely the ventral pallidum (VP), lateral
1262 hypothalamus (LH) region (includes the perifornical nucleus: PeF, PLH: peduncular part of lateral hypothalamus),
1263 the lateral preoptic area (LPO), which is closely connected with the LH and the midbrain (includes VTA: ventral
1264 tegmental area /SNc: substantia nigra compacta, and SNr: substantia nigra compacta). Related to **Figures 1, 2, 3,**
1265 **4**. See also **Supplementary Figure S6**.

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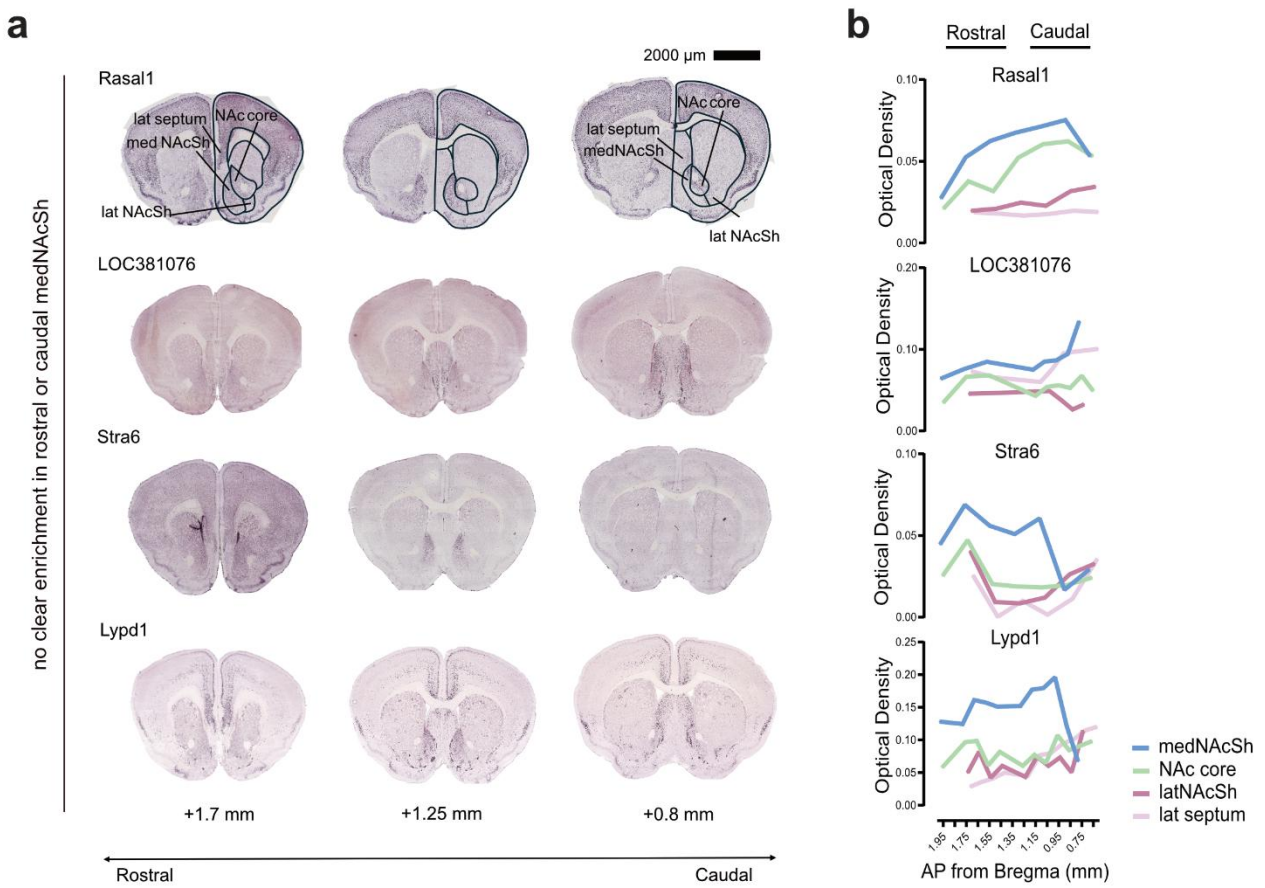
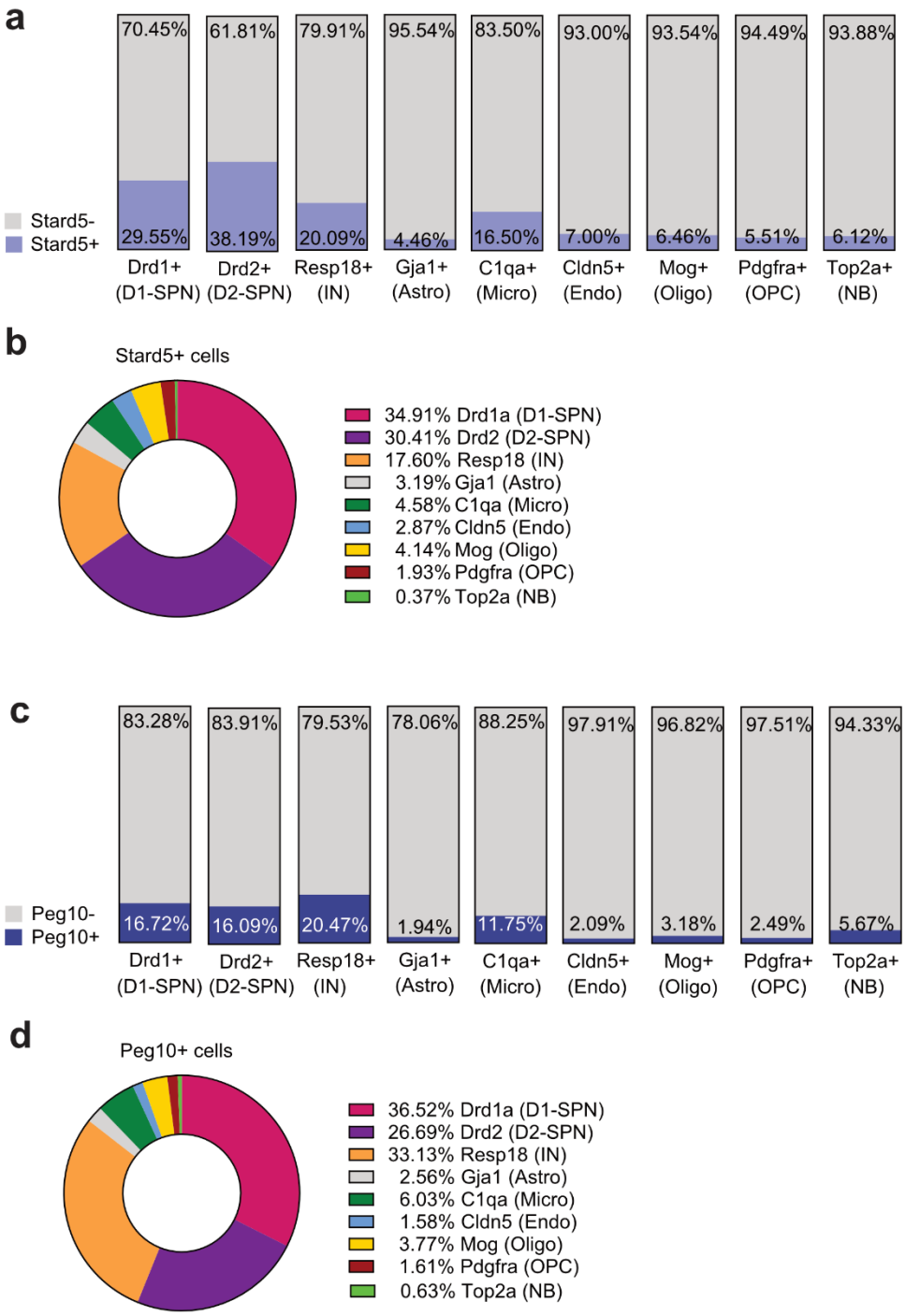


Supplementary Figure S2. Molecular markers for the medNAcSh. a. Representative in-situ hybridization images obtained from the Allen Brain Atlas (https://mouse.brain-map.org/) for markers showing no clear enriched expression in the rostral vs. caudal medNAcSh. All 48 markers available in the Allen Brain Atlas labeling the nucleus accumbens were screened, and most showed no clear enrichment pattern for the medNAcSh. Representative markers (Rasal1, LOC381076, Stra6, Lypd1) are shown here. Images on the left are more rostral (bregma +1.65 mm); images on the right are more caudal (bregma +0.85 mm). **b.** Quantification of expression levels of relevant markers in the medNAcSh along the rostral-caudal axis (bregma +1.95 mm to +0.75 mm) using optical density measurements (n = 1 mouse, 7-14 sections total, data shown is the mean from 2 measures/section) showing no clear enrichment in the rostral or caudal medNAcSh for the 4 markers. Data for the NAc core, lateral NAcSh (lat NAcSh) and lateral septum (lat septum) are also shown. Related to **Figure 5**.


a
Stard5-
Stard5+
70.45%
61.81%
79.91%
95.54%
83.50%
93.00%
93.54%
94.49%
93.88%
29.55%
38.19%
20.09%
4.46%
16.50%
7.00%
6.46%
5.51%
6.12%
Drd1+ (D1-SPN)
Drd2+ (D2-SPN)
Resp18+ (IN)
Gja1+ (Astro)
C1qa+ (Micro)
Cldn5+ (Endo)
Mog+ (Oligo)
Pdgfra+ (OPC)
Top2a+ (NB)
b
Stard5+ cells
34.91% Drd1a (D1-SPN)
30.41% Drd2 (D2-SPN)
17.60% Resp18 (IN)
3.19% Gja1 (Astro)
4.58% C1qa (Micro)
2.87% Cldn5 (Endo)
4.14% Mog (Oligo)
1.93% Pdgfra (OPC)
0.37% Top2a (NB)
c
Peg10-
Peg10+
83.28%
83.91%
79.53%
78.06%
88.25%
97.91%
96.82%
97.51%
94.33%
16.72%
16.09%
20.47%
1.94%
11.75%
2.09%
3.18%
2.49%
5.67%
Drd1+ (D1-SPN)
Drd2+ (D2-SPN)
Resp18+ (IN)
Gja1+ (Astro)
C1qa+ (Micro)
Cldn5+ (Endo)
Mog+ (Oligo)
Pdgfra+ (OPC)
Top2a+ (NB)
d
Peg10+ cells
36.52% Drd1a (D1-SPN)
26.69% Drd2 (D2-SPN)
33.13% Resp18 (IN)
2.56% Gja1 (Astro)
6.03% C1qa (Micro)
1.58% Cldn5 (Endo)
3.77% Mog (Oligo)
1.61% Pdgfra (OPC)
0.63% Top2a (NB)

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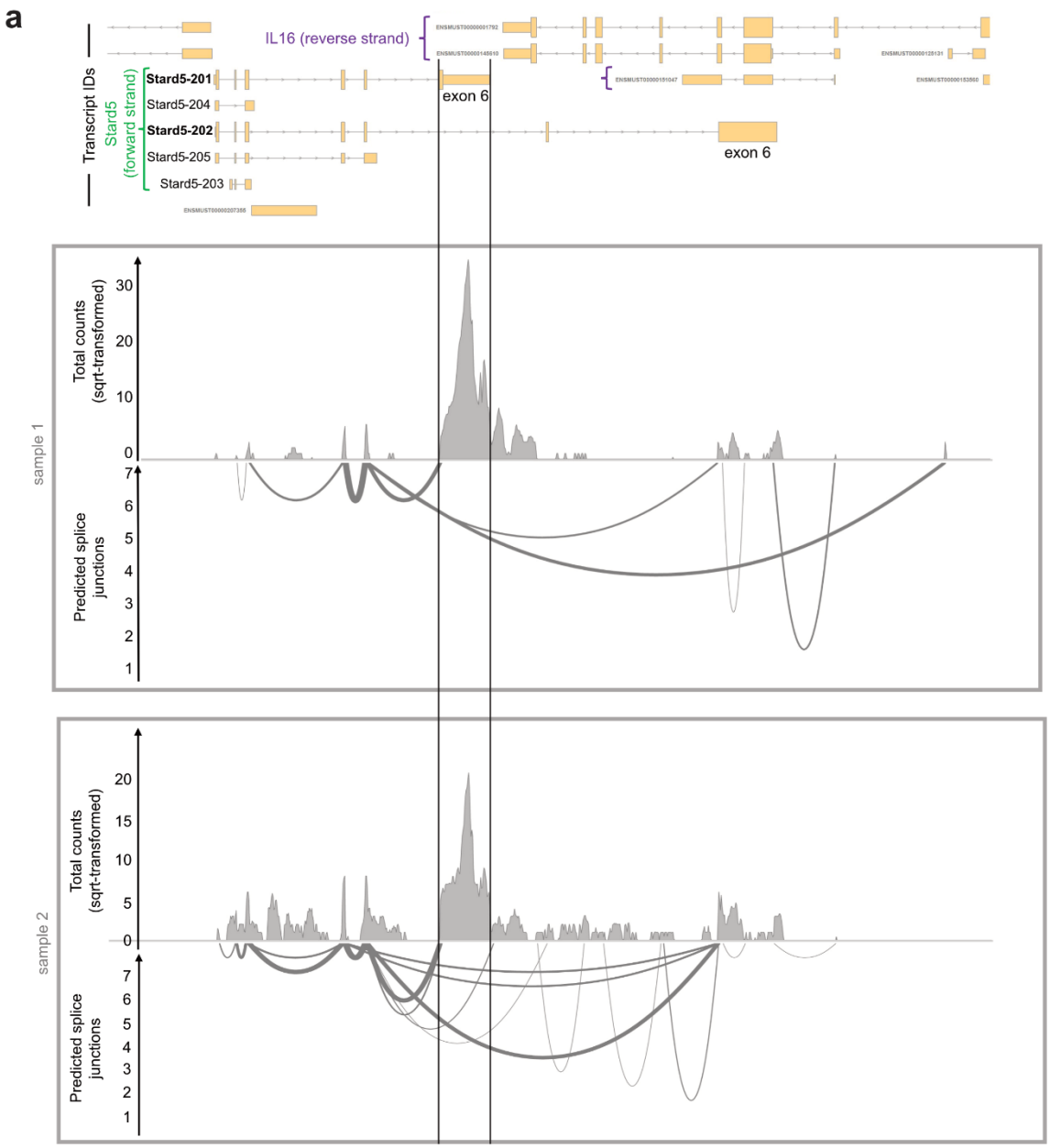
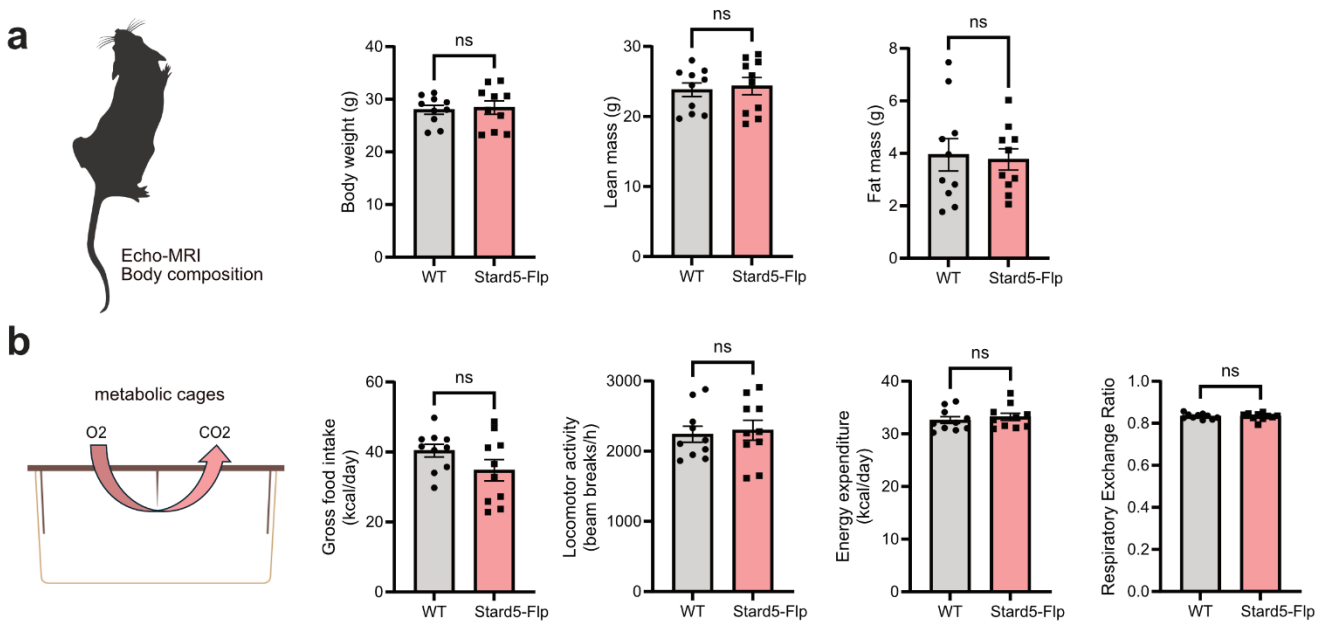
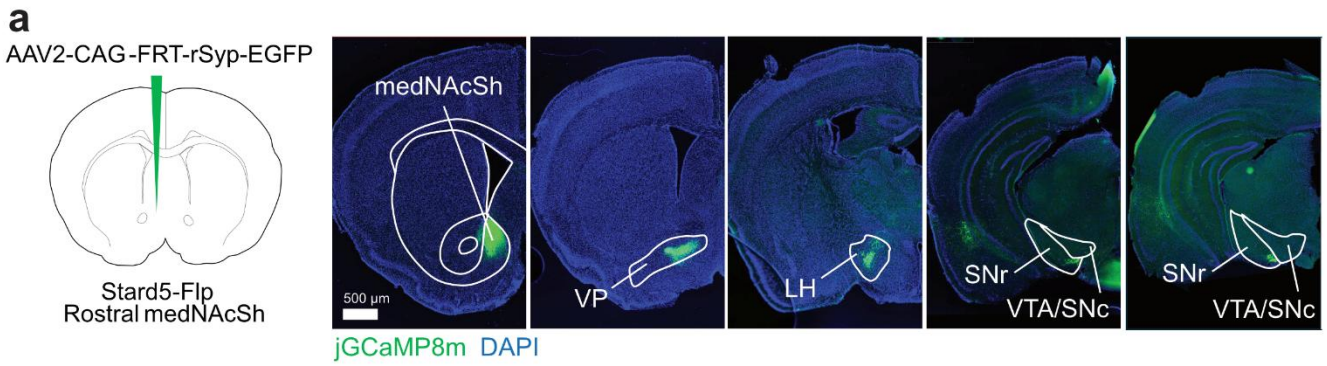
1293 **Supplementary Figure S3. Stard5 and Peg10 expression patterns in the NAc. a.** Percent expression of cells
1294 positive (+) or negative (-) for Stard5 in various cell types in the entire nucleus accumbens (NAc) (i.e. not only the
1295 medNAcSh like in our RNAscope analysis in **Fig 6d-g**), based on re-analysis of a published scRNAseq dataset:
1296 GSE118020 (Chen et al., 2021) (same dataset as in **Fig 6a-b**). This shows that ~30-40% of D1-SPNs and D2-
1297 SPNs express Stard5; ~20% of interneurons express Stard5. **b.** Expression of cell type canonical markers within
1298 all nucleus accumbens Stard5+ cells. Some cells express >1 marker; hence total % can be >100%. This shows
1299 that D1-SPNs and D2-SPNs represent the majority of Stard5+ cells (~30-35% each), while interneurons represent
1300 17%. Cell types are identified by canonical markers, the same used in (Chen et al., 2021). **c.** Percent expression
1301 of cells positive (+) or negative (-) for Peg10 in various cell types in the entire nucleus accumbens based on re-
1302 analysis of a published scRNAseq dataset: GSE118020 (Chen et al., 2021). This shows that ~20% of interneurons
1303 express Peg10; around 16% of D1-SPNs and of D2-SPNs express Peg10. **d.** Expression of cell type canonical
1304 markers within all nucleus accumbens Peg10+ cells. Some cells express >1 marker; hence total % can be >100%.
1305 This shows that D1-SPNs, D2-SPNs and interneurons represent the majority of Peg10+ cells (about 1/3 each). Cell
1306 types are identified by canonical markers, the same used in (Chen et al., 2021). Drd1 labels dopamine 1 receptor+
1307 SPNs, Drd2 labels dopamine 2 receptor+ SPNs, Resp18 labels interneurons, Gja1 labels astrocytes, C1qa labels
1308 microglia, Cldn5 labels endothelial cells, Mog labels oligodendrocytes, Pdgfra labels oligodendrocyte progenitor
1309 cells, Top2a labels neural stem cells and neuroblasts. Related to **Figure 6**.



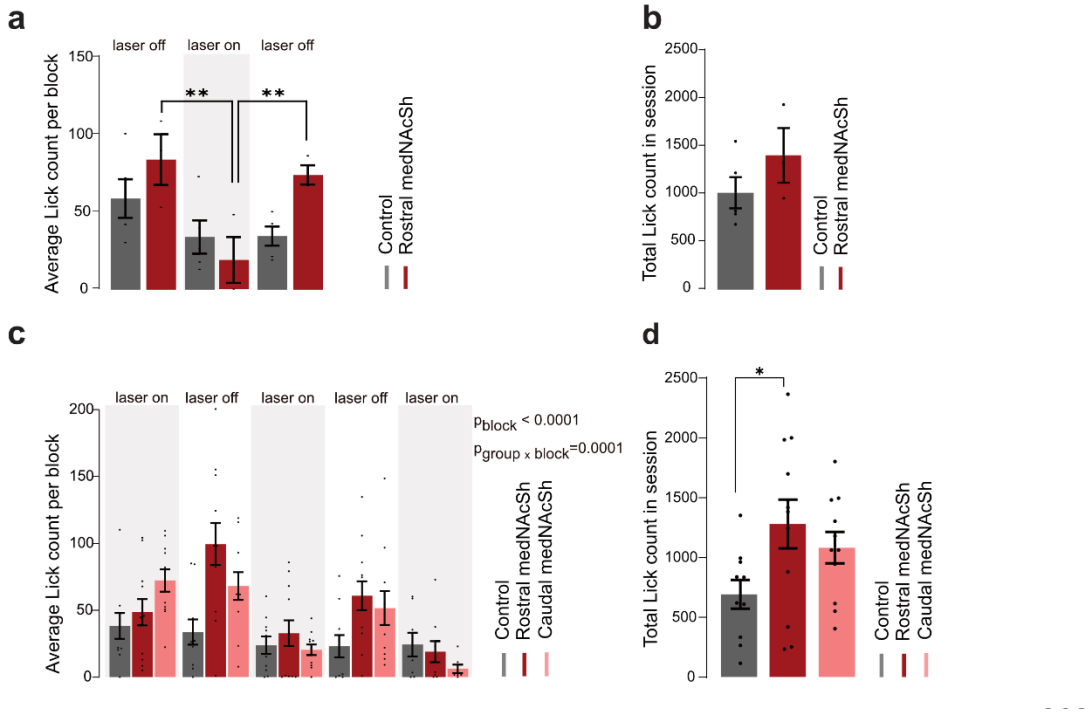
Supplementary Figure S4. Stard5-201 is the dominant splice variant expressed in the NAc, making it an adequate genomic region for 2A-Flp0 vector targeting (Stard5-Flp mouse line generation). StAR-related lipid transfer (START) domain containing 5 (Stard5) is a gene located on mouse chromosome 7, distributed over 6 exons, coding for a protein of 213 amino acid residues (transcript Stard5-201; ID: ENSMUST00000075418.15). In addition, there are 4 more registered transcripts out of which only one more has an open reading frame (transcript Stard5-202; ID: ENSMUST00000117410.2, encoding for a 206aa protein). **a.** Data is obtained from TRAP-seq data from actively transcribing transcripts in Stard5+ cells in the NAc, using a Stard5-TRAP mouse line. Sashimi plot showing the isoform model of the GENCODE GRCm39 reference genome and transcript IDs [top], the read mapping abundance (square-root transformed) [middle], and the splice junction abundance (square-root transformed) [bottom] for samples 1 and 2. A large proportion of the reads (total splice junction counts) mapped to the last exon of the Stard5-201 isoform (5742 reads mapped across both samples) rather than to the last two exons of the Stard5-202 isoform (246 reads), indicating Stard5-201 is the dominant splice variant expressed in Stard5+ cells in the NAc. Related to **Figure 7**.



Supplementary Figure S5. The Stard5-Flp transgenic mouse line genetic modification does not show off-target in vivo effects. a. Left: Scheme for the EchoMRI body composition scan and the metabolic cages (data collected across 3 days after 1-3 days habituation). Right: Bar plots showing no significant differences in body weight ($t(18) = 0.2688$, $p = 0.7912$), lean mass ($t(18) = 0.3351$, $p = 0.7415$) or fat mass ($t(18) = 0.2422$, $p = 0.8098$) between Stard-Flp positive and Stard5-Flp negative (wild-type, WT) mice. Paired t-tests, n=10 per group. **b.** Left: Scheme for the metabolic cages/indirect calorimetry. Data collected across 3 days (72 hours) after 1-3 days habituation. Right: Bar plots showing no significant differences in gross food intake ($t(18) = 1.579$, $p = 0.1317$) [this includes metabolizable and non-metabolizable energy], locomotion ($t(18) = 0.3086$, $p = 0.7612$), energy expenditure ($t(18) = 0.6488$, $p = 0.5272$) and respiratory exchange ratio ($t(18) = 0.1100$, $p = 0.9136$) between Stard-Flp positive and Stard5-Flp negative (WT) mice. This indicates a lack of effect of the Stard5-Flp transgene insertion on key in vivo parameters. Paired t-tests, n=10 per group. Data is mean ± SEM. Related to **Figure 7**.

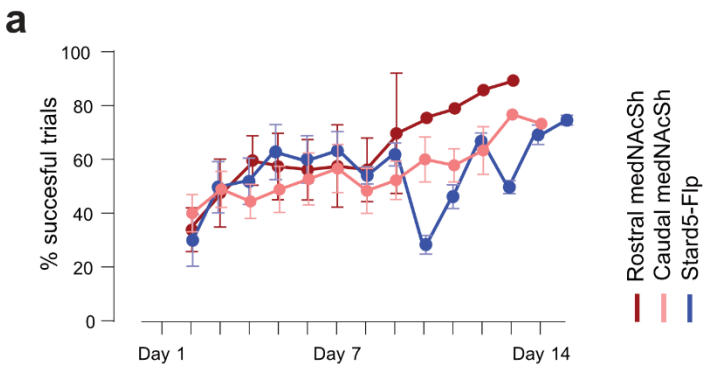


Supplementary Figure S6. The Stard5-Flp mouse line allows to identify projection sites of Stard5 cells. a. Stard5 cells send projections to the canonical projection sites of spiny projection neurons (SPNs) (i.e. D1- and D2-SPNs): the ventral pallidum (VP), lateral hypothalamus (LH) and midbrain. Left: Surgery schematic showing injection of an AAV expressing a flp-dependent fluorophore jGCaMP8m. Right: Representative coronal images showing expression of the fluorophore in the injection site (medNAcSh), as well as in identified projection sites: the VP, LH and midbrain. In the midbrain the strongest expression was in the substantia nigra reticulate (SNr) with some axons in the ventral tegmental area (VTA)/substantia nigra compacta (SNc). Related to **Figure 7**; see also **Supplementary Figure S1**.

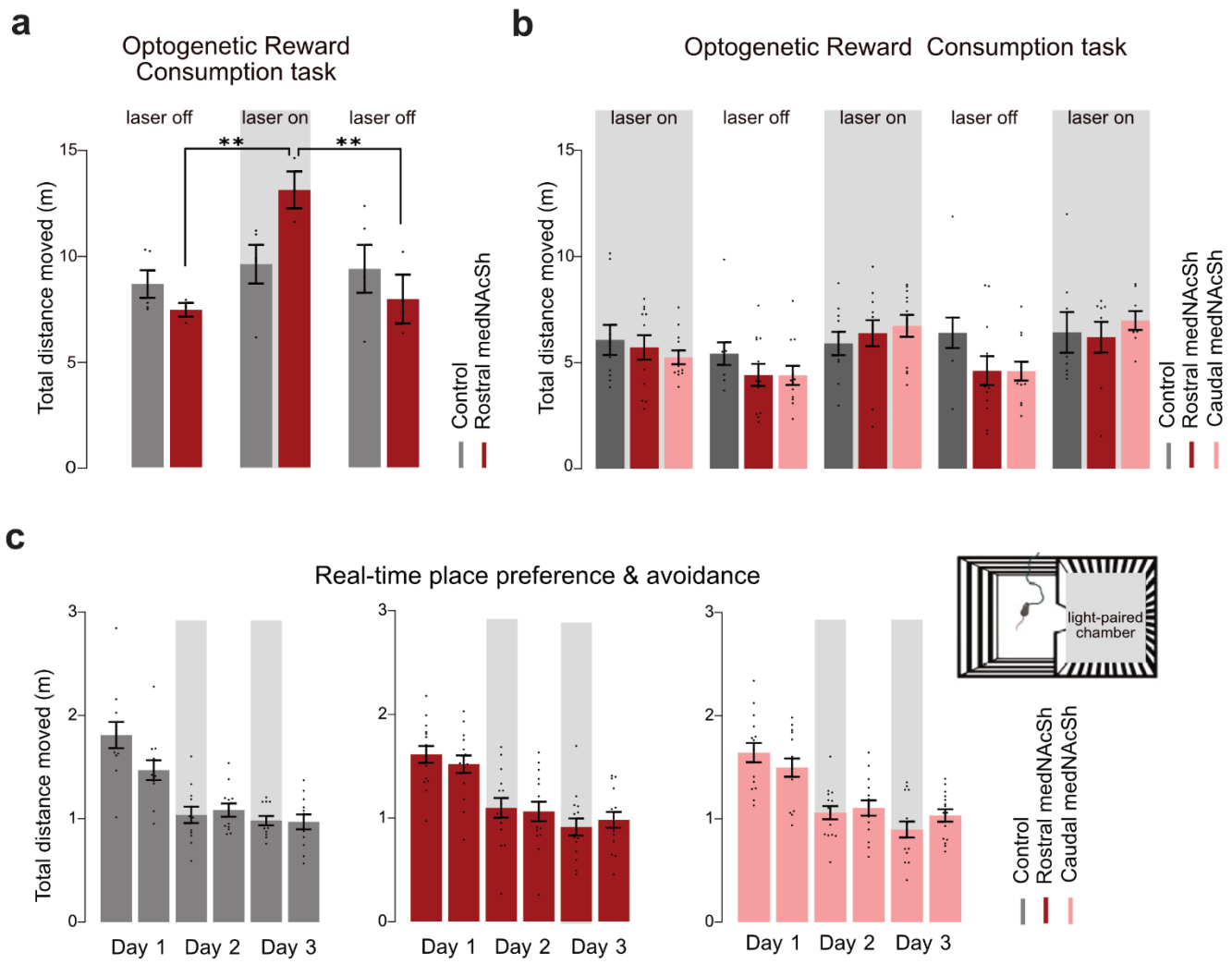


Supplementary Figure S7. Additional analyses of in vivo optogenetic stimulation of medNAcSh cells during reward consumption. a. Quantification of mean lick counts in the opto-stimulation vs. non-opto-stimulation blocks shows a significant decrease in lick counts following stimulation of rostral medNAcSh D1-SPNs (and not in mCherry controls) confirming our results in **Figure 2d-e**. 2-way RM-ANOVA (group x block). Main effects: block $F(2, 12) = 11.76$, $p=0.0015$; group $F(1, 6) = 1.693$, $p=0.2409$; group x block $F(2, 12) = 4.586$, $p=0.0332$. Sidak post-hoc opto-stimulation vs. non opto-stimulation blocks: Control off vs. on $t(12) = 2.158$, $p = 0.1477$; Control off

vs. off t(12) = 2.106, p = 0.1614; Control on vs. off t(12) = 0.05253, p >0.9999; Rostral medNAcSh off vs. on t(12) = 4.402, p= 0.0026; Rostral medNAcSh off vs. off t(12) =0.6697, p=0.8864; Rostral medNAcSh on vs. off t(12)= 3.733, p= 0.0086. N = 5 Controls; N = 3 ChrimsonR Rostral medNAcSh. Related to **Figure 2d-e**. **b**. Total lick counts during the entire 24-min session (three consecutive 8-min blocks, opto-off, opto-on, opto-off) shows no significant difference between Control and ChrimsonR Rostral medNAcSh mice. On average, Control mice licked 1017 times, while rostral ChrimsonR mice licked 1409 times. N = 5 Controls; N = 3 ChrimsonR Rostral medNAcSh. Related to **Figure 2d-e**. **c.** Quantification of mean licks per block shows a significant decrease in lick counts following stimulation of Rostral, but not Caudal medNAcSh D1-SPNs (and no change in mCherry Controls) indicating that activation of Rostral medNAcSh D1-SPNs significantly decreases reward consumption. There is a trend for a decrease in lick counts for ChrimsonR Caudal medNAcSh mice but it is not significant despite the high N (N = 11 Caudal medNAcSh, 4 female, 7 male). Results of 2-way RM-ANOVA (group x epoch) are found in **Figure 2h**. Related to **Figure 2g-i**. **d.** Total lick counts during the entire 25-min session (five consecutive 5-min blocks: opto-on, off, opto-on, off, opto-on) showed no difference between ChrimsonR Rostral and Caudal mice and between Control and Caudal medNAcSh mice, while a slight increase in lick count for the Rostral mice compared to Control (average total lick count Control: 692; Rostral medNAcSh: 1280; Caudal medNAcSh: 1082). A one-way ANOVA revealed a significant effect of the group on total licking behavior F(2,30) = 3.319, p= 0.0499). Post-hoc Sidak multiple-comparisons test showed that the Rostral ChrimsonR medNAcSh mice had a higher total lick count compared to control (t(30)=2.553, p=0.047), whereas no significant differences were observed between control and ChrimsonR Caudal medNAcSh (t(30)=1.659, p=0.29) or between Rostral and Caudal ChrimsonR mice (t(30)=0.8819, p=0.77). Related to **Figure 2g-i**.

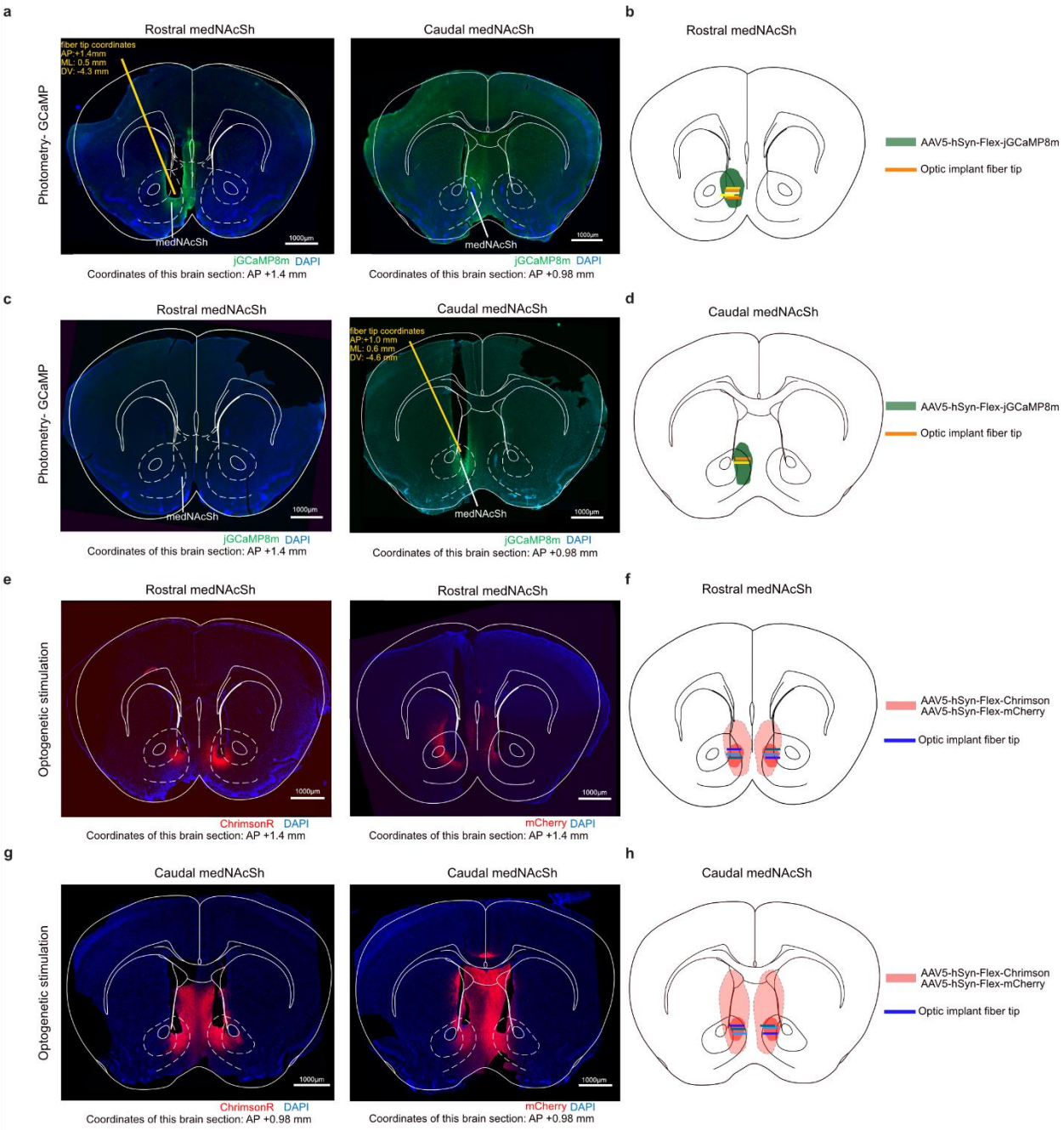


Supplementary Figure S8. Learning rate (% successful trials) during reward consumption in animals undergoing fiber photometry recordings. Mice were trained for 7–14 days prior to testing. Starting on day 7, animals that achieved at least 12 of 30 successful trials (40%) were included on the following day for *in vivo* fiber photometry recordings. Learning rate across days is shown for rostral D1-SPNs, caudal D1-SPNs, and Stard5-Flp mice. Percentages are not shown from day 1 because, during the initial training phase, mice were allowed to freely explore the arena and had unlimited access to the lick port. SEM values decrease after day 10 due to the reduced number of animals remaining in training. Related to **Figure 1** and **7**.



Supplementary Figure S9. Optogenetic stimulation of rostral medNAcSh inhibits reward consumption and promotes aversion without reducing locomotion. **a.** Total distance traveled in the operant chamber during the reward consumption experiment with an initial non-opto block, followed by an opto block and a final non-opto block. Locomotion is unchanged in Control mCherry mice, consistent with the unchanged lick counts per min shown in **Figure 2d-e**. In contrast, stimulation of rostral medNAcSh D1-SPNs increases overall movement, indicating that when mice are not consuming reward at the lick port, they spend more time exploring the chamber. This could possibly indicate anticipatory appetitive behavior. 2-way RM-ANOVA (group x epoch). Main effects: group $F(1, 6) = 0.07268$, $p=0.7965$; epoch $F(2, 12) = 10.75$, $p=0.0021$; group x epoch $F(2, 12) = 6.815$, $p=0.0105$. Sidak post-hoc non opto-stimulation vs. opto-stimulation: Controls $t(12) = 1.008$, $p = 0.7040$; Rostral medNAcSh $t(12) = 4.734$, $p = 0.0015$. Sidak post-hoc non opto-stimulation vs non opto-stimulation: Controls $t(12) = 0.7756$, $p = 0.8363$; Rostral medNAcSh $t(12) = 0.4257$, $p = 0.9666$. Sidak post-hoc opto-stimulation vs non opto-stimulation: Controls $t(12) = 0.2320$, $p = 0.9942$; Rostral medNAcSh $t(12) = 4.309$, $p = 0.0030$. N = 5 Controls, 3 female 2 male; N = 3 ChrimsonR Rostral medNAcSh, 1 female, 2 male. **b**. Quantification of the total distance moved per block (on-off-on-off-on), showing changes in locomotion across epochs are similar between Control, Rostral and Caudal ChrimsonR mice. 2-way RM-ANOVA (group x epoch). Main effects: group $F(2, 31) = 0.6620$, $p=0.5230$; epoch $F(1, 31) = 18.33$, $p=0.0002$; group x epoch $F(2, 31) = 3.035$, $p=0.0626$. **c.** Quantification of the total distance traveled in the real time place preference vs. aversion test (RTTPA). On Day 1, for all groups, the left bar corresponds to the preferred chamber and the right bar to the non-preferred chamber (identified post-hoc after analysis). The preferred chamber identified on Day 1 was subsequently designated as the light-paired chamber for

Days 2 and 3. On Days 2 and 3, the distance traveled in the light-paired chamber (marked by a gray bar) did not differ from that in the non-paired chamber, in which mice did not receive optogenetic stimulation. Distances traveled in the light-paired vs. non-paired chambers were averaged across Days 2 and 3 and analysed using a 2-way RM-ANOVA (group x chamber), which showed no significant difference in the distance moved between the two chambers for the control, ChrimsonR Rostral and ChrimsonR Caudal medNAcSh mice. Main effects: group F (2, 39) = 0.008029, p=0.9920; epoch F (1, 39) = 1.952, p=0.1702; group x epoch F (2, 39) = 0.7185, p=0.4938. Data is mean ± SEM. ns non-significant; *p<0.05; **p<0.01; ***p<0.001.



Supplementary Figure S10. Representative images of rostral and caudal optic fiber placements and viral spread. **a.** Representative coronal sections from brains in the photometry cohort. Mice were injected with a jGCaMP8m

AAV and implanted with a 400 µm optic fiber at rostral medNAcSh coordinates (AP +1.4 mm, ML 0.5 mm, DV -4.3 mm from bregma; left). The corresponding caudal section from the same brain (~AP +1.0 mm from bregma; right) shows minimal to no viral expression and no visible optic fiber track. **b**. Schematic coronal representation of the rostral medNAcSh, with the average AAV spread shown in green and optic fiber tips indicated by horizontal lines. Representative coronal section from N=6. **c.** Representative coronal section from a photometry animal injected and implanted at caudal medNAcSh coordinates (right), showing no virus or fiber track at rostral coordinates (left). **d.** Analogous to **b**, but for caudal coordinates. Representative image from N=2. **e,g.** Representative coronal sections from brains in the optogenetic cohort. Mice were injected with a ChrimsonR (left) or mCherry (right) AAV bilaterally. Representative from N=3 rostral (top, **e**) and N=3 for caudal (bottom, **g**) coordinates. **f.** Schematic coronal representation with average AAV spread in light red, highest apparent intensity of AAV in dark red and implanted with bilateral optic fiber implants (200 µm, blue lines indicate optic implant fiber tips) at rostral or **h.** caudal coordinates.

1458 **SUPPLEMENTARY DATA**

1459

1460 **Supplementary Data S1. Stard5-2A-Flp0 KI template sequence**

1461 Homology arm
1462 RAKR
1463 T2A
1464 FlpO
1465

1466 TATCATCCACAACAAGGAGGCTGAGAGGAGTCCTGGGCACTGAATCTAGAGCAGGAGTAGAAGTGACCACCATGTC
1467 TGGACCACTTCTGCTGGTCATGGCTCTGAGCTAGATGCTTCCATGGTACATATCTAACCCGCATCTGCTATTTCTT
1468 CAGGGATCCCAACAAAACCAACCTGGTCACATTCTTTCAGACAGACCTGAGTGGCTACCTCCCTCAGAGTGTGGTG
1469 GACTCCTTCTTCCCTCGAAGCATGGCTGAGTTCTACCCCAACCTTCAGAAGGCAGTGAGGAAGTTCCATCACAGGG
1470 CAAAGAGGgagggcagaggaagtcttctaacatgcggtgacgtggaggagaatcccggccctatggctcctaagaa
1471 gaagaggaaggtgatgagccagttcgacatcctgtgcaagacccccccaaggtgctggtgcggcagttcgtggag
1472 agattcgagaggcccagcggcgagaagatcgccagctgtgccgccgagctgacctacctgtgctggatgatcaccc
1473 acaacggcaccgccatcaagagggccaccttcatgagctacaacaccatcatcagcaacagcctgagcttcgacat
1474 cgtgaacaagagcctgcagttcaagtacaagacccagaaggccaccatcctggaggccagcctgaagaagctgatc
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1483 cggccctaagagccacatcggcaggcacctgatgaccagctttctgagcatgaagggcctgaccgagctgacaaac
1484 gtggtgggcaactggagcgacaagagggcctccgccgtggccaggaccacctacacccaccagatcaccgccatcc
1485 ccgaccactacttcgccctggtgtccaggtactacgcctacgaccccatcagcaaggagatgatcgccctgaagga
1486 cgagaccaaccccatcgaggagtggcagcacatcgagcagctgaagggcagcgccgagggcagcatcagatacccc
1487 gcctggaacggcatcatcagccaggaggtgctggactacctgagcagctacatcaacaggcggatcTGACAGTCGC
1488 CTATTGGCAGGGATTGCCTATGGTCCTTCAGGAACTCCAGCTGTTGAGCCTCAAAGGACACGGAAGAGCTGCTGAG
1489 GCCTTTTGAGATGCCATAGTTAACAGCAAATCTCAGGGTTCCATTCCAGAGGAGCAGCCCATCCCCCCACACCACC
1490 CTCCGTCCTCTTGGGCTTGGTGTGGAAGGAGCTAACCACTAGCTTGCCGTGGTGCACACATAGGCCTGGCAGGCCA
1491 GGGCAAGTCCCTCTATGGCCACCTGAGAGATGGCTCACGTACTCTTTGATTCCTTGTGGTCC

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Supplementary Data S2. Python code in Pynapse for unpredicted reward and unpredicted shock tasks and Optogenetic stimulation during feeding task

Unpredicted Reward Experiment

The experiment is coded in Pynapse (Python-based code in the Synapse software from Tucker Davis Technologies), driving iCON behavioral control units.

Experiment: The session lasts about 30 min. Time-out period of 3 min (180s) at start. Trial duration: 10 s (i.e. lickometer made available). Trial number: 30 times. Inter-trial interval: random intervals, 30 to 50 s. A succesful trial is defined as a trial for which the animal licked the lickometer at least once. For succesful trials, the number of licks is quantified. A lick is defined (within Synapse) as at least 3ms interacting with the lickometer.

Pynapse Code:

```
## Pynapse Source #
import random
import tdt
import numpy

class Always:   #StateID = 0
   def s_Mode_recprev():
      p_Rig.HouseFan.turnOn()
      p_Rig.HouseLight.turnOn()
      p_State.switch(PreTrial)

class PreTrial:    # StateID = 1010
   def s_State_enter():
      p_State.setTimeout(180, Trial1)

class Trial1:    # StateID = 1020
   def s_State_enter():
      p_Rig.RoundLED.turnOff()
      p_Metric.Count.inc()
      p_Metric.FirstLick.write(0)
      random.seed(56)
      p_Metric.ITI_List = []
      for n in range(0,30): #edit to change number of trials or inter-trial intervals (ITI)
         p_Metric.ITI = random.randrange(30,50,1) #edit to change range of ITI
         p_Metric.ITI_List.append(p_Metric.ITI)
      #print(p_Metric.ITI_List)
      print("Succesful trials = ", p_Metric.SuccessfulTrials.read())
      p_State.cancelTimeout()
      if p_Metric.Count.read() <= len(p_Metric.ITI_List) and p_Metric.Trials.read()<=30: #here, 30=total
number of trials (=total number of ITI)
         print("Seconds before next trial = ",p_Metric.ITI_List[p_Metric.Count.read()-1])
         p_State.setTimeout(p_Metric.ITI_List[p_Metric.Count.read()-1],Trial2)
      else:
         p_State.switch(PostTrial)
```

```
'''#TIMEOUT ONLY FOR TRAINING! REMOVE FOR ACTUAL EXPERIMENT!!
    def s_Lick_rise():
        p_Metric.ExtraLicks.inc()
        if p_Metric.ExtraLicks.read()==5: #if more than 5 licks outside the reward window, timeout initiated
            p_Metric.Timeouts.inc()
            print("lick detected outside reward window; timeout initiated")
            p_State.setTimeout(p_Metric.ITI_List[p_Metric.Count.read()-1]+60, Trial2) #timeout=60s+whatever the current ITI is
'''
class Trial2:    # StateID = 1030
    def s_State_enter():
        p_Rig.Lickometer.fire()
        p_Metric.Trials.inc()
        print("reward available")
        print("trial number = ", p_Metric.Trials.read())
        p_Metric.ExtraLicks.write(0)
        p_State.setTimeout(10, Trial1)
    def s_Lick_active():
        print("lick")
        p_Metric.Licks.inc() #at end of session, Licks=total number of valid and invalid licks from all reward windows
        p_Metric.FirstLick.inc()
        if p_Metric.FirstLick.read()==1:
            p_Rig.RoundLED.turnOn()
            p_Metric.SuccessfulTrials.inc()
            print("first lick")
    def s_Lick_fall():
        print("no lick")
    def s_Lick_fail():
        print("invalid lick")
        p_Metric.InvalidLicks.inc() #at end of sesson, InvalidLicks=total number of invalid licks from aa reward windows

class PostTrial:    # StateID = 1040
    def s_State_enter():
        p_State.setTimeout(180, EndRecording)

class EndRecording:    # StateID = 1050
    def s_State_enter():
        p_Rig.HouseFan.turnOff()
        p_Rig.HouseLight.turnOff()
        syn.setModeStr('Idle')
```

Unpredicted Shock Experiment

The experiment is coded in Pynapse (Python-based code in the Synapse software from Tucker Davis Technologies), driving iCON behavioral control units.

Experiment: Time-out period of 3 min (180s) at start, followed by a 1 s shock delivered 10 times at random intervals between 40 and 80 seconds

Pynapse Code:

```
# Pynapse Source #
import random

class Always:   #StateID = 0
   def s_Mode_recprev():
      p_Rig.HouseFan.turnOn()
      p_Rig.HouseLight.turnOn()
      p_State.switch(PreTrial)

class PreTrial:    # StateID = 1010
   def s_State_enter():
      p_State.setTimeout(180, InterTrialInterval)

class InterTrialInterval:    # StateID = 1020
   def s_State_enter():
      p_Metric.Count.inc()
      random.seed(98)
      iti_list = []
      for n in range(0,10):
         iti=random.randrange(40,80, 1)
         iti_list.append(iti)
      #print(iti_list)
      p_State.cancelTimeout()
      if p_Metric.Count.read() <= len(iti_list) and p_Metric.Shocks.read()<=10:
         print("seconds before next shock = ", iti_list[p_Metric.Count.read()-1])
         p_State.setTimeout(iti_list[p_Metric.Count.read()-1],Shock)
      else:
         p_State.switch(PostTrial)

class Shock:    # StateID = 1030
   def s_State_enter():
      p_Rig.GridShock.fire()
      print("shock given; trial number = ",p_Metric.Count.read())
      p_Metric.Shocks.inc()
      p_State.switch(InterTrialInterval)

class PostTrial:    # StateID = 1040
   def s_State_enter():
      p_State.setTimeout(180, EndRecording)

class EndRecording:    # StateID = 1050
```

```
1640        def s_State_enter():
1641            p_Rig.HouseFan.turnOff()
1642            p_Rig.HouseLight.turnOff()
1643            syn.setModeStr('Idle')
1644
1645
```

Optogenetic stimulation during reward task

Reward is available at all times. Optogenetic stimulation, triggered by TDT (5 minutes, 20 Hz)

```
## Pynapse Source #
#this code has 5 blocks:  trial 1 opto STIM,  trial 2 NO stim, trial 1 opto STIM,  trial 2 NO stim,  trial 5 OPTO STIM
#each block is 5 min long

import random
import tdt
import numpy

class Always:   #StateID = 0
    def s_Mode_recprev():
        p_Rig.HouseFan.turnOn()
        p_Rig.HouseLight.turnOn()

        p_Timer.MyTimer.turnOn()
        p_State.switch(PreTrial)
class PreTrial:    # StateID = 1010
    def s_State_enter():
        print("Baseline 2 s")
        p_Metric.TrialCounter.write(0)  # Initialize a counter to track the number of trials
        p_State.setTimeout(2, Trial1)

class Trial1:    # StateID = 1030
    def s_State_enter():
        p_Output.StartLaser.fire()  # Turn on the laser 20 Hz stim continous
        p_Metric.Trials.inc()
        print("Trial 1: OPTO stimulation, trial Nr.", p_Metric.Trials.read())
        p_State.setTimeout(300, SwitchTrial)  # After 5 minutes (300 seconds), switch to next trial

    def s_Lick_active():
        print("lick")
        p_Metric.Licks.inc()



class Trial2:    # StateID = 1031
    def s_State_enter():

        p_Metric.Trials.inc()
        print("Trial 2: NO opto stim 5 min, trial Nr. ", p_Metric.Trials.read())
        p_State.setTimeout(300, SwitchTrial)  # After 5 minutes (300 seconds), switch to next trial

    def s_Lick_active():
        print("lick")
        p_Metric.Licks.inc()


class SwitchTrial:   # StateID = 1040
    def s_State_enter():
        current_trial = p_Metric.TrialCounter.read()
        if current_trial < 4:  # Check if there are more trials to run
            if current_trial % 2 == 0:  # If the trial count is even, switch to Trial 2
```

```
            p_State.switch(Trial2)
        else:  # If the trial count is odd, switch to Trial 1 (opto stim)
            p_State.switch(Trial1)
        p_Metric.TrialCounter.inc()
    else:
        p_State.switch(PostTrial)  # After 5 trials (Trial 1 Trial 2 Trial 1 Trial 2 Trial 1), end the session

class PostTrial:    # StateID = 1050
    def s_State_enter():
        #p_Rig.Speaker2.fire()
        print("POST TRIAL: 5 seconds left!!!!")
        p_State.setTimeout(5, EndRecording)

class EndRecording:    # StateID = 1060
    def s_State_enter():
        p_Rig.HouseFan.turnOff()
        p_Rig.HouseLight.turnOff()
        syn.setModeStr('Idle')
```

1722 **Supplementary Data S3. Source Data (Separate Excel file)**

1723