# A gut-to-brain signal of fluid osmolarity controls thirst satiation

Christopher A. Zimmerman<sup>1,2,3</sup>, Erica L. Huey<sup>1,2,7</sup>, Jamie S. Ahn<sup>1,2,8</sup>, Lisa R. Beutler<sup>2,4</sup>, Chan Lek Tan<sup>1,2,9</sup>, Seher Kosar<sup>1,2</sup>, Ling Bai<sup>1,2</sup>, Yiming Chen<sup>1,2,3</sup>, Timothy V. Corpuz<sup>1,2</sup>, Linda Madisen<sup>5</sup>, Hongkui Zeng<sup>5</sup> & Zachary A. Knight<sup>1,2,3,6</sup>\*

Satiation is the process by which eating and drinking reduce appetite. For thirst, oropharyngeal cues have a critical role in driving satiation by reporting to the brain the volume of fluid that has been ingested<sup>1-12</sup>. By contrast, the mechanisms that relay the osmolarity of ingested fluids remain poorly understood. Here we show that the water and salt content of the gastrointestinal tract are precisely measured and then rapidly communicated to the brain to control drinking behaviour in mice. We demonstrate that this osmosensory signal is necessary and sufficient for satiation during normal drinking, involves the vagus nerve and is transmitted to key forebrain neurons that control thirst and vasopressin secretion. Using microendoscopic imaging, we show that individual neurons compute homeostatic need by integrating this gastrointestinal osmosensory information with oropharyngeal and blood-borne signals. These findings reveal how the fluid homeostasis system monitors the osmolarity of ingested fluids to dynamically control drinking behaviour.

Drinking influences the volume and composition of the blood<sup>1-4</sup>. However, ingestion of water and salt have opposing consequences for fluid balance, which raises the question of how the brain monitors the osmolarity of ingested fluids.

One way in which the brain controls drinking is by tracking the passage of fluids through the mouth and throat. Classic experiments have demonstrated that drinking temporarily satiates thirst even if the ingested water is immediately drained from the oesophagus<sup>5–8</sup>; recent work has identified specific populations of forebrain neurons that receive this rapid oropharyngeal signal during drinking<sup>9–12</sup>. This mechanism allows the brain to track fluid intake in real time, and thereby quench thirst and inhibit the secretion of vasopressin in anticipation of water absorption into the bloodstream (which occurs gradually over tens of minutes).

Nevertheless, this oropharyngeal signal communicates to the brain only the volume of fluid ingested and not its composition<sup>9,12</sup>, which suggests that a distinct mechanism tracks fluid osmolarity during drinking. Taste aversion prevents the consumption of highly concentrated salt solutions<sup>13,14</sup>, but there is little evidence that taste fine-tunes fluid consumption to match homeostatic need. The infusion of fluids into the gastrointestinal tract and hepatic portal circulation has previously been reported to influence drinking behaviour and vasopressin secretion in some studies and species but not others<sup>5-8,15-17</sup>, and it remains unclear where pre-absorptive fluids are monitored in the periphery, what exactly is sensed, which neurons in the brain receive this information and how they use it to regulate behaviour<sup>1-4</sup>. A fundamental source of ambiguity in these experiments is that traditional behavioural and physiologic readouts vary on the same timescale as fluid absorption (minutes), which makes it difficult to disentangle pre-absorptive signals that are sensed remotely in the periphery from systemic signals that are sensed directly in the brain.

To gain insight into these longstanding questions, we set out to monitor directly the dynamics of thirst-promoting neurons in the brain while simultaneously manipulating the fluids that were ingested or infused into peripheral tissues. We first measured how fluid osmolarity influences drinking behaviour and thirst neuron activity. Mice were equipped for fibre photometry recordings<sup>18</sup> of glutamatergic neurons in the subfornical organ<sup>9,19,20</sup> (SFO<sup>Nos1</sup> neurons; defined by expression of Nos1) that promote drinking and directly monitor blood osmolarity (Fig. 1a). These mice were dehydrated and then given access to either water or hypertonic saline. As previously shown<sup>9</sup>, the ingestion of either fluid resulted in rapid inhibition of SFO<sup>Nos1</sup> neurons that was time-locked to the act of drinking (Fig. 1b). SFO<sup>Nos1</sup> neurons remained inhibited after bouts of water consumption, but their activity increased to pre-ingestion levels after each bout of saline consumption terminated (Fig. 1c). On the basis of the kinetics of this neural activity rebound, we hypothesized that SFO<sup>Nos1</sup> neurons may receive a signal from the gastrointestinal tract that depends on the osmolarity of the ingested fluid. Consistent with this, we found that isolated changes in gastrointestinal osmolarity were sufficient to influence salt preference and drinking behaviour (Extended Data Fig. 1).

To investigate how information about the water and salt content of the gastrointestinal tract is communicated to the brain, we prepared mice with intragastric catheters for fluid infusion into the stomach<sup>21</sup> as well as fibre photometry implants for recording SFO<sup>Nos1</sup> neuron dynamics (Fig. 1d). Notably, intragastric infusion of water rapidly inhibited SFO<sup>Nos1</sup> neurons (latency  $105 \pm 13$  s, mean  $\pm$  s.e.m.), whereas intragastric infusion of hypertonic saline activated the same population of neurons (Fig. 1e). Infusions of a range of NaCl concentrations revealed a linear correlation between the osmolarity of the infused fluid and the modulation of SFO<sup>Nos1</sup> neuron activity (Fig. 1f). This response was independent of the initial hydration state of the mouse (Fig. 1g) or the identity of the infused osmolyte (Extended Data Fig. 2), and was complete before any detectable change in blood osmolarity occurred (Extended Data Fig. 2), which indicates that this response reflects local sensing within the gastrointestinal tract (probably in the proximal small intestine rather than the stomach itself) or hepatic portal circulation<sup>3,15–17</sup>. Consistent with this, intragastric infusion of hypertonic solutions strongly activated SFO<sup>Nos1</sup> neurons regardless of whether the infused osmolyte could be absorbed into the bloodstream (Extended Data Fig. 2). Together, these data reveal that osmolarity is precisely measured within the gastrointestinal tract and then communicated to thirst neurons in the brain.

We next investigated the importance of this gut–brain communication for thirst satiation. In dehydrated mice, intragastric infusion of water rapidly inhibited SFO<sup>Nos1</sup> neurons (latency  $107 \pm 15$  s, mean  $\pm$  s.e.m.) and abolished drinking when water was subsequently presented (Fig. 2a, b, Extended Data Fig. 3). This indicates that changes in gastrointestinal osmolarity (but not distension) are sufficient to

<sup>&</sup>lt;sup>1</sup>Department of Physiology, University of California San Francisco, San Francisco, CA, USA. <sup>2</sup>Kavli Institute for Fundamental Neuroscience, University of California San Francisco, San Francisco, CA, USA. <sup>3</sup>Neuroscience Graduate Program, University of California San Francisco, San Francisco, San Francisco, CA, USA. <sup>4</sup>Department of Medicine, University of California San Francisco, San Francisco, CA, USA. <sup>5</sup>Allen Institute for Brain Science, Seattle, WA, USA. <sup>6</sup>Howard Hughes Medical Institute, University of California San Francisco, CA, USA. <sup>7</sup>Present address: Graduate Program in Neuroscience, Harvard Medical School, Boston, MA, USA. <sup>8</sup>Present address: California Northstate University College of Medicine, Elk Grove, CA, USA. <sup>9</sup>Present address: Department of Neuroscience, Genentech Inc., South San Francisco, CA, USA. \*e-mail: zachary.knight@ucsf.edu



Fig. 1 | Gastrointestinal osmolarity modulates drinking behaviour and SFO thirst neuron activity. a, Schematic for fibre photometry recording of SFO neurons. Scale bar, 1 mm. b, Left, average SFO activity and drinking behaviour after dehydration. Right, quantification; n = 5 mice, two-tailed Student's t-tests. c, SFO neuron dynamics during individual water (29 bouts) or NaCl (37 bouts) drinking bouts. d, Schematic for intragastric infusion during fibre photometry recording. e, Left, SFO neuron dynamics of individual mice during infusions of water or NaCl, while hydrated. Right, average SFO activity during infusions (n = 4 mice). **f**, Correlation between infusion osmolarity (mOsm l<sup>-1</sup>) and change in SFO activity (n = 4 mice, linear regression). g, SFO activity change after infusion while hydrated or dehydrated (n = 4 mice, two-way analysis of variance (ANOVA), Holm-Šídák correction). Hyd., hydrated; dehyd., dehydrated. Error bars represent mean  $\pm$  s.e.m. Shaded areas in **b**, **e** represent mean  $\pm$  s.e.m., and in **f** represent 95% confidence interval for the line of best fit. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, \*\*\*P < 0.001. The mouse brain in this figure has been reproduced with permission from ref. 35, Copyright © 2012.

satiate thirst and, conversely, that oropharyngeal cues are not required. The behavioural response to changes in gastrointestinal osmolarity but not to systemic changes in osmolarity—was dependent on hydration state: the intragastric infusion of salt did not stimulate drinking in mice that were hydrated, but did cause an increase in water consumption by dehydrated mice (Fig. 2b, c, Extended Data Fig. 3). This state-dependent effect is reminiscent of how the inhibition of satiation signals for feeding (such as cholecystokinin) can increase meal size in hungry animals, but does not typically trigger the initiation of feeding in animals that are well-fed<sup>22</sup>.

One mechanism by which gastrointestinal osmolarity could regulate satiation is by acting as a 'stabilization signal' that determines whether SFO<sup>Nos1</sup> neurons remain inhibited when drinking terminates (Fig. 1b, c). If this were true, then delivery of salt to the gastrointestinal tract should cause the activity of SFO<sup>Nos1</sup> neurons to rebound even after mice drink pure water. To test this, we gave dehydrated mice an intragastric infusion of salt and then analysed the dynamics of SFO<sup>Nos1</sup> neurons during early bouts of water drinking (Fig. 2d–f). Indeed, we found that the activity of SFO<sup>Nos1</sup> neurons rebounded when drinking stopped, such that the neural dynamics during water ingestion now resembled those observed during the ingestion of hypertonic saline (Fig. 1b, c). This response was specific to salt infused into the stomach, as an identical dose of salt delivered by systemic injection produced no equivalent effect. This suggests that gastrointestinal osmosensation specifies whether the rapid inhibition of thirst neurons by oropharyngeal cues during drinking is transient or durable.

We next investigated whether the inhibition of SFO<sup>Nos1</sup> neurons by the gastrointestinal osmosensory signal is required for thirst satiation. We surgically equipped mice for intragastric infusion combined with



Fig. 2 | The gut-to-brain osmosensory signal controls thirst satiation. a, Example of SFO neuron dynamics during intragastric infusion after dehydration. b, Left, correlation between SFO activity change and water intake (relative to sham infusion) after 1-ml infusions into hydrated (black; n = 24 experiments from 4 mice, linear regression,  $R^2 = 0.2084$ , P = 0.0249) or dehydrated (red; n = 12 experiments from 4 mice, linear regression,  $R^2 = 0.8493$ , P < 0.0001) mice. Right, initial drinking rate of dehydrated mice after infusion (n = 4 mice, one-way ANOVA, Holm-Šídák correction). c, Water intake after systemic (intraperitoneal (i.p.)) or intragastric (i.g.) treatment with 150  $\mu$ l NaCl (n = 4 mice, one-way ANOVA, Holm-Šídák correction). d, Example of SFO neuron dynamics during water drinking after treatment with 150 µl NaCl in dehydrated mice. e, Left, average SFO activity and drinking behaviour after treatment with 150  $\mu$ l NaCl in dehydrated mice. Right, quantification (n = 4 mice, two-tailed Student's t-tests). f, SFO neuron dynamics during individual water drinking bouts after systemic (17 bouts) or intragastric (15 bouts) treatment with 150 µl NaCl. g, Schematic for intragastric infusion during optogenetic activation. Scale bar, 1 mm. h, Dehydration-induced drinking after intragastric infusion either with (right) or without (left) simultaneous photostimulation of SFO neurons (n = 4 mice). i, Quantification of **h** (n = 4 mice, two-tailed Student's *t*-test). Error bars represent mean  $\pm$  s.e.m. Shaded areas in **a**, **d** represent intragastric infusion (red) or individual licks (grey); shaded areas in b represent 95% confidence interval for the line of best fit; shaded areas in **e**, **h** represent mean  $\pm$  s.e.m. NS, not significant, \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001.

simultaneous optogenetic activation<sup>23</sup> of SFO<sup>Nos1</sup> neurons (Fig. 2g). As expected, brief (3 min) intragastric infusion of water into dehydrated mice eliminated drinking when water was subsequently presented. However, optogenetic re-activation of SFO<sup>Nos1</sup> neurons during and after water infusion completely blocked this satiating effect (Fig. 2h, i). This indicates that the SFO is a necessary site in the brain at which the osmosensory signal from the gastrointestinal tract is received to control the termination of drinking.

The peripheral mechanisms that communicate gastrointestinal osmolarity to the brain are unknown. To begin to address this question, we investigated the role of the vagus nerve (cranial nerve X), which densely innervates the stomach and intestines<sup>24</sup>. Subdiaphragmatic vagotomy significantly attenuated the satiating effect of intragastric



Fig. 3 | Vasopressin neurons bidirectionally encode gastrointestinal osmolarity. a, Schematic for fibre photometry recording of SON vasopressin neurons. Scale bar, 1 mm. b, Vasopressin neuron response to vehicle or 3 M NaCl intraperitoneal injection (n = 7 mice; two-tailed Student's *t*-test). c, Average vasopressin neuron activity and drinking behaviour after dehydration (n = 5 mice). d, Vasopressin neuron dynamics of individual mice during intragastric infusions of water or NaCl (n = 4 mice). Error bars and shaded areas represent mean  $\pm$  s.e.m. The mouse brain in this figure has been reproduced with permission from ref. <sup>35</sup>, Copyright © 2012.

water infusion and, furthermore, greatly reduced the ability of intragastric water or salt to modulate the activity of SFO<sup>Nos1</sup> neurons (Extended Data Fig. 4a–d). Genetic ablation of a subset of vagal sensory neurons had similar effects (Extended Data Fig. 4e–i). Together, these experiments indicate that vagal afferents are an essential part of the pathway by which gastrointestinal osmolarity is communicated to the brain, although they do not rule out an additional role for circulating signals released from the gut following fluid ingestion (Extended Data Fig. 4j).

We next investigated how information about the water and salt content of the gastrointestinal tract is represented within the broader neural circuit that controls fluid homeostasis. We first recorded, using fibre photometry, the dynamics of vasopressin-secreting neurons in the supraoptic nucleus (SON<sup>Avp</sup> neurons; defined by expression of *Avp*) (Fig. 3a). We confirmed that, similar to SFO<sup>Nos1</sup> neurons<sup>9</sup>, these cells are activated by increases in blood osmolarity (Fig. 3b) and are rapidly inhibited during drinking<sup>7,10,25</sup> (Fig. 3c). By combining intragastric infusions with neural recordings, we found that SON<sup>Avp</sup> neurons are also bidirectionally regulated by gastrointestinal osmolarity, with kinetics similar to those of SFO<sup>Nos1</sup> neurons (Fig. 3d, Extended Data Fig. 5). This gastrointestinal signal may function in coordination with oropharyngeal cues<sup>10</sup> to pre-emptively regulate the secretion of vasopressin during eating and drinking.

The median preoptic nucleus (MnPO) is a crucial node in the fluid homeostasis system of the brain<sup>1,2</sup> (Fig. 4a); the MnPO is bidirectionally connected to the SFO through glutamatergic neurons that promote drinking<sup>11,12,26,27</sup> (defined by expression of *Adcyap1*, *Agtr1a* and *Nxph4*) and GABAergic ( $\gamma$ -aminobutyric-acid-releasing) neurons that inhibit drinking<sup>12,26</sup> (defined by expression of *Glp1r*). Given that this classification understates the heterogeneity of MnPO cell types<sup>28</sup> and that the single-cell dynamics of these neurons during behaviour remain unknown, we used microendoscopic imaging<sup>29</sup> to investigate how these neurons encode aspects of fluid balance.

To gain genetic access to thirst-promoting, glutamatergic neurons in the MnPO, we generated knock-in mice that express Cre recombinase from the *Nxph4* locus (*Nxph4-2a-cre* mice) (Fig. 4b, Extended Data Fig. 6). We then targeted the fluorescent calcium indicator GCaMP to these neurons, and implanted a gradient-index lens above the MnPO to record their dynamics in awake, behaving mice (Fig. 4c). We first tested mice in a paradigm in which they were injected with isotonic saline, then 10 min later injected with salt to induce thirst and—another 10 min later—given access to water (Fig. 4d, e). Analysis of neural dynamics in this paradigm by *k*-means clustering revealed three distinct subpopulations of MnPO<sup>*Nxph4*</sup> neurons (defined by expression of *Nxph4*; Extended Data Fig. 7). One subpopulation (cluster 1, 17%) had no response to isotonic saline injection but showed marked and sustained activation after salt challenge, which suggests



Fig. 4 | Individual glutamatergic MnPO neurons integrate information from the oropharynx, gastrointestinal tract and blood. a, Illustration of the neural circuit that controls fluid homeostasis. b, Schematic for generation of the Nxph4-2a-cre mouse line (left) and GFP reporter recombination in the MnPO (right). Scale bar, 100 µm. c, Schematic for microendoscope imaging of glutamatergic MnPO neurons. Scale bar, 100 µm. d, Dynamics of individual neurons tracked during vehicle intraperitoneal injection, 3 M NaCl intraperitoneal injection and water drinking. **e**, Average responses of neuron clusters (n = 47 neurons; see Extended Data Fig. 7a). f, Dynamics of individual neurons tracked during intragastric infusion of water after dehydration (left), and during 500 mM NaCl intragastric infusion while hydrated (right). g, Correlation between the responses (z-score) of individual neurons to intragastric infusion of water or 500 mM NaCl (n = 38 neurons; linear regression,  $R^2 = 0.2154$ , P = 0.0033). h, Average response to 3 M NaCl intraperitoneal injection of neurons that were inhibited (red; 26% inhibited  $\geq 1\sigma$  after infusion) or unmodulated (black; 74%) by intragastric infusion of water (n = 53 neurons; see Extended Data Fig. 7d). Shaded areas in **e**, **h** represent mean  $\pm$  s.e.m., and in g represent 95% confidence interval for the line of best fit. The mouse brain in this figure has been reproduced with permission from ref. 35, Copyright © 2012.

that these neurons encode blood osmolarity. These same neurons were rapidly and uniformly inhibited during drinking. By contrast, neurons from cluster 2 (34%) showed only transient responses that we interpret as probably representing stress or pain, whereas neurons from cluster 3 (49%) were largely unresponsive. We then investigated how these neurons respond to changes gastrointestinal osmolarity. Intragastric infusion of water into dehydrated mice inhibited a subset of MnPO<sup>Nxph4</sup> neurons (24–26%), whereas infusion of hypertonic saline into hydrated mice activated a similar proportion of cells (34%) (Fig. 4f, g, Extended Data Fig. 7). Registration of neurons across trials revealed that these two populations were largely overlapping, which indicates that a specific subpopulation of MnPO<sup>Nxph4</sup> neurons is bidirectionally modulated by gastrointestinal signals. Moreover, the majority of gastrointestinal-tuned (inhibited by  $\geq 1\sigma$  after intragastric infusion of water) MnPO<sup>Nxph4</sup> neurons were robustly activated during thirst (Fig. 4h, Extended Data Fig. 7). This reveals that individual MnPO<sup>Nxph4</sup> neurons receive ingestion signals from the oropharynx, satiation signals from the gastrointestinal tract and homeostatic signals from the blood, which they integrate to estimate physiological state. Experiments that combined chemogenetic silencing<sup>30</sup> with fibre photometry recordings suggest that these MnPO neurons are required for relaying gastrointestinal osmolarity information to the SON but not to the SFO (Extended Data Fig. 8).

In contrast to MnPO<sup>*Nxph4*</sup> neurons, microendoscopic imaging of intermingled GABAergic MnPO<sup>*Glp1r*</sup> neurons (defined by expression of *Glp1r*) showed only weak and transient responses during salt challenge (Fig. 5a, Extended Data Fig. 9), which indicates that these cells



Fig. 5 | GABAergic MnPO neurons bidirectionally encode fluid ingestion. a, Schematic for microendoscope imaging of GABAergic MnPO neurons. Scale bar, 100 µm. b, Dynamics of individual neurons during water access after dehydration. c, Left, dynamics during drinking. Right, examples of tuning maps. d, Average responses of ingestionactivated (modulated  $\geq 1\sigma$  during first minute of drinking), ingestioninhibited (modulated  $\leq -1\sigma$ ) and untuned neurons during drinking (n = 77 neurons). e, Dynamics of individual neurons tracked during intragastric infusion of water while hydrated (left), and during drinking after dehydration (right). f, Correlation between the responses (z-score) of individual neurons to intragastric infusion of water or drinking (n = 46neurons; linear regression,  $R^2 = 0.1062$ , P = 0.0271). g, Correlation between the responses (z-score) to intragastric infusion of water or 500 mM NaCl (n = 45 neurons; linear regression,  $R^2 = 0.0467$ , P = 0.1514). Shaded areas in **b** represent individual licks; shaded areas in **d** represent mean  $\pm$  s.e.m.; shaded areas in **f**, **g** represent 95% confidence interval for the line of best fit. The mouse brain in this figure has been reproduced with permission from ref.<sup>35</sup>, Copyright © 2012.

do not encode blood osmolarity in their baseline activity. We therefore recorded the dynamics of these neurons during re-access to water after dehydration, which revealed strong responses during drinking (Fig. 5b-d, Extended Data Fig. 9). The majority of MnPO<sup>Glp1r</sup> neurons were either activated (28%) or inhibited (36%) during water ingestion; these responses were time-locked to the act of drinking, such that their activity returned to baseline when ingestion stopped. The cells that exhibited these varied responses were spatially intermingled (Fig. 5c), which suggests that the functional diversity of the MnPO is not anatomically organized at the scale of our recordings (500  $\mu$ m). Of note, a previous study using fibre photometry detected only activation of MnPO<sup>Glp1r</sup> neurons during drinking<sup>12</sup>, which suggests that bulk fluorescence measurements masked the existence of an equal proportion of ingestion-inhibited cells. In addition, we observed smaller subsets of MnPO<sup>Glp1r</sup> neurons that were activated or inhibited by water access alone (Fig. 5b) or by intragastric infusion of either water or hypertonic saline (Fig. 5e-g, Extended Data Fig. 9). Together, these data indicate that the majority of GABAergic MnPO neurons are strongly modulated during ingestion, with smaller subsets that encode diverse signals that are relevant to fluid balance (such as water availability, stress and gastrointestinal osmolarity).

Understanding how eating and drinking reduce appetite is one of the fundamental challenges in physiology. Traditionally, this problem has been studied by measuring the effect of peripheral manipulations on behaviour<sup>31,32</sup>. Here we have taken a complementary approach that uses the dynamics of appetite-promoting neurons in the brain as a readout to monitor, in real time, the functional implications of manipulations to peripheral tissues. Using this strategy, we have shown that the water and salt content of the gastrointestinal tract is precisely measured

during drinking and then communicated to key neurons in the brain that control fluid balance. Furthermore, we have shown that this gutto-brain signal requires the vagus nerve, and is necessary and sufficient for the satiation of thirst and regulation of vasopressin secretion during normal behaviour.

The gastrointestinal osmosensory signal described here operates alongside oropharyngeal and blood-borne cues to regulate drinking behaviour. We propose that these anatomically and temporally distinct signals cooperate to promote satiation in three steps (Extended Data Fig. 10). First, detection of liquid in the mouth generates a rapid signal that reports the volume of fluid ingested<sup>9-12</sup>. This early estimate of volume inhibits forebrain thirst neurons during the act of drinking, but is transient. Next, detection in the gastrointestinal tract generates a second signal that reports the osmolarity of the ingested fluid. This early estimate of osmolarity stabilizes the inhibition of forebrain thirst neurons if water was consumed, or causes their activity to rebound if the ingested fluid was hypertonic. Finally, absorption of water into the bloodstream alters fluid balance throughout the body, which leads to sustained changes in well-characterized signals (such as blood osmolarity) that are monitored by the brain directly<sup>1-4</sup>. This three-step mechanism enables the brain to dynamically adjust drinking behaviour to match the level of homeostatic need, regardless of the composition of ingested fluids.

The concept of a 'set point' or 'balance point' has had a dominant role in shaping how we think about homeostasis<sup>33,34</sup>. Inherent to this concept is the idea that there is an anatomical site at which measurements from the body are integrated to estimate the level of a physiological variable. Although this is commonly assumed to happen in specific neurons in the brain, it has rarely—if ever—been directly observed in vivo. We have shown here that individual, genetically defined thirst neurons in the MnPO integrate information about fluid balance that arises from the oropharynx, gastrointestinal tract and blood. This reveals that homeostatic need can be computed at the level of single neurons in a living animal. Further study of this single-cell integration may provide insights into the origin of the enigmatic set-points that characterize physiological systems.

#### **Online content**

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41586-019-1066-x.

Received: 3 September 2018; Accepted: 4 March 2019; Published online 27 March 2019.

- 1. Zimmerman, C. A., Leib, D. E. & Knight, Z. A. Neural circuits underlying thirst
- and fluid homeostasis. *Nat. Rev. Neurosci.* 18, 459–469 (2017).
  Gizowski, C. & Bourque, C. W. The neural basis of homeostatic and anticipatory
- thirst. Nat. Rev. Nephrol. 14, 11–25 (2018).
   Johnson, A. K. & Thunhorst, R. L. The neuroendocrinology of thirst and salt
- appetite: visceral sensory signals and mechanisms of central integration. Front. Neuroendocrinol. 18, 292–353 (1997).
- McKinley, M. J. & Johnson, A. K. The physiological regulation of thirst and fluid intake. *News Physiol. Sci.* 19, 1–6 (2004).
- Bellows, R. T. Time factors in water drinking in dogs. Am. J. Physiol. 125, 87–97 (1938).
- Adolph, E. F., Barker, J. P. & Hoy, P. A. Multiple factors in thirst. Am. J. Physiol. 178, 538–562 (1954).
- Thrasher, T. N., Nistal-Herrera, J. F., Keil, L. C. & Ramsay, D. J. Satiety and inhibition of vasopressin secretion after drinking in dehydrated dogs. *Am. J. Physiol. Endocrinol. Metabol.* 240, E394–E401 (1981).
- Figaro, M. K. & Mack, G. W. Regulation of fluid intake in dehydrated humans: role of oropharyngeal stimulation. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 272, R1740–R1746 (1997).
- 9. Zimmerman, C. A. et al. Thirst neurons anticipate the homeostatic consequences of eating and drinking. *Nature* **537**, 680–684 (2016).
- Mandelblat-Cerf, Y. et al. Bidirectional anticipation of future osmotic challenges by vasopressin neurons. *Neuron* 93, 57–65 (2017).
- Allen, W. E. et al. Thirst-associated preoptic neurons encode an aversive motivational drive. Science 357, 1149–1155 (2017).
- Augustine, V. et al. Hierarchical neural architecture underlying thirst regulation. Nature 555, 204–209 (2018).
- Weiner, I. H. & Stellar, E. Salt preference of the rat determined by a singlestimulus method. J. Comp. Physiol. Psychol. 44, 394–401 (1951).

### **RESEARCH LETTER**

- Oka, Y., Butnaru, M., von Buchholtz, L., Ryba, N. J. P. & Zuker, C. S. High salt recruits aversive taste pathways. *Nature* 494, 472–475 (2013).
- Maddison, S., Wood, R. J., Rolls, E. T., Rolls, B. J. & Gibbs, J. Drinking in the rhesus monkey: peripheral factors. *J. Comp. Physiol. Psychol.* 94, 365–374 (1980).
- Baertschi, A. J. & Vallet, P. G. Osmosensitivity of the hepatic portal vein area and vasopressin release in rats. J. Physiol. (Lond.) 315, 217–230 (1981).
- Stricker, E. M., Callahan, J. B., Huang, W. & Šved, A. F. Early osmoregulatory stimulation of neurohypophyseal hormone secretion and thirst after gastric NaCl loads. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 282, R1710–R1717 (2002).
- Gunaydin, L. A. et al. Natural neural projection dynamics underlying social behavior. *Cell* 157, 1535–1551 (2014).
- Oka, Y., Ye, M. & Zuker, C. S. Thirst driving and suppressing signals encoded by distinct neural populations in the brain. *Nature* 520, 349–352 (2015).
- Betley, J. N. et al. Neurons for hunger and thirst transmit a negative-valence teaching signal. *Nature* 521, 180–185 (2015).
- Ueno, A. et al. Mouse intragastric infusion (iG) model. Nat. Protocols 7, 771–781 (2012).
- Andermann, M. L. & Lowell, B. B. Toward a wiring diagram understanding of appetite control. *Neuron* 95, 757–778 (2017).
- Zhang, F. et al. Optogenetic interrogation of neural circuits: technology for probing mammalian brain structures. Nat. Protocols 5, 439–456 (2010).
- Berthoud, H.-R. & Neuhuber, W. L. Functional and chemical anatomy of the afferent vagal system. Auton. Neurosci. 85, 1–17 (2000).
- Vincent, J. D., Arnauld, E. & Bioulac, B. Activity of osmosensitive single cells in the hypothalamus of the behaving monkey during drinking. *Brain Res.* 44, 371–384 (1972).
- Abbott, S. B. G., Machado, N. L. S., Geerling, J. C. & Saper, C. B. Reciprocal control of drinking behavior by median preoptic neurons in mice. *J. Neurosci.* 36, 8228–8237 (2016).
- Leib, D. E. et al. The forebrain thirst circuit drives drinking through negative reinforcement. *Neuron* 96, 1272–1281 (2017).
- McKinley, M. J. et al. The median preoptic nucleus: front and centre for the regulation of body fluid, sodium, temperature, sleep and cardiovascular homeostasis. Acta Physiol. (Oxf.) 214, 8–32 (2015).
- Ghosh, K. K. et al. Miniaturized integration of a fluorescence microscope. Nat. Methods 8, 871–878 (2011).
- Armbruster, B. N., Li, X., Pausch, M. H., Herlitze, S. & Roth, B. L. Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. *Proc. Natl Acad. Sci. USA* **104**, 5163–5168 (2007).
- Bernard, C. Leçons de Physiologie Expérimentale Appliquée à la Médecine (J.-B. Baillière et Fils, Paris, 1856).
- 32. Richter, C. P. Total self regulatory functions in animals and human beings. *Harvey Lecture Series* **38**, 63–103 (1943).
- Cannon, W. B. Organization for physiological homeostasis. *Physiol. Rev.* 9, 399–431 (1929).

- Berridge, K. C. Motivation concepts in behavioral neuroscience. *Physiol. Behav.* 81, 179–209 (2004).
- Paxinos, G. & Franklin, K. B. J. The Mouse Brain in Stereotaxic Coordinates 4 edn (Academic, London, UK, 2012).

Acknowledgements We thank Inscopix and J. Parker for technical assistance, the René Remie Surgical Skills Center for surgical training, and members of the Knight laboratory for comments on the manuscript. C.A.Z. is supported by the NSF Graduate Research Fellowship (DGE-1144247), UCSF Discovery Fellowship, Genentech Foundation Predoctoral Fellowship, and NIH National Research Service Award (F31-HL137383). Z.A.K. is a Howard Hughes Medical Institute Investigator, and this work was supported by the New York Stem Cell Foundation, a Pathway Award from the American Diabetes Association, Rita Allen Foundation, McKnight Foundation, Alfred P. Sloan Foundation, Brain and Behavior Research Foundation, Esther A. and Joseph Klingenstein Foundation, UCSF Program for Breakthrough Biomedical Research, and the UCSF Nutrition Obesity Research Center (DK0908722). This work was also supported by an NIH New Innovator Award (DP2-DK109533), R01-DK106399, and R01-NS094781.

**Reviewer information** *Nature* thanks C. Bourque, M. McKinley and the other anonymous reviewer(s) for their contribution to the peer review of this work.

**Author contributions** C.A.Z. and Z.A.K. conceived the project and designed the experiments. C.A.Z, E.L.H. and S.K. performed stereotaxic surgery. J.S.A. and L.R.B. performed intragastric surgery. J.S.A. performed vagotomy surgery. C.L.T. generated the *Nxph4-2a-cre* mouse line. L.M. and H.Z. generated the *Ai148D* mouse line. C.A.Z., E.L.H., S.K., L.B. and Z.A.K. performed histology. E.L.H., J.S.A. and T.V.C. helped to conduct experiments. Y.C. generated code. C.A.Z. conducted the experiments, analysed the data and generated the figures. C.A.Z. and Z.A.K. prepared the manuscript with input from all authors.

Competing interests The authors declare no competing interests.

#### Additional information

**Extended data** is available for this paper at https://doi.org/10.1038/s41586-019-1066-x.

Supplementary information is available for this paper at https://doi.org/ 10.1038/s41586-019-1066-x.

**Reprints and permissions information** is available at http://www.nature.com/ reprints.

**Correspondence and requests for materials** should be addressed to Z.A.K. **Publisher's note:** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s), under exclusive licence to Springer Nature Limited 2019

### **METHODS**

Experimental protocols were approved by the University of California, San Francisco Institutional Animal Care and Use Committee, following the NIH Guide for the Care and Use of Laboratory Animals.

**Mouse strains.** Adult mice (>6 weeks old) of both sexes were used for experiments. We obtained *Nos1-ires-cre* knock-in mice<sup>36</sup> (*Nos1<sup>tm1(cre)Mgmj/J*, stock no. 017526), *Avp-ires2-cre* knock-in mice<sup>37</sup> (*Avp<sup>tm1.1(cre)Hze/J*, stock no. 023530), *Glp1r-ires-cre* knock-in mice<sup>38</sup> (*Glp1r<sup>tm1.1(cre)Lbrl/J*, stock no. 029283), *Rosa26-lsl-Gfp-Rpl10* knock-in mice<sup>39</sup> (*Gt*(*ROSA*)*26Sor<sup>tm1(CAG-EGFP/Rpl10a-birA)Wtp/J*, stock no. 022367) and wild-type mice (C57BL/6J, stock no. 000664) from the Jackson Laboratory. We obtained *Ai148D* GCaMP6f (*Igs7<sup>tm148.1</sup>(tetO-GCaMP6f,CAG-tTA2)Hze*) knock-in mice<sup>40</sup> from the Allen Institute for Brain Science, and *Trpv1-Gfp-2a-Dtr* BAC transgenic mice<sup>41</sup> from M. Hoon at the NIH.</sup></sup></sup></sup>

We generated Nxph4-2a-cre knock-in mice by CRISPR-Cas9-mediated homologous recombination as previously described<sup>27</sup>, based on published protocols<sup>42,43</sup>. In brief, homology regions were captured into a plasmid from a BAC containing the Nxph4 locus by recombination-mediated genetic engineering. The T2A-Cre sequence was inserted immediately upstream of the endogenous stop codon. The final targeting vector contained  $\sim$ 3 kb (5') and  $\sim$ 1.3 kb (3') homology arms, and was verified by restriction digest and sequencing. To generate site-specific double-stranded breaks using CRISPR, an single-guide RNA (sgRNA) sequence (GAGTGAGACTGCGATCTGGT) was selected such that the guide sequence would be separated from the PAM site in the genomic DNA by the T2A-Cre insertion. This ensured that the targeting vector and recombined Nxph4-2a-cre allele were protected from Cas9 nuclease activity. Super-ovulated female FVB/N mice were mated to FVB/N stud males, and fertilized zygotes were collected from oviducts. Cas9 protein (100 ng/µl), sgRNA (50 ng/µl) and targeting vector DNA (20 ng/µl) were mixed and injected into the pronucleus of fertilized zygotes. Injected zygotes were implanted into oviducts of pseudo-pregnant CD1 female mice. Founder pups and offspring were genotyped for the presence of the knock-in allele by quantitative PCR (qPCR). Pups positive for the knock-in allele were crossed to reporter mice, and reporter expression patterns were identical to endogenous Nxph4 expression in the brain (Extended Data Fig. 6). All Nxph4-2a-cre mice used here were maintained on a mixed FVB/C57Bl/6J background. This mouse line will be made available from the Jackson Laboratory.

All mice were maintained in temperature- and humidity-controlled facilities with 12-h light–dark cycle, and ad libitum access to water and standard chow (PicoLab 5053). *Avp-ires2-cre;Ai148D* mice were fed doxycycline (80 mg/kg) chow (BioServ F7515) until they underwent stereotaxic surgery, after which they were fed standard chow. Mice were allowed at least two weeks for recovery after stereotaxic surgery before testing. For dehydration experiments, mice were water-deprived overnight (>16 h) before testing.

**Viral vectors.** We expressed channelrhodopsin-2 variants<sup>44,45</sup>, GCaMP variants<sup>46</sup> and chemogenetic receptors<sup>30</sup> in genetically specified neurons by stereotaxic delivery of recombinant AAVs that encode Cre-dependent or *Camk2a*-promoter transgene cassettes. We obtained AAV1-CAG-FLEX-GCaMP6s, AAV5-Camk2a-GCaMP6f and AAV5-hSyn-FLEX-GCaMP6f from the Penn Vector Core. We obtained AAV5-Camk2a-hChR2(E123T/T159C)-2a-mCherry and AAV5-Ef1a-DIO-hChR2(H134R)-mCherry from the UNC Vector Core. We obtained AAV5-hSyn-DIO-hM4D(Gi)-mCherry and AAV5-Camk2a-hM4D(Gi)-mCherry from Addgene.

Stereotaxic surgery. We performed intracranial surgery using stereotaxic coordinates for the SFO, MnPO and SON<sup>35</sup> as previously described<sup>9,27</sup>. For delivery of recombinant AAVs, 100-200 nl of virus was injected (100 nl/min) at the SFO (-0.60 mm antero-posterior (AP), 0 mm medio-lateral (ML), -2.80 mm dorso-ventral (DV), relative to bregma) or MnPO (+0.35 mm AP, 0 mm ML, -4.20 mm DV). For SFO and MnPO photostimulation experiments, an optical fibre with a 200-µm inner diameter (Thorlabs FT200UMT, CFLC230-10) was placed 0.30 mm above the injection site in the same surgery. For SFO fibre photometry experiments, an optical fibre with a 400-µm inner diameter (Thorlabs BFH48-400, CF440-10) was placed 0.10 mm below the injection site in the same surgery. For SON fibre photometry experiments, an optical fibre with a 400-µm inner diameter (Thorlabs BFH48-400, CF440-10) was placed unilaterally above the SON of Avp-ires2-cre;Ai148D mice (-0.75 mm AP, -1.20 mm ML, -5.50 mm DV). For MnPO microendoscope imaging experiments, a gradient-index (GRIN) lens with a 500-µm inner diameter (6.1-mm length; Inscopix 100-000588) was placed 0.10 mm above the injection site in the same surgery.

Optical fibres and GRIN lenses were then affixed to the skull using dental cement (A-M Systems 525000) or MetaBond adhesive cement (Parkwell S380). After at least two weeks recovery from the lens implantation surgery, mice that were going to be used for microendoscope imaging were again anaesthetized, and a base-plate (Inscopix 100-000279) was placed above the lens and affixed with MetaBond adhesive cement. When these mice were not being used for imaging, a baseplate cover (Inscopix 100-000241) was attached to prevent damage to the GRIN lens.

Intragastric surgery and infusion. We prepared mice for intragastric infusion as previously described<sup>47</sup>, based on published protocols<sup>21</sup>. In brief, catheters were constructed from Silastic tubing (Silastic 508-003), Tygon tubing (Tygon AAD04119) and a curved metal connector (Component Supply Company NE-9019). Biologically compatible mesh was attached to the Silastic tubing and around the metal connector using adhesive (Xiameter RTV-3110 base; Dow Corning 4 catalyst), and a luer adaptor (Instech LS20) was placed onto the Tygon tubing. Assembled catheters were sterilized using ethylene oxide. Mice with functional photometry, optogenetic or microendoscope implants were anaesthetized with ketamine–xylazine, and the intragastric catheter was surgically implanted into the stomach, as previously described<sup>47</sup>. Mice were allowed at least one week to recover before intragastric infusion and testing.

All intragastric infusions were delivered at a rate of 200 µl/min using a syringe pump (Harvard Apparatus 70-2001). Solutions of NaCl (75, 150, 250, 375 and 500 mM), glucose (1 M) and mannitol (1 M) were prepared using deionized water, and the 1.5 M NaCl solution was prepared using phosphate-buffered saline (PBS). Previous studies have indicated that mannitol is not absorbed into the bloodstream from the intestines<sup>48</sup>. We measured the latency for infused fluids to pass through the intragastric catheter itself en route to the stomach as approximately 13 s.

Vagotomy surgery. We prepared mice for intragastric infusion and performed bilateral subdiaphragmatic resection of the vagus nerve during a single procedure. Mice with functional photometry implants were anaesthetized with ketaminexylazine and the intragastric catheter was surgically implanted as described above. In brief, a 5–7-mm incision was made along the medial line beginning at the distal edge of the sternum. Within a 3-mm radius around the incision site, the skin layer was separated from the muscular layer using blunt dissecting scissors. The liver was then retracted with sterile cotton swabs that had been moistened with saline so that the distal end of the oesophagus could be visualized. Using jeweler's forceps, both branches of the vagus nerve were isolated from the oesophagus and a 1-2-mm section of each branch of the nerve was resected with scissors. Bilateral subdiaphragmatic vagotomy was accompanied in the same surgery by pyloroplasty, in which some of the muscle that composes the pyloric sphincter was incised. In brief, a 2-mm incision was made along the longitudinal axis of the pylorus without penetrating the lumen. Then, each side of the incision was carefully approximated and sutured with two simple interrupted stitches. The purpose of the pyloroplasty is to maintain gastrointestinal flow through the pylorus<sup>49</sup> and thereby alleviate the excessive food retention, gastric distension and morbidity that accompany subdiaphragmatic vagotomy. Control mice for vagotomy experiments underwent a sham surgery that included intragastric catheter implantation and internal organ manipulation, but not vagotomy or pyloroplasty.

To validate the subdiaphragmatic vagotomy, mice received an intraperitoneal injection of wheat germ agglutinin conjugated to Alexa Fluor 555 (WGA-555; 200  $\mu$ g per mouse) and were euthanized four days later. WGA-555 is taken up by axon terminals of intact vagal motor neurons<sup>50</sup>, the somas of which are located in the brainstem and can be visualized by histology (Extended Data Fig. 4a). Labelling in the dorsal motor nucleus of the vagus was greatly reduced—but not completely eliminated—by subdiaphragmatic vagotomy. Residual vagal fibres may be due to incomplete resection during the subdiaphragmatic vagotomy, or to regeneration after surgery<sup>51</sup>.

**Behaviour.** We monitored mouse drinking behaviour as previously described<sup>9,27</sup>. All experiments were performed in sound-isolated behavioural chambers (Coulbourn Habitest Modular System) and were performed during the light cycle to control for circadian factors. Fluid consumption was monitored with an electrical lickometer and recorded using Graphic State software (v.4.2, http://www.coulbourn.com/category\_s/363.html), or using LJStreamUD software during fibre photometry experiments (v.1.17, http://www.labjack.com/support/software/applications/ud-series/ljstreamud) or nVista software during microendoscope imaging experiments (v.2.0, http://www.inscopix.com/nvista). Mice were acclimatized to the behavioural chamber for at least 10 min at the beginning of each testing session.

For two-bottle drinking experiments (Extended Data Fig. 1d), wild-type mice were dehydrated and then given access to two randomly placed bottles (1 × water, 1 × 300 mM NaCl) for >10 min before being returned to their home cages. This test was repeated four times, and the location of the bottles was randomly re-assigned on each trial. Mice were divided into two groups before testing. The first group received a gastric pre-load of hypertonic saline (1.5 M NaCl, 100  $\mu$ l) by oral gavage one minute before bottle access; the second group received a gastric pre-load of isotonic saline (150 mM NaCl, 100  $\mu$ l) by oral gavage one minute before bottle access.

For three-bottle drinking experiments (Extended Data Fig. 1e–g), SFO photometry mice were dehydrated and then given access to three randomly placed bottles ( $1 \times$  water,  $2 \times 300$  mM NaCl) for >10 min before being returned to their home cages. This test was repeated four times, and the location of the bottles was randomly re-assigned on each trial.

**Fibre photometry.** We prepared mice for in vivo fibre photometry recording as previously described<sup>9</sup>, based on published protocols<sup>18</sup>. The fibre photometry

signal was output to a lock-in amplifier (Stanford Research Systems SR810) with time-constant of 30 ms to allow filtering of noise at higher frequencies. The signal was then digitized (LabJack U6-Pro) and recorded using LJStreamUD software (v.1.17, http://www.labjack.com/support/software/applications/ud-series/ljstreamud) at a 250-Hz sampling rate. Photometry data were subjected to minimal processing, such as within-trial fluorescence normalization and temporal down-sampling. For these experiments, intragastric infusions were given in a volume of 1 ml except for Fig. 2c–f and Extended Data Fig. 2h (volume of 200  $\mu$ l, including ~50  $\mu$ l dead volume); systemic (intraperitoneal) injections were given in a volume of 150  $\mu$ l (1.5 M or 3 M NaCl) using PBS as 'vehicle' or in a volume of 150  $\mu$ l. Hormones (Extended Data Fig. 8) were given in a volume of 150  $\mu$ l) for serotonin (2, 20 mg/kg), cholecystokinin (2 mg/kg), ghrelin (2 mg/kg) and leptin (2 mg/kg), and by subcutaneous injection (volume of 400  $\mu$ l) for amylin (2 mg/kg) using PBS as vehicle.

Microendoscope imaging. We prepared mice for in vivo microendoscope imaging based on published protocols<sup>29,52</sup>. Videos were acquired at 20 Hz (20% LED power, 2.0 gain) using a miniature microscope (Inscopix) and nVista software (v.2.0, http://www.inscopix.com/nvista). After acquisition, videos were first pre-processed, spatially (binning factor of 2) and temporally (binning factor of 5) downsampled, and motion-corrected using Mosaic software (v.1.7, http://support. inscopix.com/mosaic-workflow). Activity traces for individual neurons were then extracted from these videos using the constrained non-negative matrix factorization (CNMF-E) pipeline53 (http://www.github.com/zhoupc/cnmf\_e) implemented in MATLAB. After initial CNMF-E segmentation, extracted neurons were manually refined to avoid potential confounding factors from uncorrected motion artefacts, region of interest duplication and over-segmentation of the same spatial components. For each experiment, activity traces for individual neurons were extracted from recordings from 3-4 mice and then pooled for subsequent analysis. For these experiments, intragastric infusions were given in a volume of 1 ml; systemic (intraperitoneal) injections were given in a volume of 100 µl (3 M NaCl) using PBS as vehicle.

**Optogenetics.** We prepared mice for in vivo photostimulation as previously described<sup>9,27</sup>, based on published protocols<sup>23,54</sup>. A DPSS 473-nm laser (Shanghai Laser and Optics Century BL473-100FC) was controlled by Graphic State software (v.4.2, http://www.coulbourn.com/category\_s/363.html) through a TTL signal generator (Coulbourn H03-14), and synchronized with behaviour experiments. The laser power was measured to be ~15 mW at the patch cable tip and was delivered in 10-ms pulses at 20 Hz. For these experiments, intragastric infusions were given in a volume of 600 µl.

**Chemogenetics.** We prepared mice for in vivo chemogenetic inhibition based on published protocols<sup>55,56</sup>. Clozapine *N*-oxide (CNO, 1 mg/kg) was delivered by intraperitoneal injection (volume of 125  $\mu$ l) with 0.6% DMSO in PBS as vehicle. For these experiments (Extended Data Fig. 8), CNO or vehicle was delivered >15 min before water access after dehydration or oral gavage of hypertonic NaCl.

**Diphtheria toxin receptor ablation.** We ablated genetically defined (*Trpv1*positive) sensory neurons using the diphtheria toxin (DTX) receptor-based strategy described in published protocols<sup>41,57</sup>. In brief, *Nos1-ires-cre;Trpv1-Gfp-2a-Dtr* mice were first equipped for fibre photometry recording of SFO neurons and screened for functionality. Mice with functional photometry implants then underwent intragastric catheterization surgery and, after recovery, were tested in a series of experiments ('before DTX' in Extended Data Fig. 4h, i). Mice were then given two intramuscular injections of DTX (each injection was 50 µl of 25 µg/ml DTX), separated by three days, and were then allowed at least five additional days to recover, after which they underwent the same series of experiments ('after DTX').

We confirmed the ablation of *Trpv1*-positive sensory neurons in two ways. First, *Nos1-ires-cre;Trpv1-Gfp-2a-Dtr* mice with photometry implants and intragastric catheters were given access to a single bottle of palatable sugar solution (300 mM sucrose) that also contained either capsaicin (100  $\mu$ M) or vehicle (0.15% TWEEN-80 + 1.5% DMSO). This test was conducted once before and once after DTX treatment, and intake was quantified from 1–2 h after access (Extended Data Fig. 4h). Second, *Trpv1-Gfp-2a-Dtr;hSyn-Nanobody-Rp110* mice were used for histology of the nodose ganglion and thoracic dorsal root ganglion, after DTX treatment or no treatment. This revealed a nearly complete ablation of *Trpv1*-positive vagal neurons and more-limited ablation of *Trpv1*-positive dorsal root ganglion neurons (Extended Data Fig. 4e, f). We also observed sparse labelling of *Trpv1*-positive neurons in the brain<sup>58</sup>, and this was unaffected by DTX treatment (Extended Data Fig. 4g).

**Plasma osmolality.** Mice equipped with intragastric catheters were fully hydrated and received an infusion (sham, water, 150 mM NaCl or 500 mM NaCl) at 200  $\mu$ l/min for 5 min. Beginning 3 min after the start of infusion, 125  $\mu$ l of blood was collected from the tail vein using EDTA-coated capillary tubes (RAM Scientific 07-6011). The blood collection process took approximately 3 min per mouse. Plasma was isolated by centrifugation (1,000g for 10 min), diluted in deionized water and frozen until measurement. Osmolality was then measured in triplicate for each sample using a freezing point osmometer (Fiske Associates 210). Mice were allowed one week for recovery between sessions.

Histology. Mice were transcardially perfused with PBS followed by 10% formalin. To visualize forebrain and hindbrain nuclei, whole brains were dissected, postfixed in 10% formalin overnight at 4 °C and then cryo-protected in 30% sucrose overnight at 4 °C. Free-floating sections (40 µm) were prepared with a cryostat, blocked (3% BSA, 2% NGS and 0.1% Triton-X in PBS for 2 h) and then incubated with primary antibody (chicken anti-GFP, Abcam ab13970, 1:1,000; rat anti-RFP, ChromoTek 5f8, 1:1,000; goat anti-mCherry, Acris ab0040-200, 1:1,000; rabbit anti-FOS, Santa Cruz Biotech sc52, 1:500) overnight at 4 °C. Sections were then washed, incubated with secondary antibody (Alexa Fluor 488 goat anti-chicken, Life Technologies a11039, 1:1,000; Alexa Fluor 568 goat anti-rat, Life Technologies a11077, 1:1,000; Alexa Fluor 568 goat anti-rabbit, Life Technologies a11011, 1:1,000; Alexa Fluor 568 donkey anti-goat, Life Technologies a11057, 1:1,000) for 2 h at room temperature, washed again, mounted with DAPI Fluoromount-G (Southern Biotech) and then imaged with a confocal microscope (Zeiss LSM-700). To visualize WGA-555 labelling of the dorsal motor nucleus of the vagus, sections were mounted and imaged without staining.

To visualize sensory ganglia, the ventral aspects of the skull and vertebral columns were dissected, post-fixed in 10% formalin overnight at 4 °C and then washed at room temperature. The nodose ganglion and thoracic dorsal root ganglion were further dissected, and then cryo-protected in 30% sucrose overnight at 4 °C. Sections (20  $\mu$ m) were prepared with a cryostat, mounted on slides, washed, blocked (5% NGS and 0.1% Triton-X in PBS for 30 min) and then incubated with primary antibody (chicken anti-GFP, Abcam ab13970, 1:1,000; rabbit anti-NeuN, Milliipore abn78, 1:1,000) overnight at 4 °C. Sections were then washed, incubated with secondary antibody (Alexa Fluor 488 goat anti-chicken, Life Technologies a11039, 1:500; Alexa Fluor 568 goat anti-rabbit, Life Technologies a11011, 1:500) for 2 h at room temperature, washed again, mounted with DAPI Fluoromount-G (Southern Biotech) and then imaged with the confocal microscope.

Images underwent minimal processing (such as brightness and contrast adjustments) performed using the Fiji distribution of ImageJ (v.2.0, http://www.fiji.sc/). Data analysis. We analysed behaviour data, fibre photometry data and microendoscope imaging data using custom MATLAB (v.R2018a, http://www. mathworks.com/products/matlab) scripts. Throughout the paper, a drinking bout is defined as any set of ten or more licks in which no inter-lick interval is greater than one second. For some assays, multiple trials of the same experiment were performed for an individual mouse, and then averaged and treated as a single replicate. For photometry data, all responses were normalized using the function:  $\Delta F/F_0 = (F - F_0)/F_0$ , in which F is the raw photometry signal and  $F_0$  is the median of F during the baseline period (plotted data before injection, infusion or drinking). For quantification in bar graphs, the median  $\Delta F/F_0$  of a 10-s (for long experiments with >5 min of data plotted) or 2-s (for short experiments with <5 min of data plotted) window is reported. For peri-event time histograms around the end of drinking bouts,  $F_0$  was defined as the median of F at the time  $(\pm 1 \text{ s})$  of the last lick in the bout. For microendoscope imaging data, all responses were normalized using the function  $z = (C_{\text{raw}} - \mu)/\sigma$ , in which  $C_{\text{raw}}$  is the output of the CNMF-E pipeline,  $\mu$  is the mean of  $C_{\rm raw}$  during the baseline period and  $\sigma$  is the standard deviation of C<sub>raw</sub> during the baseline period. k-means clustering was performed using the built-in MATLAB function (http://www.mathworks.com/ help/stats/kmeans.html), as illustrated in Extended Data Fig. 7a. For quantification in linear regressions, the mean z-score of the final 1 min of plotted data for intragastric infusions (9-10 min after infusion begins) or of the first 1 min after drinking onset (first lick in first bout) is reported. The mean z-score of the first 1 min after the first lick in the first drinking bout was used to classify GABAergic MnPO neuron responses during ingestion (Fig. 5c, d, Extended Data Fig. 9b, c). For timecourses of water intake, time 0 is the moment of water access unless otherwise noted. Initial drinking rate (Fig. 2b) was calculated from the first 1 min of drinking after the first lick in the experiment. Latency to inhibition of SFO<sup>Nos1</sup> neurons by intragastric infusion of water was calculated as the moment at which the fibre photometry signal crossed a threshold of four standard deviations ( $4\sigma$ ) below the mean signal level of the baseline period, and was adjusted to account for the dead volume of the intragastric catheter.

Statistics and reproducibility. We performed statistical analyses using Prism software (v.7.0, http://www.graphpad.com/scientific-software/prism). Throughout the paper, values are reported as mean  $\pm$  s.e.m. (error bars or shaded area). In figures with linear regressions, shaded areas represent the 95% confidence interval for the line of best fit. *P* values for pair-wise comparisons were performed using a two-tailed Student's *t*-test (with repeated measures when possible). *P* values for comparisons across multiple groups were performed using ANOVA (with repeated measures when possible) and corrected for multiple comparisons using the Holm–Šídák method (within-group comparison to the control condition). No statistical methods were used to predetermine sample size. The experiments were

not randomized. The investigators were not blinded to allocation during experiments and outcome assessment. Representative images were selected from one to five original biological replicates, and representative recordings were selected from three to five original biological replicates.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

#### Data availability

Data that support the findings of this study are available upon reasonable request from the corresponding author.

### Code availability

Custom MATLAB scripts that support the findings of this study are available upon reasonable request from the corresponding author.

- Leshan, R. L., Greenwald-Yarnell, M., Patterson, C. M., Gonzalez, I. E. & Myers, M. G. J. Jr. Leptin action through hypothalamic nitric oxide synthase-1-expressing neurons controls energy balance. *Nat. Med.* 18, 820–823 (2012).
- Harris, J. A. et al. Anatomical characterization of Cre driver mice for neural circuit mapping and manipulation. *Front. Neural Circuits* 8, 76 (2014).
   Williams, E. K. et al. Sensory neurons that detect stretch and nutrients in the
- digestive system. *Cell* **166**, 209–221 (2016).
- Zhou, P. et al. Interrogating translational efficiency and lineage-specific transcriptomes using ribosome affinity purification. *Proc. Natl Acad. Sci. USA* 110, 15395–15400 (2013).

40. Daigle, T. L. et al. A suite of transgenic driver and reporter mouse lines with

- enhanced brain-cell-type targeting and functionality. *Cell* 174, 465–480 (2018).
  41. Pogorzala, L. A., Mishra, S. K. & Hoon, M. A. The cellular code for mammalian thermosensation. *J. Neurosci.* 33, 5533–5541 (2013).
- Yang, H. et al. One-step generation of mice carrying reporter and conditional alleles by CRISPR/Cas-mediated genome engineering. *Cell* **154**, 1370–1379 (2013).
- Yang, H., Wang, H. & Jaenisch, R. Generating genetically modified mice using CRISPR/Cas-mediated genome engineering. *Nat. Protocols* 9, 1956–1968 (2014).

- Boyden, E. S., Zhang, F., Bamberg, E., Nagel, G. & Deisseroth, K. Millisecondtimescale, genetically targeted optical control of neural activity. *Nat. Neurosci.* 8, 1263–1268 (2005).
- Berndt, A. et al. High-efficiency channelrhodopsins for fast neuronal stimulation at low light levels. Proc. Natl Acad. Sci. USA 108, 7595–7600 (2011).
- 46. Chen, T.-W. et al. Ultrasensitive fluorescent proteins for imaging neuronal activity. *Nature* **499**, 295–300 (2013).
- Beutler, L. R. et al. Dynamics of gut–brain communication underlying hunger. Neuron 96, 461–475 (2017).
- Höber, J. & Höber, J. Experiments on the absorption of organic solutes in the small intestine of rats. J. Cell. Comp. Physiol. **10**, 401–422 (1937).
- Mordes, J. P., el Lozy, M., Herrera, M. G. & Silen, W. Effects of vagotomy with and without pyloroplasty on weight and food intake in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 236, R61–R66 (1979).
- Powley, T. L., Fox, E. A. & Berthoud, H. R. Retrograde tracer technique for assessment of selective and total subdiaphragmatic vagotomies. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 253, R361–R370 (1987).
- Powley, T. L., Chi, M. M., Baronowsky, E. A. & Phillips, R. J. Gastrointestinal tract innervation of the mouse: afferent regeneration and meal patterning after vagotomy. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 289, R563–R574 (2005).
- Resendez, S. L. et al. Visualization of cortical, subcortical and deep brain neural circuit dynamics during naturalistic mammalian behavior with head-mounted microscopes and chronically implanted lenses. *Nat. Protocols* **11**, 566–597 (2016).
- Żhou, P. et al. Efficient and accurate extraction of in vivo calcium signals from microendoscopic video data. eLife 7, e28728 (2018).
- Sparta, D. R. et al. Construction of implantable optical fibers for longterm optogenetic manipulation of neural circuits. *Nat. Protocols* 7, 12–23 (2012).
- Krashés, M. J. et al. Rapid, reversible activation of AgRP neurons drives feeding behavior in mice. J. Clin. Invest. 121, 1424–1428 (2011).
- 56. Roth, B. L. DREADDs for neuroscientists. *Neuron* **89**, 683–694 (2016).
- Saito, M. et al. Diphtheria toxin receptor-mediated conditional and targeted cell ablation in transgenic mice. *Nat. Biotechnol.* 19, 746–750 (2001).
- Cavanaugh, D. J. et al. *Trpv1* reporter mice reveal highly restricted brain distribution and functional expression in arteriolar smooth muscle cells. *J. Neurosci.* **31**, 5067–5077 (2011).



Extended Data Fig. 1 | Gastrointestinal osmolarity influences drinking behaviour and biases salt preference. a, b, Additional data related to Fig. 1b, c. a, Cumulative water or 300 mM NaCl intake after dehydration (n = 5 mice). **b**, Example of SFO neuron dynamics during drinking after dehydration. c, Ingestion of hypertonic fluids activates SFO neurons regardless of hydration state. Average SFO activity and drinking behaviour of hydrated mice given ad libitum access to isotonic (300 mM sucrose) or hypertonic (300 mM sucrose + 600 mM mannitol) sugar solutions of similar sweetness (n = 5 mice). **d**, Increases in gastrointestinal osmolarity bias salt and water preference. Left, preference in a two-bottle test after intragastric treatment with hypertonic (red; n = 8 mice) or isotonic (black; n = 9 mice) NaCl (two-way ANOVA, Holm–Šídák correction). Right, cumulative water (solid lines) and 300 mM NaCl (dashed lines) intakes in the same two-bottle test. e-g, Post-ingestive SFO neuron activity does not reflect the delayed consequences of taste or sensorimotor experience associated with an individual drinking bout. e, Mice initially do not distinguish between bottles that contain water and bottles that contain

300 mM NaCl in a three-bottle test after dehydration (n = 4 mice, linear regression,  $R^2 = 0.3163$ , P = 0.0233). f, Example of SFO neuron dynamics during drinking from water (black) and 300 mM NaCl (blue, red) bottles after dehydration. g, Left, SFO neuron dynamics during individual water (42 bouts) or NaCl (71 bouts) drinking bouts in trials 1 and 2 of the threebottle test. Right, average SFO activity after individual drinking bouts (n = 4 mice). In this experiment  $(\mathbf{e}-\mathbf{g})$ , gastrointestinal osmolarity quickly becomes hypertonic as the dehydrated mice alternate between drinking from water and NaCl bottles, such that SFO neuron activity rebounds even after water-drinking bouts; this suggests that the stabilization signal that either quenches or re-activates SFO neurons after ingestion reflects gastrointestinal osmolarity. Error bars represent mean  $\pm$  s.e.m. Shaded areas in **a**, **c**–**e**, **g** represent mean ± s.e.m.; shaded areas in **b** represent individual licks; shaded area in the linear regression (right) in e represents 95% confidence interval for the line of best fit; shaded areas in f represent individual drinking bouts. \*\*P < 0.01, \*\*\*P < 0.001.



Extended Data Fig. 2 | The gut-to-brain osmosensory signal depends on fluid tonicity but not osmolyte identity. a, b, Intragastric infusion does not rapidly alter the state of the blood. a, Schematic. b, Plasma osmolality of samples collected during approximately 3–6 min after the start of the 5-min intragastric infusion (n = 9 mice per group, one-way ANOVA, Holm–Šídák correction). c-e, The gut-to-brain osmosensory signal depends on fluid tonicity but not osmolyte identity. c, Top, SFO neuron dynamics of individual mice in response to intragastric infusion of equiosmotic concentrations of NaCl, which is absorbed into the bloodstream from the gastrointestinal tract, and mannitol, which is not absorbed (n = 4 mice). Bottom, SFO neuron dynamics of a separate cohort of individual mice in response to intragastric infusion of equiosmotic concentrations of NaCl, which does not permeate cell membranes and has high tonicity, and glucose, which does permeate cell membranes and has low tonicity

(n = 5 mice). **d**, Left, average SFO activity during intragastric infusion of NaCl or mannitol. Right, quantification (n = 4 mice, one-way ANOVA, Holm-Šídák correction). **e**, Left, average SFO activity during intragastric infusion of NaCl or glucose. Right, quantification (n = 5 mice, one-way ANOVA, Holm-Šídák correction). **f**-**h**, SFO neurons encode systemic and gastrointestinal osmosensory signals additively rather than hierarchically. **f**, Schematic. **g**, Example (left) and average (right; n = 4 mice) of SFO neuron dynamics during 1.5 M NaCl intragentric infollowed by intragastric infusion of water. **h**, Example (left) and average (right; n = 3 mice) of SFO neuron dynamics during 1.5 M NaCl intragastric infusion followed by intrageritoneal injection of water. Error bars represent mean  $\pm$  s.e.m., Shaded areas in summary traces (**d**, **e**, **g**, **h**) represent infusion. NS, not significant, \*P < 0.05, \*\*P < 0.01.



Extended Data Fig. 3 | The gut-to-brain osmosensory signal completely satiates, but only mildly stimulates, thirst. a, b, Additional data related to Figs. 1e–g and 2a, b. a, Left, average SFO activity during intragastric infusions and subsequent drinking while hydrated. Right, cumulative water intake (n = 4 mice). b, Left, average SFO activity during intragastric infusions and subsequent drinking after dehydration. Right, cumulative water intake (n = 4 mice). c, Correlation between SFO activity change and latency to drinking after 1-ml infusions into hydrated (black; n = 23experiments from 4 mice, linear regression,  $R^2 = 0.0705$ , P = 0.2208) or dehydrated (red; n = 12 experiments from 4 mice, linear regression,  $R^2 = 0.1321$ , P = 0.2456) mice. d, Additional data related to Fig. 2c. Average SFO activity after systemic (intraperitoneal) or intragastric treatment with 150 µl NaCl while hydrated. Shaded areas in a, b, d represent mean  $\pm$  s.e.m., and in c represent 95% confidence interval for the line of best fit.



Extended Data Fig. 4 | The gut-to-brain osmosensory signal involves the vagus nerve. a-d, The gut-to-brain osmosensory signal is disrupted by subdiaphragmatic vagotomy. a, Vagal motor neuron somas (located in the brainstem and labelled by intraperitoneal injection of wheat germ agglutinin (WGA-555)) were largely absent following subdiaphragmatic vagotomy (two examples per condition). Scale bar, 1 mm. b, Drinking after dehydration was less suppressed by intragastric infusion of water in vagotomized mice (middle; n = 7 mice) compared to sham mice (left; n = 6 mice). Right, quantification (two-tailed Student's *t*-test). **c**, Drinking was similarly suppressed in both groups by systemic (intraperitoneal) delivery of water (n = 4 sham and 7 vagotomy mice, two-tailed Student's *t*-test). **d**, SFO modulation by water and 500 mM NaCl intragastric infusions, but not by 1.5 M NaCl intraperitoneal injection, was attenuated in vagotomized mice compared to sham mice (n = 8 mice per group, two-tailed Student's t-tests). e-i, The gut-to-brain osmosensory signal involves Trpv1-positive sensory neurons. e, To specifically ablate Trpv1positive sensory neurons, we treated mice that contain a BAC transgene expressing GFP and the diphtheria toxin (DTX) receptor from the Trpv1

gene start codon (*Trpv1-Gfp-2a-Dtr* mice) with DTX. Scale bar, 100 μm. **f**, Quantification (n = 3 control and 2 DTX mice). NG, nodose ganglion; DRG, dorsal root ganglion. g, Treatment with DTX did not ablate Trpv1positive neurons in the brain. Scale bar, 1 mm. h, Hydrated mice avoided drinking 300 mM sucrose that contained 100 µM capsaicin (cap.) beforebut not after, DTX ablation of *Trpv1*-positive sensory neurons (n = 5mice, two-way ANOVA, Holm-Šídák correction). Veh., vehicle. i, SFO modulation by intragastric infusion of water was significantly attenuated after DTX ablation of Trpv1-positive sensory neurons, and modulation by intragastric infusion of 500 mM NaCl was slightly attenuated (n = 7 mice, two-tailed Student's t-tests). j, The response of SFO neurons to serotonin and other visceral hormones. j, SFO neuron dynamics during injection of two doses of serotonin (left; n = 5 mice) and to a single dose (2 mg kg<sup>-1</sup>) of amylin, cholecystokinin (CCK), ghrelin or leptin (right; n = 6 mice, one-way ANOVA, Holm-Šídák correction) in hydrated mice. Error bars and shaded areas represent mean  $\pm$  s.e.m. NS, not significant, \**P* < 0.05, \*\**P* < 0.01.



**Extended Data Fig. 5** | Vasopressin neurons integrate systemic and gastrointestinal osmosensory signals and are stress-responsive. a, b, Additional data related to Fig. 3a, b. a, Schematic for fibre photometry recording of vasopressin neurons. Scale bar, 1 mm. b, Vasopressin neuron dynamics (average, left; individual mice, right) during vehicle or NaCl intraperitoneal injection (n = 7 mice). c, Vasopressin neurons are stress-responsive. Vasopressin neuron activity during tail suspension (n = 7 mice). d–j, Additional data related to Fig. 3d. d, Schematic. e, Change in vasopressin neuron activity after infusion, while hydrated or dehydrated (n = 4 mice, two-way ANOVA, Holm–Šídák correction). f, Vasopressin neuron activity during intragastric infusions, while hydrated (n = 4 mice). g, Vasopressin neuron dynamics of individual mice (left) and distribution

of  $\Delta F/F_0$  values before and after intragastric infusion with 500 mM NaCl (right). **h**, Vasopressin neuron dynamics during intragastric infusions after dehydration (n = 4 mice). **i**, Vasopressin neuron activity of individual mice (left) and distribution of  $\Delta F/F_0$  values before and after intragastric infusion with water (right). **j**, Gastrointestinal osmolarity modulates both the median of  $\Delta F/F_0$  (left; used here as a proxy for tonic activity) and the standard deviation ( $\sigma$ ) of  $\Delta F/F_0$  (right; used here as a proxy for bursting activity) of vasopressin neurons (n = 4 mice, two-tailed Student's *t*-tests). Error bars represent mean  $\pm$  s.e.m. Shaded areas in **b**, **c**, **f**, **h** represent mean  $\pm$  s.e.m., and in **g**, **i** represent 'before' and 'after' infusion periods. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001. The mouse brain in this figure has been reproduced with permission from ref. <sup>35</sup>, Copyright © 2012.



Extended Data Fig. 6 | Nxph4-expressing MnPO neurons are activated by dehydration and drive thirst. a, Additional data related to Fig. 4b. a, The Nxph4-2a-cre recombination pattern (bottom; crossed to a GFP reporter line) recapitulates the endogenous Nxph4 mRNA expression pattern (top; Allen Institute for Brain Science ISH #73521000) in the organum vasculosum of the lamina terminalis (OVLT), MnPO, SFO and paraventricular hypothalamus (PVH). b, MnPO<sup>Nxph4</sup> neurons are activated by dehydration. Nxph4-2a-cre recombination (green; crossed to a GFP reporter line) and the immediate early gene product FOS (red; induced by 3 M NaCl intraperitoneal injection) co-localize in the MnPO during dehydration. Scale bar, 100  $\mu$ m. c, d, MnPO<sup>*Nxph4*</sup> neurons drive thirst. **c**, Schematic for optogenetic activation of MnPO<sup>*Nxph4*</sup> neurons. Scale bar, 1 mm. d, Left, water intake in response to photostimulation. Right, quantification (n = 4 mice, two-tailed Student's *t*-test). Error bars and shaded areas represent mean  $\pm$  s.e.m. \* P < 0.05. The mouse brains in this figure have been reproduced with permission from ref. <sup>35</sup>, Copyright © 2012.



Extended Data Fig. 7 | In vivo imaging of individual glutamatergic MnPO neurons during thirst, drinking and gastrointestinal manipulation. a, Additional data related to Fig. 4d, e. a, Workflow for *k*-means clustering of individual MnPO<sup>Nxphd</sup> neurons based on their activity during intraperitoneal injection of vehicle or 3 M NaCl, and water drinking. **b**-**d**, Additional data related to Fig. 4f–h. **b**, Schematic. **c**, Dynamics of individual neurons during intragastric infusion of water while hydrated. **d**, Dynamics of individual neurons tracked during intragastric infusion of water after dehydration (left) and 3 M NaCl intraperitoneal injection (right). Neurons inhibited  $\geq 1\sigma$  after intragastric infusion of water were classified as 'gastrointestinal-tuned' (red; 26%) and the remaining neurons were plotted in Fig. 4h.



Extended Data Fig. 8 | Glutamatergic MnPO neurons relay the gastrointestinal osmosensory signal to vasopressin neurons. **a-d**, Glutamatergic MnPO neurons are necessary for relaying gastrointestinal osmosensory information to SON vasopressin neurons. a, Schematic for simultaneous fibre photometry recording of vasopressin neurons and chemogenetic inhibition of glutamatergic MnPO neurons. Scale bar, 100 µm. b, Injection of CNO inhibited water intake after dehydration (n = 5 mice). c, Example of vasopressin neuron dynamics during intraperitoneal injection of CNO or vehicle (left) and subsequent intragastric infusion of 1.5 M NaCl by oral gavage (right). Inset, water intake after dehydration for the example mouse. d, Quantification of vasopressin neuron response to intraperitoneal injection (left) and NaCl intragastric infusion (right) (n = 5 mice, two-tailed Student's *t*-tests). **e**-**h**, Glutamatergic MnPO neurons are not necessary for relaying gastrointestinal osmosensory information to SFO thirst neurons. e, Schematic for simultaneous fibre photometry recording of SFO neurons and chemogenetic inhibition of glutamatergic MnPO neurons. Scale bar,

100 µm. f, Injection of CNO inhibited water intake after dehydration (n = 5 mice). g, Example of SFO neuron dynamics during intragastric infusion of 1.5 M NaCl by oral gavage, after intraperitoneal injection CNO or vehicle. Inset, water intake after dehydration for the example mouse. h, Quantification of SFO neuron response to intraperitoneal injection (left) and intragastric infusion of NaCl (right) (n = 5 mice, two-tailed Student's t-tests). i, CNO inhibits drinking in mice that express the Gicoupled human M4D designer receptor (hM4D(Gi)) in glutamatergic MnPO neurons but not in control mice that lack hM4D(Gi). i, Injection of CNO significantly inhibited water intake after dehydration in MnPO<sup>*Camk2a::hM4D*(*Gi*)</sup> + SON mice with photometry implants (n = 5 mice; quantified from **b**) and MnPO<sup>*Nos1::hM4D*(*Gi*)</sup> + SFO mice with photometry implants (n = 5 mice; quantified from **f**) but not in control mice (n = 6mice, two-way ANOVA, Holm-Šídák correction). Error bars and shaded areas represent mean  $\pm$  s.e.m. n.s., not significant; \**P* < 0.05, \*\**P* < 0.01. The mouse brains in this figure have been reproduced with permission from ref. <sup>35</sup>, Copyright © 2012.



Extended Data Fig. 9 | In vivo imaging of individual GABAergic MnPO neurons during thirst, drinking and gastrointestinal manipulation. a, Individual GABAergic MnPO neurons do not encode systemic osmolarity in their baseline activity. Left, dynamics of individual neurons during intraperitoneal injection of 3 M NaCl while hydrated. Right, comparison to thirst-activated MnPO<sup>Nxph4</sup> neurons (cluster 1 from Fig. 4e). **b**, **c**, Dynamics of ingestion-tuned GABAergic MnPO neurons during hypertonic NaCl drinking. **b**, Left, dynamics of individual neurons during 300 mM NaCl drinking after dehydration (left). Right, proportion of ingestion-activated (red, modulated  $\geq 1\sigma$  during first min of drinking), ingestion-inhibited (blue, modulated  $\leq -1\sigma$ ) and untuned

(black) neurons during water (top; n = 77 neurons from Fig. 5c, d) or 300 mM NaCl (bottom; n = 95 neurons) drinking. c, Average responses of ingestion-activated, ingestion-inhibited and untuned neurons during 300 mM NaCl drinking (n = 95 neurons). Note that ingestion of NaCl persists for much longer than ingestion of water after dehydration (see Fig. 5d, Extended Data Fig. 1a), which may explain differences in the dynamics of ingestion-tuned GABAergic MnPO neurons when mice drink these fluids. **d**, **e**, Additional data related to Fig. 5e–g. **d**, Schematic. **e**, Dynamics of individual neurons during intragastric infusion of 500 mM NaCl while hydrated.



Extended Data Fig. 10 | Model of the neural control of thirst and satiation. Anatomically and temporally distinct peripheral sensory signals encode information about the current hydration state of the body (blood) as well as the volume (oropharynx) and osmolarity (gastrointestinal tract) of recently ingested fluids. These signals converge on the thirst circuit of the brain to generate an integrated central representation of fluid balance at the level of individual neurons, which use this information to dynamically control drinking behaviour and vasopressin secretion in real time. Illustration from iStock/artsholic.

## natureresearch

Corresponding author(s): Zachary A. Knight

Last updated by author(s): Feb 22, 2019

### **Reporting Summary**

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

### Statistics

| For         | all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.   |
|-------------|---|
| n/a         | Confirmed   |
|             | The exact sample size ( <i>n</i> ) for each experimental group/condition, given as a discrete number and unit of measurement  |
|             | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly   |
|             | The statistical test(s) used AND whether they are one- or two-sided Only common tests should be described solely by name; describe more complex techniques in the Methods section.  |
|             | A description of all covariates tested  |
|             | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons   |
|             | A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
|             | For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>                       |
| $\ge$       | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings  |
| $\ge$       | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes  |
| $\boxtimes$ | Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i> ), indicating how they were calculated  |
|             | Our web collection on statistics for biologists contains articles on many of the points above   |

### Software and code

| Policy information about <u>availability of computer code</u> |  |  |  |  |  |
|---|--|--|--|--|--|
| Data collection   | Coulbourn Graphic State (v4.2), Inscopix nVista (v2.0), LabJack LJStreamUD (v1.7)  |  |  |  |  |
| Data analysis   | GraphPad Prism (v7.0), Inscopix Mosaic (1.7), MathWorks Matlab (vR2018a), Fiji/ImageJ (v2.0)   |  |  |  |  |
| For manuscripts utilizing c                                   | ustom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. |  |  |  |  |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

### Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets

- A list of figures that have associated raw data
- A description of any restrictions on data availability

Data that support the findings of this study are available from the corresponding author upon reasonable request.

### Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences

Behavioural & social sciences

Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

### Life sciences study design

| Sample size     | Statistical methods to predetermine sample size were not used. Sample sizes were based on previous studies from our lab and others.  |
|-----------------|--|
| Data exclusions | Mice for fiber photometry (>10% ΔF/F response to 3 M NaCl i.p.) and optogenetics (>100 licks during photostimulation) experiments were included based on functional validation. Recordings from microendoscope experiments were included based on the visibility of fluorescent cells. |
| Replication     | The main findings of the paper were confirmed by multiple complementary experiments. We performed recordings and behavioral experiments with multiple animals to confirm reproducibility. All attempts at replication were successful.   |
| Randomization   | For recording experiments, trials were not randomized, but within-animal controls were used whenever possible. For behavioral experiments, mice were pseudorandomly assigned to experimental and control groups before surgery/experiment.   |
| Blinding        | Blinding was not used. All data analysis was performed automatically using Matlab with the same scripts run for each experimental group.   |

#### All studies must disclose on these points even when the disclosure is negative.

### Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

### Methods

| n/a         | Involved in the study       | n/a   | Involved in the study  |
|-------------|-----------------------------|-------|------------------------|
|             | Antibodies                  | $\ge$ | ChIP-seq               |
| $\ge$       | Eukaryotic cell lines       | $\ge$ | Flow cytometry         |
| $\ge$       | Palaeontology               | $\ge$ | MRI-based neuroimaging |
|             | Animals and other organisms |       |                        |
| $\ge$       | Human research participants |       |                        |
| $\boxtimes$ | Clinical data               |       |                        |

### Antibodies

| Antibodies used | chicken anti-GFP (Abcam ab13970, 1:1000), rat anti-RFP (ChromoTek 5f8, 1:1000), goat anti-mCherry (Acris ab0040-200, 1:1000), rabbit anti-Fos (Santa mCruz Biotech sc52, 1:500), rabbit anti-NeuN (Millipore abn78, 1:1000), Alexa Fluor 488 goat anti-chicken (Life Technologies a11039, 1:500 or 1:1000), Alexa Fluor 568 goat anti-rat (Life Technologies a11077, 1:1000), Alexa Fluor 568 goat anti-rabbit (Life Technologies a11011, 1:500 or 1:1000), Alexa Fluor donkey anti-goat (Life Technologies a11057, 1:1000). |
|-----------------|--|
| Validation      | Antibodies were validated for use in mouse brain sections in pilot experiments in our lab and by the manufacturers.  |

### Animals and other organisms

| Policy information about <u>stud</u> | lies involving animals; ARRIVE guidelines recommended for reporting animal research  |
|--------------------------------------|--|
| Laboratory animals                   | Nos1-Ires-Cre (stock no. 017526), Avp-Ires2-Cre (stock no. 023530), Glp1r-Ires-Cre (stock no. 029283), Rosa26-IsI-Gfp-RpI10 (stock no. 022367), and wild type (stock no. 000664) mice were obtained from the Jackson Laboratory. Ai148D mice were obtained from the Allen Institute for Brain Science and Trpv1-Gfp-2a-Dtr mice were obtained from Mark Hoon at the National Institutes of Health. Nxph4-2a-Cre mice were generated by CRISPR/Cas9-mediated homologous recombination and maintained on a mixed FVB/C57BI/6J background. Adult mice (>6 weeks old) of both sexes were used for experiments. |
| Wild animals                         | No wild animals were used.   |
| Field-collected samples              | No field-collected samples were used.  |
| Ethics oversight                     | Experimental protocols were approved by the University of California, San Francisco IACUC following the NIH Guide for the Care and Use of Laboratory Animals.  |

Note that full information on the approval of the study protocol must also be provided in the manuscript.