

Neural circuits underlying thirst and fluid homeostasis

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Abstract | Thirst motivates animals to find and consume water. More than 40 years ago, a set of interconnected brain structures known as the lamina terminalis was shown to govern thirst. However, owing to the anatomical complexity of these brain regions, the structure and dynamics of their underlying neural circuitry have remained obscure. Recently, the emergence of new tools for neural recording and manipulation has reinvigorated the study of this circuit and prompted re-examination of longstanding questions about the neural origins of thirst. Here, we review these advances, discuss what they teach us about the control of drinking behaviour and outline the key questions that remain unanswered.

Why do we feel thirsty? Early theories posited that thirst is the local sensation of dryness in the mouth and throat^{1,2}, but we now know that thirst is a homeostatic response to changes in the blood: increases in plasma osmolality^{3–6} or decreases in plasma volume^{7,8} or pressure⁹ trigger the sensation of thirst, which motivates animals to find and consume water and thereby restore these parameters to their physiological set points. In addition, animals have evolved complex anticipatory mechanisms that predict impending changes in fluid balance to enable the regulation of drinking pre-emptively.

A set of three deep forebrain nuclei known as the lamina terminalis (LT) monitors the state of the blood and coordinates homeostatic responses to fluid imbalance (described in more detail below). Although the importance of the LT in the control of drinking has been appreciated for decades (for reviews, see REFS 10–12), our understanding of the underlying neural circuitry remains limited. For example, we still do not know the genetic identity of most of the cell types that reside in the LT; the dynamics of those cells during behaviour; or the anatomical pathways by which they transmit information to other brain regions. This knowledge gap reflects, in part, the complexity of the LT, which contains a diversity of intermingled neural cell types distributed across three

small nuclei. Although these features have traditionally made the thirst circuit challenging to dissect, the recent application of genetically targeted techniques in mice has led to renewed progress.

In this Progress article, we summarize our current understanding of the neural circuitry underlying thirst and drinking behaviour in mammals. First, we briefly overview the well-established roles of the LT and various circulating hormones in the regulation of fluid balance. Second, we describe recent insights from the application of genetically targeted methods — including optogenetics, *in vivo* recordings and viral tracing — that have been used to probe discrete elements of the thirst circuit and characterize their function, dynamics and connectivity in freely behaving animals^{13–21}. Last, we highlight some of the key questions that remain unanswered.

The lamina terminalis

Our modern understanding of the neural control of thirst originated with the discovery by Bengt Andersson in the 1950s that infusion of hypertonic saline into the anterior hypothalamus of goats stimulates intense drinking and water retention (antidiuresis)^{22–24}. James Fitzsimons later discovered that infusion of the hormone angiotensin II (ANGII) into the same area of rats also produces thirst^{25,26}. Together, these and subsequent^{27–33} experiments identified a small forebrain region (the LT)

that monitors homeostatic signals of fluid balance (plasma osmolality and ANGI) and translates these signals into appropriate counter-regulatory responses.

The LT is composed of three small, interconnected structures that lie adjacent (anterior and/or dorsal) to the third ventricle. Two of these structures — the subfornical organ (SFO) and the organum vasculosum of the LT (OVLT) — are sensory circumventricular organs, meaning that they lie outside the blood–brain barrier and therefore have direct access to the circulation³⁴. Information about fluid balance enters the LT primarily through specialized interoceptive neurons in the SFO and OVLT. Some of these interoceptive SFO and OVLT neurons are intrinsically osmosensitive, meaning that their firing rate increases in response to elevations in the tonicity of the extracellular fluid^{35–38}, and many of these osmosensitive neurons are also activated by the hormone ANGI^{39–43}, which is rapidly generated in the circulation when plasma volume or pressure falls. In addition, some SFO or OVLT neurons may receive ascending neural signals from peripheral blood pressure sensors (baroreceptors)^{44,45}. Thus, SFO and OVLT neurons are poised to integrate signals about plasma osmolality, volume and pressure and to use this information to control thirst. The third component of the LT is the median preoptic nucleus (MnPO), which cannot access the blood directly and is thought to be an integratory centre⁴⁶. Together, these three structures form a forebrain hub for the regulation of fluid balance.

Signals detected in the SFO and OVLT are shared with each other and the MnPO through an extensive network of bidirectional projections^{47–51}. Activation of this network triggers a coordinated set of homeostatic responses that restores fluid balance. These responses include: behavioural mechanisms that motivate water and sodium consumption (that is, thirst and salt appetite)^{28,52–54}; autonomic mechanisms that modulate sympathetic outflow and thereby alter blood pressure and heart rate^{55,56}; and neuroendocrine mechanisms that modulate water and sodium retention by the kidneys^{57,58}. The neuroendocrine responses are mediated primarily by the hormones arginine vasopressin (AVP) and, in

rodents^{59,60} but not in humans^{61,62}, oxytocin, both of which are secreted into the circulation by specialized posterior pituitary-projecting magnocellular neurosecretory cells in the paraventricular (PVH) and supraoptic (SON) nuclei of the hypothalamus that are under

direct control of descending input from the LT (for reviews, see REFS 63,64).

Distinct neural cell types

The LT is neurochemically heterogeneous, but how this molecular diversity maps onto

functional populations of neurons has been unclear. Experiments using optogenetics and chemogenetics have begun to define functional subsets of neurons in the LT on the basis of their genetic and anatomic features^{13–16,21} (FIG. 1; TABLE 1).

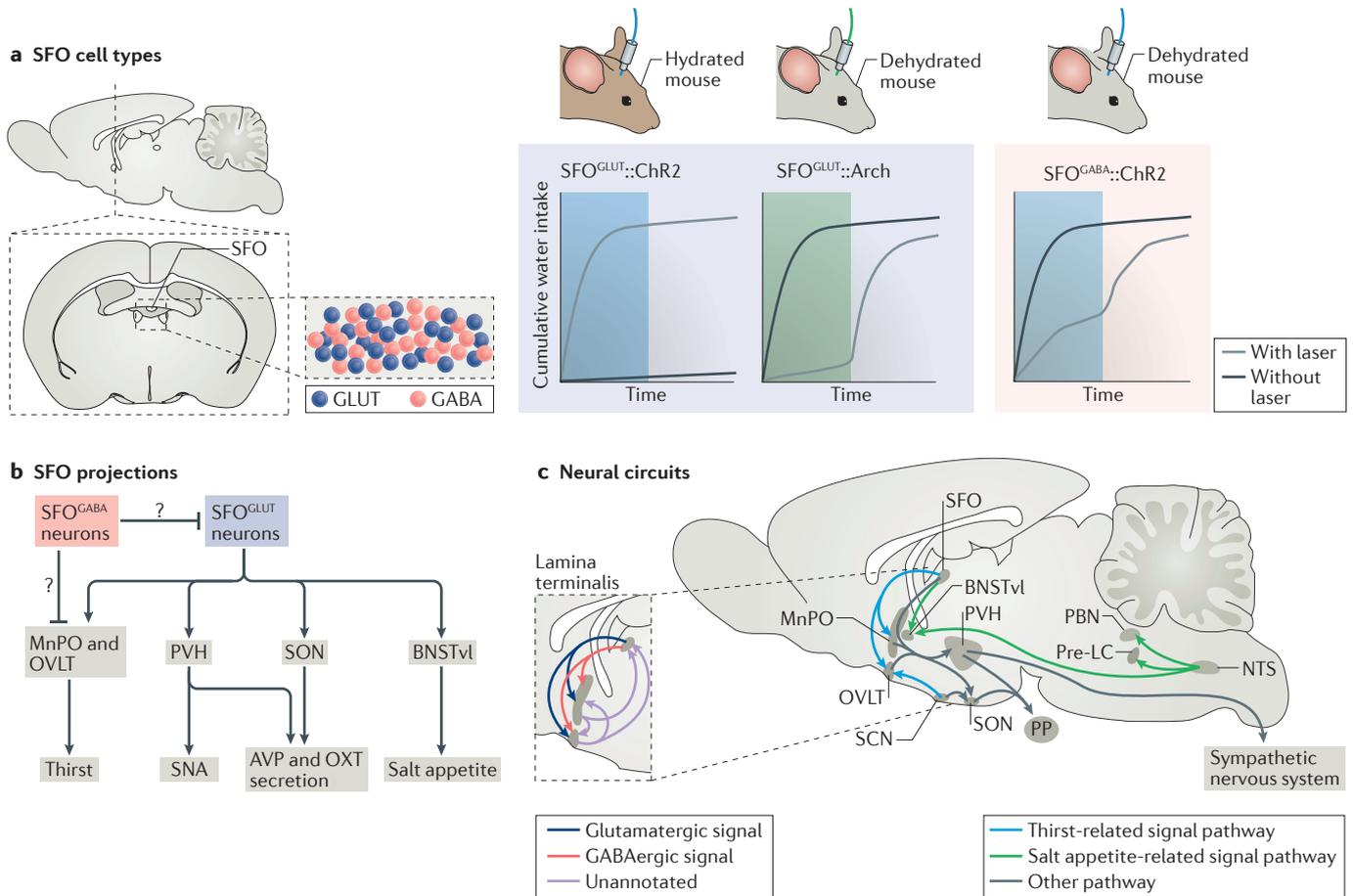


Figure 1 | Structure of the neural circuits underlying thirst and fluid homeostasis in mammals. **a** | The subforaminal organ (SFO) is situated immediately dorsal to the third ventricle and contains intermingled populations of glutamatergic (SFO^{GLUT}) and GABAergic (SFO^{GABA}) neurons with opposing effects on drinking behaviour. Optogenetic activation of channelrhodopsin-2 (ChR2)-expressing SFO^{GLUT} neurons (using 473 nm blue light for 30 min) stimulates intensive drinking in hydrated mice, whereas optogenetic silencing of archaerhodopsin (Arch)-expressing SFO^{GLUT} neurons (using 532 nm green light for 15 min) suppresses drinking in dehydrated mice. By contrast, optogenetic activation of SFO^{GABA} neurons (using 473 nm blue light for 15 min) suppresses drinking in dehydrated mice. **b** | SFO neurons innervate several brain regions that are involved in the regulation of fluid balance. SFO^{GLUT} projections to the median preoptic nucleus (MnPO) and organum vasculosum of the lamina terminalis (OVLT) drive thirst, whereas SFO^{GLUT} projections to the ventrolateral part of the bed nucleus of the stria terminalis (BNSTvl) promote sodium consumption. SFO^{GLUT} projections to the paraventricular (PVH) and supraoptic (SON) nuclei of the hypothalamus have not yet been functionally annotated with cell-type specificity, but classic models suggest that these projections mediate secretion of arginine vasopressin (AVP) and, in rodents, oxytocin (OXT) into the circulation by posterior pituitary (PP)-projecting magnocellular neurosecretory cells. Classic models also suggest that SFO^{GLUT} projections to the PVH may modulate sympathetic nerve activity (SNA), and thereby alter blood pressure, heart rate and renal function by engaging brainstem-projecting parvocellular preautonomic

neurons. However, projections from neurons in the MnPO and OVLT to the PVH and SON probably also contribute to these neuroendocrine and autonomic responses. Projections from SFO^{GABA} neurons to the MnPO or OVLT, as well as locally within the SFO, have not yet been functionally annotated (marked '?' in the figure). **c** | Sagittal illustration of the cell type-specific neural circuits that underlie thirst and fluid homeostasis in the mouse brain. The lamina terminalis (LT) consists of two sensory circumventricular organs (the SFO and OVLT) and an integratory structure (the MnPO). Information about plasma osmolality, volume and pressure enters the LT through specialized interoceptive neurons in the SFO and OVLT, some of which are intrinsically osmosensitive and angiotensin II-sensitive (for example, SFO^{GLUT} neurons). The LT nuclei communicate with each other through an extensive network of bidirectional projections that has not yet been fully mapped with cell-type specificity. Outside the LT, SFO^{GLUT} neurons project to the PVH, SON and BNSTvl; projections from the MnPO and OVLT have not yet been mapped with cell-type specificity, but projections from these regions to the PVH and SON are well established. AVP neurons in the supraoptic nucleus (SON) project to the OVLT and SON to mediate circadian regulation of thirst and AVP secretion, respectively. Information about plasma sodium enters the circuit through specialized aldosterone-sensitive neurons in the nucleus of the solitary tract that express 11 β -hydroxysteroid dehydrogenase type 2 (NTS^{HSD2} neurons), which promote salt appetite and project to the pre-locus coeruleus (pre-LC), parabrachial nucleus (PBN) and BNSTvl.

Table 1 | Cell type-specific manipulations of the thirst circuit

Pathway	Genetic markers	Manipulation	Neural activity	Water intake	Food intake	Sodium intake
SFO ^{GLUT} somas	<i>Camk2</i> (REFS 13, 16), <i>Etv1</i> (REF. 13) or <i>Nos1</i> (REFS 14, 16)	ChR2	↑	↑ ^{13,14,16}	↔ ^{13,14,16}	↑ ¹³
	<i>Camk2</i> (REF. 15) or <i>Nos1</i> (REF. 14)	G _q -DREADDs	↑	↑ ^{14,15}	↔ ¹⁴	↑ ¹⁵
	<i>Nos1</i> (REF. 16)	Arch	↓	↓	↑	N.R.
SFO ^{GLUT} → MnPO	<i>Camk2</i> (REF. 16)	ChR2	↑	↑	N.R.	N.R.
SFO ^{GLUT} → PVH	<i>Camk2</i> (REF. 16)	ChR2	↑	↔	N.R.	N.R.
SFO ^{GLUT} → OVLT	<i>Slc17a6</i> (REF. 21)	ChR2	↑	↑	N.R.	↔
SFO ^{GABA+GLUT} → OVLT	No marker ²¹	Arch	↓	↓	N.R.	↔
SFO ^{GLUT} → BNSTvl	<i>Slc17a6</i> (REF. 21)	ChR2	↑	↔	N.R.	↑
	No marker ²¹	Arch	↓	↔	N.R.	↓
SFO ^{GABA} somas	<i>Slc32a1</i> (REFS 13, 21)	ChR2	↑	↓ ^{13,21}	↔ ¹³	↔ ¹³ , ↓ ²¹
MnPO ^{GLUT} and OVLT ^{GLUT} somas	<i>Slc17a6</i> (REF. 17)	ChR2	↑	↑	N.R.	N.R.
MnPO ^{GABA} and OVLT ^{GABA} somas	<i>Slc32a1</i> (REF. 17)	ChR2	↑	↓	↔	N.R.
SCN ^{AVP} → OVLT	<i>Avp</i> ¹⁸	ChR2	↑	↑	N.R.	N.R.
		Arch	↓	↓	N.R.	N.R.
NTS ^{HSD2} somas	<i>Hsd11b2</i> (REF. 20)	G _q -DREADDs	↑	↔	↓	↑
		G _r -DREADDs	↓	↔	N.R.	↓
		TetTox	↓	Fatal (>20% body weight lost by day 10)		

Arch, archaerhodopsin; AVP, arginine vasopressin; BNSTvl, ventrolateral part of the bed nucleus of the stria terminalis; *Camk2*, gene encoding calcium/calmodulin-dependent protein kinase type 2; ChR2, channelrhodopsin 2; DREADDs, designer receptors exclusively activated by designer drugs; *Etv1*, gene encoding ETS translocation variant 1; GABA, GABAergic; GLUT, glutamatergic; HSD2, 11β-hydroxysteroid dehydrogenase type 2 (encoded by *Hsd11b2*); MnPO, median preoptic nucleus; *Nos1*, gene encoding neuronal nitric oxide synthase, brain; N.R., not reported; NTS, nucleus of the solitary tract; OVLT, organum vasculosum of the lamina terminalis; PVH, paraventricular nucleus of the hypothalamus; SCN, suprachiasmatic nucleus; SFO, subfornical organ; *Slc17a6*, gene encoding solute carrier family 17 member 6 (vesicular glutamate transporter 2); TetTox, tetanus toxin system; ↑, increase; ↓, decrease; ↔, no change.

The SFO. The SFO contains at least two molecularly distinct populations of neurons with opposing effects on drinking behaviour: a glutamatergic population (SFO^{GLUT} neurons) that promotes drinking^{13–16} and modest sodium consumption¹⁵, and a GABAergic population (SFO^{GABA} neurons) that suppresses drinking¹³. There is evidence that these glutamatergic and GABAergic SFO populations may be further subdivided into additional functional classes. For example, some SFO^{GABA} neurons have been electrophysiologically characterized as sensitive to either cholecystokinin or lactate²¹. However, at present, molecular markers that distinguish these subsets have not been identified.

SFO^{GLUT} neurons are activated *in vivo* by circulating signals that stimulate thirst, including increases in plasma osmolality and (via ANGII) decreases in plasma volume or pressure¹⁶. Optogenetic silencing of SFO^{GLUT} neurons under these conditions suppresses drinking in thirsty mice¹⁶, indicating that SFO^{GLUT} neuron activity is necessary for physiological drinking behaviour. Notably, this finding contrasts with the effects of electrolytic

ablation of the entire SFO, which does not consistently block drinking^{65–73}. One possible explanation for this discrepancy is that lesioning destroys both SFO^{GLUT} and SFO^{GABA} neurons, which have opposing effects on thirst¹³. This combined ablation may therefore result in a milder behavioural impairment than does silencing of thirst-promoting SFO^{GLUT} neurons alone.

SFO neurons innervate several brain regions that are involved in fluid homeostasis^{13,16,21}, including the MnPO, OVLT, PVH, SON and the ventrolateral part of the bed nucleus of the stria terminalis (BNSTvl), and SFO neurons can be further subdivided on the basis of their projections to these structures. Optogenetic stimulation of SFO^{GLUT} projections to the MnPO¹⁶ and OVLT²¹ promotes thirst, whereas simultaneous optogenetic silencing of SFO^{GLUT} and SFO^{GABA} neurons that project to the OVLT suppresses drinking in thirsty mice²¹. This suggests that the MnPO and OVLT are the main targets through which SFO^{GLUT} neurons drive thirst.

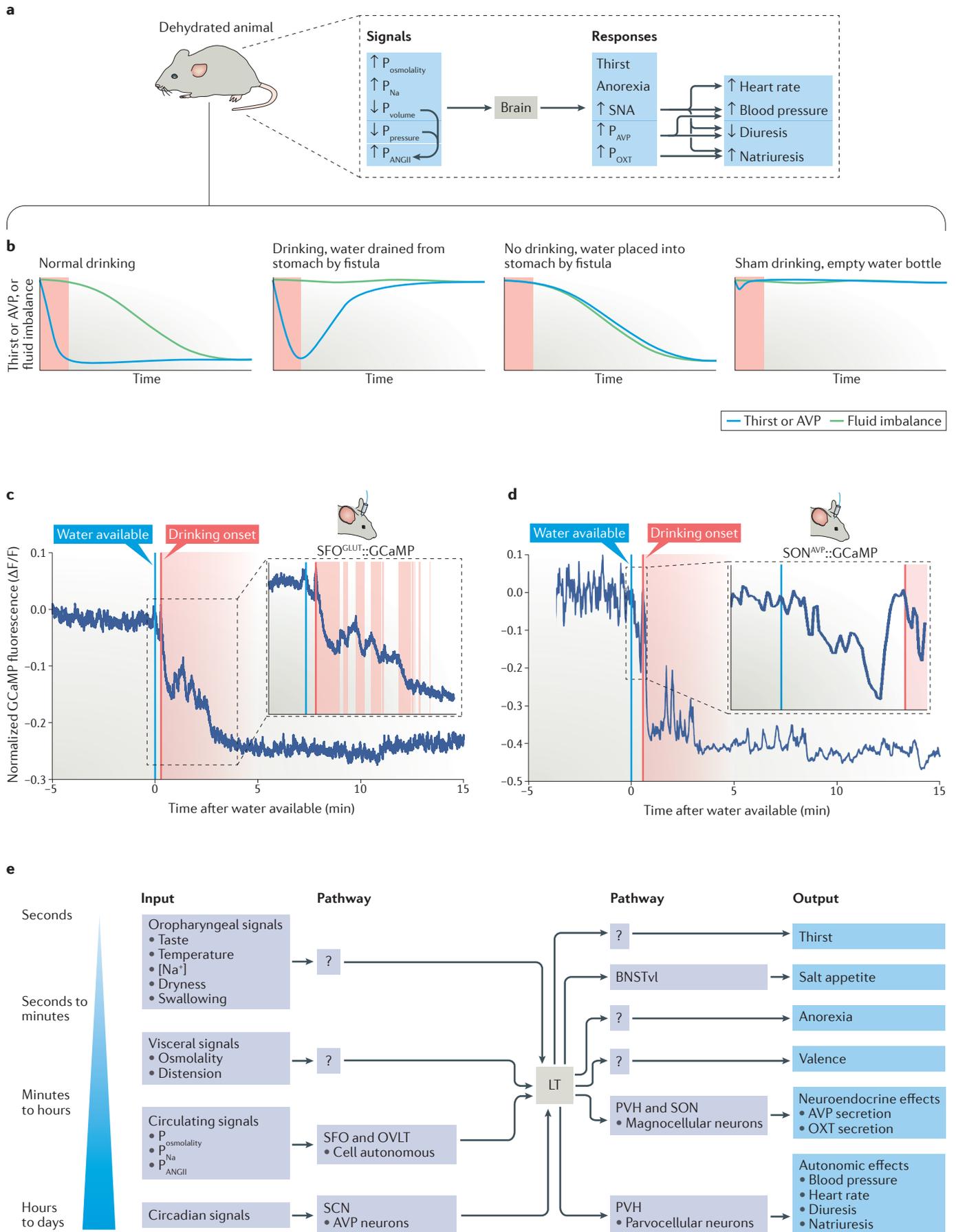
Other SFO projections seem to mediate different functions. Optogenetic stimulation of SFO^{GLUT} neurons that project to the

BNSTvl specifically increases sodium consumption²¹, suggesting that this pathway is involved in salt appetite. Optogenetic stimulation of SFO^{GLUT} projections to the PVH does not promote thirst¹⁶, consistent with a primary role for the PVH in neuroendocrine⁶⁴ and cardiovascular⁷⁴, rather than behavioural, responses to fluid imbalance.

In contrast to their glutamatergic counterparts, SFO^{GABA} neurons innervate only the MnPO and OVLT^{13,21}, and the function of these projections has not yet been tested. Similarly, little is known about the microcircuits within the SFO that control drinking behaviour, although there is some evidence that SFO^{GLUT} neurons receive inhibitory input from neighbouring SFO^{GABA} neurons^{16,21}.

The MnPO and OVLT. Relative to the SFO, cellular diversity in other regions of the LT has been less explored. One challenge is that the MnPO and OVLT are located in close proximity, making it difficult to selectively target neurons in only one of these structures by viral injection. Optogenetic stimulation of either glutamatergic or GABAergic

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◀ **Figure 2 | Anticipatory and homeostatic regulation of the thirst circuit. a** | Challenges to fluid homeostasis, such as water deprivation, cause deviations in the composition of the blood. These deviations can include increases in plasma osmolality ($P_{\text{osmolality}}$) and plasma sodium (P_{Na}), as well as decreases in plasma volume (P_{volume}) and pressure (P_{pressure}) that stimulate renin secretion by the kidneys and, consequently, angiotensin II (ANGII) production (P_{ANGII}) in the blood¹⁸¹. These homeostatic circulating signals are translated by the brain into counter-regulatory responses, including thirst, anorexia, arginine vasopressin (AVP) and oxytocin (OXT) secretion (leading to increases in P_{AVP} and P_{OXT} respectively), and sympathetic nerve activation (SNA). Although thirst-motivated drinking behaviour is ultimately necessary to restore fluid homeostasis, this coordinated set of responses also promotes water retention (antidiuresis) and sodium excretion (natriuresis) by the kidneys; suppresses ingestion of food (and therefore of sodium and other osmolytes); and modulates blood pressure and heart rate to maintain fluid homeostasis until water can be ingested. **b** | When thirsty animals are allowed to drink (red area), thirst and AVP secretion are rapidly inhibited before the composition of the blood is corrected by ingested water. Historical experiments^{75,76,79,97–100,104,107,182,183} using oesophageal or gastric fistulae and sham drinking suggest that this anticipatory regulation involves both immediate oropharyngeal and delayed visceral signals; however, the neural mechanisms underlying rapid anticipatory regulation of thirst and AVP secretion remained unexplored until recently. **c** | Fibre photometry recordings revealed that glutamatergic neurons in the subformal organ (SFO^{GLUT} neurons) are rapidly inhibited during drinking to coordinate the anticipatory control of thirst and AVP secretion¹⁶. In this example recording, a mouse deprived of water for 48 hours is given access to water. The inset highlights that rapid inhibition of SFO^{GLUT} neurons is time-locked to individual drinking bouts (red areas). **d** | Fibre photometry recordings demonstrated that AVP neurons in the supraoptic nucleus (SON^{AVP} neurons) are also rapidly inhibited during drinking¹⁹. In this example recording, a mouse deprived of water for 24 hours is given access to water. The inset highlights that SON^{AVP} neurons are transiently inhibited by water-predicting cues before drinking is initiated and that this inhibition is rapidly reset in the seconds immediately preceding water ingestion. The neural mechanism and physiological importance of this transient pre-ingestive inhibition remain unclear. **e** | In addition to homeostatic circulating signals (such as $P_{\text{osmolality}}$, P_{Na} and P_{ANGII}) that were canonically thought to activate the lamina terminalis (LT) (see part **a**), recent experiments have revealed that a diverse set of anticipatory oropharyngeal, visceral and circadian signals also influences SFO^{GLUT} neurons and the LT on different timescales. This convergence of homeostatic and anticipatory signals allows SFO^{GLUT} neurons and the LT to control a diverse set of behavioural, neuroendocrine and autonomic outputs both in response to, and in anticipation of, deviations from fluid homeostasis. Many of the neural pathways carrying this information to and from the LT have not yet been identified (marked '?' in the figure). BNSTvl, ventrolateral part of the bed nucleus of the stria terminalis; OVLT, organum vasculosum of the LT; PVH, paraventricular hypothalamus; SCN, suprachiasmatic nucleus. Part **c** is adapted from REF. 16, Macmillan Publishers Limited. Part **d** is adapted from REF. 19, Elsevier.

neurons to closely track the amount of water ingested. This suggests that SFO^{GLUT} neurons control drinking in real-time by making a comparison between two parameters: the level of physiological need, which they measure by monitoring the blood, and the amount of water recently ingested, which they measure by tracking signals from the oropharynx. This model explains how drinking can rapidly quench thirst^{75–80}, yet also be properly metred to match the physiological deficit of the animal. Notably, early functional MRI and positron emission tomography studies of thirsty humans^{81–86} did not detect changes in neural activity in the LT during drinking and consequently attributed thirst satiation to higher cortical centres. This discrepancy may reflect the difficulty of monitoring activity changes in small, heterogeneous structures such as the LT (which is smaller than 10 mm³ in humans⁸³) using functional imaging.

How water ingestion is detected in the oral cavity and then communicated to SFO^{GLUT} neurons is not well understood. One mechanism for water detection seems to involve temperature sensing in the oral cavity, as ingestion of cold water inhibits SFO^{GLUT} neurons more efficiently than does ingestion of warm water and, indeed, isolated oral cooling (using cold, dry metal) can transiently inhibit the activity of SFO^{GLUT} neurons¹⁶. This finding suggests a neural basis for the everyday phenomenon that cold liquids are experienced as more thirst quenching^{87–93} and raises the possibility that sensory fibres innervating the mouth that express the cold-activated transient receptor potential cation channel subfamily M member 8 (TRPM8)^{94–96} may participate in water detection. Studies in several species have suggested that sensory fibres innervating the stomach, duodenum and hepatic blood vessels also participate in the rapid control of drinking behaviour^{97–100}. Consistent with these observations, SFO^{GLUT} neurons receive a second, delayed presystemic signal during ingestion that may arise from the early gastrointestinal tract and encodes the osmolality of ingested fluids¹⁶. The neural pathway by which peripheral sensory fibres communicate these distinct anticipatory signals to the LT remains unclear, but sensory relay nuclei in the hindbrain probably participate^{101–103}.

Anticipatory control of AVP secretion. The PVH and SON are important downstream targets of the LT that control secretion of AVP into the circulation and thus modulate water retention by the kidneys.

neurons spanning both the MnPO and OVLT promotes or suppresses drinking, respectively¹⁷. However, the broad extent of viral transduction in these experiments complicates their interpretation¹⁷, and further progress will require identification of molecular markers for subsets of MnPO and OVLT neurons with specific functions.

The MnPO and OVLT share an overlapping set of projection targets with the SFO, including the PVH, SON and BNSTvl, as well as reciprocal connections within the LT^{39,41,43}. These projections have yet to be functionally annotated or mapped with cell-type specificity. Given the high degree of interconnectedness among structures in the LT¹⁶, it will be crucial to dissect the degree of collateralization, redundancy and necessity of efferents from each region with cell-type specificity to fully understand how information flows through the thirst circuit.

Anticipatory signals

Neurons in the LT have a well-established role in monitoring the state of the blood, but whether these cells receive other types

of signals that control drinking has not been explored. The recent development of methods for optical recording of deep brain calcium dynamics has made it possible to measure for the first time how these neurons are regulated during behaviour^{16,19} (FIG. 2).

Anticipatory control of thirst satiation.

The SFO was the first structure in the thirst circuit to be examined using genetically targeted methods for *in vivo* neural recording in freely behaving animals¹⁶. Optical recording of calcium dynamics in SFO^{GLUT} neurons confirmed that these cells monitor changes in plasma osmolality, volume and pressure, consistent with classic models^{10–12}. Unexpectedly, these recordings also revealed that SFO^{GLUT} neurons are rapidly inhibited when thirsty mice begin to drink, before any changes in the composition of the blood, such that they effectively 'predict' the impending restoration of homeostasis¹⁶. This rapid feedback is triggered by the detection of water in the oral cavity and is time-locked to the act of licking, enabling SFO^{GLUT}

Classic studies showed that AVP concentration in the blood rapidly falls during drinking^{104–107}, but little has been reported about the *in vivo* dynamics of the AVP neurons themselves^{105,108}. Recently, recordings of calcium dynamics and electrophysiological activity in thirsty mice demonstrated that AVP neurons in the PVH and SON are rapidly inhibited during drinking before any change in the blood can occur¹⁹. Presystemic inhibition of AVP neurons seems to occur in two parts: a first phase, in which these neurons are transiently inhibited by water-predicting cues alone, and a second phase, in which they are durably inhibited by drinking. This second phase is probably mediated through inhibition of upstream SFO^{GLUT} neurons¹⁶. The rapid inhibition of AVP neurons explains why circulating levels of AVP begin to decline at the outset of drinking, in anticipation of the restoration of homeostasis.

Interactions with feeding

Many animals, including humans^{109,110} and rodents^{111–113}, tightly coordinate eating and drinking to ensure that adequate water is available for food ingestion^{112–115} and digestion¹¹⁶ and also to counteract increases in plasma osmolality due to the absorption of osmolytes in food¹¹⁷. This stimulation of drinking by eating is known as prandial thirst. Conversely, dehydration potently suppresses food intake when water is unavailable^{111,118–120}, known as dehydration anorexia. Recent experiments have begun to reveal how these interactions between eating and drinking are controlled by the brain^{16,19} (FIG. 3).

Prandial thirst. Eating stimulates thirst within seconds, even though ingested food does not begin to alter the composition of the blood for several minutes. This raises the question of how the brain generates thirst at the outset of a meal. Optical recordings of SFO^{GLUT} neuron activity in hungry mice revealed that these cells are progressively activated by eating in a manner that tracks the amount of food consumed and precedes any change in plasma osmolality¹⁶. This observation suggests that activation of SFO^{GLUT} neurons during feeding is driven by a signal originating from the oral cavity or from the early gastrointestinal tract that anticipates impending changes in the blood. Importantly, blocking this rapid activation by optogenetic silencing of SFO^{GLUT} neurons is sufficient to abolish meal-associated drinking. Thus, SFO^{GLUT} neurons are activated by a presystemic signal generated

during eating, and this seems to be a primary cause of prandial thirst. The nature of the signal that communicates information about food ingestion to SFO^{GLUT} neurons is not known and may involve both neural^{112–115} and endocrine^{121–132} components.

In addition to generating thirst, eating also triggers AVP secretion to promote renal water retention. Optical recordings of SON^{AVP} neurons in hungry mice revealed that these cells are also rapidly activated by eating¹⁹, with kinetics very similar to those observed in upstream SFO^{GLUT} neurons¹⁶. This suggests that the presystemic signal communicated to the LT during eating controls both prandial thirst and AVP secretion.

Dehydration anorexia. The discovery that SFO^{GLUT} neurons are persistently activated by eating when water is unavailable¹⁶ suggests that activation of these cells may also underlie dehydration anorexia. Indeed, optogenetic silencing of SFO^{GLUT} neurons during feeding eliminates the drive for prandial drinking and restores food intake to normal levels when water is unavailable¹⁶, confirming that activation of SFO^{GLUT} neurons is necessary for the dehydration-induced suppression of food intake. The neural pathway through which SFO^{GLUT} neurons modulate the feeding circuit is not known, but one possible downstream target is the PVH, which is a convergence point for signals that regulate energy and fluid balance.

Circadian regulation

Most drinking occurs during subjective daytime when animals are most active¹³³. Whereas some of the circadian regulation of thirst is probably secondary to the circadian regulation of other behaviours, such as eating, activity and wakefulness^{134,135}, certain aspects of drinking behaviour seem to be controlled directly by the brain's 'central clock', the suprachiasmatic nucleus (SCN)¹³⁶. It was recently reported that SCN^{AVP} neurons that project to the OVLT are more active in the hours immediately preceding sleep and that this increase in synaptic AVP release can stimulate thirst by activating downstream OVLT neurons¹⁸. This brief increase in presomnial drinking prevents small (1–2%) increases and decreases in plasma osmolality and volume, respectively, that would otherwise occur during sleep¹⁸. Some SCN^{AVP} neurons also project directly to the SON, and this projection has been proposed to facilitate an increase in OVLT-stimulated secretion of AVP into the circulation by SON^{AVP} neurons

during late sleep¹³⁷. Complementary control of the thirst circuit by the SCN may therefore prevent night-time dehydration by stimulating water intake before sleep and, later, by promoting water retention during sleep.

Salt appetite

Sodium is a major determinant of plasma osmolality, and animals therefore must consume both water and sodium to maintain fluid homeostasis. The motivational drive to consume sodium, known as salt appetite (for a review, see REF. 138), is innate and therefore probably controlled by genetically hard-wired neural circuits, but the organization of these circuits has been unclear. Recently, a framework has emerged in which two circulating hormones, ANGII^{52,139} and aldosterone^{140–142}, act synergistically^{54,143} to control salt appetite.

Aldosterone acts both at the kidneys to promote sodium conservation^{144,145} and in the brain to stimulate salt appetite^{54,142,143}. Aldosterone can activate the mineralocorticoid receptor (MR) only in cells that express the enzyme 11 β -hydroxysteroid dehydrogenase type 2 (HSD2)¹⁴⁶, and co-expression of MR and HSD2 in the brain is restricted to a small population of glutamatergic¹⁴⁷ neurons in the nucleus of the solitary tract (NTS^{HSD2} neurons)¹⁴⁸. This unique population of aldosterone-sensitive neurons is specifically activated during sodium deprivation¹⁴⁹, and recent chemogenetic manipulations demonstrated that activation of NTS^{HSD2} neurons is both necessary and sufficient to drive sodium consumption²⁰. Projections from NTS^{HSD2} neurons are restricted to three brain regions that have been previously implicated in the control of salt appetite^{20,150,151}; NTS^{HSD2} neurons innervate *Foxp2*-expressing neurons in the pre-locus coeruleus and lateral parabrachial nucleus, and an unidentified population of neurons in the BNSTvl.

ANGII stimulates aldosterone secretion during sodium deficiency^{152–154} and also acts in the brain to directly stimulate salt appetite^{52,54,139,143}, presumably by activating ANGII-sensitive LT neurons. Whereas electrolytic ablation of the entire SFO has variable effects on sodium consumption^{71,73,155–157}, activation of SFO^{GLUT} neurons by ANGII was recently shown to be necessary for salt appetite²¹. Furthermore, these neurons were found to drive sodium consumption via their projection to the BNSTvl. Indeed, electrolytic ablation of the amygdala and BNST potently suppresses salt appetite^{158–160}. Thus, together, recent

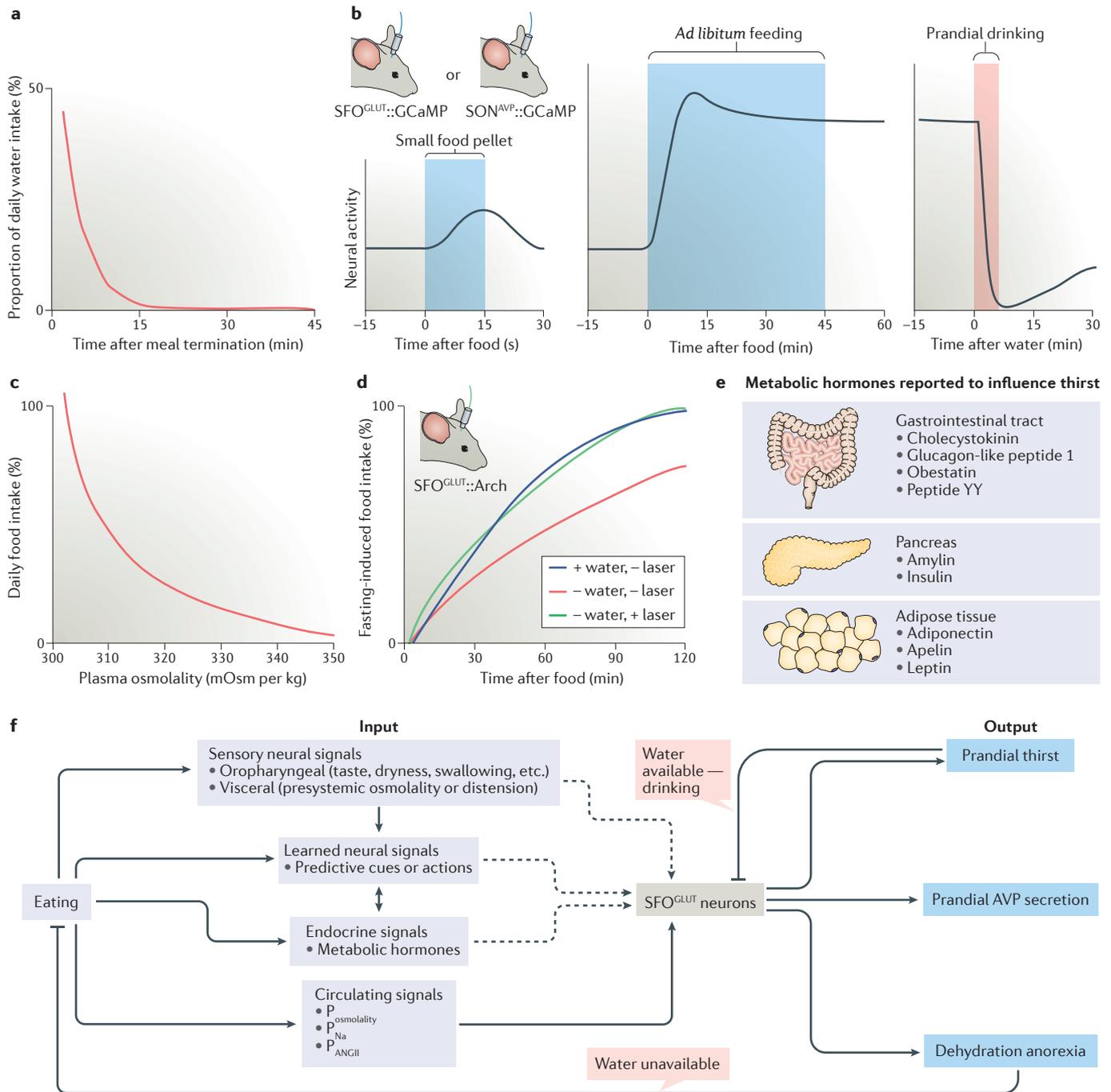


Figure 3 | The thirst circuit monitors and controls feeding behaviour.

a | Eating potently stimulates prandial thirst, and the majority of daily water intake therefore occurs in close temporal proximity to eating. Schematic is based on data in REF. 113. **b** | Glutamatergic neurons in the subfornical organ (SFO^{GLUT} neurons) and arginine vasopressin (AVP) neurons in the supraoptic nucleus (SON^{AVP} neurons) are rapidly activated on both short (seconds) and long (minutes) timescales during feeding to promote prandial thirst and AVP secretion. Schematics are based on data in REFS 16, 19. **c** | Dehydration potently suppresses feeding, and increases in plasma osmolality therefore inhibit total daily food intake. Schematic is based on data in REF. 184. **d** | Dehydration anorexia causes hungry mice (here, following 24 hours of food deprivation) to consume less food when water is absent (red line) than when water is available (blue line). However, this suppression of food intake is completely alleviated by optogenetic silencing of SFO^{GLUT} neurons during feeding (green

line), indicating that activation of SFO^{GLUT} neurons promotes dehydration anorexia in addition to prandial thirst. Schematic is based on data in REF. 16. **e** | Metabolic hormones secreted during feeding by the gastrointestinal tract^{121–124}, pancreas^{125–129} and adipose tissue^{130–132} have been proposed to regulate the electrical activity of SFO neurons *ex vivo*. However, it remains unclear whether such endocrine signals contribute to the natural coordination of eating and drinking by SFO^{GLUT} neurons *in vivo*. **f** | Eating rapidly activates SFO^{GLUT} neurons to promote prandial thirst and AVP secretion, as well as dehydration anorexia. The mechanism of this presystemic activation remains unclear but may involve learned or sensory neural signals or endocrine signals (dashed lines). If water is unavailable during eating, then ingested food will eventually alter the composition of the blood, and circulating signals will additionally activate SFO^{GLUT} neurons. P_{osmolality}, plasma osmolality; P_{ANGII}, plasma angiotensin II; P_{Na}, plasma sodium.

experiments^{20,21} have identified the BNSTvl as a convergence point for the synergistic control of salt appetite by discrete ANGII-sensitive forebrain and aldosterone-sensitive hindbrain circuits.

Open questions

Despite recent progress, many fundamental questions about the thirst circuit remain unresolved. Here, we highlight some of the key unanswered questions.

How is water in the oral cavity detected and signalled? The finding that the LT is potently modulated by signals from the oral cavity during eating and drinking¹⁶ re-emphasizes the importance of understanding how food and water are detected in the mouth. However, at present, little is known about the specific molecules and cell types that detect oral signals that are relevant to fluid balance. In *Drosophila melanogaster*, water detection is mediated by a discrete population of gustatory neurons expressing the water taste receptor Pickpocket protein 28 (Ppk28)¹⁶¹, but it is unclear whether water constitutes a distinct taste modality in mammals¹⁶². It is likewise unclear how the peripheral sensations that influence drinking behaviour, including osmolality, temperature, dryness and pressure, are detected in the oropharynx and early gastrointestinal tract and communicated to the LT.

What are the molecular identities of the osmosensor and baroreceptor? Plasma osmolality and pressure are thought to be detected by mechanosensitive ion channels expressed within osmosensitive and pressure-sensitive neurons, but the identity of these channels has remained elusive. A number of candidates have been advanced^{163–171}, but whether any of these proteins serve as direct sensors remains controversial. For example, although early studies suggested a role for the cation channels TRPV1 (REFS 163,164) and TRPV4 (REFS 165–167) in osmosensation, later work found that rodents lacking the genes encoding these channels display normal regulation of drinking and fluid balance^{172–175}, implying that other molecules are involved. Similarly, although acid-sensing ion channel 2 (ASIC2) seems to play a part in blood pressure sensing by peripheral nerves that innervate the aortic arch and carotid sinus^{170,171}, it may not be the mechanosensitive ‘baroreceptor’ that directly detects changes in pressure^{176,177}. The ability to access molecularly defined populations of interoceptive neurons will provide new insights into these questions.

How does thirst engage learning, motivation and motor systems? Thirst is one of the most potent motivational drives in mammals, yet it remains almost completely unknown how thirst neurons in the LT connect to brain regions that are important for reinforcement and learning¹⁷⁸; how this input generates a motivational drive that is specific for water consumption relative to competing homeostatic needs; and how conflicts between various homeostatic drives are resolved in the brain. It is similarly unclear how changes in LT neuron activity are communicated to motor pattern generators in the brainstem that control licking and swallowing^{179,180}, which are ultimately the sites in the brain where drinking behaviour is controlled. Maps of functional connectivity between the LT and various cortical and subcortical brain regions during thirst in humans^{81–86} may guide efforts to dissect the anatomical pathways connecting the LT to learning, motivation and motor systems in rodents.

Implications and conclusions

Genetic tools for circuit analysis have reinvigorated the study of thirst^{13–21}, enabling new insight into the cells, pathways and dynamics that underlie drinking behaviour. Much still remains to be discovered; however, one sign of recent progress is the emergence of neural circuit mechanisms that can potentially explain elements of everyday human experience, including prandial and circadian thirst; dehydration anorexia; rapid thirst satiation; and the effects of oral cooling. Although none of these phenomena is yet understood in detail, we now have a foothold at the level of neural circuits that can serve as an entry point for further investigation. We expect rapid further progress towards understanding these and other aspects of drinking behaviour over the next few years.

Note added in proof

A recent paper reports that acid-sensing taste receptor cells expressing the cation channel PKD2L1 (polycystic kidney disease 2-like 1 protein), the putative sour-taste sensor, also contribute to water detection in the mouth¹⁸⁵. These cells may contribute to part of the presystemic signal that rapidly regulates drinking behaviour and thirst satiation.

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