Central mechanisms of osmosensation and systemic osmoregulation

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Abstract | Systemic osmoregulation is a vital process whereby changes in plasma osmolality, detected by osmoreceptors, modulate ingestive behaviour, sympathetic outflow and renal function to stabilize the tonicity and volume of the extracellular fluid. Furthermore, changes in the central processing of osmosensory signals are likely to affect the hydro-mineral balance and other related aspects of homeostasis, including thermoregulation and cardiovascular balance. Surprisingly little is known about how the brain orchestrates these responses. Here, recent advances in our understanding of the molecular, cellular and network mechanisms that mediate the central control of osmotic homeostasis in mammals are reviewed.

Osmolality
A quantitative measure of the total solute concentration in a solution expressed in moles per kilogram of solution. Osmolality is not the same as osmolarity, which is the number of moles of total solutes per litre of solution.

Osmolyte
Any dissolved substance that contributes to the osmolality of a solution.

Hypertonic conditions
Conditions in which the ECF contains a higher concentration of membrane-impermeant solutes than is observed at rest in that particular species.

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Changes in extracellular fluid (ECF) osmolality cause water to flow across cell membranes to equilibrate the osmolality of the cytoplasm with that of the ECF[1]. By altering cell volume and intracellular ionic strength, large changes in ECF osmolality can affect the physical integrity of cells and tissues[2] and the biological activity of life-sustaining macromolecules[3]. This is a threat that most animals face as they interact with their habitat. Some aquatic animals, termed osmoconformers, seemingly make little effort to resist osmotic forces and adopt ECF osmolality values that are comparable to those of their external environment[4,5]. Osmoconformers tolerate such conditions because they have evolved molecular and biochemical mechanisms that optimize cell volume regulation[1,6,7] and minimize increases in ionic strength by synthesizing osmolytes under hypertonic conditions[8–11]. By contrast, animals termed osmoregulators have evolved mechanisms that maintain ECF osmolality near a stable value. These animals engage physiological responses that actively oppose osmotic perturbations and serve to restore ECF osmolality towards a seemingly fixed osmotic 'set-point'. Although it is not known whether this value represents a true singular physical entity or whether it is a balance point between different feedback systems that participate in the control of body fluid balance, the term set-point is retained to designate the value that is observed at rest. As a class, the mammals stand out because they maintain a common ECF osmotic set-point (near 300 mosmol kg^{-1}) (FIG. 1; Supplementary information S1 (table)). This is important because, as it is encased in a rigid cranium, the mammalian brain can be damaged by shrinking or swelling. Indeed, large changes in ECF osmolality can cause severe neurological symptoms in these species owing to the effects of altered electrolyte concentrations on neuronal excitability[12,13] and the physical trauma that can be caused by such conditions[14,15] (BOX 1). In this Review I address the mechanisms by which mammals defend against large changes in ECF osmolality. Following a brief overview of the processes that are involved in systemic osmoregulation as a whole, I focus on the location and function of the osmoreceptors that mediate osmosensation and on the neural pathways through which relevant homeostatic responses can be modulated by these unique sensory elements.

Systemic osmoregulation in mammals

Although mammals strive to maintain a constant ECF osmolality, values measured in an individual can fluctuate around the set-point owing to intermittent changes in the rates of water intake and water loss (through evaporation or diuresis) and to variations in the rates of Na^+ intake and excretion (natriuresis). In humans, for example, 40 minutes of strenuous exercise in the heat[16,17] or 24 hours of water deprivation[18,19] causes plasma osmolality to rise by more than 10 mosmol kg^{-1}. In a dehydrated individual, drinking the equivalent of two large glasses of water (~850 ml) lowers osmolality by approximately 6 mosmol kg^{-1} within 30 minutes[20]. Analogously, ingestion of 13 g of salt increases plasma osmolality by approximately 5 mosmol kg^{-1} within 30 minutes[21]. Although osmotic perturbations larger than these can be deleterious to health, changes in the 1–3% range play an integral part in the control of body-fluid homeostasis. In fact, differences between the ECF osmolality and the desired

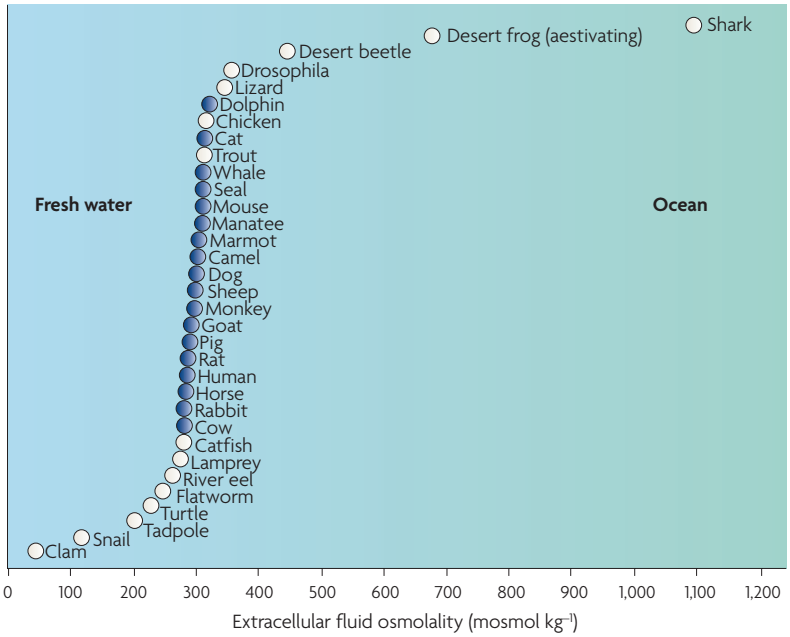


Figure 1 | **Extracellular fluid osmolality in animals.** The plot shows values of extracellular fluid (ECF) osmolality observed in various animals. The values, organized in ascending order along the y-axis, were taken from published studies (Supplementary information S1). Although different types of organisms (empty circles) can display values that span the full range of environmental osmolalities, mammals (filled circles) display osmotic set-points that cluster around 300 mosmol kg^{-1}. Aestivating frogs spend the summer in a state of dormancy.

set-point induce proportional homeostatic responses according to the principle of negative feedback (FIG. 2). Functionally speaking, therefore, it would seem that sensory osmoreceptors actively generate a basal signal at the desired set-point and have the ability to modulate this signal in a manner that encodes both the polarity and the magnitude of a change in osmolality.

Homeostatic responses to hyperosmolality. Studies in mammals[22–24], including in humans[25–27], have established that ECF hyperosmolality stimulates the sensation of thirst, to promote water intake, and the release of vasopressin (VP, also known as antidiuretic hormone), to enhance water reabsorption in the kidney. Hyperosmolality resulting from infusions of either NaCl or non-NaCl hypertonic solutions into the carotid artery also increases the rate of natriuresis from the kidney in various mammals[28–32], including man[33]. In rats[31,34] (but not in man[35]), this effect is mediated in part by the release of oxytocin into the bloodstream[36]. Hyperosmolality also inhibits salt appetite in sheep[37] and in rats, in which the effect is mediated in part by central release of oxytocin[38,39]. Finally, it is worth noting that increases in ECF osmolality have been shown to inhibit panting in animals[40,41] and exercise-induced sweating in humans[42–44]. Although these effects might support water conservation by reducing evaporative water loss under these conditions, the quantitative extent of their impact on osmoregulation remains to be defined. Therefore the osmotic control of sweating and panting will not be considered further in the present Review.

Diuresis
An increase in the flow of urine produced by the kidney.

Natriuresis
The excretion of Na^+ in urine.

Homeostatic responses to hypo-osmolality. ECF hypo-osmolality suppresses basal VP secretion in rats[22] and humans[45,46]. Because renal water reabsorption is partly stimulated by VP levels at rest[45], this inhibition of VP release effectively stimulates diuresis (FIG. 2). Intravenous infusion of hypo-osmotic solutions reduces thirst in dehydrated humans[47]. Moreover, the threshold for osmotically modulated thirst in water-replete individuals seems to lie a few milliosmoles below the osmotic set-point[48]. Thus, ECF hypo-osmolality can also promote homeostasis by inhibiting any desire to drink that might prevail under basal conditions (FIG. 2). As mentioned above, natriuresis is stimulated by systemic release of oxytocin in rats[31,34]. Interestingly, the basal electrical activity of hypothalamic oxytocin-releasing neurosecretory neurons is inhibited by hypo-osmolality[36]. It is therefore possible that a reduction in basal circulating oxytocin levels might reduce natriuresis under these conditions (FIG. 2). Analogously, a decrease in central oxytocin release might enhance salt appetite during ECF hypotonicity (FIG. 2). Whether ECF hypo-osmolality specifically inhibits natriuresis or stimulates salt appetite has yet to be determined.

Osmoreceptors in the brain and the periphery

The fact that there is feedback control of osmoregulatory responses implies the existence of a sensory mechanism that can detect osmotic perturbations. Early studies[49–52] provided clear evidence that "cellular dehydration" (that is, cell shrinking) was required for thirst and VP release to be stimulated during ECF hyperosmolality: these responses could be induced by infusions of concentrated solutions containing membrane-impermeable solutes, which extract water from cells, but not by infusions of solutes that readily equilibrate across the cell membrane (such as urea). Verney coined the term osmoreceptor to designate the specialized sensory elements. He further showed that these were present in the brain[50,53] and postulated that they might comprise "tiny osmometers" and "stretch receptors" that would allow osmotic stimuli to be "transmuted into electrical" signals[50]. Osmoreceptors are therefore defined functionally as neurons that are endowed with an intrinsic ability to detect changes in ECF osmolality. As such, it is reasonable to posit that the osmotic set-point is encoded by the resting electrical activity of these cells, and that the magnitude and polarity of ambient osmotic perturbations is signalled to downstream neurons by proportional changes in the action-potential firing rate (or firing pattern). Although cerebral osmoreceptors have a determinant role in the control of osmoregulatory responses (FIG. 2), it is now known that both cerebral and peripheral osmoreceptors contribute to the body fluid balance.

Peripheral osmoreceptors. Experiments in animals and humans have indicated that there are peripheral osmoreceptors along the upper regions of the alimentary tract and in the blood vessels that collect solutes absorbed from the intestines (FIG. 3). Specifically, such receptors are located in the oropharyngeal cavity[54], the gastrointestinal tract[21,55,56], the splanchnic mesentery[57], the hepatic portal vein[58] and the liver[59,60]. In rats, delivery

Box 1 | **Pathophysiology of osmotic perturbations in mammals**

Increases in plasma osmolality of ~10 mosmol kg^{-1} (which evoke a concurrent hypernatraemia) are associated with feelings of headache, reduced levels of alertness and difficulty in concentrating[19]. Larger perturbations can also lead to lethargy, weakness, irritability, hyperflexia, spasticity, confusion, coma and seizures[179,180]. Acute increases in plasma osmolality exceeding 80 mosmol kg^{-1} (for example, resulting from excessive salt ingestion during failed attempts to induce emesis) usually cause seizures and death[181–183]. Analogously, extracellular fluid (ECF) hypo-osmolality (also termed dilutional hyponatraemia) is commonly induced by excessive water intake. Marathon runners, for example, can develop hypo-osmolar hyponatraemia if their water intake exceeds the body's need for fluid replacement[184]. Hypo-osmolality can also occur as a result of excessive voluntary drinking[185,186] or compulsive drinking (for example, in some schizophrenic patients)[187] or from accidental over-hydration in the hospital setting[184]. The clinical symptoms associated with ECF hypo-osmolality are mainly neurological, progressing from headache, nausea and vomiting to mental confusion, seizures, coma and death[179,184,188].

of a gastric salt load increases the osmolality of blood in the hepatic portal vein within 7 minutes, whereas systemic osmolality remains unchanged for up to 15 minutes[56]. Osmoreceptors in these areas can therefore detect the osmotic strength of ingested materials and, through afferent connections to the CNS (FIG. 3), induce anticipatory responses that might buffer the potential impact of ingestion-related osmotic perturbations[61]. Indeed, water intake causes satiety in thirsty humans and animals before ECF hyperosmolality is fully corrected[27,62] (FIG. 4b). Similarly, gastric water loading has been shown to lower osmotically stimulated VP release long before any detectable reduction in ECF osmolality is observed[18,63–65]. Furthermore, gastric salt loading inhibits salt appetite in Na^+-depleted rats[66] and stimulates both VP release[56,57,67] and thirst[62] before ECF osmolality is enhanced by absorption of the salt. Although osmoreceptors located on the luminal side of the alimentary tract seem to mediate many of these effects, peripheral receptors located at post-absorption sites might also be important because infusions of hypertonic saline directly into the hepatic portal vein can provoke anticipatory osmoregulatory responses in the absence of significant changes in ECF osmolality[68,69].

The molecular and cellular structure of peripheral osmoreceptors is unknown. However, the information that they collect has been shown to reach the CNS through fibres that ascend in the vagus nerve[59,60,64,68,70,71]. A spinal pathway that relays afferent signals from the splanchnic nerves also mediates responses to hyperosmotic stimulation of the mesenteric-portal area[72]. Thus, as illustrated in FIG. 3, osmosensory afferents reach the CNS through the same anatomical routes as other visceral sensory signals[73–77]. Indeed, osmosensitive neurons have been found in the nodose ganglia[78], from which axons that ascend in the vagus nerve can make synapses in the nucleus tractus solitarius (NTS). Osmosensory fibres that course through the splanchnic nerves are presumably peripheral projections of dorsal root ganglion neurons that provide input to the thoracic spinal cord, where first order synapses are made onto ascending relay neurons in the superficial layers of the dorsal horn (FIG. 3).

Hypernatraemia
A condition in which a solution has a higher concentration of free Na^+ than is normal for the species in question.

Central osmoreceptors. Classic studies[23,50,52,53] provided clear evidence that the brain possesses an intrinsic osmosensor that responds poorly to infusions of hypertonic urea into the internal carotid artery. However, because urea only weakly permeates across the blood–brain barrier, its infusion into the bloodstream can effectively withdraw water and thus cause cellular dehydration within the brain compartment[79]. The primary cerebral osmoreceptors that modulate thirst and VP release, therefore, seem to be located in regions of the brain that are devoid of a blood–brain barrier[23,24], such as the circumventricular organs[80] (FIG. 3). Previous studies have shown that hypertonic solutions injected into the anterior ventral region of the third ventricle can provoke thirst and VP release[81,82], and lesions of this area prevent these responses during ECF hyperosmolality[83,84]. This area encloses the organum vasculosum laminae terminalis (OVLT; FIG. 3), one of the brains' circumventricular organs[80]. The OVLT has therefore been proposed to serve as one of the key osmosensing sites in the mammalian brain[85]. In agreement with this hypothesis, functional MRI studies have shown that the anterior region of the third ventricle becomes activated during the onset of ECF hypertonicity in animals[86] and humans[27,87] (FIG. 4a,b). Moreover, electrophysiological studies indicate that the rate of action-potential discharge in a subset of OVLT neurons varies as a positive function of fluid osmolality[88,89] (FIG. 4c,d), a behaviour that is retained when synaptic transmission is blocked[90] or when individual neurons are physically isolated from the surrounding cells[89]. Thus, there are neurons in the OVLT that seem to serve as primary osmoreceptors.

Mechanisms of osmosensory transduction

Osmoreceptors are specialized neurons. Osmoreceptor neurons lie at the heart of the central systems that mediate osmosensation and osmoregulation. These neurons must detect differences between ECF osmolality and a pre-established set-point, and they must encode this information into electrical signals that can persist even during prolonged perturbations[50]. Studies involving electrophysiological recording (FIG. 4c,d), functional imaging (FIG. 4a,b) or the expression of activity-dependent immediate-early genes such as *Fos*[91] have shown that many subsets of neurons in the CNS are osmoresponsive[92]. Although osmoresponsive neurons might display changes in firing frequency during osmotic stimulation, this alone does not identify such cells as osmoreceptors. By definition, osmoreceptor neurons must display an intrinsic ability to transduce osmotic perturbations into changes in the rate or pattern of action-potential discharge. Previous studies using *in vitro* preparations in which synaptic transmission was blocked by chemical means have suggested that osmoreceptor neurons might be present in the OVLT[90], the supraoptic nucleus[93,94], the subfornical organ[95], the medial preoptic area[96] and the caudal part of the NTS[97]. However, because glial cells can confer osmosensitivity through the release of taurine (see below), chemical blockade of synaptic transmission is not sufficient to prove that responsive neurons are intrinsically osmosensitive.

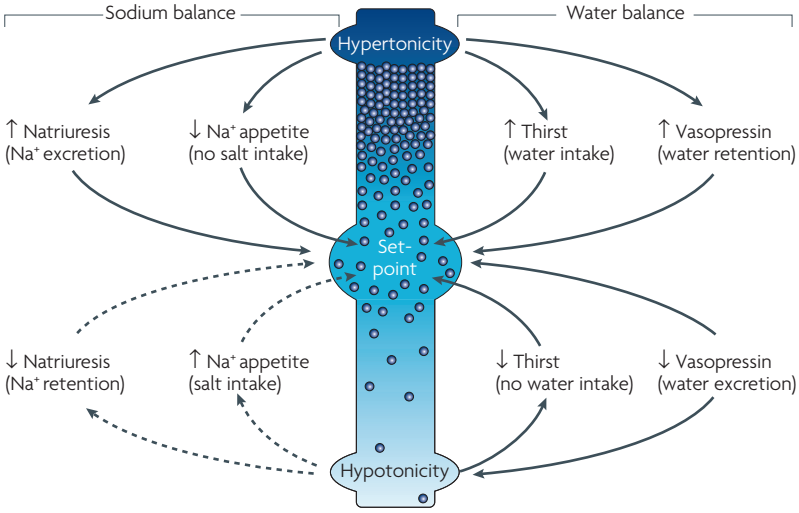


Figure 2 | **Basic mechanisms of osmoregulation.** Changes in extracellular fluid (ECF) osmolality modulate homeostatic responses that affect the Na^+ balance (left) and the water balance (right) to promote homeostasis according to the principle of negative feedback. Hypertonic and hypotonic conditions lead to proportional changes in the intake or excretion of water and sodium to maintain ECF osmolality near a constant set-point. Dashed lines illustrate potential homeostatic responses for which experimental data is presently unavailable.

Studies performed on cells that had been acutely isolated from specific brain regions have indicated that neurons in the OVLT[89] and in the subfornical organ[98], as well as magnocellular neurosecretory cells (MNCs) in the supraoptic nucleus (SON)[99,100], can operate as intrinsic osmoreceptors. Although experiments on osmoresponsive neurons have indicated that different subtypes of neurons can be either excited or inhibited by hyperosmotic stimuli, studies on osmoreceptor neurons suggest that most of these cells are proportionally excited by hypertonic stimuli (FIG. 4c,d) and inhibited by hypo-osmotic stimuli[101] (FIG. 5a). Thus, the basal electrical activity of these cells effectively encodes the osmotic set-point.

Osmotic stimuli modulate non-selective cation channels in MNCs. Most of what we know about the cellular mechanism of osmosensory transduction has come from work that was performed on MNCs in the rodent SON, and a recent study has indicated that MNCs in the paraventricular nucleus (PVN) sense changes in ECF osmolality through similar mechanisms[102]. Recordings in hypothalamic slices or explants have shown that hyperosmotic stimuli increase the firing rate in MNCs by depolarizing the membrane potential[93], and that this effect is caused by the activation of a non-selective cation current[94,102]. These findings have been confirmed by studies performed on MNCs that were acutely isolated from the SON of adult rats[99], which further revealed that hypo-osmotic stimuli inhibit firing by hyperpolarizing the membrane potential, an effect that is caused by the inhibition of a cation conductance that is active under resting conditions[100,103]. Indeed, individual neurons have been shown to respond to both increases and decreases in osmolality (FIG. 5a), and steady-state current-voltage analysis has shown that MNCs encode dynamic changes in ECF osmolality through proportional changes in the probability of opening of non-selective cation channels[100,103].

Osmosensory transduction is a mechanical process. Studies on isolated MNCs have revealed that increases in cation conductance that are caused by hypertonicity are temporally linked to a decrease in cell volume[100]. Indeed, unlike many cells in the body, which resist changes in volume through various regulatory mechanisms[1,7,10], MNCs behave as osmometers: they display reversible changes in volume that are inversely proportional to ECF osmolality (FIG. 5b) and that can be sustained for many minutes[104]. Thus, in principle, the modulation of the osmosensory transduction channels could be mediated by a mechanical effect associated with an osmotically evoked change in cell volume, the concentration or dilution of a specific cytoplasmic solute, or a change in intracellular ionic strength (FIG. 5c). However, decreases in cell volume provoked by applying suction to the inside of a patch-clamp pipette can depolarize and excite MNCs by enhancing a cation current, whereas inflating cells with positive pressure attenuates a basal cation conductance and inhibits action-potential firing by causing hyperpolarization[100,105]. Moreover, as shown in FIG. 5d,e, responses evoked by osmotic stimuli can be reversed by restoring the cell volume through changes in pipette pressure[105]. Because responses that are evoked during changes in pipette pressure occur without concurrent changes in ionic strength and without concentration–dilution effects, these results suggest that osmosensory transduction in MNCs is essentially a mechanical process. Indeed, when they are normalized to the degree of volume change, changes in cation conductance measured in MNCs are quantitatively equivalent whether they are evoked by changes in pipette pressure or by osmotic stimuli[105].

The osmosensory transducer might be a TRPV channel. The first clue concerning the molecular identity of the osmosensory transduction channel came from the discovery[106] that *osm-9*, a gene that is mutated in a mutant line of *Caenorhabditis elegans* that lacks an avoidance response to strongly hyperosmolar solutions, encoded a member of the superfamily of transient receptor potential (TRP) channels[107]. Indeed, many subtypes of TRP channels can be blocked by the nonspecific inhibitors gadolinium and ruthenium red[108], compounds that are also potent inhibitors of osmosensory transduction in MNCs[109,110] and OVLT neurons[89]. Moreover, most subtypes of TRP channels are permeable to Ca^{2+} (REF. 107), and the osmosensory transduction current of MNCs is known to be mediated by a non-selective cation conductance that features a significant degree of permeability to Ca^{2+} (P_{Ca}/P_{Na} = ~5)[111]. Although members of the TRP vanilloid (TRPV) family of cation channels seem to have important roles in osmosensation and osmoregulation, the molecular architecture and composition of the mammalian central osmoreceptor transduction channel remains unknown (BOX 2).

Patch-clamp pipette
A glass pipette with a tip diameter of approximately 1 μm. To make patch-clamp recordings, it is filled with a medium that approximates the composition of the cytoplasm. It is held by a plastic holder that makes a contact between this fluid and a silver electrode attached to an amplifier. A flexible tube connected to the same holder is used to alter the hydrostatic pressure inside the pipette and the cell to which it is connected.

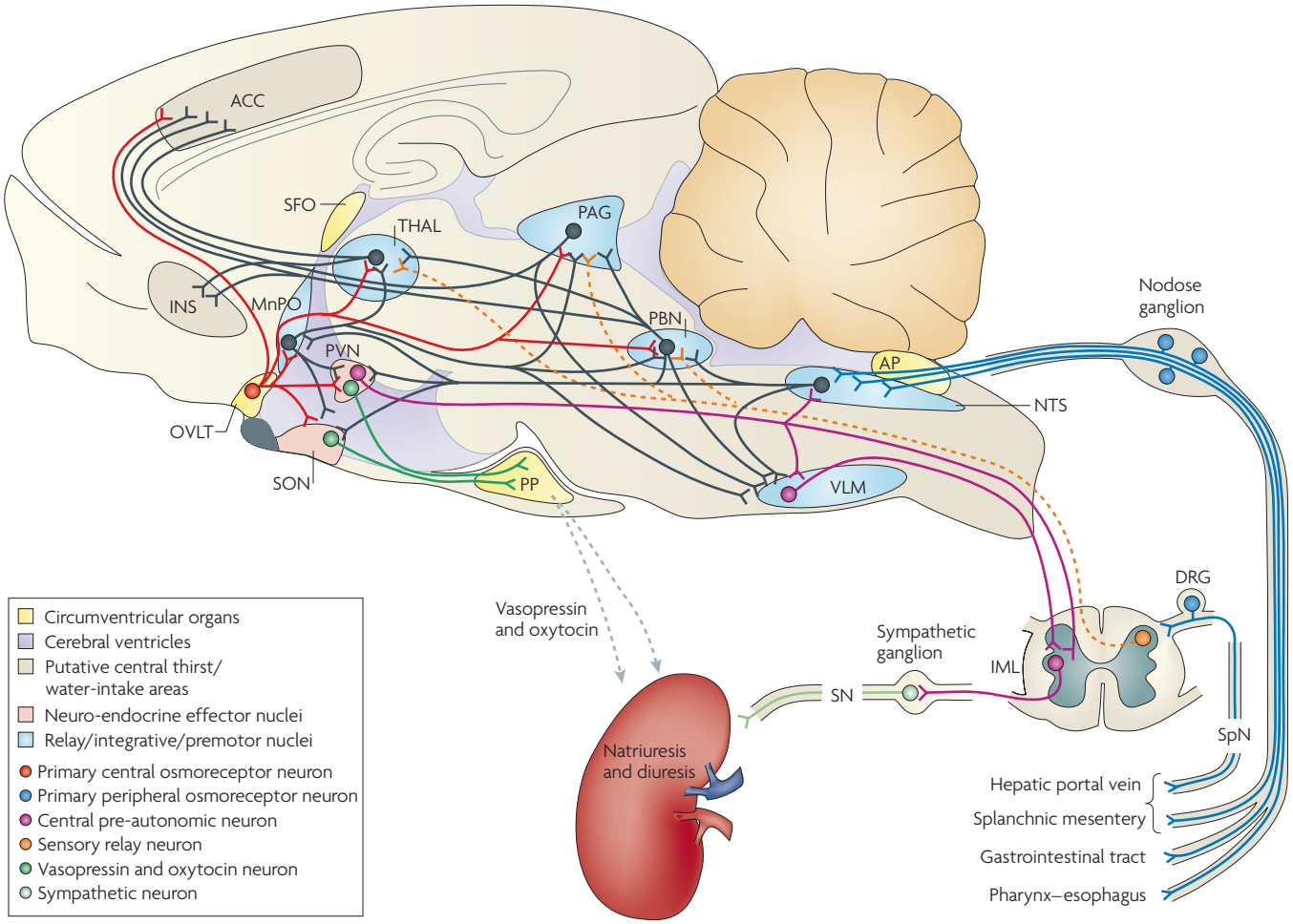


Figure 3 | **Osmoregulatory circuits in the mammalian brain and the periphery.** Sagittal illustration of the rat brain, in which the relative positions of relevant structures and nuclei have been compressed into a single plane. Only structures that have been directly implicated in the osmotic control of osmoregulatory responses are shown. Neurons and pathways are colour-coded to distinguish osmosensory, integrative and effector areas. Specific references documenting evidence for the pathways that are illustrated can be found in the Supplementary information S2 (Box). Although visceral sensory pathways that relay information from dorsal root ganglion neurons are known to ascend through the spinal cord, specific evidence that peripheral osmosensory information ascends through this route is only partial[72]; this tract is therefore illustrated as a dashed line. ACC, anterior cingulate cortex; AP, area postrema; DRG, dorsal root ganglion; IML, intermediolateral nucleus; INS, insula; MnPO, median preoptic nucleus; NTS, nucleus tractus solitarius; OVLT, organum vasculosum laminae terminalis; PAG, periaqueductal grey; PBN, parabrachial nucleus; PP, posterior pituitary; PVN, paraventricular nucleus; SFO, subfornical organ; SN, sympathetic nerve; SON, supraoptic nucleus; SpN, splanchnic nerve; THAL, thalamus; VLM, ventrolateral medulla.

Molecular basis of osmosensory transduction. Studies on acutely isolated MNCs have shown that they have a plasma-membrane area that is almost 50% greater than would be required if they had a smooth surface, and capacitance measurements have shown that the total surface area of these cells does not change significantly during osmotically evoked changes in cell volume[104]. These observations imply that variations in volume are accompanied by changes in the shape of membrane-surface features (such as folds) in osmotically stimulated cells. The nature of these changes and their physical relationship to the transduction channels have not been studied, but they might play an important part in the channels' mechanical gating. In addition, actin filaments have been found to be required for osmosensory transduction in MNCs, and the magnitude of the transducer current varies in proportion with subcortical actin density in these neurons[105]. How actin filaments mediate these effects is not known. Actin filaments could serve as tethers that impart volume-dependent strain through physical links to the ion channel, or as scaffolds in specialized membrane domains in which untethered channels could experience shape-induced forces at the protein–lipid interface[112,113]. Another possibility is that the channels might be gated indirectly, through the action of an actin-dependent mechanosensitive enzyme. In such a scenario, increases and decreases in channel activity during hypertonicity and hypotonicity would require rapid and bidirectional changes in the basal activity of the enzyme[113].

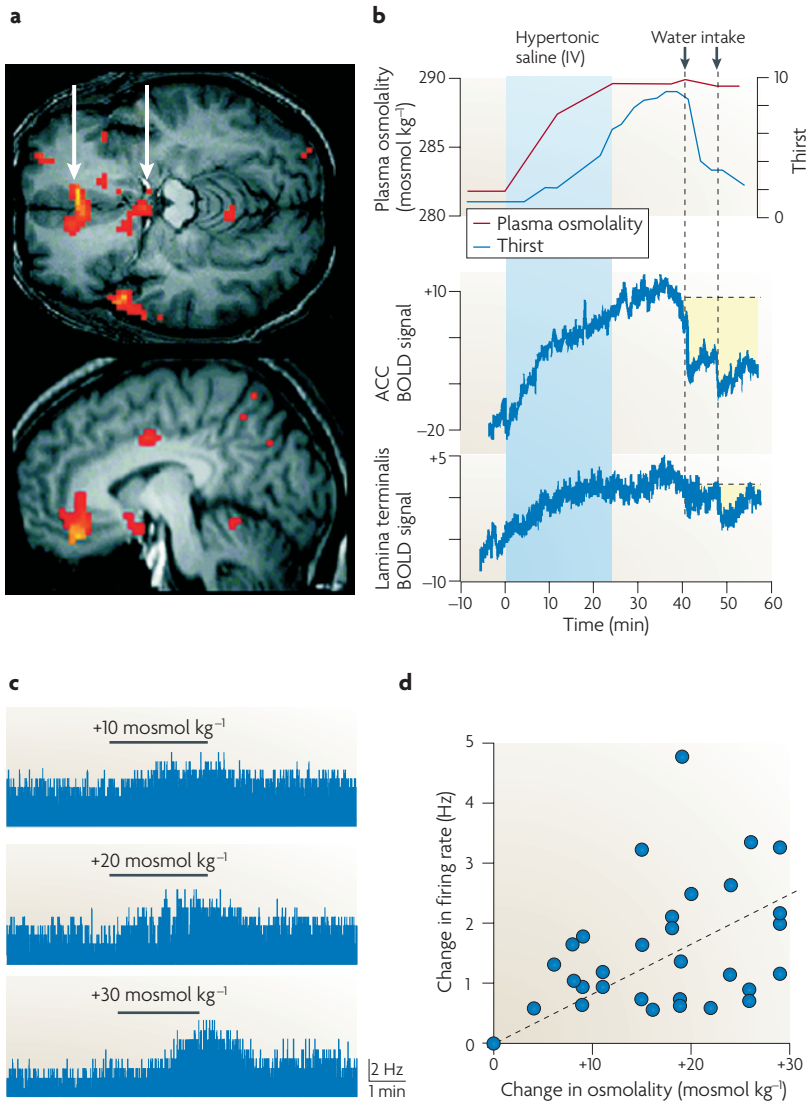


Figure 4 | **Cerebral osmosensors are excited by hypertonicity. a** | MRI images in the horizontal (upper image) and sagittal (lower image) planes, highlighting areas that show a significantly increased blood-oxygen-level-dependent (BOLD) signal under conditions in which thirst was stimulated in a healthy human by infusion of hypertonic saline. The arrows point to increased BOLD signals in the anterior cingulate cortex (ACC; left-hand arrow) and in the area of the lamina terminalis (right-hand arrow) that encompasses the organum vasculosum laminae terminalis (OVLT). **b** | Plots showing changes in thirst (upper plot) and changes in the BOLD signals in voxels of interest in the ACC (middle plot) and the lamina terminalis (lower plot) of the subject imaged in part **a**. The values of plasma osmolality shown in the upper plot represent average changes that were observed in a group of subjects that all underwent the same treatment. The traces show that osmoreceptors in the OVLT stay activated as long as plasma osmolality remains elevated, whereas the activation of cortical areas correlates with the sensation of thirst. **c** | Frequency plots showing examples of changes in firing rate that were detected during extracellular single-unit recordings obtained from three OVLT neurons in superfused explants of mouse hypothalamus. **d** | A scatter plot showing the changes in firing rate (relative to baseline) that were recorded from many mouse OVLT neurons during the administration of hyperosmotic stimuli of various amplitudes. The data indicate that osmoreceptor neurons in the OVLT encode increases in extracellular fluid osmolality through proportional increases in firing rate. This plot only shows data from osmoresponsive neurons (approximately 60% of the total neuronal population in the OVLT). Part **a** modified, with permission, from REF. 27 © (2003) National Academy of Sciences. Part **b** modified, with permission, from REF. 27 © (2003) National Academy of Sciences and REF. 197 © (1999) National Academy of Sciences. Parts **c** and **d** reproduced, with permission, from REF. 89 © (2006) Society for Neuroscience.

Role of taurine release from glial cells. Many cells release osmolytes as a mechanism to promote regulatory volume decreases in response to hypo-osmotic swelling[1,6,7]. The amino acid taurine is one of the primary organic osmolytes that is released during the brain's adaptive response to hyponatraemia *in vivo*[10,11]. Previous studies have shown that hypo-osmotic conditions promote taurine release from the astrocytes that surround MNCs in the SON[114,115]. Taurine is a potent agonist at extra-synaptic glycine receptors expressed on these MNCs[115,116], and its release has been shown to contribute to the inhibitory effect of hypotonicity on the firing rate of MNCs *in vivo*[115]. Although pharmacological studies suggest that taurine release by glial cells is mediated by a volume-regulated anion channel[117], the molecular identity of this channel remains to be established.

Role of other ion channels in osmoreception. In principle, the inward current that mediates depolarizing responses to hypertonicity could be assisted by the activation of other Na^+ or Ca^{2+} channels, by the inhibition of a resting K^+ conductance or by the modulation of an electrogenic transporter. Analogously, the outward current that mediates the hyperpolarizing effect of hypotonicity could be supported by actions opposite to those listed above. Patch-clamp recordings from acutely isolated MNCs[118] have revealed that these cells express a number of stretch-activated K^+ channels, the properties of which are consistent with those of Tandem P-domain weak inward-rectifying K^+ (TWIK)-related (TREK) channels and TWIK-related arachidonic-acid-stimulated K^+ (TRAAK) channels[119]. In principle, the activation of such channels during membrane stretching caused by cell swelling could assist hyperpolarizing responses to hypotonicity. This hypothesis has yet to be investigated. A recent study has shown that acute hyperosmotic conditions can upregulate a slow time- and voltage-dependent K^+ current in MNCs[120]. The activation threshold of this current (near –60 mV) is not affected by hyperosmolality, but the absolute magnitude of the outward current recorded at more positive voltages is significantly enhanced under these conditions. Although the enhancement of an outward current cannot cause a depolarizing response, the upregulation of a slow voltage-gated K^+ current might play an important part in promoting the phasic bursting activity that emerges in VP-releasing MNCs during ECF hyperosmolality[36,121–124] and that facilitates VP release from the axon terminals of these cells in the neurohypophysis[125,126]. The identity, the functional role of and the basis for osmotic modulation of this channel remain to be defined.

CNS osmoregulatory mechanisms

Information derived from peripheral and cerebral osmoreceptors is transmitted either directly or indirectly to many parts of the brain, where integration with other visceral sensory modalities (such as blood volume, blood pressure, ECF Na^+ concentration and body temperature) coordinates the activation or

inhibition of individual osmoregulatory responses in a manner that optimizes overall homeostasis[127]. Different types of osmotic perturbations require different combinations of physiological responses.

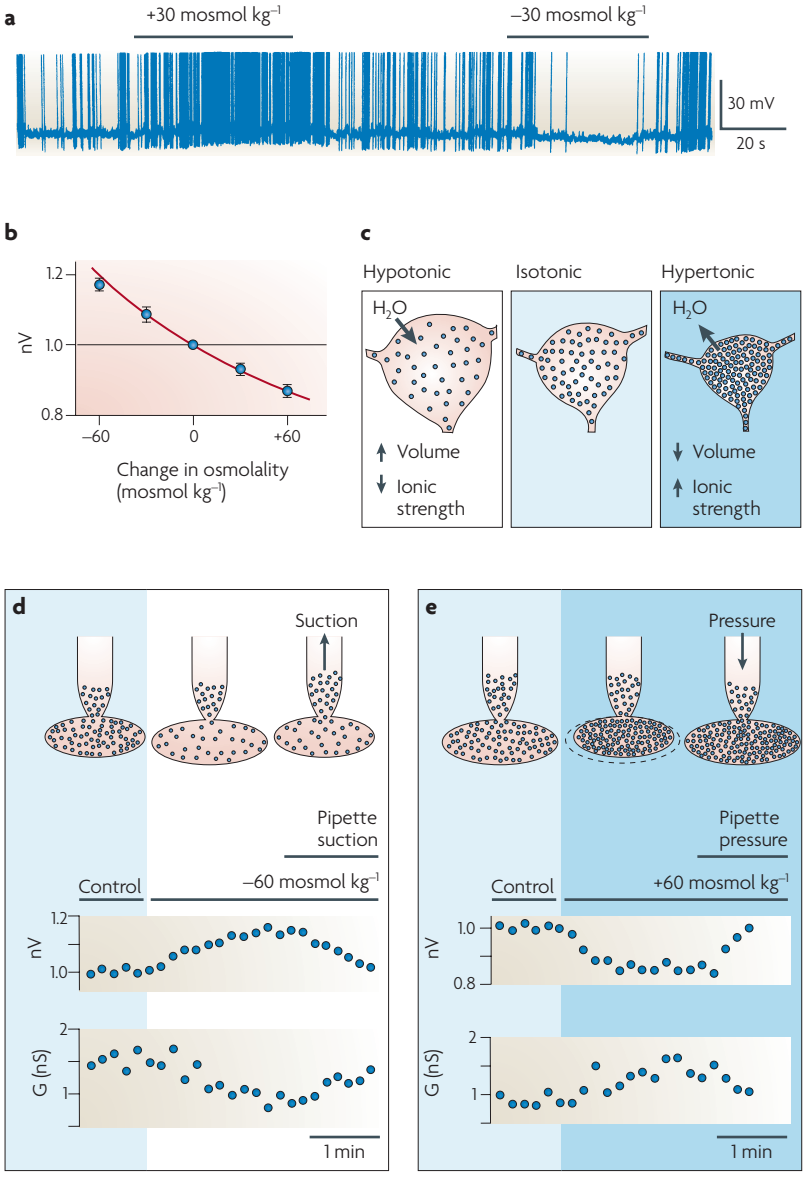


Figure 5 | **Osmosensory transduction is a mechanical process. a** | Whole-cell voltage recording from an acutely isolated rat magnocellular neurosecretory cell (MNC). Note that hyperosmolality causes membrane depolarization and increased action-potential firing, whereas hypo-osmolality induces hyperpolarization and reduced firing frequency. **b** | Isolated MNCs show changes in cell volume (nV, normalized to control volume) that are inversely proportional to extracellular fluid (ECF) osmolality. **c** | Cells exposed to hypotonic conditions show an increase in volume and a decrease in intracellular ionic strength. The opposite changes are observed under hypertonic conditions. **d** | During whole-cell patch-clamp recording, the decrease in membrane cation conductance (G) that is caused by a hypo-osmotic stimulus can be reversed by restoring the cell volume through suction applied to the recording pipette. **e** | Analogously, the increase in G that is caused by a hyperosmotic stimulus can be reversed by restoring the cell volume through an increase in pipette pressure. Part **a** reproduced, with permission, from REF. 103 © (1993) American Physiological Society. Part **b** reproduced, with permission, from REF. 104 © (2003) Macmillan Publishers Ltd. Parts **d** and **e** modified, with permission, from REF. 105 © (2007) Society for Neuroscience.

For example, dilutional hyponatraemia is corrected by a combination of salt intake and diuresis, whereas hypovolaemic hyponatraemia (such as that following intake of a diuretic compound[128]) requires intake of both salt and water. To implement the correct homeostatic programme, the osmotic control of the mechanisms shown in FIG. 2 is modulated by non-osmotic perturbations. For example, the VP release that is normally induced by ECF hyperosmolality is inhibited during concurrent hypervolaemia[22,45]. This effect is appropriate because these conditions require a net loss of salt and fluid that can best be achieved by stimulating natriuresis and suppressing water retention. As might be expected, CNS osmoregulatory circuits interact intimately with other homeostatic networks (such as inputs from baroreceptors). For simplicity, the present Review focuses specifically on the transmission of osmosensory information towards effector sites in the CNS. Readers should consult other reviews for complementary information regarding the non-osmotic control of osmoregulatory responses (for example, see REFS 127,129,130). Little is known about the mechanisms by which osmotic and non-osmotic signals are integrated; however, previous work has shown that at least six areas of the CNS participate in this process (FIG. 3): the NTS, the median preoptic nucleus (MnPO), the lateral parabrachial nucleus (PBN), the thalamus, the hypothalamic PVN and parts of the ventrolateral medulla (VLM). Information that is gathered and processed in these areas is presumably transmitted to effector sites that generate individual osmoregulatory responses. The network connections involving these areas that are illustrated in FIG. 3 are based on extensive anatomical studies (see Supplementary information S2 (box)). These parts of the brain are discussed below in the context of individual homeostatic responses.

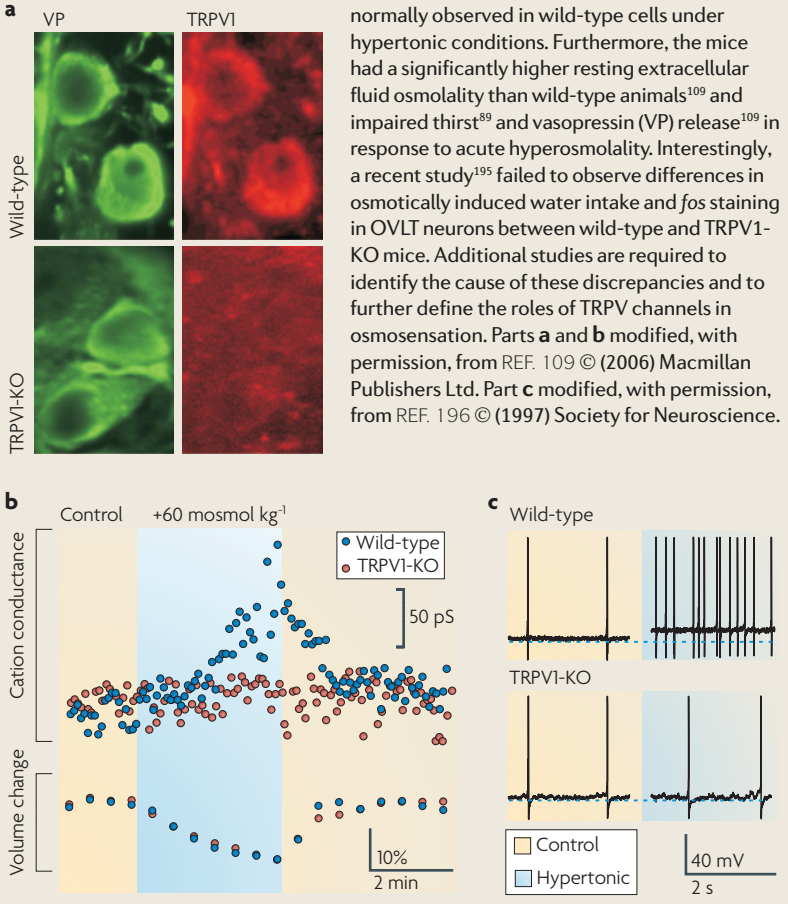
Controlling VP release. VP is synthesized by a subset of MNCs located in the PVN and SON of the hypothalamus. These MNCs project axons into the neurohypophysis (FIG. 3), where hormone release occurs when action potentials stimulate voltage-gated Ca^{2+} influx and exocytosis[125,126]. VP-releasing MNCs in the SON and PVN are thus the 'command' neurons that regulate diuresis. Indeed, the rate of action-potential discharge by MNCs varies as a positive function of ECF osmolality[36,101,121,124]. The firing rate of MNCs that prevails under resting conditions (~1–3 Hz) mediates basal VP secretion, whereas decreases and increases in firing frequency (respectively) inhibit and enhance hormone release during ECF hypotonicity and hypertonicity[101,131]. As discussed above, various local factors, including MNCs' intrinsic osmosensitivity and taurine release from glia, contribute to the osmotic control of firing rate in MNCs. However, these neurons also receive synaptic afferents from the OVLT[132–134], the MnPO[132,133], the PBN[135,136] and the NTS[135] (FIG. 3), and previous studies have established that the osmotic modulation of MNCs *in situ* depends in large part on afferent signals derived from peripheral osmoreceptors[58] and from neurons in the OVLT and MnPO[137,138]. Notably, experiments

Box 2 | **TRPV channels as osmosensory transducers**

There are four heat-sensitive mammalian transient receptor potential vanilloid (TRPV) channels — TRPV1, TRPV2, TRPV3 and TRPV4 — and two highly Ca^{2+}-selective, heat-insensitive TRPV channels (TRPV5 and TRPV6)[107,108]. The mammalian TRPV genes are orthologues of the *Caenorhabditis elegans* gene *osm-9*, which encodes an ion channel that is involved in hyperosmolality-avoidance behaviour[106]. Osmosensory neurons in the organum vasculosum laminae terminalis (OVLT) express TRPV1 (REFS 89,144) and TRPV4 (REFS 189,190), and magnocellular neurosecretory cells (MNCs) in the supraoptic nucleus (SON) express TRPV1 (REFS 109,144) (see figure, part **a**) and TRPV2 (REF. 191). Whether OVLT neurons also express TRPV2 and TRPV3, and whether MNCs also express TRPV3 and TRPV4, is unknown. When they are transfected into heterologous cells, TRPV2 and TRPV4 form cation channels that can be activated by hypo-osmolality[189,192]. Experiments on TRPV1-knockout (TRPV1-KO) mice have also indicated that the *Trpv1* gene is required for hypotonicity-induced ATP release from urothelial cells[193].

Importantly, this activation by hypotonicity is the reverse of the osmosensory responses of MNCs and OVLT neurons (FIG. 4). However, transgenic expression of mammalian TRPV4 can rescue the hyperosmolality-avoidance phenotype of *osm-9*-mutant *C. elegans*[194]. This suggests that TRPV4 is important for osmosensitivity, but that additional proteins expressed in native osmosensory neurons might be required to generate a channel that is activated by hyperosmolality. Two groups have generated mice that lack *Trpv4*. One reported an impairment in the stimulation of *fos* expression in OVLT neurons, as well as thirst and VP release in response to hyperosmolality[190]. However, the other study found no difference in water intake, and an exaggerated VP response to hypertonicity. Further work is required to clarify the role of TRPV4 in osmoreceptor neurons.

Although wild-type OVLT neurons[144] and MNCs[109,144] contain the carboxy terminus of TRPV1, they are insensitive to capsaicin[89,109]. The molecular structure of the TRPV1 variant that is expressed in these neurons has yet to be determined, but it seems to lack part of the amino terminus[109]. Interestingly, deletion of the *Trpv1* gene in mice abolished the responsiveness of MNCs and OVLT neurons to hypertonicity[89,109]. The neurons displayed normal shrinking (lower half of part **b**), but lacked the accompanying increase in cation conductance (upper half of part **b**), the membrane depolarization and the increased action-potential firing (part **c**) that are normally observed in wild-type cells under hypertonic conditions. Furthermore, the mice had a significantly higher resting extracellular fluid osmolality than wild-type animals[109] and impaired thirst[89] and vasopressin (VP) release[109] in response to acute hyperosmolality. Interestingly, a recent study[195] failed to observe differences in osmotically induced water intake and *fos* staining in OVLT neurons between wild-type and TRPV1-KO mice. Additional studies are required to identify the cause of these discrepancies and to further define the roles of TRPV channels in osmosensation. Parts **a** and **b** modified, with permission, from REF. 109 © (2006) Macmillan Publishers Ltd. Part **c** modified, with permission, from REF. 196 © (1997) Society for Neuroscience.



in superfused explants of rat hypothalamus have shown that glutamatergic afferents from OVLT neurons play an important part in the osmotic control of MNCs in the SON[101,139].

Regulating thirst. In hyperosmolar subjects, water ingestion satiates the sensation of thirst within seconds, many minutes before the absorption of water can correct ECF osmolality[27] (FIG. 4b). As mentioned above, this effect is mediated in part by peripheral osmoreceptors, but it is also mediated by other subtypes of oropharyngeal receptors[63,140] and gastrointestinal distension sensors[62], which together monitor the pre-systemic impact of ingested fluids. Functional imaging studies in humans have shown that the area that encompasses the OVLT remains activated in satiated hyperosmolar individuals[27] (FIG. 4b). This indicates that osmoreceptors in this region continue to monitor ECF osmolality under these conditions, and that the conscious perception and satiation of thirst must occur elsewhere in the CNS. Imaging studies in humans have offered a unique opportunity to define the brain regions that are activated in response to ECF hyperosmolality, the de-activation of which correlates with the onset of satiety[27,141]. These approaches have revealed that parts of the anterior cingulate cortex (ACC) and the insular cortex (INS) show changes in activity that correlate with the progressive intensification of thirst and its satiation upon drinking across different subjects (see REF. 87 for a review). It has been proposed that the activation of parts of the INS might be involved in the genesis of specific homeostatic sensations (such as pain, hunger and thirst), whereas the activation of specific sites in the ACC might serve to motivate behavioural responses that are demanded by particular homeostatic disturbances[73,74,142]. In agreement with this hypothesis, electrical stimulation in parts of the ACC has been found to elicit drinking within seconds of stimulus onset in awake monkeys[143]. Moreover, studies in rats have shown that the INS and the ACC receive information from osmoreceptors (FIG. 3), and immunohistochemical detection of Fos, a product of the activity-dependent immediate-early gene *Fos*, has suggested that neurons in the INS become activated under conditions that stimulate thirst[144,145]. Interestingly, cortical lesions that encompass the ACC and/or the INS do not completely prevent water intake[146]. Thus, although a conscious perception of the sensation of thirst might require cortical tissue, drinking behaviour might also be commanded from subcortical structures (such as the periaqueductal grey (PAG); for a review see REF. 147).

Regulating salt appetite. Although many parts of the CNS are known to participate in the control of salt appetite during changes in ECF volume[62,127,129], little is known about the central pathways that specifically inhibit salt appetite during ECF hyperosmolality. Recent studies have shown that the PBN exerts a powerful inhibitory influence on salt intake[127], and that inhibition of PBN neurons stimulates salt intake in water-replete[148] or hyperosmolar rats[149]. Moreover, lesions that encompass parts of the NTS have been shown to remove an

Organic osmolyte
An organic molecule that is synthesized by a cell to increase the effective osmolality of the intracellular compartment and thus resist the shrinking that would otherwise be caused by extracellular hypertonicity.

Hyponatraemia
A condition in which the plasma has a lower concentration of free Na^+ ions than is normal for the species in question.

Neurohypophysis
The posterior pituitary gland, also known as the pars nervosa of the pituitary.

Dilutional hyponatraemia
A condition in which the plasma becomes hyponatraemic as a result of excessive water intake, as opposed to as a result of sodium loss.

Hypovolaemic hyponatraemia
A condition in which the plasma becomes hyponatraemic in combination with a significant reduction in total blood volume.

Superfused explant
A small explant of adult brain tissue that is kept functional by the superfusion of an oxygenated artificial cerebrospinal fluid.

inhibitory influence on salt appetite in resting animals[150,151]. It is therefore possible that peripheral osmoreceptors mediate an inhibitory influence on salt appetite through ascending inputs that are relayed directly to the PBN or through the NTS (FIG. 3). Similarly, inputs from cerebral osmoreceptors might also inhibit salt appetite through projections from the OVLT and the MnPO to the PBN. As mentioned earlier, centrally released oxytocin inhibits salt appetite under hyperosmotic conditions[38,39]. The neural source of the oxytocin and the CNS targets that mediate these effects have yet to be identified. However, oxytocin-containing parvocellular neurons in the PVN are known to project to parts of the dorsal medulla that include the NTS[152], and subsets of parvocellular PVN neurons can be activated by ECF hyperosmolality[153,154]. Thus, osmotically activated PVN neurons might inhibit salt intake by releasing oxytocin into the NTS. Although circuits involving neurons in the OVLT, the MnPO, the PVN and the NTS might modulate salt appetite through the PBN, salt-intake behaviour is known to involve other transmitters and depend on additional forebrain areas, including the amygdala, the bed nucleus of the stria terminalis and the lateral hypothalamus (for reviews see REFS 127,129). Specific cortical areas, other than taste-associated regions[155], that might in part be activated by cognitive perception of salt appetite have yet to be identified.

Controlling natriuresis. Renal Na^+ excretion is regulated by various hormones and by sympathetic innervation from the renal nerves (for reviews see REFS 130,156–158). Previous studies have shown that lesions of the lamina terminalis that encompass the OVLT impair the increase in natriuresis that is normally provoked by ECF hyperosmolality[32]. Although the primary hormones that regulate natriuresis (aldosterone, angiotensin II and atrial natriuretic peptide) are secreted by tissues located outside the brain[156], oxytocin released by MNCs has been shown to act as a natriuretic hormone[159] and to stimulate natriuresis under hypertonic conditions in rats[34]. As mentioned earlier, oxytocinergic rat MNCs are excited and secrete this hormone into the blood during ECF hyperosmolality[36,131]. Oxytocin-releasing MNCs therefore represent command neurons through which natriuresis can be modulated during osmotic perturbations in rats. Humoral factors that mediate hyperosmolality-induced natriuresis in humans remain to be identified. As illustrated in FIG. 3, sympathetic outflow is also modulated by osmoreceptor signals through descending pre-autonomic neurons in the PVN and the VLM. Indeed, ECF hyperosmolality has been shown to influence sympathetic outflow in both humans[160] and rats[161–165], and changes in renal sympathetic-nerve activity that would otherwise be evoked by ECF hyperosmolality can be blocked by lesions of the lamina terminalis that encompass the OVLT[166,167]. Moreover, water deprivation has been shown to activate PVN neurons that project to the spinal cord and the VLM[168], and inactivation of PVN neurons reduces renal sympathetic-nerve activity in water-deprived rats[169]. Studies using retrograde propagation of the pseudorabies virus have confirmed that the kidney is innervated by polysynaptic projections involving the OVLT, the MnPO, the PVN, the VLM, the IML and sympathetic neurons[170,171] (FIG. 3). Thus, pre-autonomic neurons in the hypothalamus and the brainstem are additional command neurons that might regulate natriuresis through neurogenic influences on renal function[156–158].

Concluding comments and future directions

Several issues remain unresolved. First, the molecular structure of the osmoreceptor remains unknown. Although TRPV-channel subunits represent strong candidate components of the transduction channel (BOX 2), much work needs to be done before any formal structure can be proposed. Second, the nature of the cytoskeleton's involvement in osmosensory transduction remains to be elucidated. Is an enzyme involved? Do actin filaments serve as channel tethers or scaffolds? Addressing these fundamental questions will require a combination of biochemical, molecular and genetic approaches and, ultimately, the heterologous reconstitution of a functional osmosensor. Third, the osmosensory mechanisms described herein have been shown to operate during acute osmotic perturbations (stimuli that last less than 1 hour). However, osmosensory signalling *in vivo* can last for days without significant adaptation[121,172]. It is therefore possible that additional mechanisms are recruited under such conditions. For instance, recent studies have indicated that specific Na^+ sensors can modulate osmoregulatory responses independently from osmoreceptors[173,174]. The involvement of these sensors and their interactions with osmoreceptors remain to be elucidated. Moreover, chronic hyperosmolality causes dramatic changes in gene expression in MNCs[175], resulting in changes in the density of subtypes of *N*-methyl-D-aspartate receptors[176], Na^+ channels[177] and Ca^{2+} channels[178]. The contribution of such changes to osmosensing deserves further attention. It is also important to emphasize that most of the work on osmosensory transduction has been performed on MNCs, and that equivalent work has to be performed on other osmoreceptor neurons, such as those in the OVLT and those that relay peripheral osmosensory signals. Fourth, another unresolved aspect regards the neural mechanisms whereby thirst and salt appetite become perceived at a conscious level. Recent studies have highlighted the cortical structures that might be activated during the emergence of thirst. We must now turn our attention to the network mechanisms by which inputs from the periphery promote satiety under conditions in which cerebral osmoreceptors remain engaged. Analogous studies are also required to provide information concerning the conscious emergence of salt appetite. Fifth, studies are now required to define precisely how osmotic and non-osmotic signals are integrated to recruit individual effector responses. The cellular and network interactions that underlie the polymodal optimization of homeostasis remain largely unexplored, yet disruption of these mechanisms probably contributes to the aetiology of many homeostatic disorders of unknown origin.

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