**Supplementary Methods**

**Preparation and perfusion of isolated cells**

ONs from the supraoptic nucleus and HNs from hippocampus CA1 region were acutely isolated from the brains of adult (100-200 g) male Long Evans rats according to procedures reported previously. Briefly, brain slices containing ONs or HNs were cut and placed in oxygenated (100% O\textsubscript{2}) PIPES (piperazine-N,N’-bis[2-ethanesulfonic acid]) buffered saline (pH 7.4; 22°C) comprising (in mM): NaCl 120, KCl 3, MgCl\textsubscript{2} 1, CaCl\textsubscript{2} 1, dextrose 25, and PIPES 10. Kynurenic acid (0.5 mM) was added to solutions incubating HNs to increase viability. Blocks (~1 mm\textsuperscript{3}) containing either ONs, or HNs were then cut and incubated for 60-90 min in oxygenated PIPES solution (33.5 °C; pH 7.0) containing 0.7 mg/ml trypsin (type XI, Sigma Chemicals Corp., St-Louis MO.). All blocks were then transferred to a trypsin-free PIPES solution (pH 7.4; 22 °C) and dispersed, when required. Resulting cell suspensions were plated onto plastic petri dishes and used within 30-120 min. Dishes were mounted to the stage of an inverted phase contrast microscope (Nikon Diaphot) for imaging or electrophysiology. There, dishes were perfused with a [n-(2-Hydroxyethyl)piperazine-N’-(2-ethanesulfonic acid)] (HEPES)-buffered saline contained (in mM): Na\textsubscript{2}SO\textsubscript{4} 75, KCl 3, MgCl\textsubscript{2} 1, HEPES 10, CaCl\textsubscript{2} 1, glucose 10. Different amounts of mannitol were added to bring all solutions to the desired osmolality (isotonic, hypertonic, hypotonic).

**Preparation and perfusion of acute brain slices**

Coronal slices (300 μm thick) were cut using a vibratome from blocks of adult rat hypothalamus or hippocampus immersed in carbogenated (95% O\textsubscript{2}; 5%CO\textsubscript{2}) isotonic artificial cerebrospinal fluid (ACSF; pH7.4) at 4°C comprising (mM): Na\textsubscript{2}SO\textsubscript{4} 50, KCl 4.96, MgCl\textsubscript{2} 1.48, NaH\textsubscript{2}PO\textsubscript{4} 1.23, CaCl\textsubscript{2} 1, NaHCO\textsubscript{3} 26, Glucose 10, and mannitol as required to reach the desired osmolality. Slices stored in isotonic ACSF at room temperature were transferred to an imaging chamber as required. There, slices were perfused with ACSF at 32°C at a rate of ~2ml /min. Images of single cells were captured via a BX-51 upright Olympus microscope equipped for 80x infrared differential interference contrast video.

**Osmotic stimulation**

Slow osmotic ramps were applied according to methods by Lohr and Grantham (1986) and Van Driessche (1997). Briefly, two bottles of exactly the same size were interconnected through a control valve. Bottle A contained 100 ml (isolated cells) or 200ml (slices) isotonic control solution (295mOsmol/kg) and was constantly agitated with a magnetic stirrer. Bottle B contained an equal amount of +80 or –80mOsmol/kg solution. A circulating pump delivered
solution from bottle A to the preparation at a flow rate of ~2ml/min. Both ONs and HNs were first perfused with isotonic solution as control. The slow osmotic ramp was started with the opening of the control valve and continued for 60min. This procedure caused fluid osmolality to increase or decrease at a rate of 0.67mOsm/kg min⁻¹. Note that upon switching back to another reservoir containing control solution at the end of the ramp, a period of ~10min was required to fully return the osmolality of the fluid perfusing the dish back to control.

Fast osmotic steps were delivered to isolated cells by gravity at a rate of 1.0-1.2 ml min⁻¹. A fast stepper device (SF-77B, Warner Instrument Co. Hamden, CT) was used to control the fast switching (τ < 5ms) between the adjacent barrels of a glass capillary assembly that contained different solutions. In all experiments, the over-flowing solution was sucked away through a vacuum system.

**Measurements of relative cell volume**

We determined relative changes in cell volume from measurements of maximal cross-sectional area (CSA, from digitized video images) as performed elsewhere⁴⁻⁵. For each image captured before and during the stimulus, the perimeter of the cell was traced via a mouse using Scion Image for Windows 4.02 (Scion Corp., Frederick MA). The CSA (in pixels) was determined by the software. All values of CSA measured in the control period were averaged (CSAo) and values of relative (normalized) volume at each individual time point (nVt) were calculated from the CSA value at that time point (CSAt) using the equation; nVt = [(CSAτ)⁰.⁵/(CSAo)⁰.⁵]. This formula assumes that the cell is a simple ellipsoid and that evoked changes in volume occur uniformly in all planes. Cells with the simplest ellipsoid morphologies and shortest processes were thus selected for analysis. Three dimensional computer reconstruction of 6 individual cells, each made from a consecutive (z-plane) series of x-y images obtained by confocal microscopy, confirmed that the cells acutely isolated using our procedure retain a uniform ellipsoid morphology in all dimensions. Cells were retained for analysis only if their morphology was not overtly damaged by the osmotic shock, and if their volume recovered completely upon return to isosmotic conditions.

**Boyle-van’t Hoff fit**

Volume data was analyzed for osmometry using the Boyle-van’t Hoff osmotic pressure equation⁶:

\[ \pi (V - b) = \pi_0 (V_0 - b) \quad \text{or} \quad V = \frac{1}{\pi} [\pi_0 (V_0 - b)] + b \]

where \( \pi_0 \) is the control osmotic pressure, \( V_0 \) is the initial cell volume, \( \pi \) is the test osmotic pressure and \( V \) is the cell volume at steady state in test solution. Constant \( b \) represents the
fraction of the cell content that is presumed to be osmotically inactive. Data for ONs were fitted
with the Boyle-van’t Hoff equation using SigmaPlot for Windows Version 5.00 (SPSS Inc.,
Chicago, IL). The best fit was obtained with the value of \( b \) set at 0.224±0.0244 (mean±s.e.m.).
Values of \( b \) published for other cell types are similar (e.g. 0.25 for human islets\(^7\), 0.19 for human
mature oocytes\(^8\)).

**Measurements of cell capacitance and surface area**

Cells were patch-clamped with glass pipettes (1.2 mm o.d. glass, A-M Systems Inc.,
Carlsborg, WA) containing a solution (pH 7.2) comprising (in mM): K Gluconate 120, HEPES
10, MgCl\(_2\) 1, EGTA 1. Recordings were performed in the whole-cell mode using an Axopatch-1D
amplifier (Axon Instruments Inc., Union City CA). Membrane current (d.c.-2 kHz) was digitized
(10 kHz) via a digidata 1200B interface coupled to a PC running Clampex 8 (Axon Instruments
Inc.). Whole cell capacitance was not compensated electronically. Values of cell input
capacitance (\( C_\text{i} \)) were obtained by integration of the area beneath the current charging transients
evoked by 5mV voltage steps using the "membrane test" routine of Clampex. Data were stored on
a computer for offline analysis.

Changes in capacitance (i.e. \( nC \), normalized capacitance) predicted by the normalized
volume (nV; **Fig. 3b**) were calculated as \( nC = nV^{2/3} \). This calculation assumes that the starting
membrane surface is smooth, and that changes in volume are accompanied by increases or
decreases in membrane area required to comply.

Total membrane surface area (TMSA) was measured electronically as \( \text{TMSA} = C_\text{i} / C_m \),
where the constant \( C_m \) is 1 \( \mu \)F / cm\(^2\). TMSA estimated morphometrically was computed as the
sum of the surface areas of the cell soma and processes, if present. Soma area was calculated as 4
\times CSA (in \( \mu \)m\(^2\)). The surface area of processes was calculated assuming a cylindrical shape with
only one base using equation \( \pi r^2 + 2\pi rl \), where \( r \) is the midline radius of the process and \( l \) is the
length of the process.

This approach was validated by two observations. First, there was a close correspondence
between morphometrically- and electronically-determined TMSA in HNs (**Fig. 3a**). Second, there
was also a close correspondence between the amount of membrane reserve in ONs that was
estimated by comparing morphometric and electronically determined TMSA (54 ± 8%; \( n = 12; 
**Fig. 3a**), and membrane reserve estimated from the maximal enlargement in cell size observed
prior to lysis upon exposure to distilled water (55 ± 6%; \( n = 15, \) data not shown).
Statistical analysis

All values in this paper are reported as mean plus or minus the standard error of the mean (± s.e.m.). Linear regressions and monoexponential fits through the data were performed using Sigmaplot 5 (SPSS Inc.). Comparisons between groups were performed using Student's t-test.

References