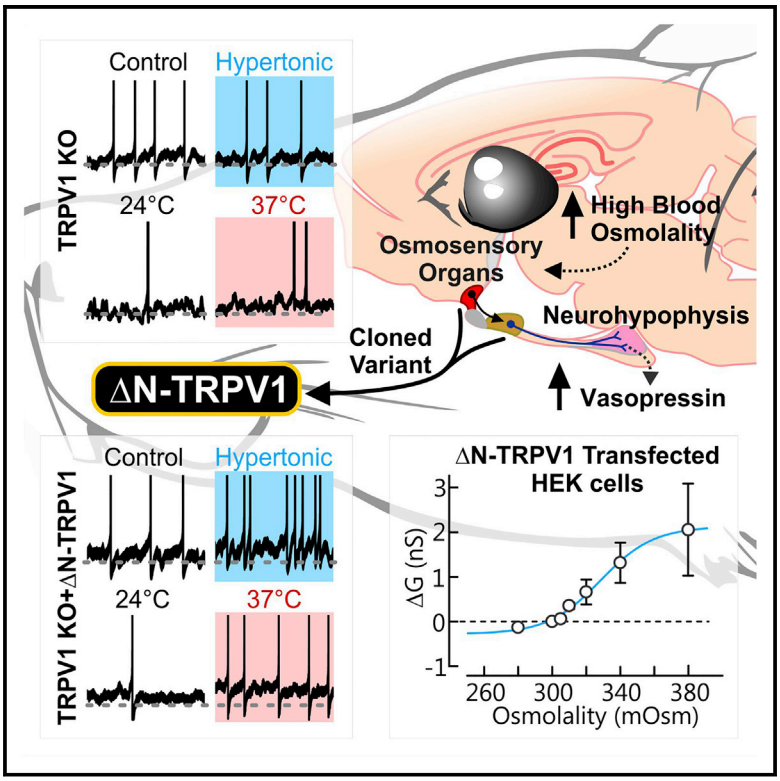
Report

Cell Reports

ΔN-TRPV1: A Molecular Co-detector of Body Temperature and Osmotic Stress

Graphical Abstract



Authors

Cristian Zaelzer, Pierce Hua, Masha Prager-Khoutorsky, ..., Daniel L. Voisin, Wolfgang Liedtke, Charles W. Bourque

Correspondence

charles.bourque@mcgill.ca

In Brief

Osmoregulation requires that thirst and vasopressin release be evoked in response to a rise in body temperature or fluid tonicity. In this study, Zaelzer et al. reveal that single hypothalamic osmoregulatory neurons can detect physiologically relevant increases in osmolality and temperature through the expression of an alternate transcript of *Trpv1*.

Highlights

- Osmoregulatory neurons express ΔN-TRPV1, a capsaicin-insensitive cation channel
- ΔN-TRPV1 channels are activated by physiological heat and cell shrinking
- Expression of *Trpv1dn* rescues osmoreception and thermosensing in *Trpv1*$^{-/-}$ mice

Accession Numbers

KP277509.1
KP277510.1

Zaelzer et al., 2015, Cell Reports *13*, 23–30
October 6, 2015 ©2015 The Authors
http://dx.doi.org/10.1016/j.celrep.2015.08.061

Cell Reports
Report

ΔN-TRPV1: A Molecular Co-detector of Body Temperature and Osmotic Stress

Cristian Zaelzer,[1] Pierce Hua,[1] Masha Prager-Khoutorsky,[1] Sorana Ciura,[1,2] Daniel L. Voisin,[1,3] Wolfgang Liedtke,[4] and Charles W. Bourque[1,*]
[1]Centre for Research in Neuroscience, Research Institute of the McGill University Health Centre, 1650 Cedar Avenue, Montreal, QC H3G1A4, Canada
[2]Institut du Cerveau et de la Moelle Épinière, Hôpital Pitié Salpêtrière, 47 Boulevard de l'Hôpital, 75013 Paris, France
[3]Neurocentre Magendie, INSERM U862, 146 Rue Léo Saignat, 33077 Bordeaux, France
[4]Department of Neurology and Center for Translational Neuroscience, 201G Bryan Research Building, Box 2900, Duke University Medical Center, Durham, NC 27710, USA
*Correspondence: charles.bourque@mcgill.ca
http://dx.doi.org/10.1016/j.celrep.2015.08.061

SUMMARY

Thirst and antidiuretic hormone secretion occur during hyperthermia or hypertonicity to preserve body hydration. These vital responses are triggered when hypothalamic osmoregulatory neurons become depolarized by ion channels encoded by an unknown product of the transient receptor potential vanilloid-1 gene (*Trpv1*). Here, we show that rodent osmoregulatory neurons express a transcript of *Trpv1* that mediates the selective translation of a TRPV1 variant that lacks a significant portion of the channel's amino terminus (ΔN-TRPV1). The mRNA transcript encoding this variant (*Trpv1dn*) is widely expressed in the brains of osmoregulating vertebrates, including the human hypothalamus. Transfection of *Trpv1dn* into heterologous cells induced the expression of ion channels that could be activated by either hypertonicity or by heating in the physiological range. Moreover, expression of *Trpv1dn* rescued the osmosensory and thermosensory responses of single hypothalamic neurons obtained from *Trpv1* knockout mice. ΔN-TRPV1 is therefore a co-detector of core body temperature and fluid tonicity.

INTRODUCTION

In terrestrial mammals, systemic water deficits experienced during heat- or exercise-induced dehydration are detected by hypothalamic osmoregulatory neurons (ONs) whose excitation triggers the sensation of thirst and secretion of antidiuretic hormone to prevent dehydration (Bourque, 2008; McKinley et al., 2006; Sladek and Johnson, 2013). Previous work has shown that the electrical activity of rodent ONs is regulated in a feedback manner by their cell autonomous ability to detect changes in extracellular fluid osmolality (Ciura and Bourque, 2006; Mason, 1980; Oliet and Bourque, 1992, 1993a; Qiu et al., 2004; Vivas et al., 1990). Moreover, ONs have been shown to be intrinsically thermosensitive, a property that mediates feedforward anticipatory responses that prevent dehydration (Sharif-Naeini et al., 2008; Sudbury and Bourque, 2013). These observations suggest that the feedforward and feedback signals regulating ONs might be detected and integrated at the level of single neurons (Sladek and Johnson, 2013), a concept that remains hitherto untested. Moreover, the molecular identity of the ion channels transducing heat and osmotic signals in ONs remain unknown. Indeed, although several studies have implied the involvement of channels encoded by *Trpv1* (Ciura and Bourque, 2006; Sharif-Naeini et al., 2008; Sharif Naeini et al., 2006), the gene coding for the heat- and capsaicin-activated channel TRPV1 (transient receptor potential vanilloid 1), it is presently unclear whether these channels are encoded as a full-length product of *Trpv1* (Moriya et al., 2015; Nishihara et al., 2011) or as a shorter variant of this ion channel (Ciura and Bourque, 2006; Sharif Naeini et al., 2006; Sudbury et al., 2010). This study shows that ONs express a truncated product of *Trpv1* that encodes an ion channel that enables the co-detection of osmotic and thermal signals within the hypothalamus.

RESULTS

Trpv1dn Is Selectively Expressed in ONs

In rat and mouse, mRNA coding for wild-type (WT) TRPV1 protein (*Trpv1*) comprises 15 exons flanked by UTRs at the 5′ and 3′ ends and a translation start site within exon 1 (Xue et al., 2001) (Figure 1A). Previous work has shown that alternate transcripts of *Trpv1* can be generated, notably by production of mRNAs that exclude exons 1–4 and retain intron 4 within the 5′ UTR (Figure 1B) (Schumacher et al., 2000; Xue et al., 2001). One such variant, VR.5′sv, does not form a functional channel but serves as a negative regulator of TRPV1 (Eilers et al., 2007; Schumacher et al., 2000). However, other variants lacking exons 1–4 could potentially encode functional channels. In order to ascertain the molecular identity of *Trpv1* variants expressed in ONs, we subjected osmoregulatory nuclei from mouse and rat to PCR cloning. Mouse paraventricular (PVN) and supraoptic nuclei (SON) were subjected to PCR with primers positioned at the 3′ and 5′ ends of the previously described VR.5′sv cDNA

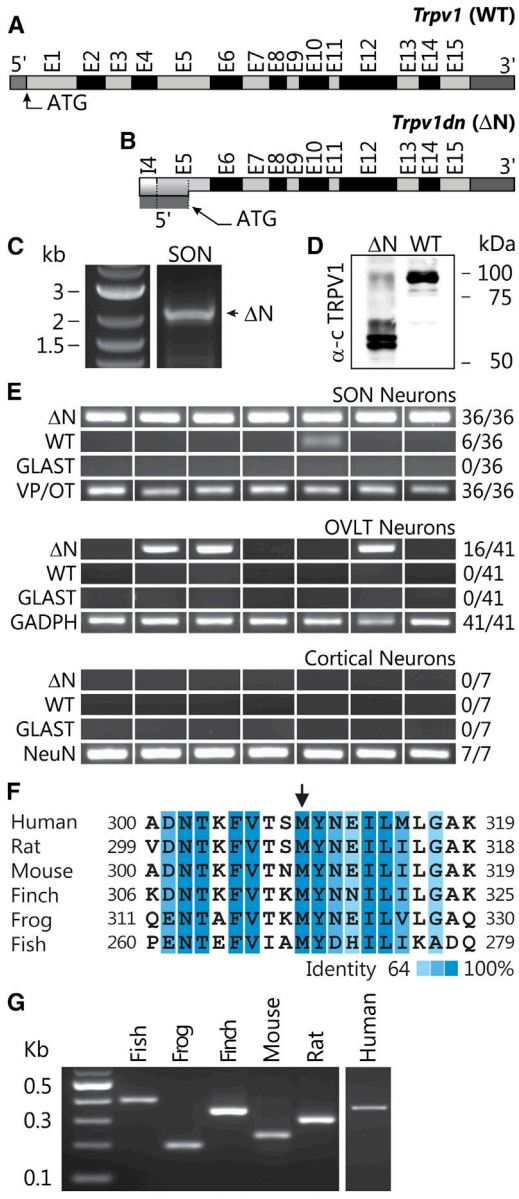


Figure 1. Osmoregulatory Neurons Express *Trpv1dn*

(A) Schema of *Trpv1* mRNA (upper), including 5′ and 3′ UTRs, translation start site (ATG), and 15 exons (Ex).

(B) Schema of *Trpv1dn* mRNA isolated from mouse and rat osmoregulatory nuclei. The transcript lacks E1–4 and incorporates intron 4 (I4) within the 5′ UTR (5′).

(C) RT-PCR detection of mRNA in rat supraoptic nucleus (SON) using primers specific for *Trpv1dn* (ΔN).

(D) Western blot shows proteins detected using an antibody directed against the C terminus of Trpv1 (α-cTRPV1) in lysates of HEK293 cells transfected with *Trpv1dn* (ΔN) or *Trpv1* (WT). High-molecular-weight bands presumably reflect glycosylation of the third extracellular loop (Veldhuis et al., 2012).

(E) Single-cell RT-PCR detection of transcripts specific to ΔN or WT and various markers (GADPH, glyceraldehyde 3-phosphate dehydrogenase; NeuN, hexaribonucleotide binding protein-3; VP/OT, vasopressin or oxytocin). Absence of GLAST (glutamate aspartate transporter) was used to confirm absence of astrocyte material. Each panel shows data from seven neurons isolated from the areas indicated. Numbers indicate positive/total tested.

(Schumacher et al., 2000; see Supplemental Experimental Procedures). This led to the isolation of *Trpv1dn*, a cDNA containing an open reading frame encompassing all of exons 5–15 of *Trpv1*, including an ATG start site (GenBank: KP277509.1; Figure 1B). A corresponding transcript was also isolated from rat SON (Figure 1C) and OVLT (*organum vasculosum lamina terminalis*) using the same strategy (GenBank: KP277510.1). *Trpv1dn* is predicted to encode a protein comprising 531 amino acids, corresponding to residues 308–838 of rat TRPV1 and residues 309–839 of mouse TRPV1 (Figure S1). Indeed, transfection of rat *Trpv1dn* into HEK293 cells induced the expression of a 58-Kd protein, significantly smaller than TRPV1 (92 Kd; Figure 1D).

A recent study indicated that TRPV1 may also be expressed in central osmosensory nuclei and co-localize with glia-specific markers (Mannari et al., 2013). In agreement with this observation, we detected transcripts encoding parts of the N terminus of *Trpv1* (exons 1–5) in rat SON and OVLT, together with products specific for *Trpv1dn*. To determine whether ONs selectively express *Trpv1dn* rather than *Trpv1*, we performed single-cell RT-PCR on subsets of neurons acutely isolated from adult brains. Whereas products corresponding to *Trpv1* were not found, transcripts specific for *Trpv1dn* were readily detected in ONs isolated from SON and OVLT, but not in neurons from non-osmosensitive brain regions including piriform cortex and hippocampus (Figure 1E). Alignment analysis of the amino acid sequences of TRPV1 orthologs from different species revealed that the methionine that provides the alternate translation start site in ΔN-TRPV1 is highly conserved throughout osmoregulating vertebrates (Figure 1F), and transcripts specific for *Trpv1dn* mRNA were also detected in the brains of fish, amphibians, and birds, as well as human hypothalamus (Figure 1G).

Trpv1dn Encodes an Osmoreceptor

Previous work has shown that the cell-autonomous osmosensory phenotype of hypothalamic ONs is lost in *Trpv1* knockout mice (*Trpv1*$^{-/-}$ mice; Ciura and Bourque, 2006; Ciura et al., 2011; Sharif Naeini et al., 2006; Sudbury et al., 2010). We therefore examined whether directed expression of *Trpv1dn* into hypothalamic neurons cultured from *Trpv1*$^{-/-}$ mice could rescue their osmosensory behavior. Immunostaining of *Trpv1*$^{-/-}$ neurons transfected with *Trpv1dn* using an antibody directed against the C terminus of TRPV1 (α-cTRPV1) revealed a high degree of expression along the cell perimeter whereas no staining was observed in untransfected controls (Figure 2A). Bath application of a hypertonic solution (+20 mOsm) caused a sustained and significant increase in the average action potential firing rate (FR) ($p = 0.00033$) and resting membrane potential (RMP)

(F) Alignment of amino acid sequences spanning the region containing the alternate start site for ΔN in various TRPV1 orthologs (arrow). Colors indicate the degree of identity of corresponding residues across the group. Human (*Homo sapiens*; GI:74315354), rat (*Rattus norvegicus*; GI:14010883), mouse (*Mus musculus*; GI:46309115), finch (*Taenopygia guttata*; GI:224076526), frog (*Xenopus laevis*; GI:298676437), and fish (*Danio rerio*; GI:187607882) are shown.

(G) PCR detection of *Trpv1dn*-specific transcripts in brains from various vertebrates. The different weights of bands reflect differences in the sizes of the products predicted for primer sets used for each species (see Supplemental Experimental Procedures).

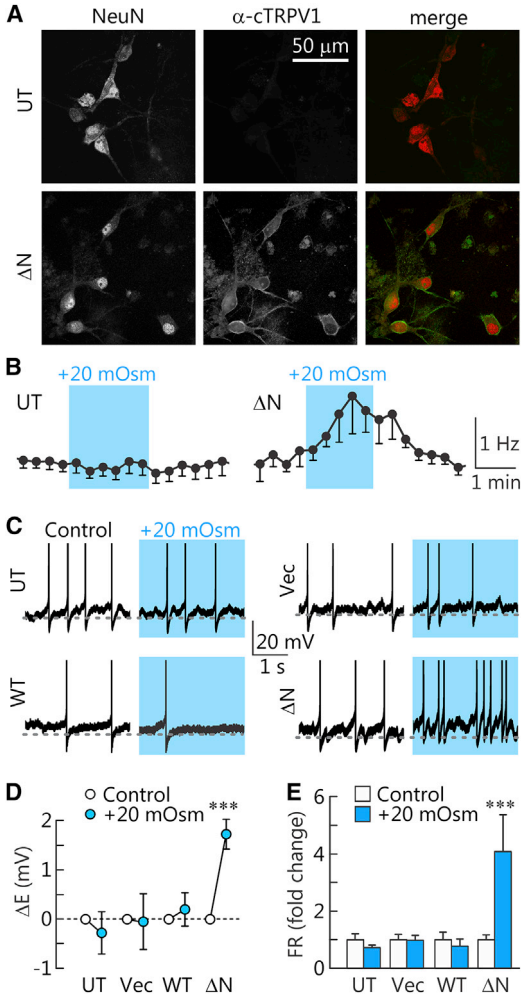


Figure 2. *Trpv1dn* Rescues Osmosensitivity in Neurons from *Trpv1*$^{-/-}$ Mice

(A) Immunostaining of neurons cultured from the hypothalamus of *Trpv1*$^{-/-}$ mice using antibodies directed against the neuronal marker NeuN and against the C terminus of TRPV1 (α-cTRPV1). Upper panels show staining in untransfected (UT) neurons, and lower panels show neurons transfected with *Trpv1dn* (ΔN).

(B) Plots show the time course of mean (±SEM) changes in firing rate (FR) induced by hypertonicity (blue area) in UT (n = 21) and ΔN-expressing neurons (n = 18).

(C) Excerpts of voltage recordings obtained before (control) and after increasing osmolality (blue area) in *Trpv1*$^{-/-}$ neurons that were UT or transfected with vector (Vec), ΔN, or *Trpv1* (WT).

(D) Plots show mean (±SEM) steady-state osmotically induced changes in membrane voltage (ΔE) in different groups of neurons.

(E) Bars plot mean (±SEM) steady-state osmotically induced changes in FR in the various groups of neurons tested. ***p < 0.005.

(p = 0.0000024) of *Trpv1*$^{-/-}$ neurons transfected with *Trpv1dn* (n = 33) but had no effect on untransfected neurons (FR, p = 0.088; RMP, p = 0.53; n = 20), neurons expressing vector alone (FR, p = 0.955; RMP, p = 0.927; n = 21), or neurons expressing *Trpv1* (FR, p = 0.259; RMP, p = 0.580; n = 6; Figures 2B–2E).

We next examined the effect of expressing *Trpv1dn* in heterologous cells. Dishes containing HEK293 cells transfected with *Trpv1dn* displayed a significantly lower population density compared to untransfected controls (p < 0.005), and these cells were rounded and loosely attached, as commonly observed when basal Ca^{2+} permeability is high (Caterina et al., 1997; Zhang and Hao, 2013). Moreover, only a small proportion (9.3%) of cells present in such dishes could be stained using α-cTRPV1 (Figure S2), suggesting that expression of *Trpv1dn* had a toxic effect on these cells. Under voltage-clamp (V_{hold} −60 mV), HEK293 cells that were either untransfected (n = 21) or transfected with *Trpv1* (n = 20) did not respond to the application of a hyperosmotic stimulus (+40 mOsm; p > 0.05; Figure S2). In contrast, the same stimulus caused the appearance of a slow inward current in a significant proportion (21%; Z test; p = 0.002) of the HEK293 cells transfected with either rat *Trpv1dn* (8/37) or mouse *Trpv1dn* (34/156; Figure S2). Current-voltage analysis revealed that this current was associated with a sustained increase in membrane conductance and a reversal potential (E_{rev}) of 2.5 ± 3.1 mV (Figures S2 and S3), consistent with the involvement of a nonselective cation conductance. Equivalent results were obtained in neuroblastoma Neuro-2A (N-2A) cells; where 18% (6/34) of cells transfected with mouse *Trpv1dn* responded to hypertonicity with a slow inward current reversing at 1.5 ± 3.2 mV (n = 14), whereas no response was observed in untransfected N2A cells (0/21) or in N2A cells transfected with *Trpv1* (0/18). As found in native ONs (Ciura and Bourque, 2006; Ciura et al., 2011; Sharif Naeini et al., 2006; Sudbury et al., 2010), responses to hypertonicity were significantly reduced by the TRPV1 antagonists Ruthenium red and SB 366791 (p < 0.01; n = 9; Figure S2), and capsaicin had no effect on neurons expressing *Trpv1dn* (Figure S3).

An analysis of the osmotically responsive HEK293 cells expressing mouse *Trpv1dn* revealed that hypertonic stimuli caused dose-dependent increases in membrane conductance, whereas a decrease in basal membrane conductance was observed when the cells were exposed to a hypo-osmotic stimulus (Figure 3A). Fitting the dose-response data with a sigmoidal equation revealed a 2% threshold for osmotic activation at 268 mOsm (Figure 3A). Concurrent measurements of digital images captured during the experiments revealed that the volume of HEK293 cells varies as an inverse function of fluid osmolality (Figure S4). Moreover, as observed in native ONs (Ciura et al., 2011; Oliet and Bourque, 1993a; Zhang et al., 2007), osmotically induced changes in conductance were inversely related to changes in cell volume (Figure 3B).

ΔN-TRPV1 Is Activated by Cell Shrinking

Native ONs display osmometric changes in cell volume (Ciura et al., 2011; Zhang and Bourque, 2003), and the electrical responses of these neurons to osmotic stimuli reflect a mechanical modulation of channel activity that varies as an inverse function of cell volume (Ciura et al., 2011; Prager-Khoutorsky et al., 2014; Zhang and Bourque, 2008; Zhang et al., 2007). We therefore examined whether expression of *Trpv1dn* can confer shrinkage-dependent responses to heterologous cells and neurons cultured from *Trpv1*$^{-/-}$ mice. In osmoresponsive HEK293 cells expressing ΔN-TRPV1, shrinking induced by application of negative pressure to the recording pipette caused a significant

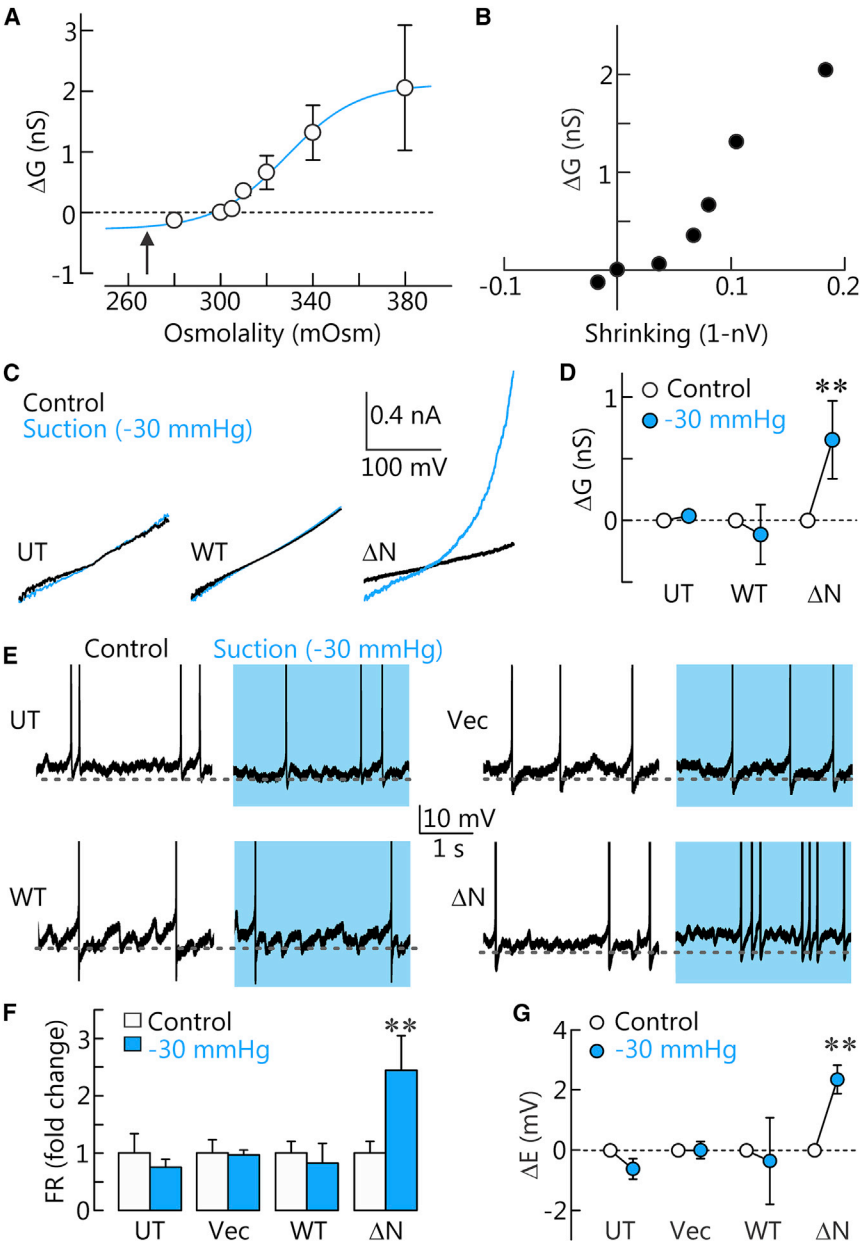


Figure 3. *Trpv1dn* Encodes a Shrinking-Activated Channel

(A) Plot shows mean (±SEM) changes in membrane conductance (ΔG) as a function of osmolality in osmoresponsive HEK293 cells. The solid line is a sigmoidal fit. Arrow indicates where ΔG is 2% above baseline.

(B) Mean values of osmotically induced ΔG in responsive HEK293 cells as a function of normalized volume change (nV) plotted as 1 nV (positive and negative values indicate shrinking and swelling, respectively).

(C) Whole-cell current-voltage relations show effects of suction (−30 mmHg) applied to the recording electrode in HEK293 cells that were either UT or transfected with WT or ΔN.

(D) Plots show mean (±SEM) suction-induced ΔG in the three groups (ΔN group includes only osmoresponsive cells).

(E) Effects of suction on cultured *Trpv1*$^{-/-}$ neurons that were either UT or transfected with Vec, WT, or ΔN.

(F) Bars plot mean (±SEM) suction-induced changes in FR observed in various groups (**p < 0.01).

(G) Plots show mean (±SEM) suction-induced changes in ΔE in different groups (**p < 0.01).

increase in membrane conductance and activated an ion current reversing near 0 mV (Figures 3C and 3D). Although it caused an equivalent decrease in cell volume (p > 0.05), the application of negative pressure had no effect on the membrane conductance of untransfected HEK293 cells or cells transfected with *Trpv1* (Figures 3C and 3D).

We next examined the effects of volume changes on hypothalamic *Trpv1*$^{-/-}$ neurons. Application of negative pressure (−30 mmHg) to the recording pipette had no effect on untransfected neurons (FR, p = 0.0567; RMP, p = 0.202; n = 14) or neurons transfected with vector (FR, p = 0.350; RMP, p = 0.957; n = 13) or *Trpv1* (FR, p = 0.437; RMP, p = 0.823; n = 4). However, an equivalent degree of suction-evoked shrinkage caused a significant increase in FR (p = 0.00525) and RMP (p = 0.000539; n = 11) in neurons transfected with *Trpv1dn* (Figures 3E–3G). Thus, *Trpv1dn* encodes an osmosensory channel whose activity is enhanced to cause neuronal excitation in response to cell shrinking.

Trpv1dn Encodes a Physiological Thermosensor

A channel encoded by the *Trpv1* gene is also required for thermosensation and anticipatory homeostatic osmoregulatory responses controlled by ONs (Sharif-Naeini et al., 2008; Sudbury et al., 2010). However, unlike the TRPV1 channel, which displays a threshold near 42°C (Caterina et al., 1997; Kauer and Gibson, 2009), ONs are gradually and proportionally activated by increases in temperature across the physiological range (~35°C–39°C; Sudbury and Bourque, 2013). To determine whether *Trpv1dn* can confer this phenotype, we first examined the effects of heat on HEK293 cells. Raising the temperature of the perfusing solution from 30°C to 42°C had no effect on untransfected cells (n = 24) or cells transfected with vector alone (n = 10) but caused a progressive and sustained increase in inward membrane current in 21.3% (17/80) of HEK293 cells expressing *Trpv1dn* (e.g., Figures 4A and 4B). Cells transfected with *Trpv1* also displayed a modest increase in current at a temperature of 37°C; however, these cells displayed a much larger and sharp increase in current at temperatures above 41°C (Figures 4A and 4B).

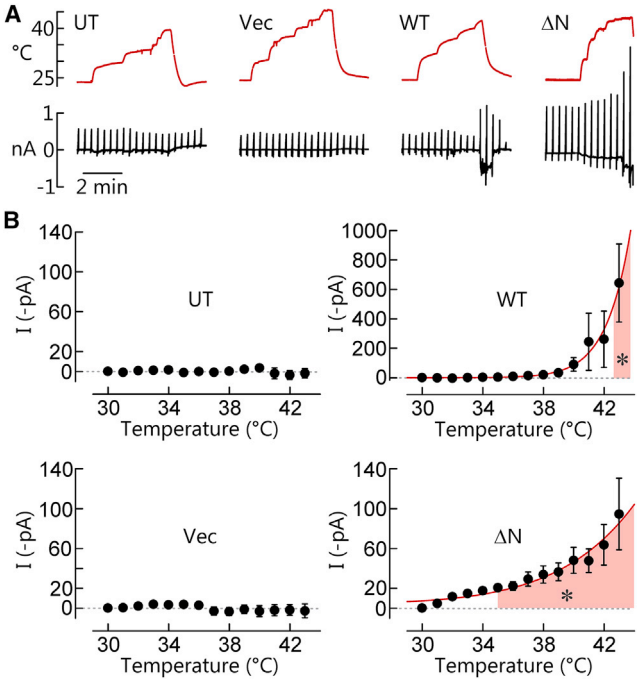


Figure 4. *Trpv1dn* Encodes a Thermosensitive Channel

(A) Effects of temperature (upper) on membrane current (lower; V_{hold} −60 mV) in HEK293 cells that were UT or transfected with Vec, WT, or ΔN. Vertical deflections are current responses to voltage ramps (−100 to +100 mV) used to generate I-V relations at different time points.

(B) Graphs plot mean (±SEM) changes in holding current (−pA; inward goes up) versus temperature (relative to 30°C) in all groups; shaded area indicates range where $p < 0.05$ (*).

ONs Co-detect Heat and Osmotic Stress via ΔN-TRPV1

We next examined whether *Trpv1dn* could rescue physiological thermosensitivity in hypothalamic *Trpv1*$^{-/-}$ neurons. In agreement with the findings reported above, *Trpv1*$^{-/-}$ neurons expressing *Trpv1dn* (n = 11) were significantly excited and depolarized by heating from 24°C to 37°C, whereas neurons transfected with vector alone (n = 13) or untransfected neurons (n = 8) were unaffected by this stimulus (Figures 5A–5C). Moreover, the vast majority of *Trpv1*$^{-/-}$ neurons transfected with *Trpv1dn* (81.8%) displayed significant thermosensitivity across the physiological range. Therefore, ΔN-TRPV1 can confer physiological thermosensing properties upon otherwise thermally non-responsive heterologous cells and neurons from *Trpv1*$^{-/-}$ mice.

Although separate studies have shown that subsets of ONs can detect changes in osmolality (Ciura and Bourque, 2006; Ciura et al., 2011; Oliet and Bourque, 1993a; Qiu et al., 2004) and temperature stimuli in the physiological range (Sharif-Naeini et al., 2008; Sudbury and Bourque, 2013), it remains unclear whether individual ONs can respond to both types of stimuli and, if so, via a signaling pathway that relies on one sensory molecule or rather on diverse pathways and/or diverse molecular sensors. To address this fundamental issue, we obtained single-unit extracellular recordings from ONs in situ. As illustrated in Figures 5D and 5E, the FR of native ONs recorded under conditions blocking synaptic transmission was reversibly and significantly increased by small increases in temperature and osmolality. Moreover, as shown in Figures 5F and 5G, significant excitatory responses were also induced by these stimuli in single hypothalamic *Trpv1*$^{-/-}$ neurons transfected with *Trpv1dn*.

DISCUSSION

Our data show that single hypothalamic ONs can cell-autonomously detect physiological increases in temperature and fluid osmolality and that the ΔN-TRPV1 channel by itself is sufficient to bestow these critical cell physiological properties on ONs. Although previous studies have shown that ion channels can operate as polymodal sensors (Caterina et al., 1997; Ramsey et al., 2006), our work establishes that this feature can serve homeostatic sensory functions under physiological conditions in the mammalian brain.

When expressed in heterologous cells, *Trpv1dn* formed an ion channel that was active under basal conditions and whose activity was proportionally increased, in a non-desensitizing manner, by mechanically or hypertonicity-induced shrinking. Conversely, basal channel activity was suppressed by mechanically or hypotonicity-induced swelling. This behavior, together with the apparent 2% threshold for activation near 270 mOsm, is consistent with the features of the osmosensory transduction current measured in native ONs (Oliet and Bourque, 1992, 1993a, 1993b) and with the sensitivity of osmoregulatory responses observed in vivo (Bourque, 2008). These features of ΔN-TRPV1 make us confident that we have isolated a critical constituent of the osmosensory transduction channel operative in hypothalamic ONs, a long-sought-after molecular entity. Although ΔN-TRPV1 is required for cell-autonomous heat and osmolality sensing in ONs, it is likely that additional factors are involved in the osmotic control of ONs in vivo, including other types of ion channels (Hiyama et al., 2002; Tanaka et al., 1999; Zhang et al., 2009) and chemical mediators released by glial cells (Choe et al., 2012; Hussy et al., 2000; Shimizu et al., 2007).

Since its discovery, the *Trpv1* gene has been implicated in the detection of nociceptive heat by peripheral thermosensitive neurons (Caterina et al., 1997). Subsequent loss-of-function studies using knockout animals and specific TRPV1 antagonists have supported a role for this gene in thermoregulation (Caterina, 2007). However, it has been argued that TRPV1 does not itself operate as a physiological thermosensor (Romanovsky et al., 2009). The identification of *Trpv1dn* and its product, ΔN-TRPV1, as an alternate product of *Trpv1* with physiologically relevant temperature sensitivity suggests that this channel could also contribute to the central control of thermoregulation. Additional studies are required to explore this possibility.

The molecular basis for the different thermosensitive properties of TRPV1 and ΔN-TRPV1 remains to be determined; however, modest changes in specific regions of TRPV1 have been shown to affect its temperature sensitivity (Gracheva et al., 2011). Interestingly, the N-terminal region that is missing in ΔN-TRPV1 includes an amino acid residue previously shown to be required for the activation of TRPV1 by capsaicin (Jung et al., 2002) (Figure S3), providing a likely explanation for the lack of responsiveness of native ONs to this compound. Importantly, ΔN-TRPV1 retains an intact C terminus, including regions

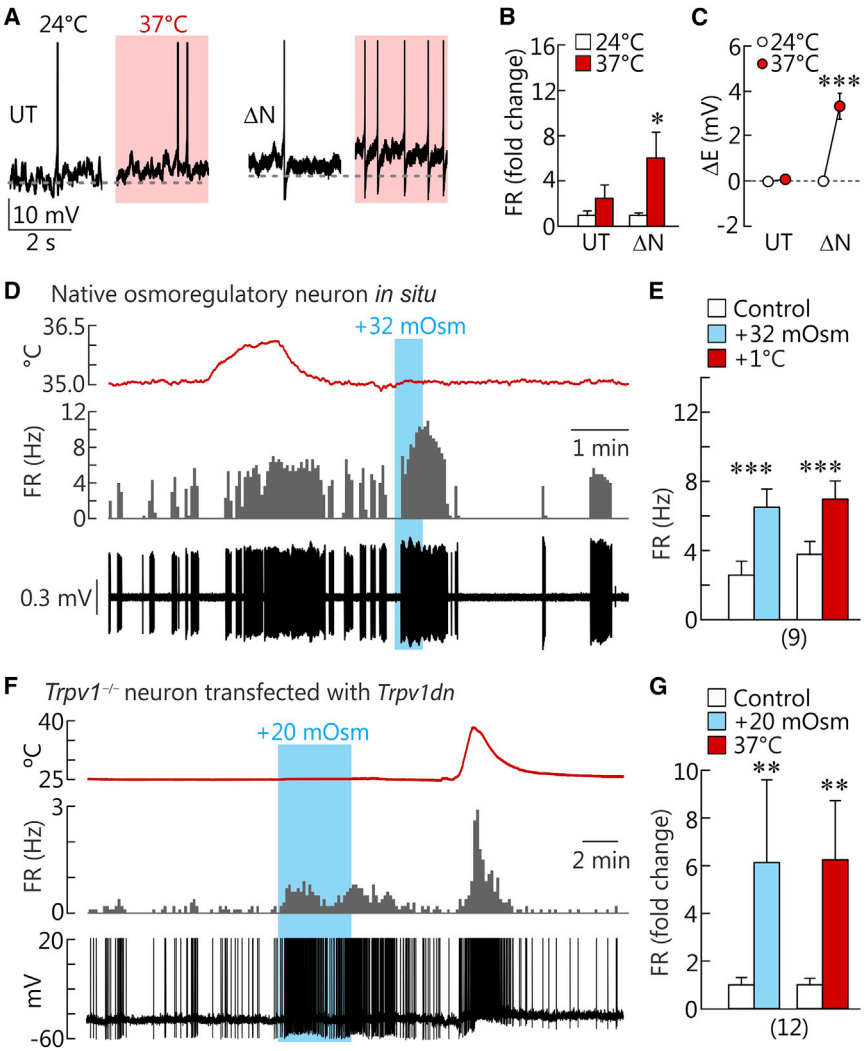


Figure 5. ΔN-TRPV1 Enables Cell-Autonomous Co-detection of Heat and Hypertonicity

(A) Voltage traces show the effect of heating (red shaded area) on cultured hypothalamic *Trpv1*$^{-/-}$ neurons (UT or transfected with ΔN). Dashed lines shown as reference.

(B) Bars plot mean (±SEM) heat-induced changes in FR (*$p < 0.05$).

(C) Plots show mean (±SEM) heat-induced ΔE (***$p < 0.005$).

(D) Extracellular activity (lower) and FR (middle) of a native ON in a rat hypothalamic explant under conditions blocking synaptic transmission. Red trace (upper) shows temperature, and the blue shaded area indicates when a hyperosmotic stimulus was applied.

(E) Bars plot mean (±SEM; n = 9) FR observed before (control) and during each form of stimulation (***$p < 0.005$).

(F) Whole-cell voltage (lower) and FR (middle) shows the effect of heat (upper) and hypertonicity (shaded area) on a cultured *Trpv1*$^{-/-}$ neuron transfected with ΔN.

(G) Plots show mean (±SEM; n = 12) FR before (control) and during each form of stimulation (**$p < 0.01$).

required for tetramerization, thermosensitivity, and binding domains for β-tubulin (Figure S3). Our group has recently shown that parts of the C terminus are required for microtubule-dependent push activation of the channel during cell shrinking (Prager-Khoutorsky et al., 2014). Lastly, it is interesting to note that the N-terminal portion of TRPV1, which is absent in ΔN-TRPV1, includes domains that mediate rapid desensitization of the full-length channel (Lishko et al., 2007). The absence of a desensitizing module in ΔN-TRPV1 may be a critical feature for the generation of non-desensitizing thermosensory and osmosensory currents in ONs, a subject worthy of future investigations.

EXPERIMENTAL PROCEDURES

Tissue Collection

Adult Long Evans rats (Charles River Canada) or mice (Jackson Laboratory) were anesthetized with isoflurane and decapitated. The animals were treated in strict accordance with the guidelines outlined by the Canadian Council on Animal Care (http://www.ccac.ca/en), and experiments adhered to protocols approved by the Facility Animal Care Committee of McGill University (protocol no. 1190). Brains were removed and immersed in chilled (2°C–4°C), oxygenated (95% O_2 and 5% CO_2) artificial cerebrospinal fluid (ACSF) (pH 7.35) comprising (in mM) NaCl 120, KCl 3, NaH_2PO_4 1.23, $NaHCO_3$ 26, $MgCl_2$ 1.5, $CaCl_2$ 2, and glucose 10; osmolality adjusted to 300 mOsm with mannitol. Blocks of tissue (~1 mm^3) were removed to prepare acutely isolated neurons or submerged in RNA-later for isolation of total RNA.

Preparation of Acutely Isolated Neurons

Tissue blocks were incubated in oxygenated PIPES solution (pH 7.3; 295 mOsm; 100% O_2) comprising (in mM) NaCl 120, KCl 5, $MgCl_2$ 1, PIPES 10, $CaCl_2$ 1, and glucose 10, supplemented with protease XIV (8 mg/ml; Sigma-Aldrich). After incubation at room temperature for 30 min, blocks were rinsed in enzyme-free PIPES solution, triturated in ~0.4 ml of PIPES solution, and plated onto Petri dishes (BD Falcon).

Culture and Transfection of Heterologous Cells

HEK293 and N2A cells were kept in an incubator at 37°C (5.0% CO_2) in DMEM supplemented with 10% heat-inactivated fetal bovine serum (both from Wysent) and 1% penicillin/streptomycin (Sigma Chemical). Cells were transfected with p-IRES2-eGFP vector alone, pcDNA3-*Trpv1*, or *Trpv1dn* cloned on pIRES2-eGFP between EcoRI and BamHI restriction sites, using non-liposomal jetPRIME (PolyPlus Transfection) reagent.

Culture and Transfection of Hypothalamic Neurons

WT and *Trpv1*$^{-/-}$ pregnant female mice (day 18) were anesthetized with CO_2 and sacrificed according to a protocol approved by the FACC of McGill University. Fetus brains were collected and submerged in Hank's balanced salt solution (GIBCO). After removing the meninges, hypothalami were dissected and placed in DMEM (37°C) and dissociated using Pasteur pipettes and plated onto UV-treated poly-lysine (70,000–15,000 MW; Sigma) coated glass coverslips and placed in an incubator (37°C and 5% CO_2) for 3 to 4 hr. Coverslips were then transferred to a 24-well plate and covered with Neurobasal medium containing 0.25% GlutaMAX-1, 2% B-27 Supplement (all from GIBCO-BRL), and 1% penicillin/streptomycin, with osmolality adjusted to 300 mOsm with mannitol. Half of the medium was exchanged every

4 days. Neurons were transfected 9 to 10 days after isolation with pIRES2-eGFP (vector alone) or pcDNA3-*Trpv1* or p-*Trpv1dn*-IRES2-eGFP adhered to magnetic nanoparticles (Magnetofection; Neuromag; OZ Biosciences).

Whole-Cell Recording

Whole-cell current (DC 0.5 kHz) and voltage (DC 2 kHz) were measured using glass pipettes (2–4 MΩ) containing (in mM; pH 7.2) K gluconate 120, $MgCl_2$ 1, EGTA 10, and HEPES 10 and connected to an Axopatch 200B amplifier (Molecular Devices). ATP (2 mM) was added to this solution when recording from neurons. Cells were perfused (~0.8 ml/min) with a HEPES solution comprising (in mM; pH 7.3) NaCl 150, KCl 3, $MgCl_2$ 1, HEPES 10, $CaCl_2$ 1, and glucose 10. Osmolality was adjusted to 300 mOsm with mannitol. Temperature was regulated with a PTC-20 temperature control system (NPI Electronic). Pressure in the recording pipette was monitored using a PM015D pressure monitor (World Precision Instruments).

Osmotic Stimulation

After recording baseline values for >2 min, solutions of different osmolalities (adjusted with mannitol) were applied for 2–5 min. Effects were quantified using values recorded during the last 30–60 s of each condition. Recordings were analyzed only if membrane conductance or FR and cell volume were stable during the baseline period. HEK293 and N2A cells were considered osmoresponsive if they showed ΔG values exceeding twice the SD of basal values in response to a +40-mOsm stimulus. Neurons were considered osmoresponsive if they showed an increase in FR greater than the average change observed in untransfected neurons +2 SD in response to a +20-mOsm stimulus.

Measurement of Changes in Cell Volume

Normalized volume (nV) (fraction of control volume) was determined from changes in maximal cell cross-sectional area (CSA) from images captured during the recordings. For each image, CSA (in pixels) was determined using ImageJ (NIH). CSA at baseline (CSA_0) and different times (t) (CSA_t) were used to calculate nV using the equation $nV_t = (CSA_t)^{1.5}/(CSA_0)^{1.5}$. Normalized shrinking was expressed as $1 - nV$ or as percent shrinking ($\% = 100 \times [nV_t - nV_0]$).

cDNA Preparation and PCR

For single-cell analysis, micropipettes (autoclaved) were filled with 1.5 μl of a solution comprising HEPES internal solution and RNaseIN (10 U/μl; Invitrogen-Life Technologies; 10:1). Upon contact, suction was applied and the cell was lifted and completely suctioned into the electrode. The content was then expelled by positive pressure into a 250-μl microcentrifuge tube containing 0.5 μl DNase I (1 U/μl; Fermentas; Fisher Scientific) and 1× $MgCl_2$ buffer and stored over dry ice. Tubes were incubated at 37°C for 30 min, and the reaction was stopped by addition of 1 μl EDTA (25 mM) and incubation at 65°C for 10 min. The RT reaction was then performed by adding 1 μl 50 μM Random Hexamer primers (Applied Biosystems-Life Technologies), 0.25 μl RNaseIN (10 U/μl), 1 μl 0.1M DTT, 1 μl 50 mM $MgCl_2$, 1 μl 10 mM (each) dNTPs mix (QIAGEN), 2 μl 5× First Strand Buffer, and 0.25 μl SuperscriptIII (200 U/μl; Invitrogen). The mix was incubated at 50°C for 2 hr and then the cDNA was stored at −20°C. For tissue analysis, blocks of tissue were submerged in five to ten volumes of RNAlater (Ambion-Applied Biosystems-Life Technologies) and kept at 4°C until use. Total purified RNA was isolated and retro-transcribed to cDNA using RiboPure Kit and RETROscript Kit, respectively (Ambion). Nested PCR and nested multiplex single-cell PCR were performed as specified in Supplemental Experimental Procedures.

Immunostaining

Cultured neurons were fixed for 15 min with PBS containing 3% paraformaldehyde, 0.2% glutaraldehyde, and 0.25% Triton X-100 and washed with PBS at room temperature. Next, cells were treated for 15 min in 0.1% $NaBH_4$. After a 1 hr incubation with 2% normal goat serum, cells were washed and incubated overnight at 4°C with a mixture of anti-NeuN chicken polyclonal antibody (EMD Millipore; 1:500) and anti c-TRPV1 rabbit polyclonal antibody (Alomone Labs; 1:200). Following wash, cells were incubated for 1 hr with fluorescently labeled secondary antibodies (Life Technologies; 1:500), mounted in SlowFade Gold Antifade reagent (Life Technologies), and imaged using an FV1000 confocal microscope (Olympus Canada).

Immunoblotting

Cells expressing WT-Trpv1 or ΔN-Trpv1 were lysed on ice for 10 min in Triton lysis buffer (in mM; pH 7.4), Tris 20, NaCl 137, and EDTA 2, supplemented with Triton X-100 1%, glycerol 10%, SDS 0.1%, protease inhibitors, and sodium orthovanadate (Jones et al., 2011). The lysate was separated from cellular debris by centrifugation at 13,000 rpm for 10 min at 4°C. Samples were stored in 1× sample buffer (β-mercaptoethanol 5.3%) and warmed to 60°C just prior to being resolved by SDS page (acrylamide 7.5%). Samples were immunoblotted with anti c-TRPV1 (1:10,000; Alomone Labs) and -GAPDH (1:100,000; EMD Millipore) and exposed using Amersham ECL Prime Western Blotting Detection Reagent (GE).

Statistical Analysis

Group values, expressed as means plus or minus the SEM, were compared using the t test or paired t test, as appropriate. The Z test was applied to assess differences in the proportions of responsive cells between groups. Differences were considered significant when $p < 0.05$. Curve fitting was performed using Sigmaplot 12 (Systat Software).

ACCESSION NUMBERS

The accession numbers for the isolated transcripts reported in this paper are GenBank: KP277509.1 and GenBank: KP277510.1 for mouse and rat, respectively.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.08.061.

ACKNOWLEDGMENTS

This work was supported by grants FRN82818 and MOP9939 from the Canadian Institutes of Health Research to C.W.B. and by a James McGill Chair awarded to C.W.B. by McGill University. W.L. was supported by funds from Duke University, a George Ebert Fellowship by the Klingenstein Fund, and the Mathers Foundation. D.L.V. was supported by a Congé pour recherche ou conversion thématique from Université de Bordeaux. The Research Institute of the McGill University Health Center receives support from Le Fonds de Recherche du Québec-Santé. We thank Chris Salmon and Huashan Peng for technical assistance with western blotting and tissue culture, Suk Hee Lee for providing plasmids, and Katrina Y. Choe for help with tissue collection, as well as Pierre Drapeau, Edward Ruthazer, and Jon Sakata for providing brain tissue used for PCR experiments.

Received: October 23, 2014
Revised: July 25, 2015
Accepted: August 21, 2015
Published: September 17, 2015

REFERENCES

Bourque, C.W. (2008). Central mechanisms of osmosensation and systemic osmoregulation. Nat. Rev. Neurosci. *9*, 519–531.

Caterina, M.J. (2007). Transient receptor potential ion channels as participants in thermosensation and thermoregulation. Am. J. Physiol. Regul. Integr. Comp. Physiol. *292*, R64–R76.

Caterina, M.J., Schumacher, M.A., Tominaga, M., Rosen, T.A., Levine, J.D., and Julius, D. (1997). The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature *389*, 816–824.

Choe, K.Y., Olson, J.E., and Bourque, C.W. (2012). Taurine release by astrocytes modulates osmosensitive glycine receptor tone and excitability in the adult supraoptic nucleus. J. Neurosci. *32*, 12518–12527.

Ciura, S., and Bourque, C.W. (2006). Transient receptor potential vanilloid 1 is required for intrinsic osmoreception in organum vasculosum lamina terminalis

neurons and for normal thirst responses to systemic hyperosmolality. J. Neurosci. *26*, 9069–9075.

Ciura, S., Liedtke, W., and Bourque, C.W. (2011). Hypertonicity sensing in organum vasculosum lamina terminalis neurons: a mechanical process involving TRPV1 but not TRPV4. J. Neurosci. *31*, 14669–14676.

Eilers, H., Lee, S.Y., Hau, C.W., Logvinova, A., and Schumacher, M.A. (2007). The rat vanilloid receptor splice variant VR.5'sv blocks TRPV1 activation. Neuroreport *18*, 969–973.

Gracheva, E.O., Cordero-Morales, J.F., González-Carcacía, J.A., Ingolia, N.T., Manno, C., Aranguren, C.I., Weissman, J.S., and Julius, D. (2011). Ganglion-specific splicing of TRPV1 underlies infrared sensation in vampire bats. Nature *476*, 88–91.

Hiyama, T.Y., Watanabe, E., Ono, K., Inenaga, K., Tamkun, M.M., Yoshida, S., and Noda, M. (2002). Na(x) channel involved in CNS sodium-level sensing. Nat. Neurosci. *5*, 511–512.

Hussy, N., Deleuze, C., Desarménien, M.G., and Moos, F.C. (2000). Osmotic regulation of neuronal activity: a new role for taurine and glial cells in a hypothalamic neuroendocrine structure. Prog. Neurobiol. *62*, 113–134.

Jones, E.V., Bernardinelli, Y., Tse, Y.C., Chierzi, S., Wong, T.P., and Murai, K.K. (2011). Astrocytes control glutamate receptor levels at developing synapses through SPARC-beta-integrin interactions. J. Neurosci. *31*, 4154–4165.

Jung, J., Lee, S.Y., Hwang, S.W., Cho, H., Shin, J., Kang, Y.S., Kim, S., and Oh, U. (2002). Agonist recognition sites in the cytosolic tails of vanilloid receptor 1. J. Biol. Chem. *277*, 44448–44454.

Kauer, J.A., and Gibson, H.E. (2009). Hot flash: TRPV channels in the brain. Trends Neurosci. *32*, 215–224.

Lishko, P.V., Procko, E., Jin, X., Phelps, C.B., and Gaudet, R. (2007). The ankyrin repeats of TRPV1 bind multiple ligands and modulate channel sensitivity. Neuron *54*, 905–918.

Mannari, T., Morita, S., Furube, E., Tominaga, M., and Miyata, S. (2013). Astrocytic TRPV1 ion channels detect blood-borne signals in the sensory circumventricular organs of adult mouse brains. Glia *61*, 957–971.

Mason, W.T. (1980). Supraoptic neurones of rat hypothalamus are osmosensitive. Nature *287*, 154–157.

McKinley, M.J., Denton, D.A., Oldfield, B.J., De Oliveira, L.B., and Mathai, M.L. (2006). Water intake and the neural correlates of the consciousness of thirst. Semin. Nephrol. *26*, 249–257.

Moriya, T., Shibasaki, R., Kayano, T., Takebuchi, N., Ichimura, M., Kitamura, N., Asano, A., Hosaka, Y.Z., Forostyak, O., Verkhratsky, A., et al. (2015). Full-length transient receptor potential vanilloid 1 channels mediate calcium signals and possibly contribute to osmoreception in vasopressin neurones in the rat supraoptic nucleus. Cell Calcium *57*, 25–37.

Nishihara, E., Hiyama, T.Y., and Noda, M. (2011). Osmosensitivity of transient receptor potential vanilloid 1 is synergistically enhanced by distinct activating stimuli such as temperature and protons. PLoS ONE *6*, e22246.

Oliet, S.H., and Bourque, C.W. (1992). Properties of supraoptic magnocellular neurones isolated from the adult rat. J. Physiol. *455*, 291–306.

Oliet, S.H., and Bourque, C.W. (1993a). Mechanosensitive channels transduce osmosensitivity in supraoptic neurons. Nature *364*, 341–343.

Oliet, S.H., and Bourque, C.W. (1993b). Steady-state osmotic modulation of cationic conductance in neurons of rat supraoptic nucleus. Am. J. Physiol. *265*, R1475–R1479.

Prager-Khoutorsky, M., Khoutorsky, A., and Bourque, C.W. (2014). Unique interweaved microtubule scaffold mediates osmosensory transduction via physical interaction with TRPV1. Neuron *83*, 866–878.

Qiu, D.L., Shirasaka, T., Chu, C.P., Watanabe, S., Yu, N.S., Katoh, T., and Kannan, H. (2004). Effect of hypertonic saline on rat hypothalamic paraventricular nucleus magnocellular neurons in vitro. Neurosci. Lett. *355*, 117–120.

Ramsey, I.S., Delling, M., and Clapham, D.E. (2006). An introduction to TRP channels. Annu. Rev. Physiol. *68*, 619–647.

Romanovsky, A.A., Almeida, M.C., Garami, A., Steiner, A.A., Norman, M.H., Morrison, S.F., Nakamura, K., Burmeister, J.J., and Nucci, T.B. (2009). The transient receptor potential vanilloid-1 channel in thermoregulation: a thermosensor it is not. Pharmacol. Rev. *61*, 228–261.

Schumacher, M.A., Moff, I., Sudanagunta, S.P., and Levine, J.D. (2000). Molecular cloning of an N-terminal splice variant of the capsaicin receptor. Loss of N-terminal domain suggests functional divergence among capsaicin receptor subtypes. J. Biol. Chem. *275*, 2756–2762.

Sharif Naeini, R., Witty, M.F., Séguéla, P., and Bourque, C.W. (2006). An N-terminal variant of Trpv1 channel is required for osmosensory transduction. Nat. Neurosci. *9*, 93–98.

Sharif-Naeini, R., Ciura, S., and Bourque, C.W. (2008). TRPV1 gene required for thermosensory transduction and anticipatory secretion from vasopressin neurons during hyperthermia. Neuron *58*, 179–185.

Shimizu, H., Watanabe, E., Hiyama, T.Y., Nagakura, A., Fujikawa, A., Okado, H., Yanagawa, Y., Obata, K., and Noda, M. (2007). Glial Nax channels control lactate signaling to neurons for brain [Na+] sensing. Neuron *54*, 59–72.

Sladek, C.D., and Johnson, A.K. (2013). Integration of thermal and osmotic regulation of water homeostasis: the role of TRPV channels. Am. J. Physiol. Regul. Integr. Comp. Physiol. *305*, R669–R678.

Sudbury, J.R., and Bourque, C.W. (2013). Dynamic and permissive roles of TRPV1 and TRPV4 channels for thermosensation in mouse supraoptic magnocellular neurosecretory neurons. J. Neurosci. *33*, 17160–17165.

Sudbury, J.R., Ciura, S., Sharif-Naeini, R., and Bourque, C.W. (2010). Osmotic and thermal control of magnocellular neurosecretory neurons–role of an N-terminal variant of trpv1. Eur. J. Neurosci. *32*, 2022–2030.

Tanaka, M., Cummins, T.R., Ishikawa, K., Black, J.A., Ibata, Y., and Waxman, S.G. (1999). Molecular and functional remodeling of electrogenic membrane of hypothalamic neurons in response to changes in their input. Proc. Natl. Acad. Sci. USA *96*, 1088–1093.

Veldhuis, N.A., Lew, M.J., Abogadie, F.C., Poole, D.P., Jennings, E.A., Ivanusic, J.J., Eilers, H., Bunnett, N.W., and McIntyre, P. (2012). N-glycosylation determines ionic permeability and desensitization of the TRPV1 capsaicin receptor. J. Biol. Chem. *287*, 21765–21772.

Vivas, L., Chiaraviglio, E., and Carrer, H.F. (1990). Rat organum vasculosum laminae terminalis in vitro: responses to changes in sodium concentration. Brain Res. *519*, 294–300.

Xue, Q., Yu, Y., Trilk, S.L., Jong, B.E., and Schumacher, M.A. (2001). The genomic organization of the gene encoding the vanilloid receptor: evidence for multiple splice variants. Genomics *76*, 14–20.

Zhang, Z., and Bourque, C.W. (2003). Osmometry in osmosensory neurons. Nat. Neurosci. *6*, 1021–1022.

Zhang, Z., and Bourque, C.W. (2008). Amplification of transducer gain by angiotensin II-mediated enhancement of cortical actin density in osmosensory neurons. J. Neurosci. *28*, 9536–9544.

Zhang, Y., and Hao, H. (2013). Conserved glycine at position 45 of major cochlear connexins constitutes a vital component of the Ca^{2+} sensor for gating of gap junction hemichannels. Biochem. Biophys. Res. Commun. *436*, 424–429.

Zhang, Z., Kindrat, A.N., Sharif-Naeini, R., and Bourque, C.W. (2007). Actin filaments mediate mechanical gating during osmosensory transduction in rat supraoptic nucleus neurons. J. Neurosci. *27*, 4008–4013.

Zhang, W., Wang, D., Liu, X.H., Kosala, W.R., Rajapaksha, J.S., and Fisher, T.E. (2009). An osmosensitive voltage-gated K+ current in rat supraoptic neurons. Eur. J. Neurosci. *29*, 2335–2346.