The verified ion channelome of the rat subfornical organ.

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Background

Sensory circumventricular organs (CVOs) lack a blood-brain-barrier, and they express a high density and wide variety of receptors. Therefore, CVOs can detect numerous circulating hormones and signalling molecules, and communicate this information to homeostatic control centres.

The subfornical organ (SFO) is an AV3V CVO which is well known to play critical roles in osmoregulation[1], cardiovascular regulation[2], and energy balance[3]. The electrical behaviour of SFO neurons is determined by the ion channels and receptors present. With the aim of improving our understanding this unique and important structure, we have characterised the transcriptome of the SFO using RNA sequencing.

Here we present data outlining the ion channel and G-protein coupled receptors (GPCR) expressed.

Methods

Animals: All procedures complied with CCAC and were approved by the University of Manitoba.
Pups were from timed-pregnant Sprague Dawley dams and randomly assigned to dams in litters of 12. At age 6 weeks, rats were sacrificed, SFO carefully dissected out and stored in RNAlater at -20 °C until time of RNA extraction. Six samples from the same litter were pooled.

Library Prep, RNAseq, and Analysis:
RNA was extracted using PureLink RNA Mini Kit (Thermo Fisher), and analysed using a Bioanalyzer (Agilent). Two µg of RNA were prepared for library contruction using the TruSeq Standard mRNA Library Prep Kit (Illumina), then sequenced on the NextSeq 500 platform with 2x 75 bp end sequencing. Reads aligned to RNOR 6.0 genome assembly (Hisat2). FPKM was used as measure of transcript levels (Stringtie). Ion channels and G protein coupled receptors were identified by comparison to the IUPHAR-DB. Data were compared to previously published microarray data for validation, where possible[7].

Neuron Culture and Electrophysiology
Rats were sacrificed, brain removed, and cultures of dissociated cells prepared. Cells were plated on 35mm glass culture dishes in B-27 supplemented Neurobasal media and cultured up to 5 days at 37℃ with 5% CO_2.
Whole cell patch-clamp of dissociated neurons was conducted in the current clamp and voltage clamp configuration using using HEKA EPC 10 amplifier and PatchMaster V2x90 software.

Literature

1. Felix, D. *Naunyn-Schmiedebergs Arch Pharmacol.* **292** 15–20 (1976)
2. Mangiapane, M.L., and Simpson, J.B. AJP Regul Integr Comp Physiol. **244** R508-13 (1983)
3. Smith, P.M., *et* al. 2010. Physiol Behav. **99** 534–7 (2010)
4. Miselis, R.R. *Peptides*. **3** 501–502. (1982)
5. Whyment, A., *et* al. *Nat Neurosci.* **7** 493–494 (2004)
6. Burton, M.J., *et* al. *Exp Neurol.* **51** 668–677 (1976)
7. Hindmarch, C. *et* al. *Am J Physiol.* **295** R1914-R1920 (2008)

Results

Voltage-gated Na+ channels

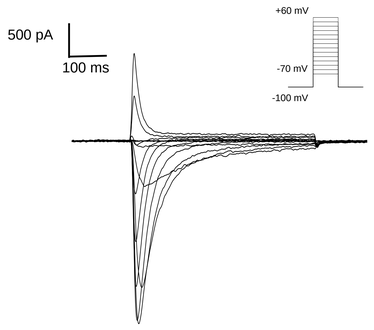

Figure 1. Voltage-gated Na+ currents from SFO neurons.

Table 1. Voltage-gated Na+ channels in the SFO.

Present	Absent
SCN1A	SCN4A
SCN2A	SCN5A
SCN3A	SCNA10A
SCN8A	SCN11A
SCN9A	

****bold** indicates only detected by RNAseq*

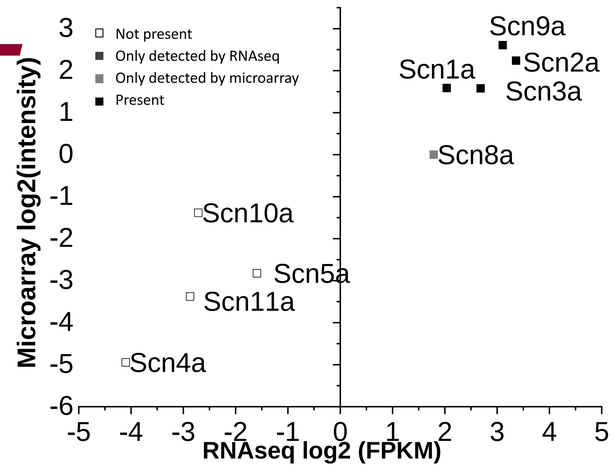

Figure 2. Correlation between RNAseq FPKM and microarray intensity for voltage-gated Na+ channels. R^2: 0.9247. n = 10.

Voltage-gated K+ channels

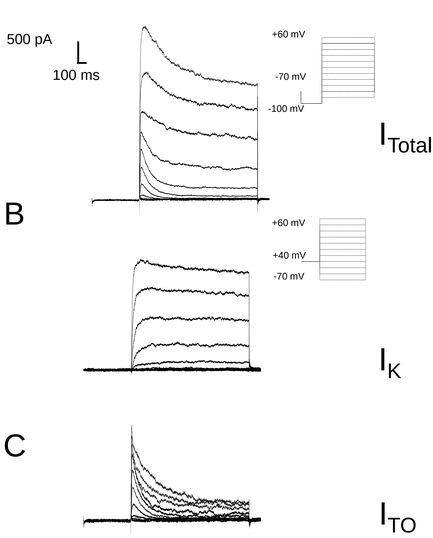

Figure 3. Voltage-gated K+ currents from SFO neurons.
(A) Total K+ current.
(B) Non-inactivating K+ current.
(C) Transient K+ current. (B-A)

Table 2. Voltage-gated K+ channels in the SFO.

Present	Absent
KCNA **1**, 2, **3**-6	KCNA 7, 10
KCNB 1, **2**	
KCNC 1-4	
KCND **1**, 3	
KCNF 1	
KCNG **1**, 2, **4**	KCNG3
KCNH 1, 2, **4**, 5, 6, **7**	KCNH3, 5, 8
KCNJ **2**, **3**, 5, **8-10**, **12-14**, 16	KCNJ1, 4, 15
KCNK 1-3, **5**, **9**, **10**, **13**,**16**	KCNK4, 6, 7, 12, 15, 17
KCNMA1	
KCNN 1-3	
KCNQ 1, **2-4**	KCNQ1
KCNS2	KCNS1, 3
KCNT 1, 2	
	KCNV2
HCN 1, 3, **4**	

****bold** indicates only detected by RNAseq*

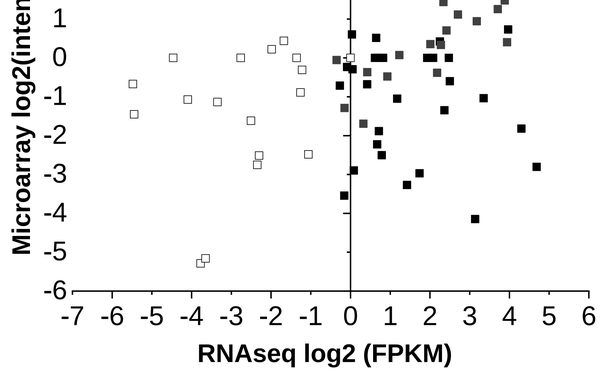

Figure 4. Correlation between RNAseq FPKM and microarray intensity for voltage-gated K+ channels. R^2: 0.2619. n = 69.

G-protein-coupled receptors

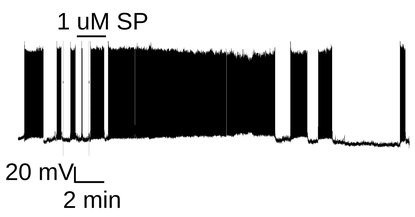

Figure 5. Response of a dissociated SFO neuron to 1µM substance P. *TACR1* is expressed in rat SFO.

Figure 6. Response of dissociated SFO neuron to 100nM neurotensin. *NTSR2 and SORT1* are highly expressed in rat SFO.

For a full list of GPCRs and ion channels, please visit this website:
sites.google.com/site/sfochannelome

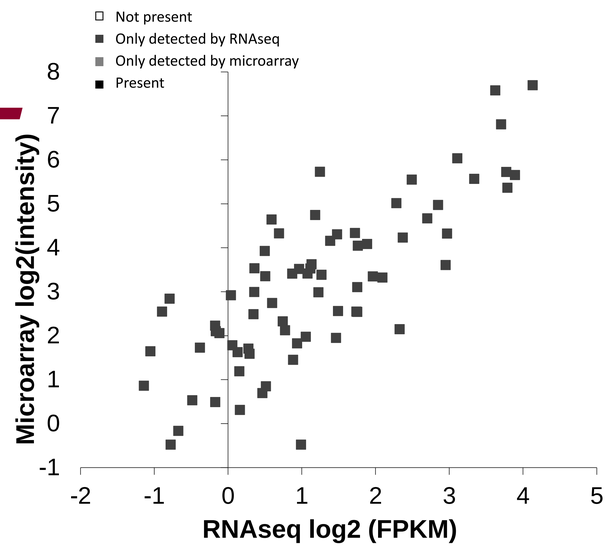

Figure 7. Correlation between RNA-seq FPKM and microarray intensity for GPCRs. R^2: 0.6274. n = 73.