Non-pit snake TG

TRPA1
TRPV1
other TRPs

Rattlesnake TG

TRPA1
TRPV1
other TRPs

Non-pit snake DRG

TRPA1
TRPV1
other TRPs

Rattlesnake DRG

SFig. 1
**SFig. 1**  
**Anatomy of the pit organ and comparison of gene expression in snake sensory ganglia**

(a) Longitudinal projection showing trigeminal innervation of rattlesnake pit organ. (adapted from Terashima and Liang, J Neurophysiol 66, 623-634 (1991)).  
(b) Python head showing location of nostril and labial pits (black and red arrows, respectively)(from Wikimedia Commons).  
(c - d) Number of mRNA-Seq reads from snake ganglia that align to the chicken proteome. TRPA1 and TRPV1 are highlighted, as are other TRP family channels. The blue line indicates the expected number of sequencing reads for genes with similar expression levels in the two samples based on the total number of aligned reads obtained in each. Signals below 20 reads are within the statistical noise of the RNA-seq protocol and therefore considered as non-expressed sequences. Rattlesnake refers to *C. atrox* and non-pit refers to a combination of Texas Rat (*Elaphe obsolete lindheimerii*) and Western Coachwhip (*Masticophis flagellum testaceus*) snakes.
SFig. 2    Sequence alignment of TRPA1 orthologues

a Alignment of deduced protein sequences for rattlesnake versus rat snake TRPA1 channels showing 81% identity. b Alignment of deduced protein sequences for rattlesnake versus human TRPA1 channels showing 63% identity. Predicted transmembrane regions are denoted by black bars over relevant sequences; cysteine residues critical for activation of human TRPA1 by AITC are denoted with asterisks; N-terminal region containing ankyrin repeats is indicated by green bar (top panel only).
SFig. 3  Heat sensitivity of cloned snake TRPA1 channels

a  Temperature-response profile for transfected HEK293 cells expressing cloned rattlesnake and rat snake TRPA1 channels as determined by ratiometric calcium imaging. Data collected from 41 cells for rattlesnake and 31 cells for rat snake channels; threshold = 28.0 ± 1.1 and 36.3 ± 0.3°C, respectively; P < 0.0015. b  Representative current-voltage and temperature-response relationships for rattlesnake and rat snake TRPA1 channels expressed in oocytes (left and middle). Current-voltage relationships showing relative responses to heat or AITC (1 mM) (right).
RT Heat AITC

Human TRPA1

Zebras/fish TRPA1

Zebras/fish TRPA1b

SFig. 4
SFig. 4 Heat and AITC sensitivity of human and zebrafish TRPA1

HEK293 cells transfected with human TRPA1 or zebrafish TRPA1 or TRPA1b cDNAs were subjected to live cell ratiometric calcium imaging to measure responses to heat (45°C maximal stimulus) or AITC (200 µM). Note lack of heat-evoked responses in all cases.
a) RT, Heat, and AITC images.

b) Graph showing current vs. voltage with specific temperatures indicated.

c) Graph showing normalized current vs. temperature with AITC and 22°C marked.

SFig. 5
SFig. 5  Thermal and chemical sensitivity of fly TRPA1 channel

a Thermal and chemical sensitivity of Drosophila TRPA1 (dTRPA1) as measured by live-cell ratiometric calcium imaging. Cloned receptors were transiently expressed in HEK293 cells and subjected to a temperature ramp, followed by application of mustard oil (AITC; 200 µM) at 24°C. b Current-voltage relation determined by two-electrode voltage clamp recording from dTRPA1-expressing oocyte in response to heat or AITC (1 mM). c Normalized curve showing heat response profile for dTRPA1 channel in oocytes. Response at any given temperature was normalized to the maximal heat-evoked current obtained at 45°C.
Python log$_2$ ratio TG/DRG

Rattlesnake log$_2$ ratio TG/DRG

TRPA1

Voltage (mV)

Current (µA)

45°C

AITC

80

22°C

SFig. 6

c

10

Current (µA)

Voltage (mV)

80

45°C

AITC

22°C

32°C

SFig. 6
a Comparison of rattlesnake and python transcriptomes showing relative expression levels in TG versus DRG based on number of aligned sequencing reads. b Current-voltage relationships from oocytes expressing python TRPA1 in response to heat or AITC (1 mM). c Current-voltage relationships from oocytes expressing boa TRPA1 in response to heat or AITC (1 mM).
a

% of total cells

Python TRPA1
Rat snake TRPA1
Rat snake TRPV1

b

% of total cells

Cell diameter (μm)

Python
Rat snake

10 - 20
21 - 30
31 - 40
41 - 50

c

F_{340}/F_{380}

Temperature (°C)

24 26 28 30 32 34 36 38 40 42 44 46

1 2 3 4

d

Heat

2 nA

5 s

SFig. 7
SFig. 7  Anatomy and physiology of python and rat snake neurons

**a** Quantitative analysis of TRPA1 or TRPV1 mRNA expression in python and rat snake TG as determined from histological sections shown in Fig. 5a (mean ± s.d.; n = 726 neurons from 16 sections for python TRPA1; 795 neurons from 10 sections for rat snake TRPA1, and 1174 neurons from 10 independent sections for rat snake TRPV1).  

**b** Quantification of neuronal cell size from histological sections of python (grey) or rat snake (black) TG (n ≥ 200 cells from 5 sections each).  

**c** Average temperature-response profile for capsaicin-sensitive trigeminal neuron from rat snake as determined by calcium imaging (n = 11 neurons). Thermal response threshold = 38.7 ± 1.4ºC (n = 28 neurons).  

**d** Representative patch-clamp recording from cultured rat snake neuron shows heat-evoked current with a threshold of 35.6 ± 1.2ºC (n = 3).
a Thermal and chemical sensitivity of rattlesnake TRPV1 as measured by live-cell ratiometric calcium imaging. Cloned receptors were transiently expressed in HEK293 cells and subjected to a temperature ramp, followed by application of capsaicin (Cap; 1 µM) at 24°C. b Current-voltage relation determined by two-electrode voltage clamp recording from rattlesnake TRPV1-expressing oocyte in response to heat or Cap (1 µM).