Thermogenetics, a tool to study neurophysiology in Drosophila

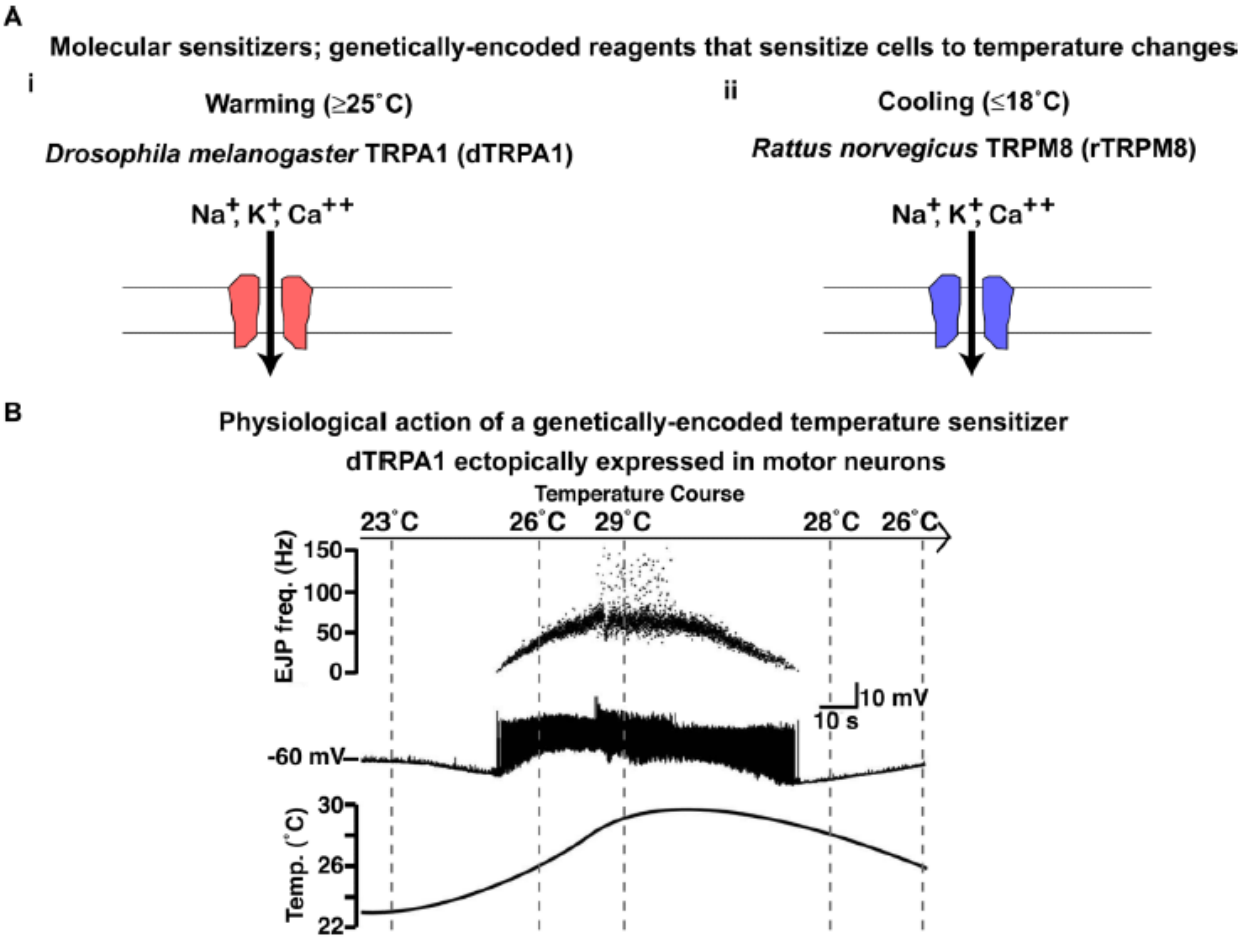
Journal club

Henning Leske

Technologies for brain activity manipulation

- Optogenetics
- Thermogenetics
- Features:
  - temporally precise control of electric activity
  - Cell specific (no neighboring cells)
- Requirement:
  - Focused energy delivered to the brain
  - Molecular sensitizer reacting to energy by inhibition or activation
  - Ideally no influence on neighboring cells

Thermogenetics

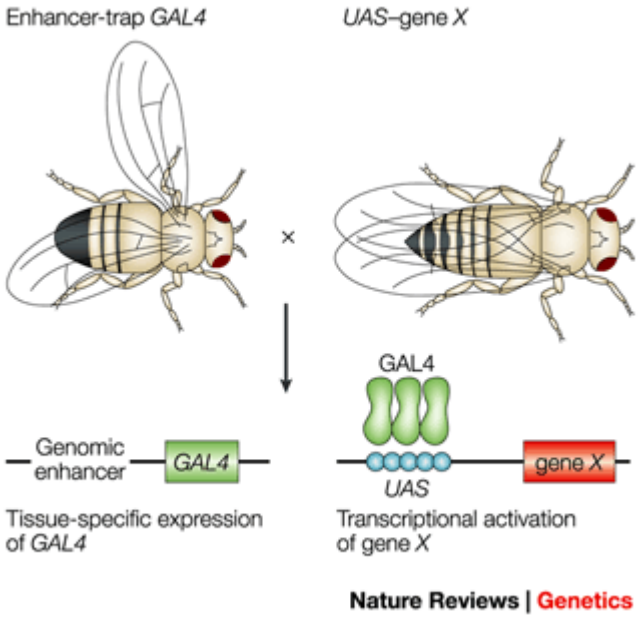


Curr Opin Neurobiol. 2012 February ; 22(1): 61–71. doi:10.1016/j.conb.2011.10.023.

Features of thermogenetics compared to optogenetics

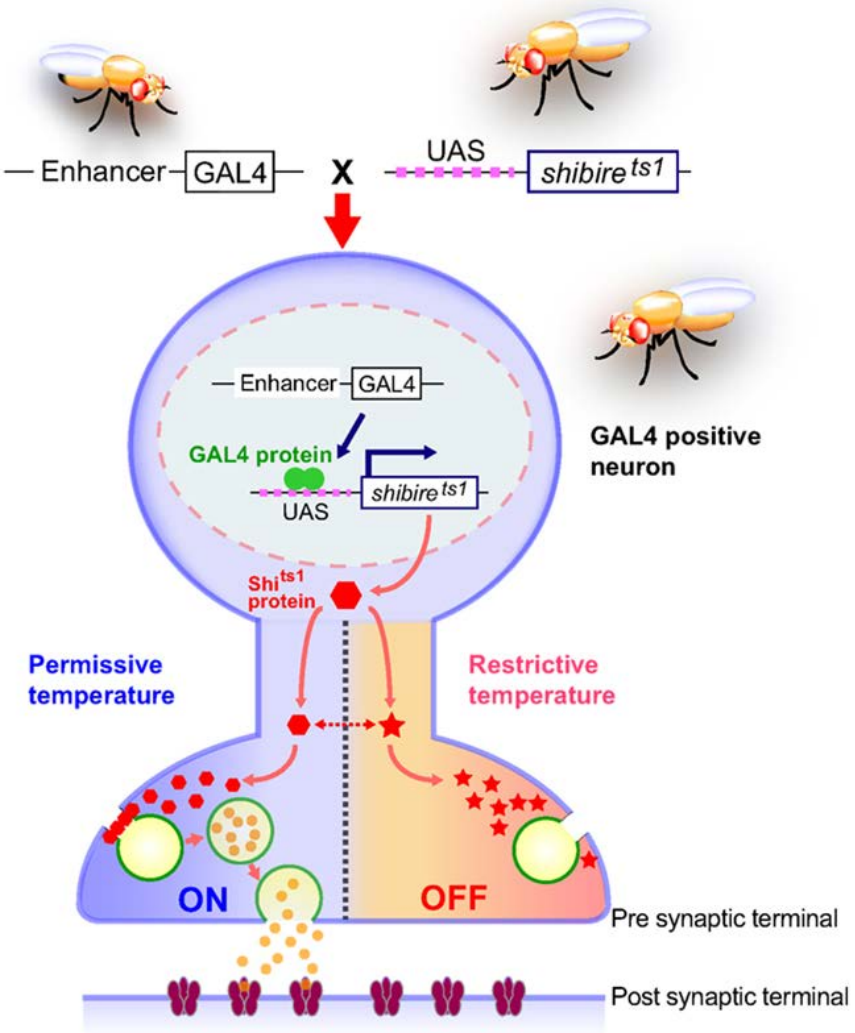
Thermogenetics	
Advantages	**Disadvantages**
Thermo Transient receptor potentials (TRPs): 1000 fold greater activity than channelrhodopsins	Still not suitable for warm blooded animals (Temperature must be compatible with physiology of the manipulated organism)
Relatively weak promotors possible	Other physiologic reaction due to increased temperature possible
Low level expression minimizes potential toxicity	Temporal resolution (dTrpA1 - sec)
Non invasive in non transparent animals (fruit fly)	Function so far restricted to neuronal activation
Multiple sites simultaneously	
Easy application	
robust	

Genetic modification in Drosophila



Nature Reviews Genetics 3, 176-188 (March 2002) | doi:10.1038/nrg751

Concept in case of shibire



GAL4: yeast transcription activator
UAS: upstream activation sequence
Shibire: encodes dynamin
Shibire^{ts1}: pointmutation G273D

Front. Mol. Neurosci., 20 August 2009 | doi: 10.3389/neuro.02.011.2009

Effect of Shibire^{ts1}

http://www.endocytosis.org/Dynamin/Shibire.html

Vol 454|10 July 2008|doi:10.1038/nature07001 nature

LETTERS

An internal thermal sensor controlling temperature preference in *Drosophila*

Fumika N. Hamada[1], Mark Rosenzweig[1], Kyeongjin Kang[1], Stefan R. Pulver[1], Alfredo Ghezzi[1], Timothy J. Jegla[2] & Paul A. Garrity[1]

Rational behind the study

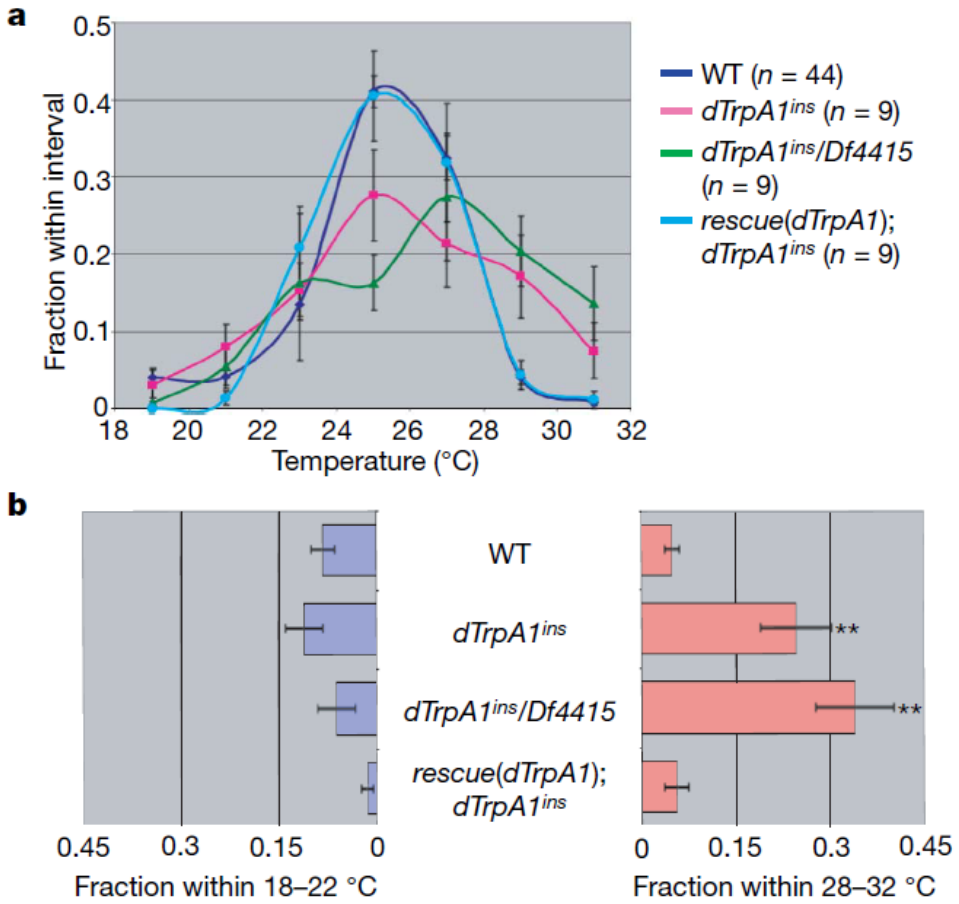
- Animals show affinity to environments of optimal temperature
- Neural circuits and strategies through which annimals select a preferred temperature are mainly unknown

→explore the mechanisms of temperature affinity or avoidance in Drosophila

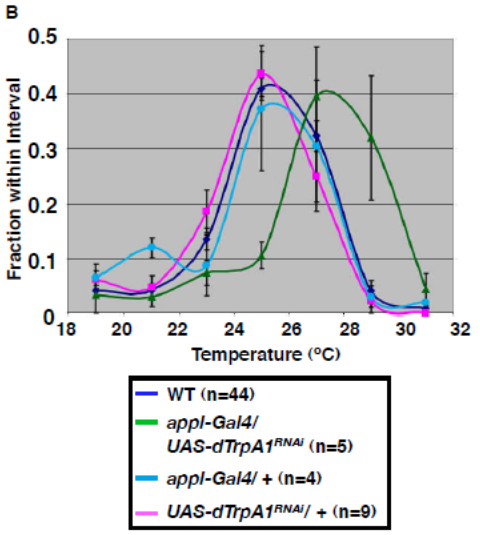
Background

- dTrpA1: temperature dependent cation channel in Drosophila
- Physiologic function of dTrpA1: larval heat avoidance
- Preferred Temperature of Drosophila is 25°C

Function of dTrpA1 in adult Drosophila



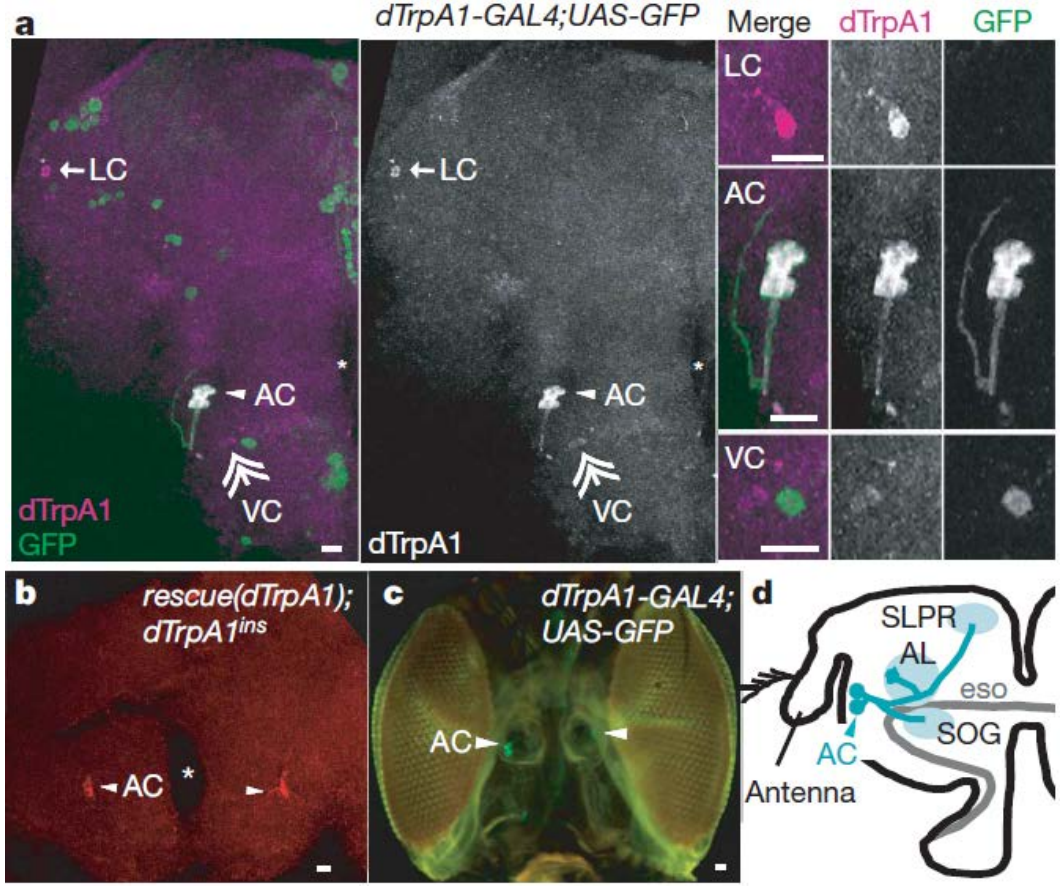
30 min
Wt: wildtype
dTrpA1^{ins}: unfunctional dTrpA1
Rescue: dTrpA1 coding minigene



→ dTrpA1 contributes for avoidance of warm regions

Localization of thermosensors

- dTrpA1 expression in the brain

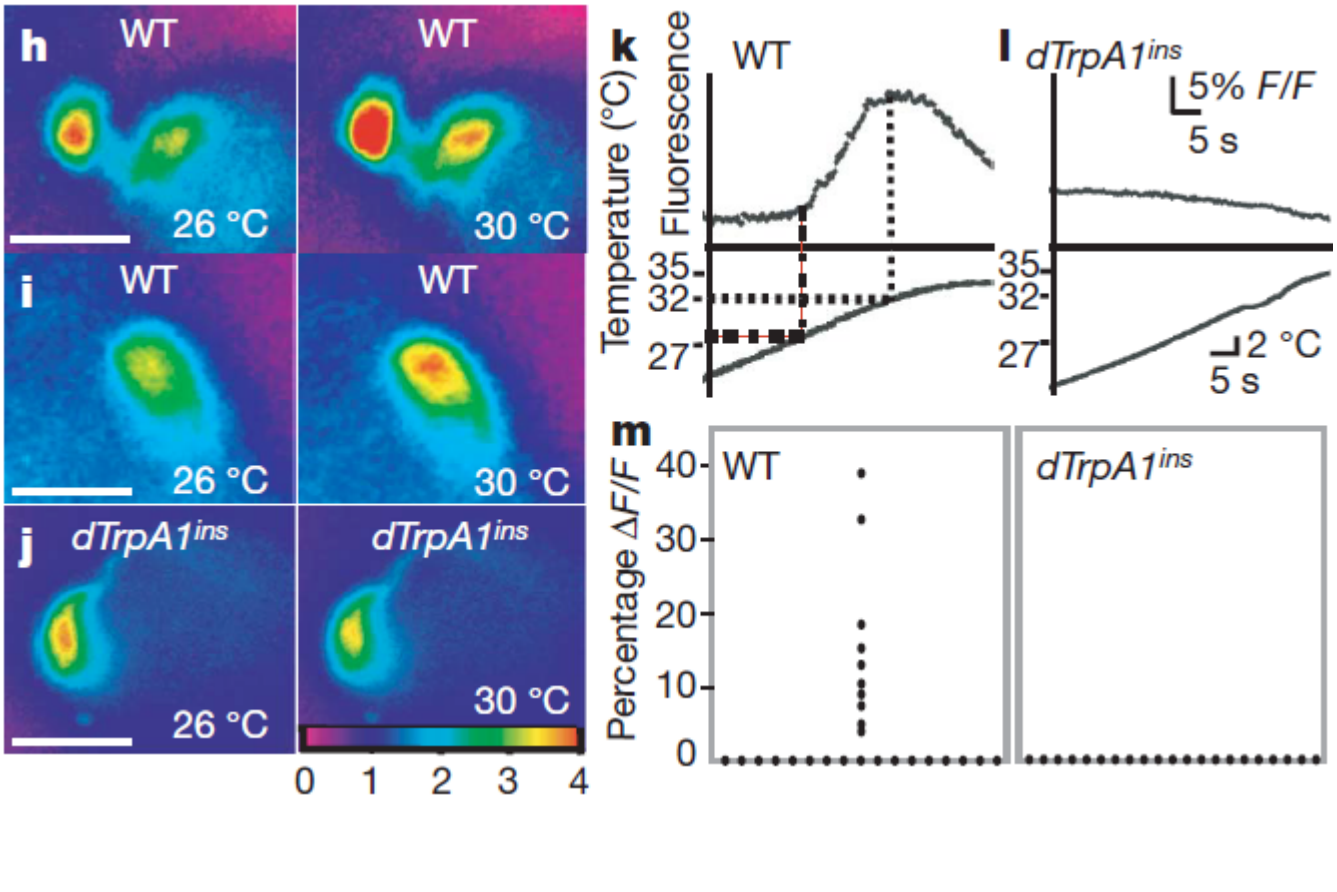


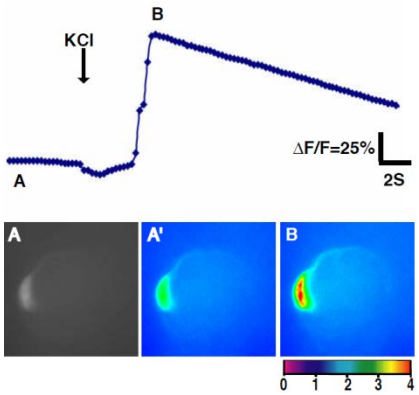
LC: lateral cell
VC: ventral cell
AC: anterior cell

AL: antennal lobe
Eso: esophagus
SOG: subesophageal ganglion
SLPR: superior lateral protocerebrum

→ Anterior cell neurons might act as thermosensors

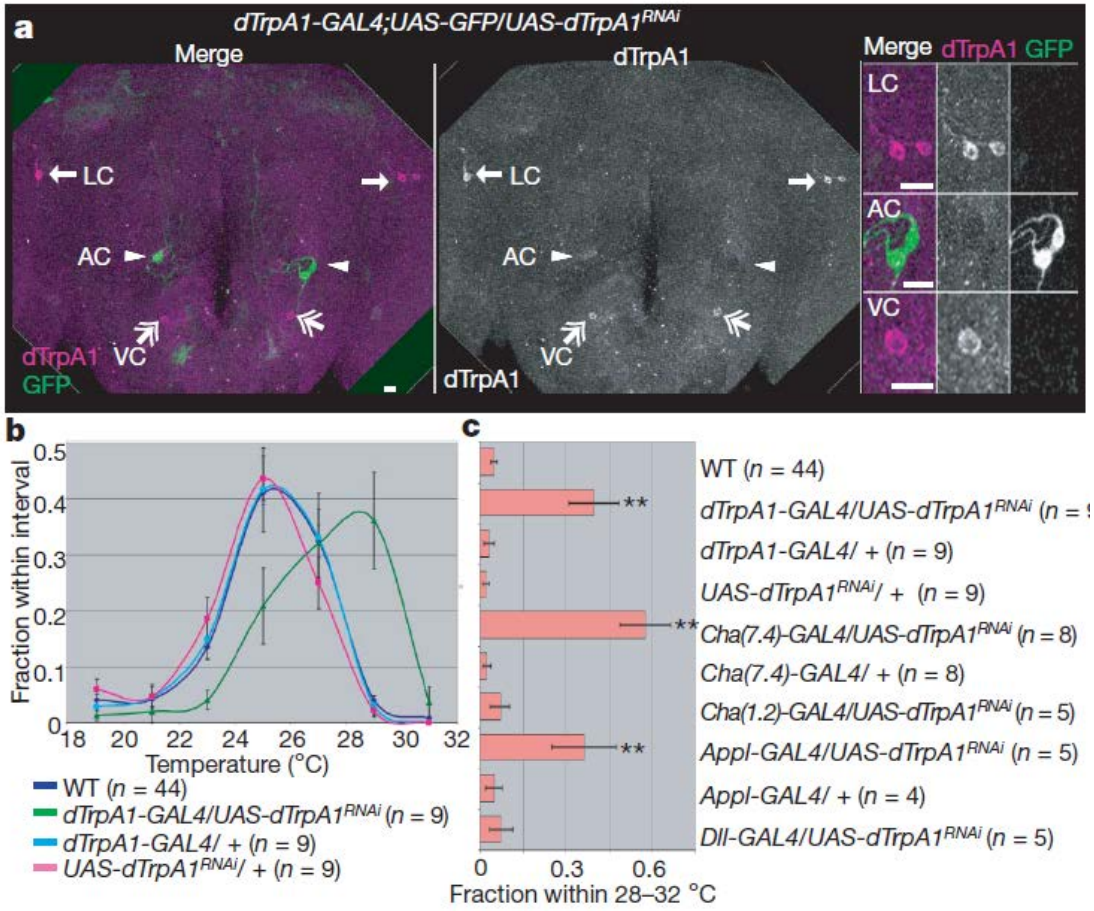
Physiology of AC neurons





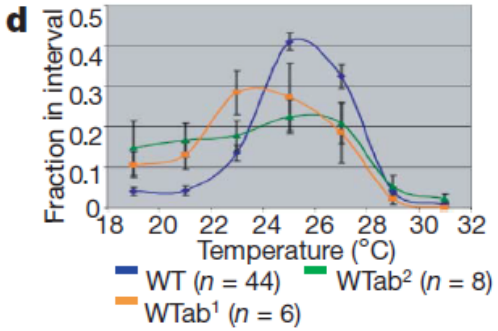
Calcium imaging revealed increase of fluorescence with increase of temperature

Blocking of TrpA1 in AC neurons

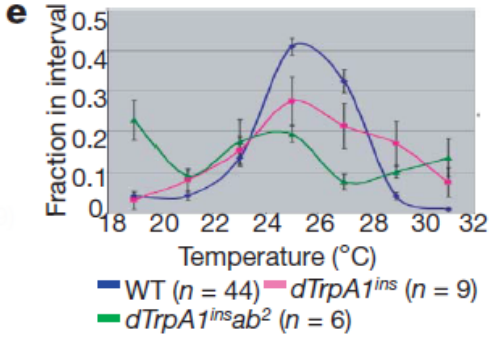


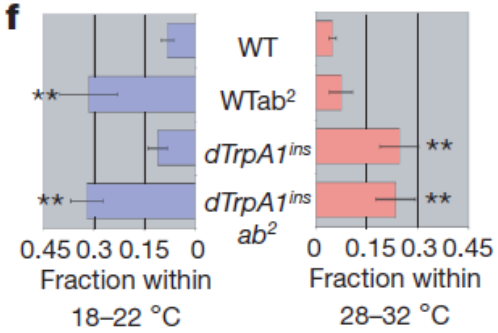
dTrpA1^{SH}: AC specific RNAi expression → dTrpA1 knockdown
Cha (7.4): general cholinergic neuron promotor
Cha (1.2): not expressed in AC neurons
Appl: broad neuronal promotor

Surgical ablation of antennae



- antennal ablation showed preference at cool temperatures





- antennal ablation in dTrpa1 mutants showed no preference in terms of temperatures

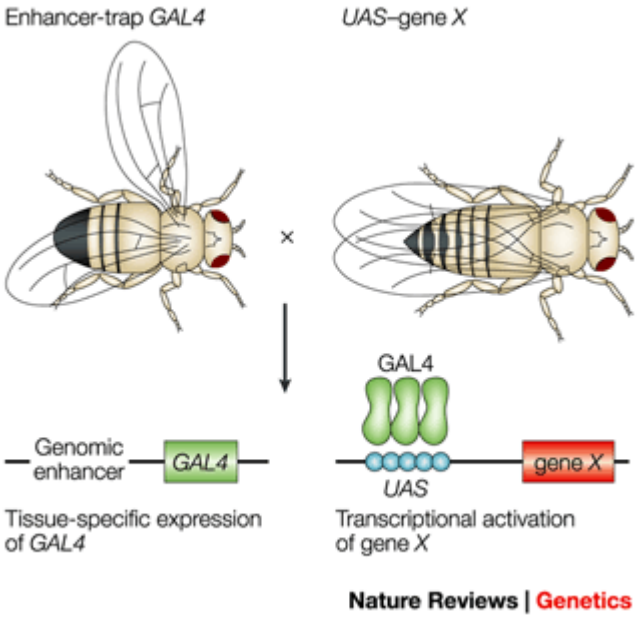
→ dTrpA1 expressing cells and antennal cells function additively to set preferred temperature

Conclusion 1

- dTrpA1 contributes for avoidance of warm regions when expressed in the anterior cells
- Antennae might act on avoidance of cold temperatures
- Antennae and anterior cells are crucial to set the preferred temperature.

Neuronal Control of *Drosophila* Walking Direction
Salil S. Bidaye *et al.*
Science **344**, 97 (2014);
DOI: 10.1126/science.1249964

Screen for neuron specific walking pattern



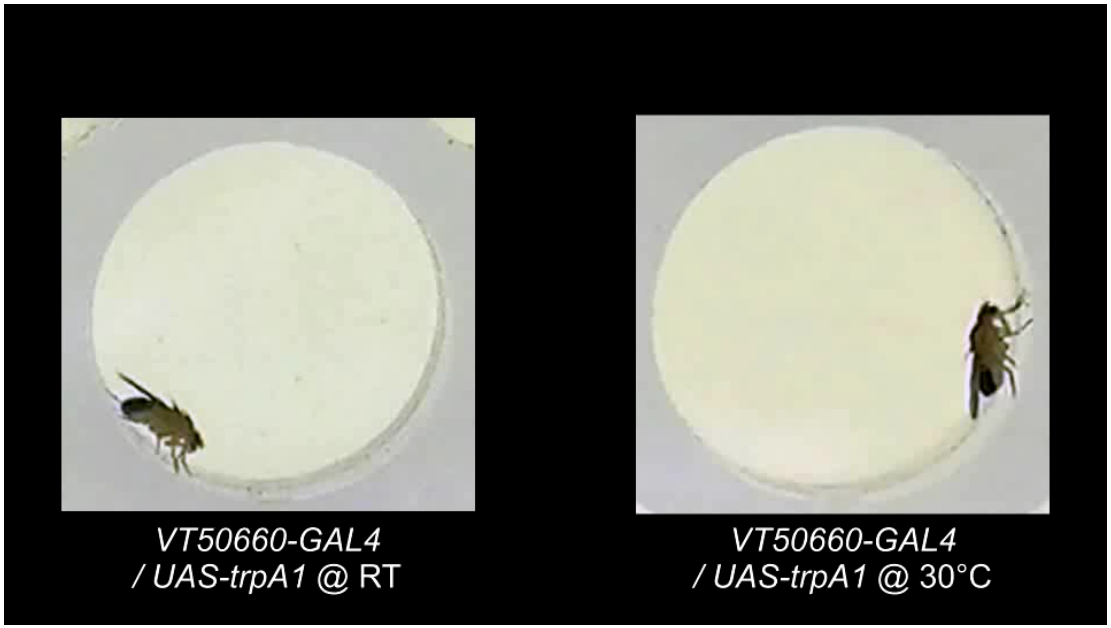
Nature Reviews Genetics 3, 176-188 (March 2002) | doi:10.1038/nrg751

Vienna tiles GAL4 driver lines (6738 viable constructs)

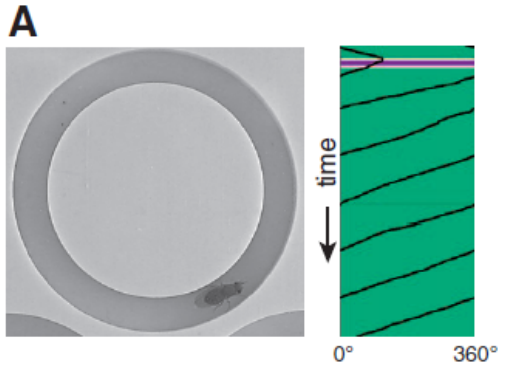
→ 3470 lines were screened (GAL4 – UAS-trpA1)
→ 4 lines showed a backward locomotion upon 30°C but not at 24°C

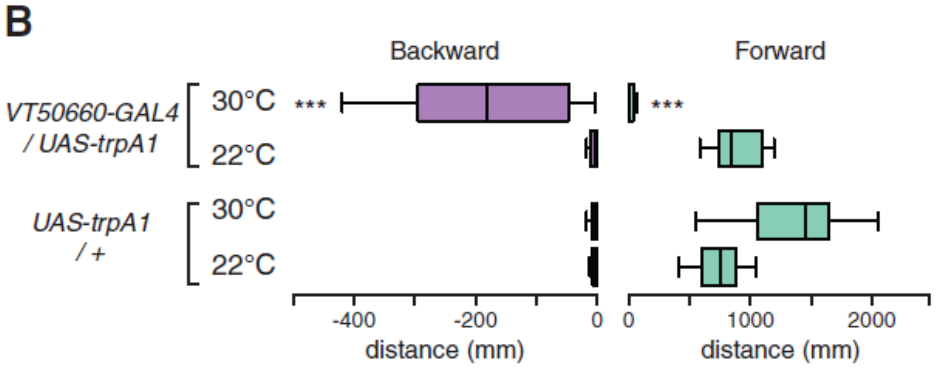
Choosing the line

- VT50660 showed most pronounced backward walking („moonwalker“) → Line for further analyses



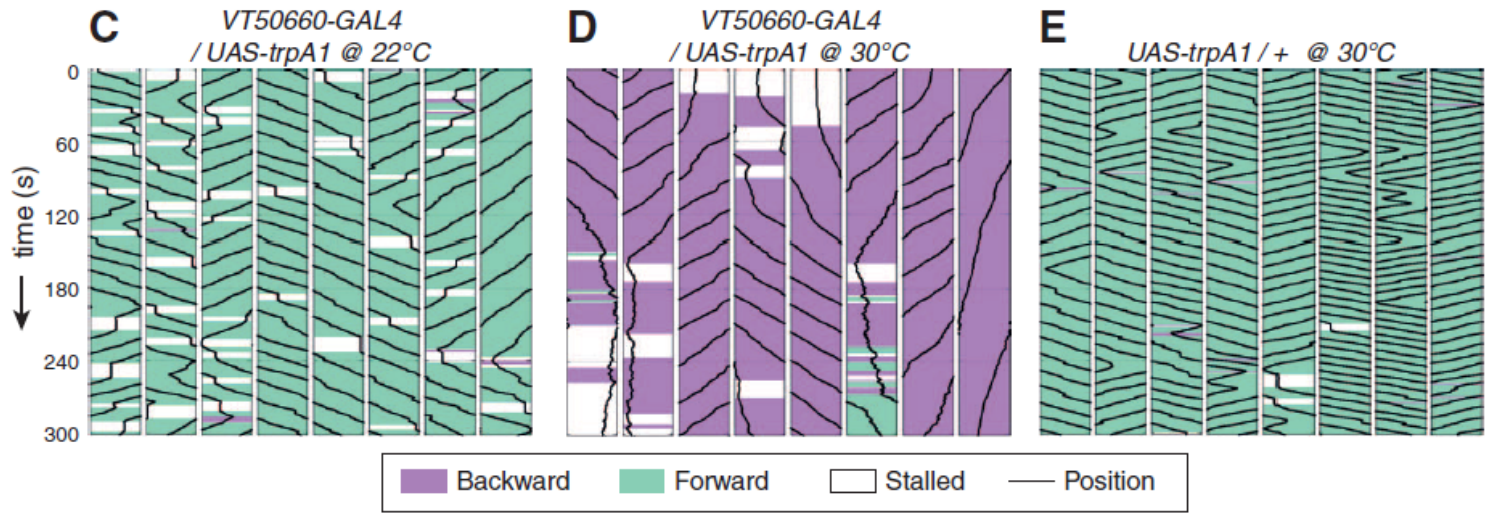
Direction and quantification of distance

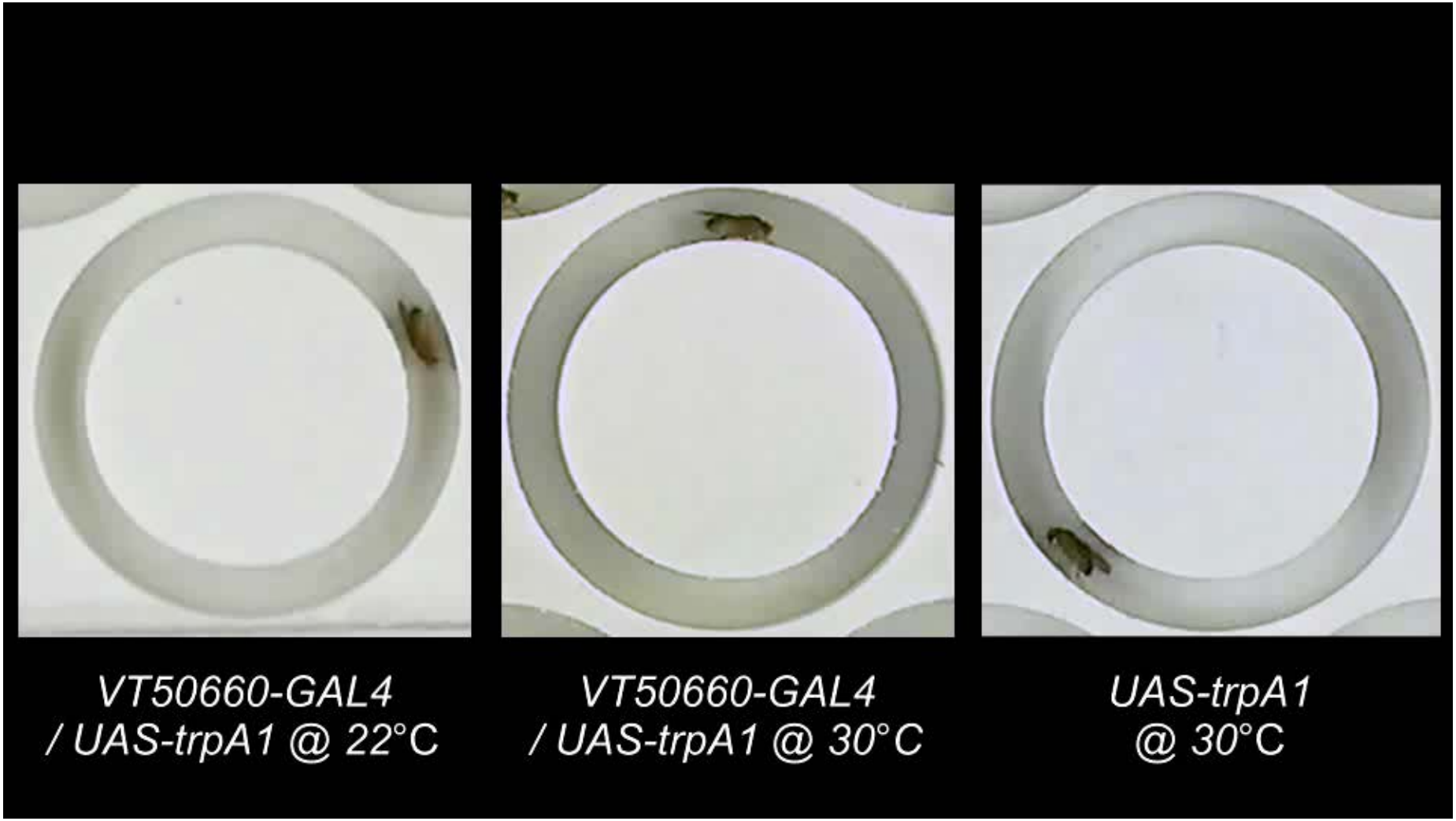




Width 2.5mm
Diameter 16mm

n = 20
t = 5min




VT50660-GAL4
/ UAS-trpA1 @ 22°C
VT50660-GAL4
/ UAS-trpA1 @ 30°C
UAS-trpA1
@ 30°C

Inducing the „moonwalk“

- Test whether moonwalker neurons contribute to backward locomotion

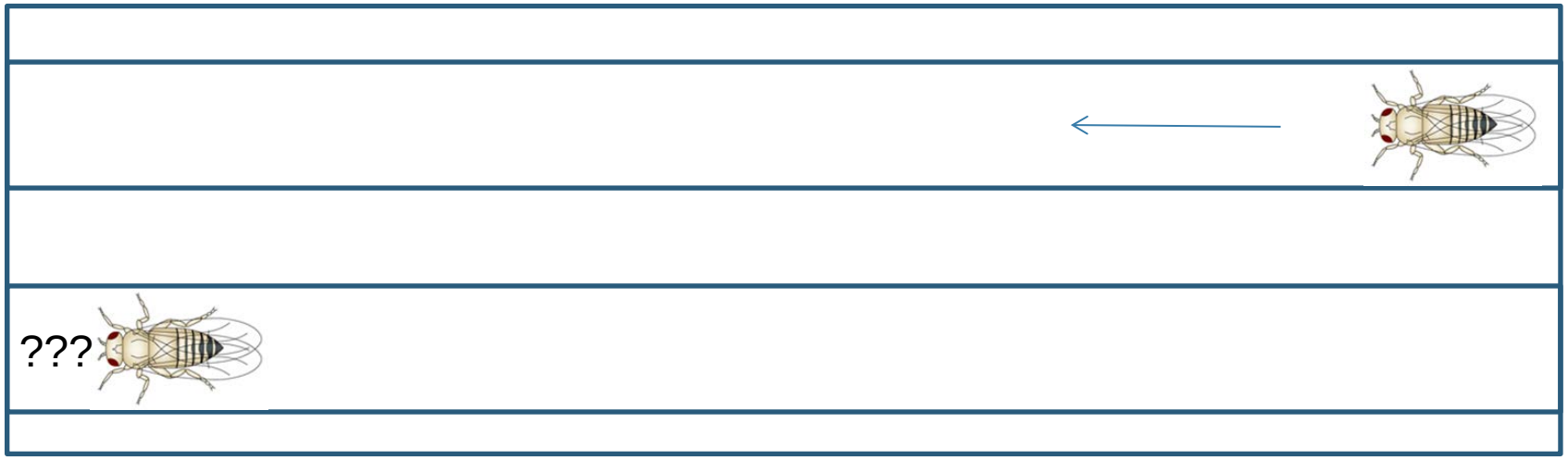
→ Inhibition of „moonwalker neurons“

VT50660-GAL4 + UAS TNT

TNT: tetanus toxine light chain → synaptobrevin↓ → inhibition of synaptic transmission

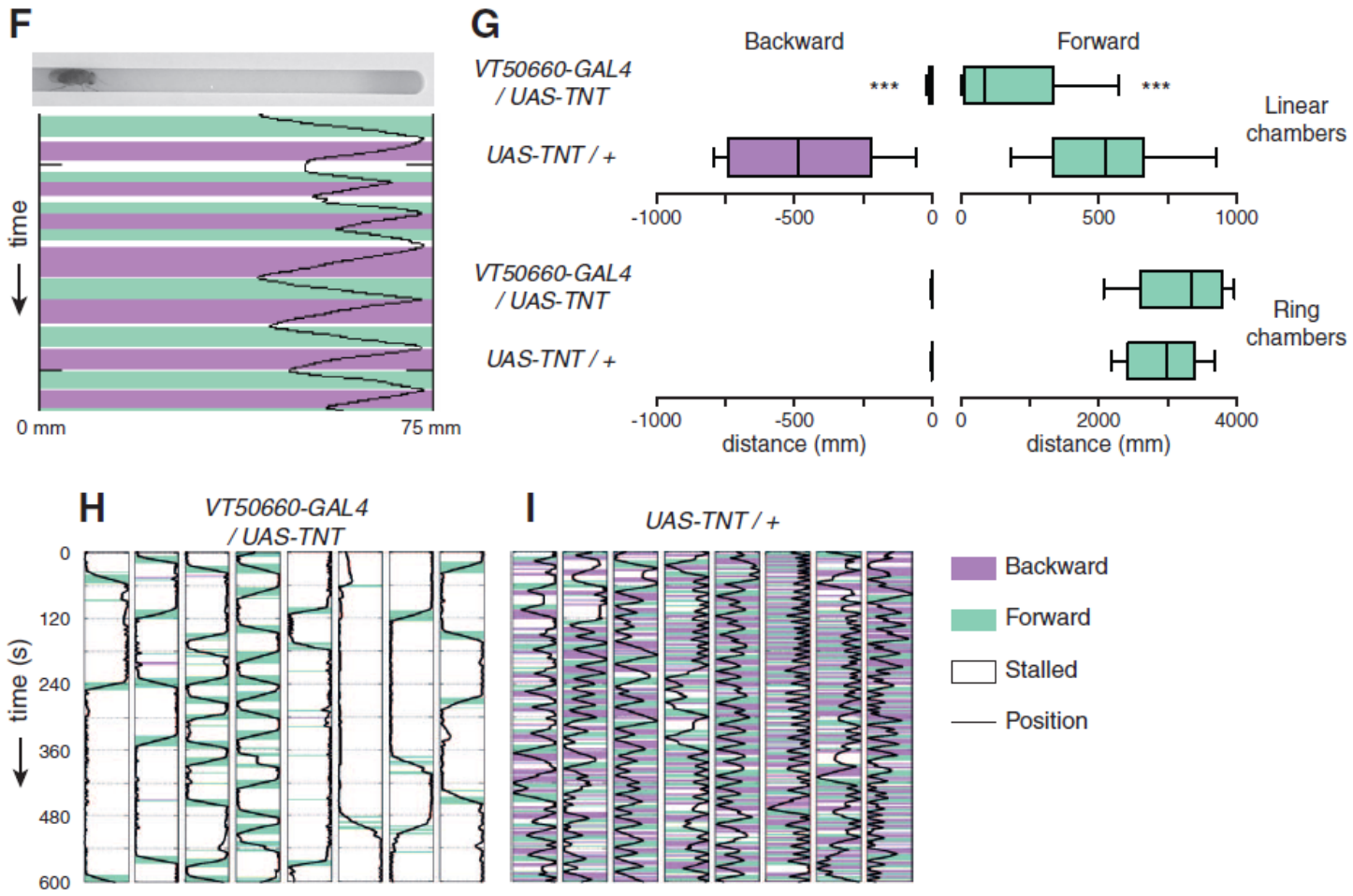
Inducing the „moonwalk“

- Linear chamber (1.5mm wide, 75mm long)
- At the end of the chamber, flies are confronted with a „dead end“
- Grooves are too narrow to easily turn and walk forward again

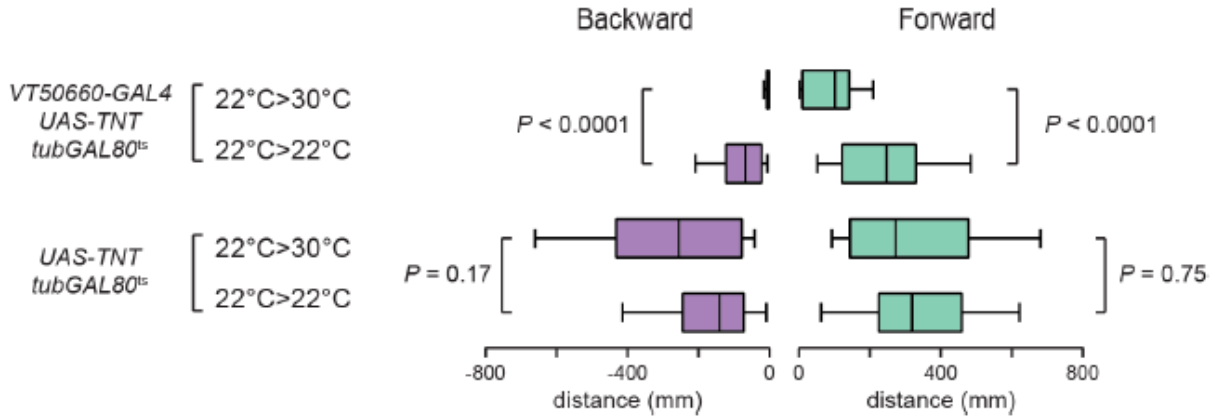



UAS-TNT / +
VT50660-GAL4 UAS-TNT

Inducing the „moonwalk“



Rescuing the phenotype



tubGAL80^{ts}: ubiquitiously expressed
thermosensitive inhibitor of GAL4 (active at 22°C)

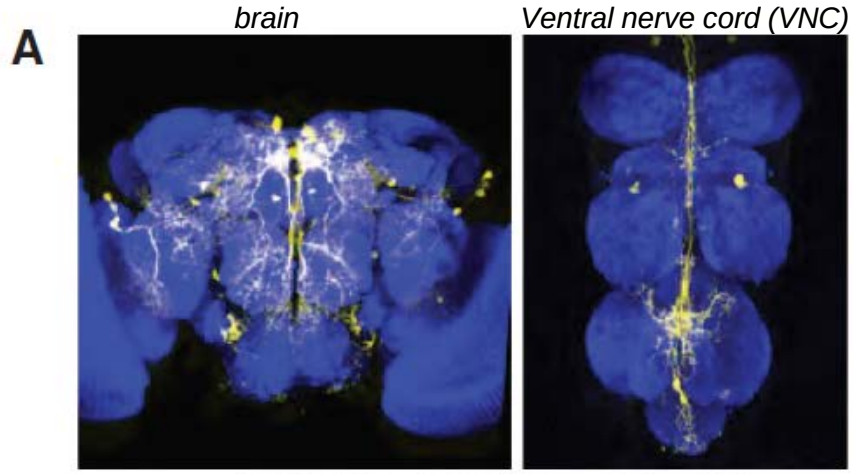
Conclusion 2

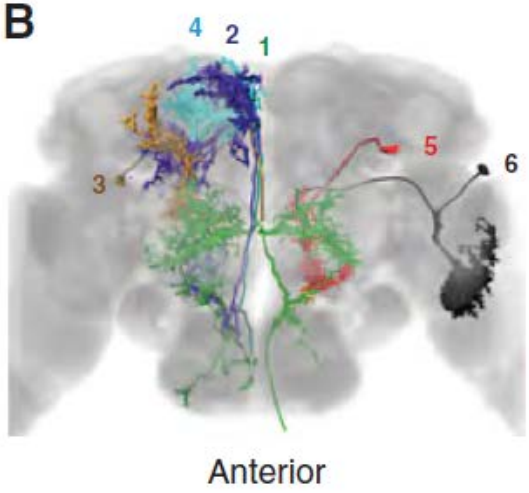
- VT50660 GAL4 UAS trpA1 leads to sponateous backward locomotion upon activation (30°C)
- VT50660 GAL4 UAS TNT leads to inhibition of backward walking
- VT50660 GAL4 UAS TNT tubGAL80 partially rescues the phenotype
- Forward movement is unaltered

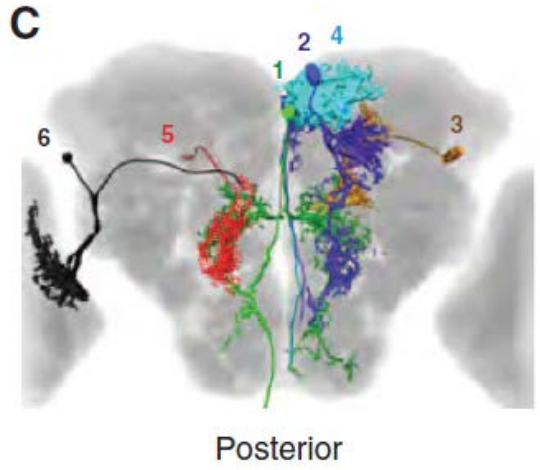
→ Activity of moonwalker neurons is essential for backward, but not forward locomotion

Specification of moonwalker neurons

- VT50660-GAL4 labels 7 distinct cell types in the CNS

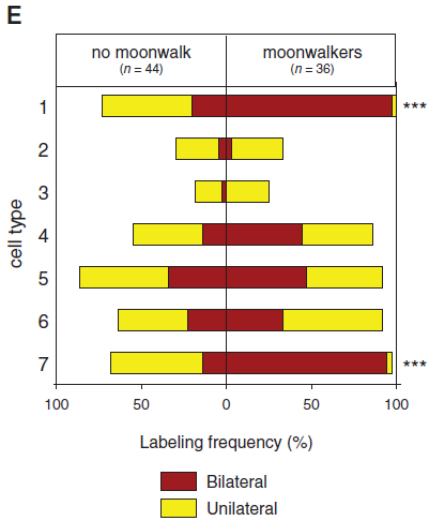


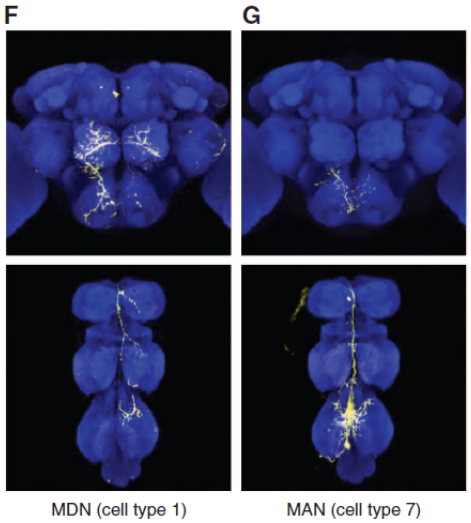


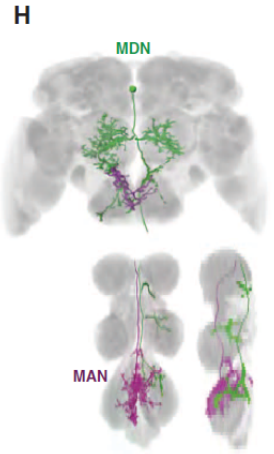


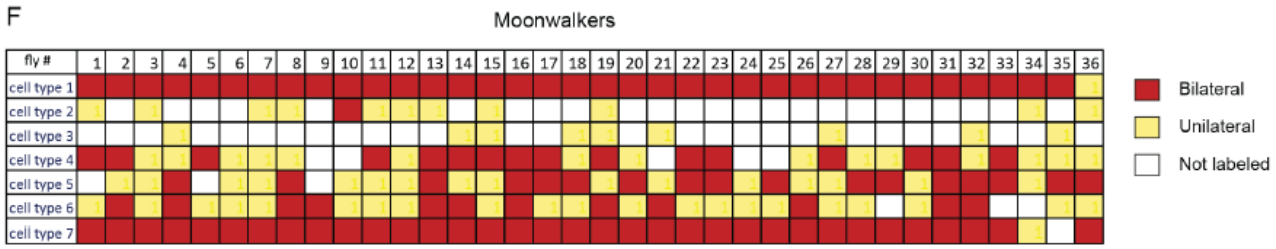
UAS >stop> mCD8-GFP
UAS >stop> TrpA1$^{c\text{-}myc}$
> FLP recombinase sites
hs FLP recombinase TG in larvae

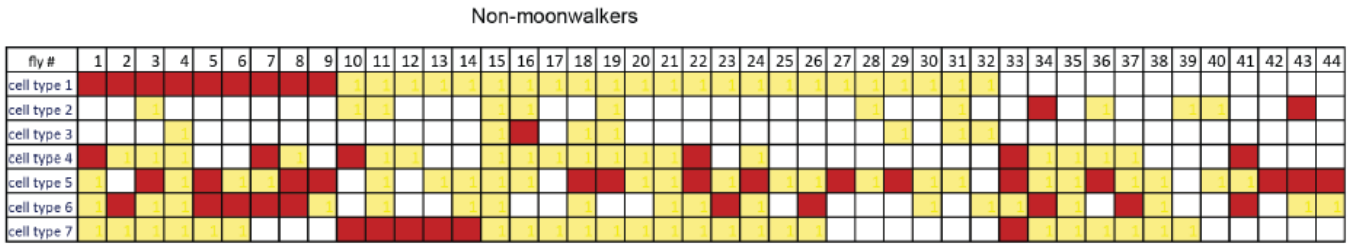
Mutants tested at 30°C









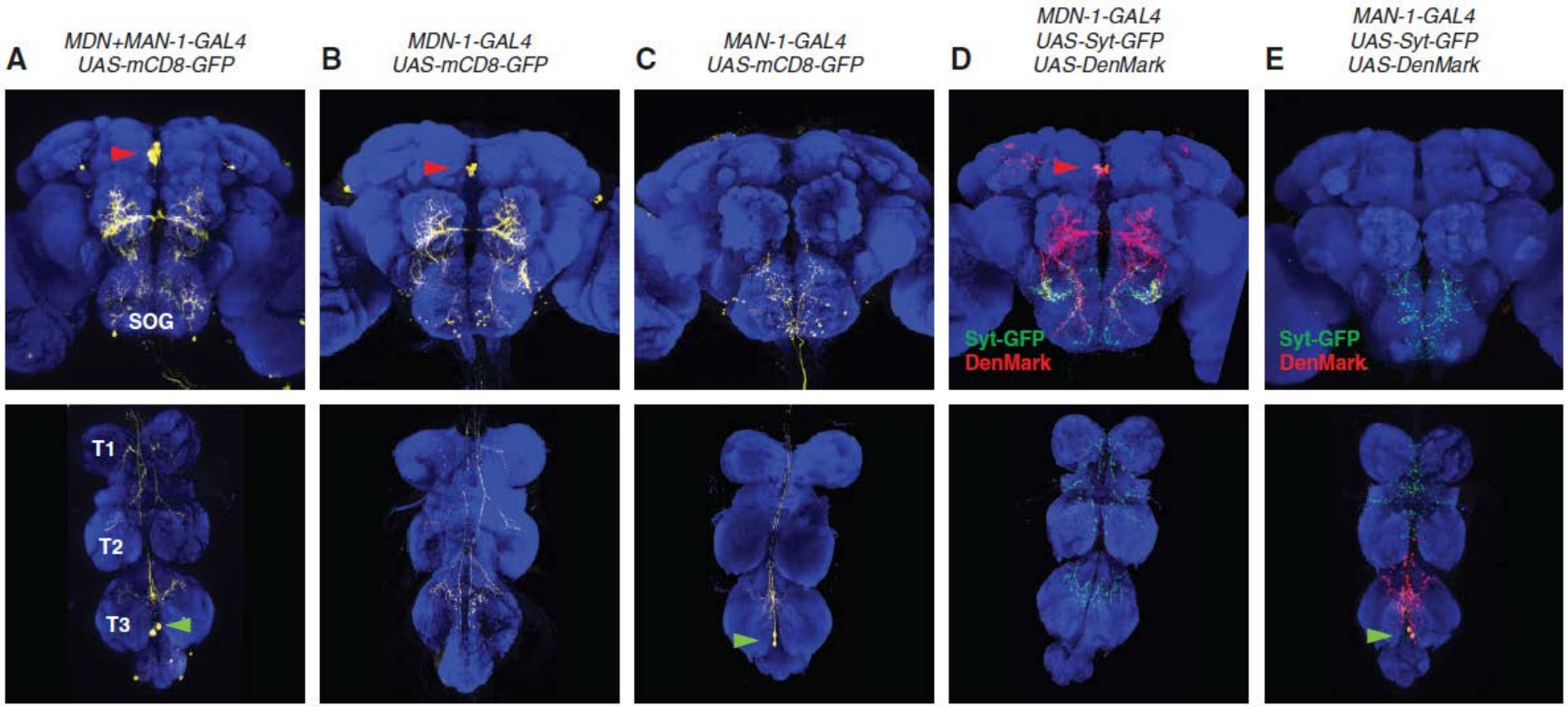


MDN: moonwalking descending neuron
MAN: moonwalking ascending neuron

Specifically target MDN and MAN

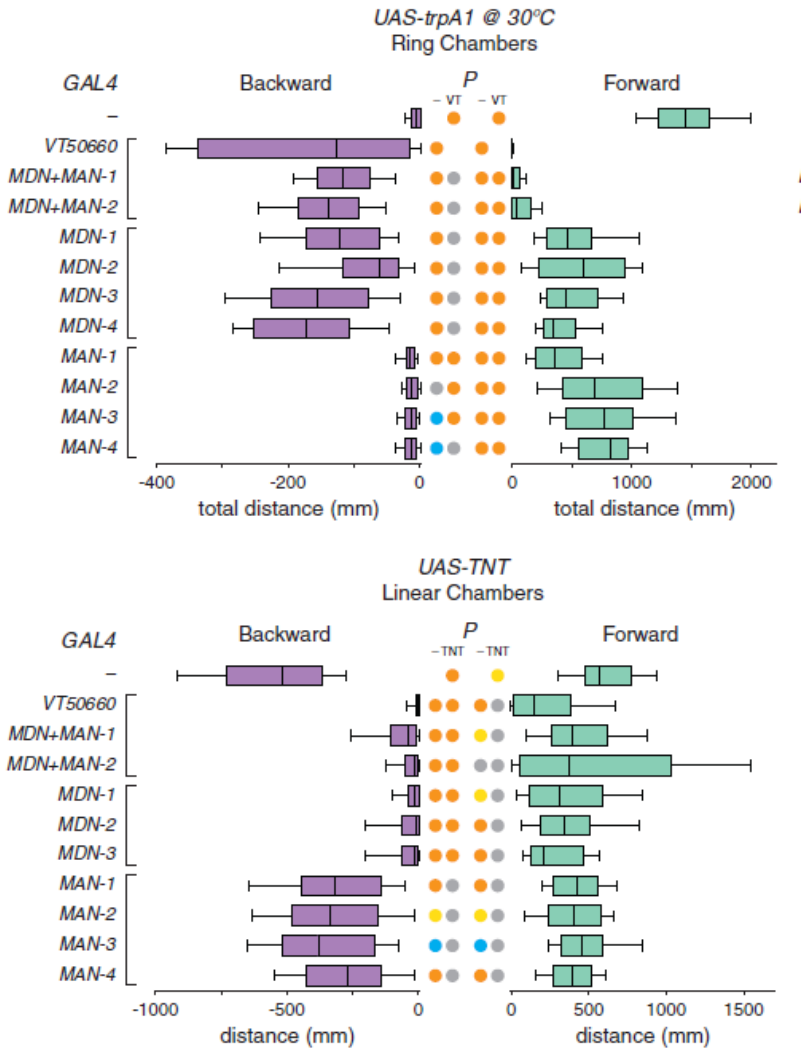
- Split GAL4 system
- 2 complementary Leucine zipper linked to inert GAL4 halves
- 4 drivers targeting MDN and not MAN
- 4 drivers targeting MAN and not MDN
- 2 drivers targeting MAN and MDN

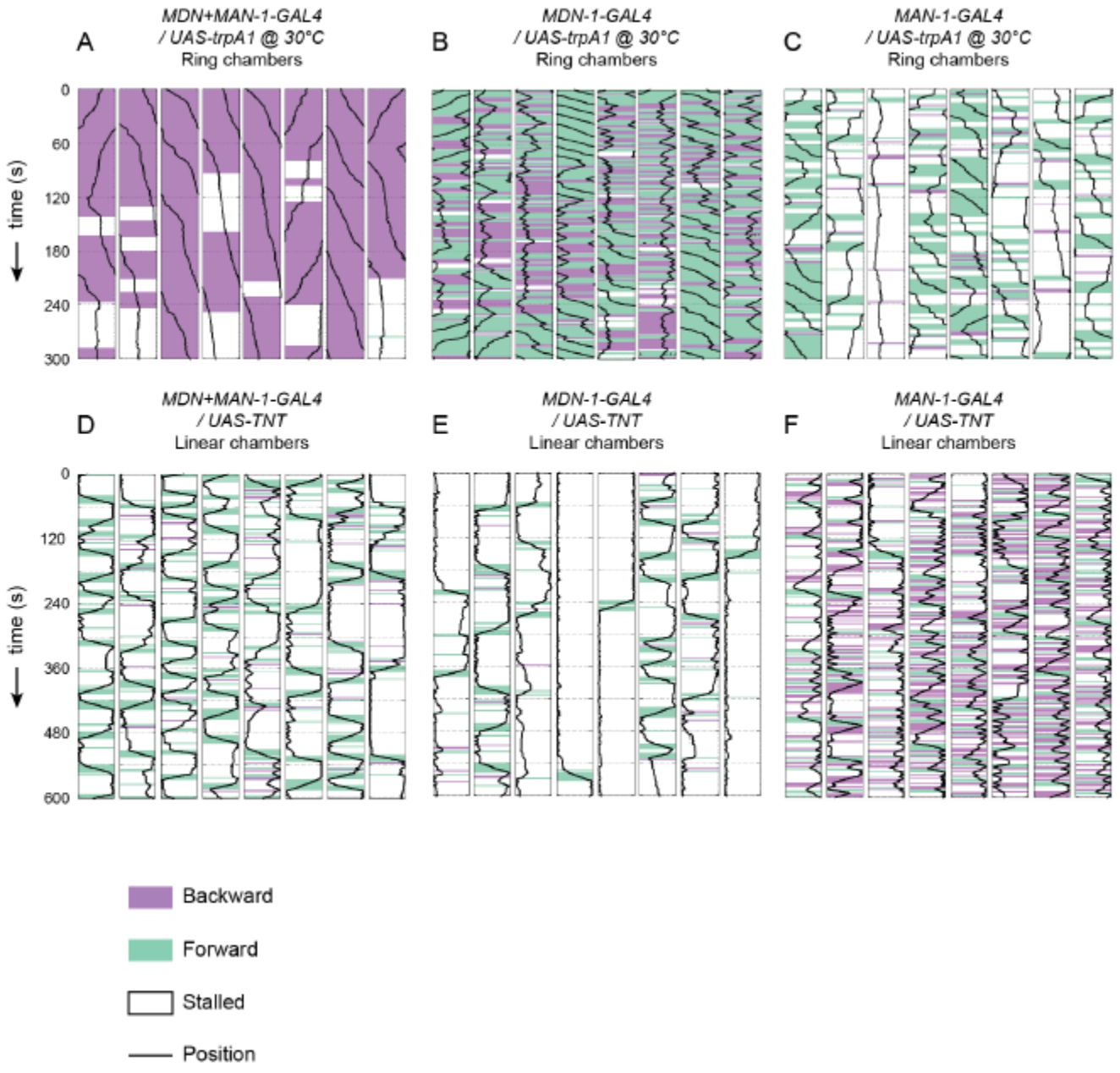
Anatomy of these neurons

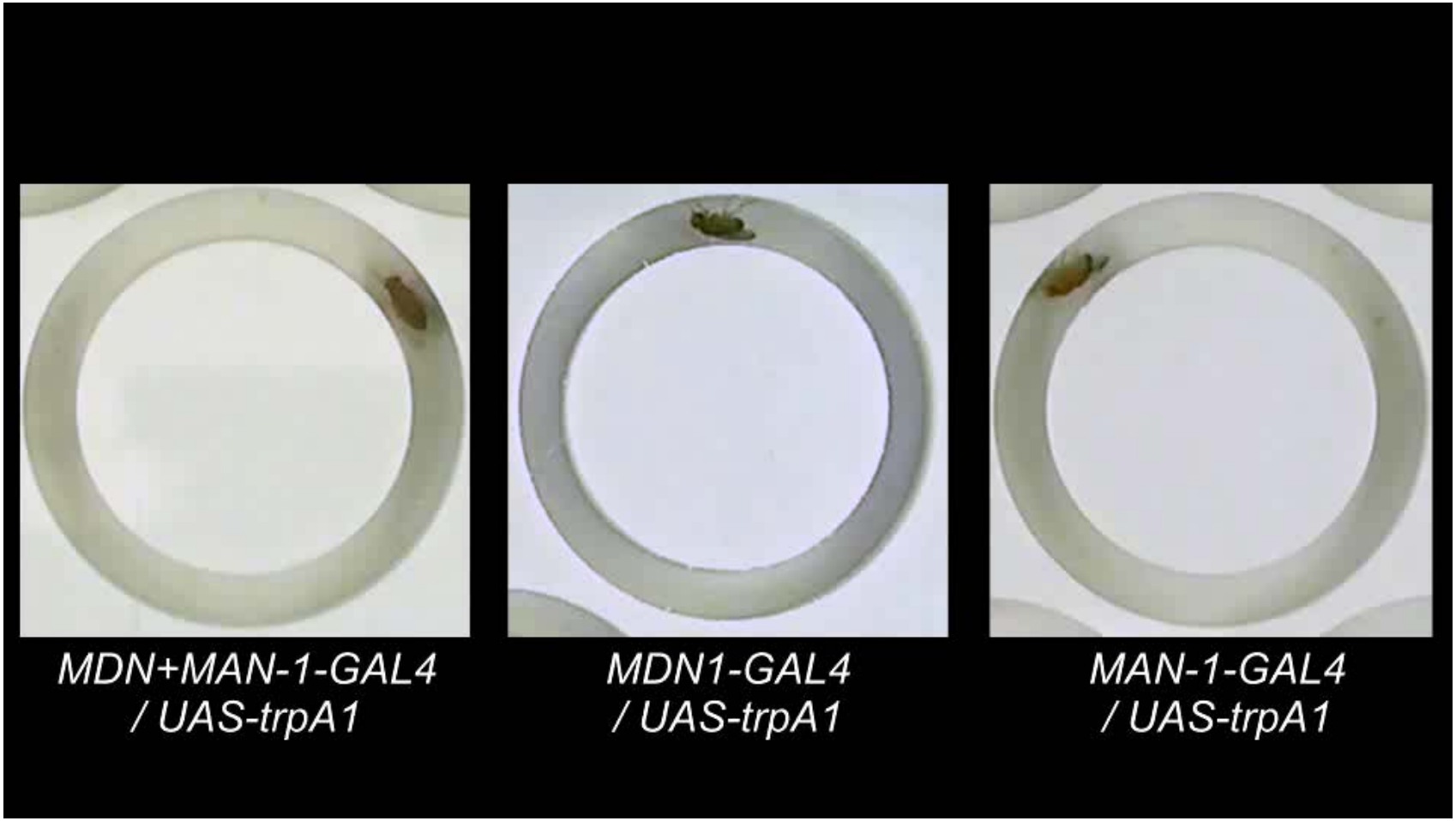


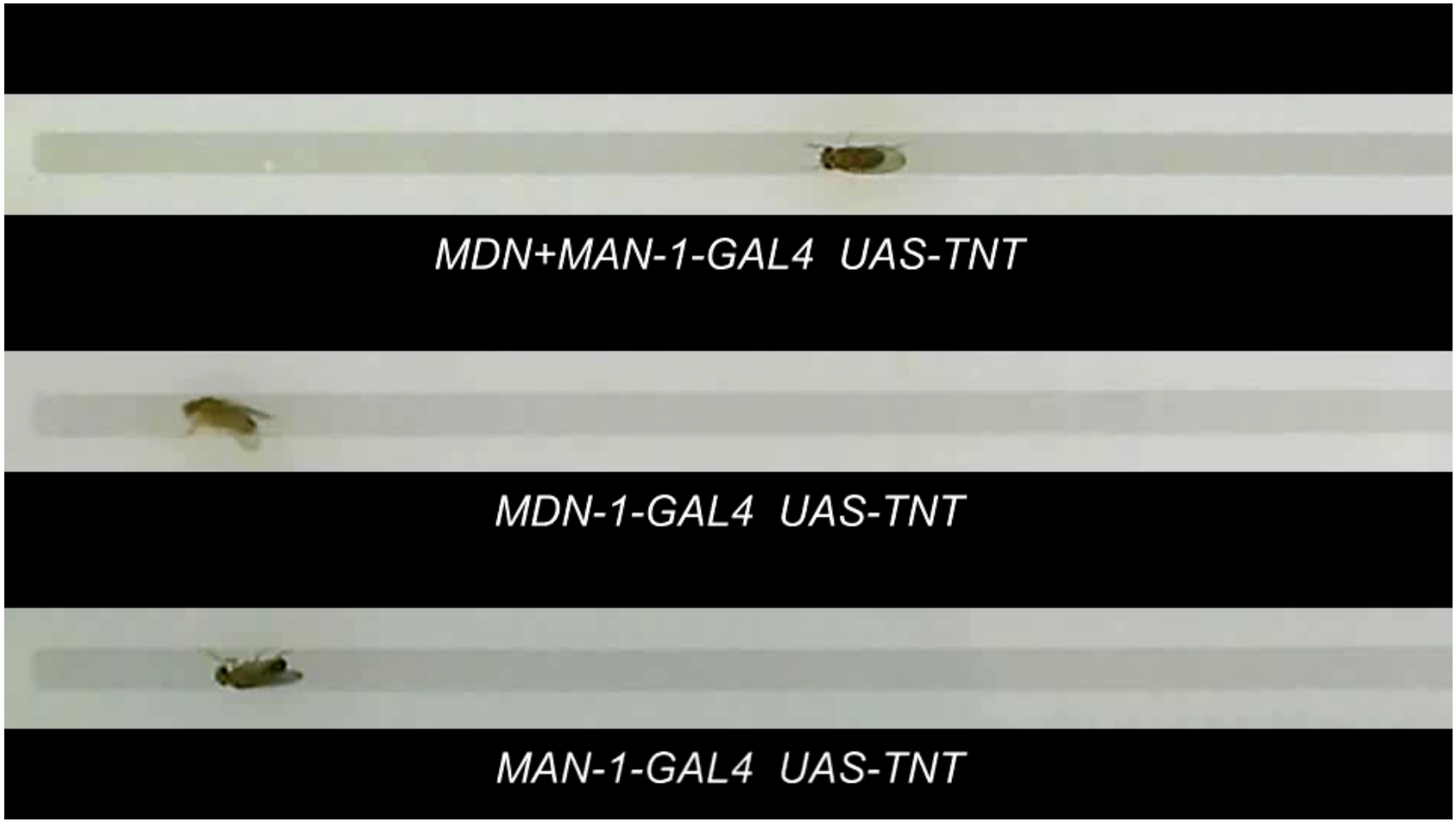
SOG: subesophageal region
mCD8-GFP: membrane marker
DenMark: dendritic marker
Syt GFP: presynaptic marker
T1-T3: leg neuropils

Functional study of mutants




A
MDN+MAN-1-GAL4
/ UAS-trpA1 @ 30°C
Ring chambers
B
MDN-1-GAL4
/ UAS-trpA1 @ 30°C
Ring chambers
C
MAN-1-GAL4
/ UAS-trpA1 @ 30°C
Ring chambers
time (s)
0
60
120
180
240
300
D
MDN+MAN-1-GAL4
/ UAS-TNT
Linear chambers
E
MDN-1-GAL4
/ UAS-TNT
Linear chambers
F
MAN-1-GAL4
/ UAS-TNT
Linear chambers
time (s)
0
120
240
360
480
600
Backward
Forward
Stalled
Position


MDN+MAN-1-GAL4
/ UAS-trpA1
MDN1-GAL4
/ UAS-trpA1
MAN-1-GAL4
/ UAS-trpA1


MDN+MAN-1-GAL4 UAS-TNT
MDN-1-GAL4 UAS-TNT
MAN-1-GAL4 UAS-TNT

Conclusion 3

- MDN seems to be crucial for moonwalking
- Still, the strong correlation with MAN suggests that MAN might promote backward movement
- the pronounced forward walking inactivity in activated MAN suggests that those neurons play a role in inhibition of forward walking

Thank you for your attention