

Supplementary material for:

Rapid threat assessment in the Drosophila thermosensory system

by

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Included:

Supplementary Figure 1, related to Figure 2

Supplementary Figure 2, related to Figure 4

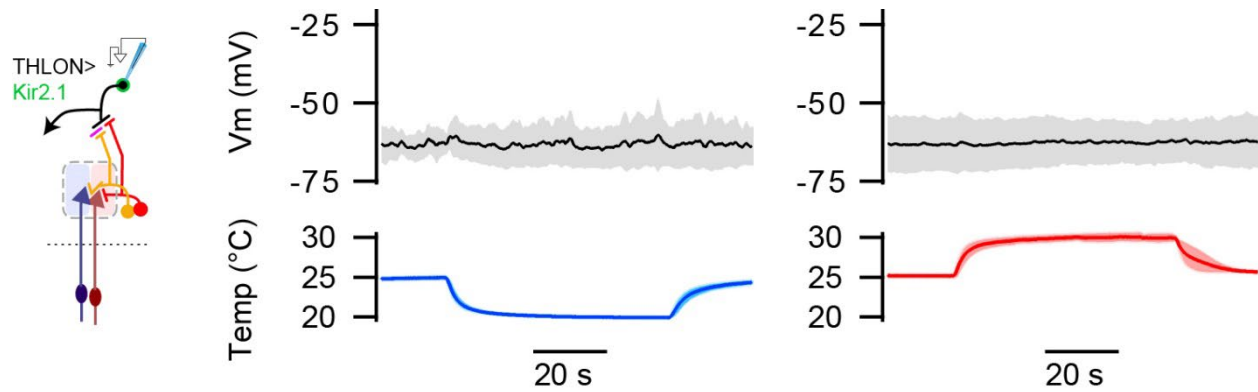
Supplementary Figure 3, related to Figure 5

Supplementary Table 1, related to Figure 2

Supplementary Table 2, related to Figure 3

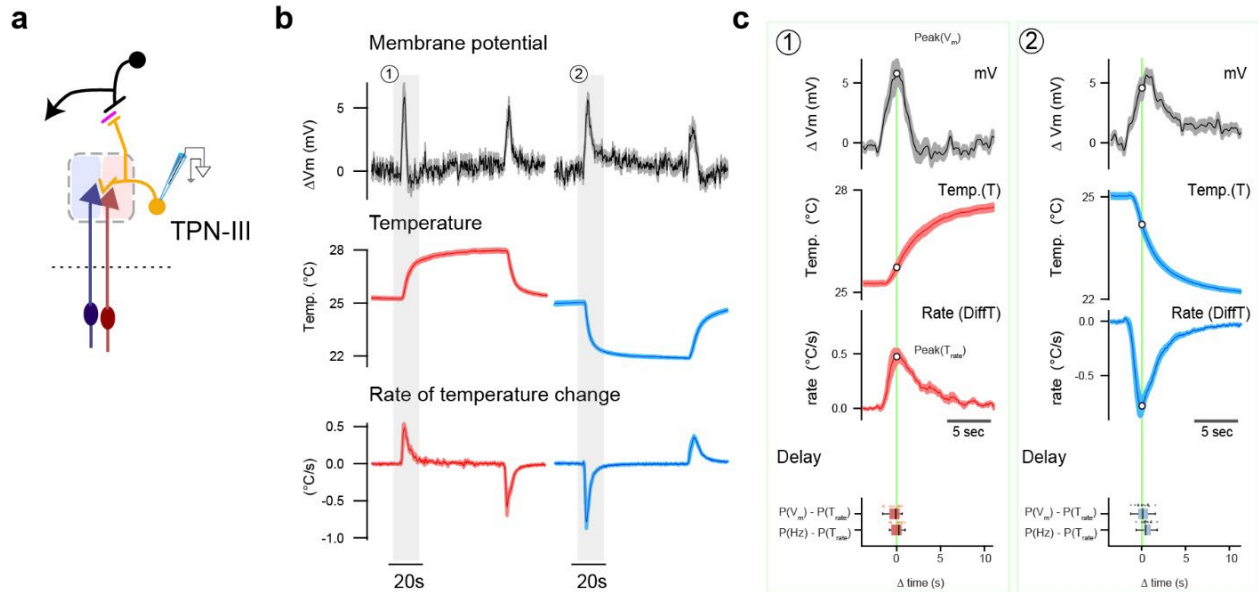
Supplementary Table 3, related to Figure 7

Supplementary Table 4, Table of Genotypes



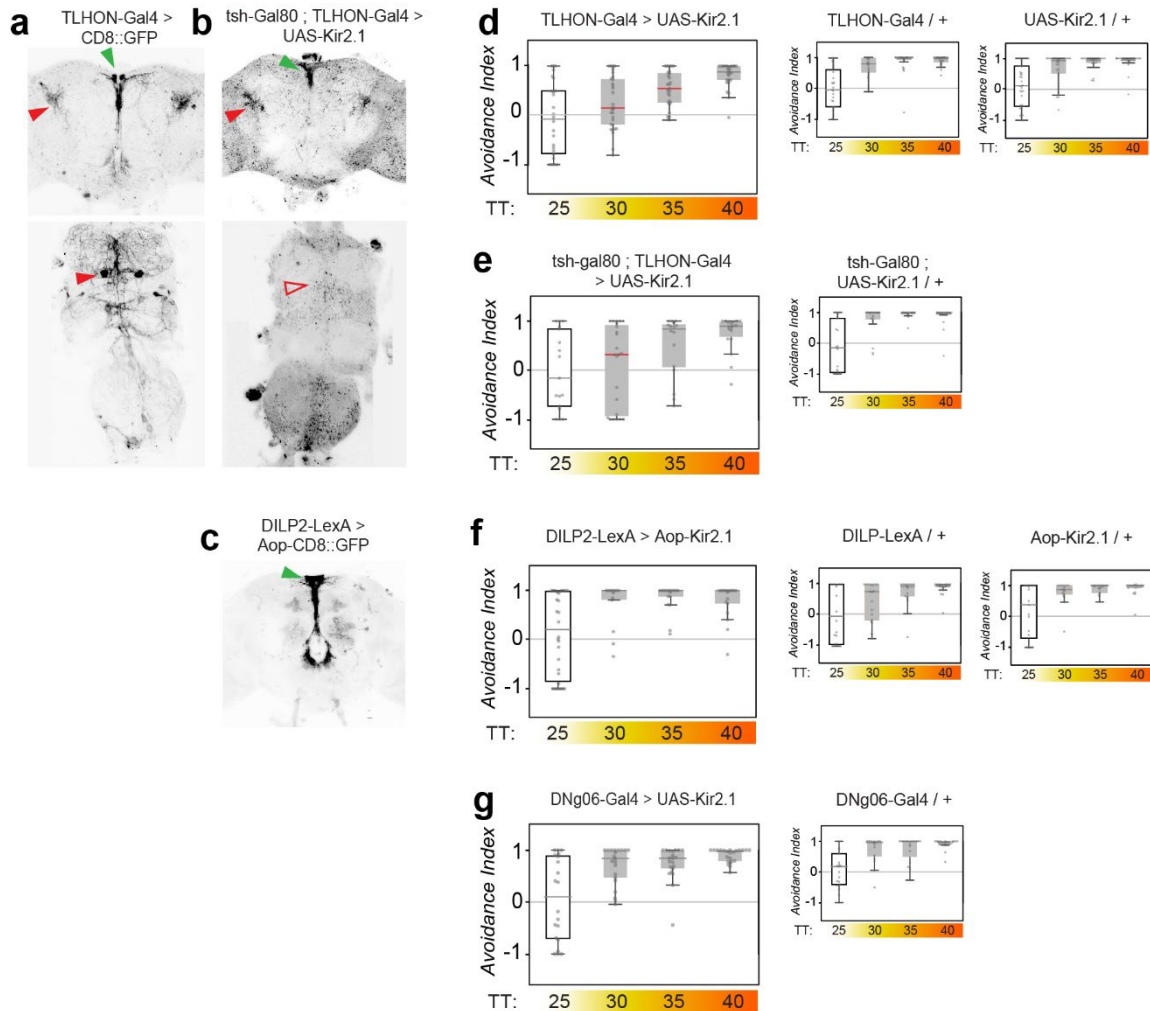
Supplementary Figure 1, related to Figure 2. Expression of Kir2.1 in TLHONs produces hyperpolarization and silences responses to thermal stimuli.

TLHONs were recorded in 2-photon guided patch-clamp in flies of the genotype: TLHON-Gal4, UAS-Kir2.1, UAS-CD8:GFP. (Left) schematic of recording configuration. (Right) traces acquired in whole cell patch clamp mode while challenging the preparation with cold (blue, left) or hot (red, right) step stimuli. (Top) average membrane potential; (Bottom) temperature recording (cold $n = 4$ cells, 2 animals; and hot $n = 3$ cells, 2 animals; bold line = mean; shading = $\pm$ SEM).



Supplementary Figure 2, related to Figure 4. TPN-III ON responses correlate with peak rate of temperature change.

TPN-III ON responses peak in correspondence with the fastest rate of heating or cooling. **(a)** Experiment schematic. **(b)** TPN-III filtered membrane potential responses (top traces), stimulus temperature (middle traces), and rate of thermal change (temporal derivative of temperature stimuli; bottom traces). **(c)** Green boxes are x-axis expansions of grey shaded regions in **(b)** as indicated. Delay (shown as boxplots) is quantified as the difference between the timing of the peak membrane potential or the peak firing rate and the timing of the peak rate of thermal change and shows temporal locking between peak response and peak stimulus rate (N=14 cells/7 animals, trace line and shading indicate mean $\pm$ SEM; boxplots: black line = median, box = interquartile range, whiskers = range, dots = individual cells).



Supplementary Figure 3, related to Figure 5. TLHON-Gal4 full expression pattern and controls for off-target expression

(a) TLHON-Gal4 expression pattern in the brain and ventral nerve cord revealed by expression of CD8:GFP. Brain: Green arrowheads = Insulin-producing cells (IPCs). Red arrowheads = TLHONs. VNC: Red arrowheads = cell bodies. (b) tshirt-Gal80 (tsh-Gal80) effectively suppresses VNC expression (empty arrowhead). (c) The expression pattern of Dilp2-LexA > CD8:GFP is shown for reference. (d-g) Single fly temperature preference behavior suggests the phenotype of TLHON-Gal4 mediated silencing is not due to off-target expression in the VNC or DILPs. Single fly heat avoidance indexes for (d) TLHON-Gal4 > UAS-Kir2.1 and controls, (e) tshirt-gal80; TLHON-gal4/UAS-Kir2.1 and controls, (f) Dilp2-LexA > Aop-Kir2.1, and controls (see expression pattern of Dilp2-LexA in d), (g) DNg06-Gal4 > UAS-Kir2.1 and controls (note that, while TLHON-Gal4 mediated expression of GFP in DNg06 is at the detection limit, we tested this cell type out of an abundance of caution). In (d-g) gray dots each represent the avoidance index in 1 trial for 1 fly. In all boxplots, the edges of the boxes are the first and third quartiles, a solid line marks the median, and whiskers delimit the data range, a solid red median line denotes a significant interaction between experimental and control animals (2-way ANOVA, $p < 0.05$, $N = 14-38$, see Statistics table for precise N values for each test). TT = test temperature in °C; white to orange scale = 25 to 40°C.

		LHPV2a									
		724820565	758903321	822005494	822009511	822684007	853717974	886130319	5813014218	5813041244	5813090530
		LHPV2a postsynaptic									
TPN-III pre-	819993149	0	1	2	0	0	1	6	0	2	1
	850703925	1	13	7	0	1	18	4	12	8	7
	850708902	1	8	8	0	1	16	3	27	11	10
	1037293275	1	0	0	0	20	0	0	3	1	0
	1630734360	3	2	2	0	0	4	11	5	4	1
	1733677514	2	0	0	3	9	0	1	2	1	0
	1943811736	2	0	0	0	0	0	0	0	0	0
	5813044177	0	2	5	0	0	2	17	1	9	0
	5813083723	0	1	0	3	0	0	0	0	0	0
LHPV2g pre-	882995659	0	8	1	0	0	37	1	0	0	10
	914027038	0	7	5	0	0	26	3	3	0	6
		LHPV2a presynaptic									
TLHON post-	882995659	0	2	20	1	2	1	15	4	12	0
	914027038	0	4	11	0	1	1	11	7	6	0
		LHAV		LHPV							
		574377845	1037510115	1006146837	913341138						
TPN pre-	TPN ID	postsynaptic									
	1755556097	13	0	4	0						
	1975878958	124	23	1	10						
	2065197353	0	0	18	4						
	2069644133	0	0	10	4						
	5813040515	16	0	7	0						
		presynaptic									
TLHON post-	882995659	16	8	13	8						
	914027038	14	6	3	4						
		TLHON post-									
		882995659	914027038								
TPN-V pre-											
	1975878958	26	30								

Supplementary Table 1, related to Figure 2. Connectivity between TPN-IIIs, LHPV2as and TLHONs (LHPV2gs) and additional thermosensory drive to TLHONs. (See Figure 2 and methods for details). Synaptic connections between individual neurons for TPN-III thermosensory circuit with TLHONs: TPN-IIIs to LHPV2a, TLHONs to LHPV2a, LHPV2a to TLHONs. Synaptic connections for direct and indirect thermosensory pathways to TLHONs: TPNs to LHAV/LHPV, LHAV/LHPV to TLHONs, TPN-V to TLHONs.

			TLHON post-	
			882995659	914027038
OPN pre-	VL2p_adPN	1944507292	27	30
	VL2a_adPN	5813069089	24	4
	VM4_lvPN	1850024931	10	12
	VM4_lvPN	726207450	6	6

LHPV pre-	LHPV4i1	699515201	8	12
	LHPV4a6	729867427	13	4
	LHPV6k1	758239379	6	4
	LHPV2a3	759582415	14	6
	LHPV4j1_a	759888294	5	6
	LHPV4i1	759888465	5	6
	LHPV4j1_b	760570054	7	5
	LHPV2a3	789934899	10	5
	LHPV2a1_d	822005494	20	11
	LHPV4k1_a	851314221	11	3
	LHPV2a1_c	886130319	15	11
	LHPV4g1	913341138	8	4
	LHPV4f1_a	942677658	10	5
	LHPV1d1	1006146837	13	3
	LHPV2a1_c	5813014218	4	7
	LHPV2a1_c	5813041244	12	6
	LHPV6g1	5813049920	18	7

LHAV pre-	LHAV3f1	574377845	16	14
	LHAV4a2	637121971	5	5
	LHAV1a3	823999645	27	12
	LHAV2b2_a	851961337	22	9
	LHAV2b2_a	852302504	16	8
	LHAV1a3	855439675	18	4
	LHAV2b2_b	1037510115	8	6
	LHAV4f1	1259528984	11	8

OPN Class pre-	LHPV/LHAV post-
VA1v_vPN	393
VL2p_adPN	349
DA1_IPN	190
DA4l_adPN	183
DP1m_adPN	148
VC5_adPN	145
DC2_adPN	132
VL2a_adPN	118
VC3m_lvPN	98
VM1_IPN	95
DA4m_adPN	85
VA6_adPN	84
VL1_vPN	77
VM7d_adPN	75
VL2a_vPN	70
VA1v_adPN	63
VM4_lvPN	59
VA7l_adPN	52
DP1l_adPN	44
DA2_IPN	40
VC1_IPN	37
DC1_adPN	33
DL1_adPN	28
VL1_ilPN	27
DC3_adPN	25
DC4_adPN	23
DL2d_adPN	23
VL2p_vPN	20
VA2_adPN	19
VM7v_adPN	17
DL4_adPN	16
V_I2PN	14
VC5_lvPN	14
VC4_adPN	10

Supplementary Table 2, related to Figure 3. Olfactory drive to TLHONs. (See Figure 3 and methods for details). Synaptic connections between individual neurons for direct and indirect olfactory PN: OPNs to TLHONs, OPNs to LHAV/LHPV, LHAV/LHPV to TLHONs. (Right) Combined synaptic connections for identified OPN glomerulus to LHAV/LHPV.

		LH/VLP post-						
		7923262 06	821612285	851961337	852302504	915105429	103751011 5	581302153 3
TLHON pre-	882995659	31	22	20	18	39	39	51
	914027038	18	20	23	21	32	52	39

		LHAD1 7923262 06
LH/VL P pre-	821612285	50
	851961337	40
	852302504	44
	1037510115	22
	5813021533	66

		DN post										
		8871959 02	122688776 3	128132495 8	140523147 5	140696687 9	146699897 7	156584663 7	230702772 9	581302332 2	581302401 5	581305045 5
LH/VL P pre-	792326206	0	8	25	27	0	0	15	201	336	24	3
	915105429	0	41	5	6	18	16	1	0	145	27	0
	5813021533	29	0	0	0	0	0	0	0	3	0	22

Supplementary Table 3, related to Figure 7. Connectivity from TLHONs to LHAD1, LHAV2b, PVLP076, AVLP053 and to downstream DNs. (See Figure 7 and methods for details). Synaptic connections between individual neurons downstream of TLHONs: TLHONs to LH/VLP, LH/VLP feedback to LHAD1, LH/VLP to DNs

Figure	Genotype	Panel
1	VT040053-GAL4 > UAS.CD8::GFP	b
	R22C06-GAL4 > UAS.CD8::GFP	b
	VT040053-GAL4.DBD; R22C06 AD> UAS.CD8::GFP	b,g,h,j-l
	VT040053-GAL4.DBD; R22C06 AD> UAS.Kir2.1	d
	UAS.Kir2.1/ +	e
	VT040053-GAL4.DBD; R22C06 AD/ +	e
	GR28B.d-LexA> LexAop.CsChrimson, VT040053-GAL4.DBD; R22C06 AD> UAS.CD8::GFP	g,h
	R77C10-GAL4 > UAS.CsChrimson, R22C06-LexA>LexAop::GFP	g,h
2	MB247dsRed, R22C06-LexA > LexAop::GFP, nSyb-GAL4 > UAS.C3PA	a,b
	VT061933-GAL4 > UAS.CD8::GFP	g
	VT061933-GAL4 > UAS.DenMark,Syt::GFP	h
	R22C06-LexA>LexAop.P2X2, VT061933-GAL4>UAS.GCamp6m	i
	R22C06-LexA/ +, VT061933-GAL4>UAS.GCamp6m	i
	VT061933-GAL4 > UAS.Kir2.1	k,l
	VT061933-GAL4/ +	k,l
	UAS.Kir2.1/ +	j
3	VT061933-GAL4 > UAS.Kir2.1	d,g
	UAS.Kir2.1/ +	e,g
	VT061933-GAL4/ +	e,g
	WT	h
	Orco ²	h
4	VT061933-GAL4 > UAS.CD8::GFP	b-e,g,h,k
	VT040053-GAL4.DBD; R22C06 AD> UAS.CD8::GFP	i,j
5	VT061933-GAL4/ +	b-e,g
	VT061933-GAL4 > UAS.Kir2.1	b-d,f,g
	UAS.Kir2.1/ +	c-e,g
7	VT019018 AD; VT017411-GAL4.DBD / +	e
	VT019018 AD; VT017411-GAL4.DBD > UAS.Kir2.1	e
	VT023490 AD; R38F04-GAL4.DBD > UAS.Kir2.1	e

Supplementary Table 4. Table of Genotypes