Cinnamaldehyde-evoked scratching in mice requires direct or indirect activation of TRPV4- and TRPV1- but not TRPA1-expressing sensory neurons

E. Carstens, Taylor Follansbee, Dan Domocos, Amanda Nguyen, Zihao Cai, Mirela Iodi Carstens

Neurobiology, Physiol. And Behavior, Univ of California Davis CA

INTRODUCTION

Cinnamaldehyde (CA) elicits itch sensation in humans.[1,2] We presently investigated the involvement of TRPV1, TRPA1 and TRPV4 channels in CA-evoked itch behavior, as well as activation of dorsal root ganglion (DRG) cells by CA.

[1]Namer et al., *Neurorep* 16:955-9, 2005

[2]Hojland et al., *Acta Derm Venereol* 95 :798-803, 2015

METHODS

Female and male C57BL6 wildtype (WT) and TRPV1 knockout (KO), TRPA1KO and TRPV4KO mice were used. **Behavior:** CA (1-5% in 5% Tween80) was delivered unilaterally to the cheek via intradermal (id) injection (10 uL). Mice were videotaped and numbers of hindlimb scratch bouts, forelimb wipes directed to the injected cheek, and groom bouts, were scored offline by blinded observers. **Calcium imaging:** DRG cells from each genotype were cultured, loaded with Fura-2 and imaged ratiometrically. A gravity flow system delivered CA (300 uM) and other chemicals (AITC 100 uM; capsaicin 300 nM, histamine 50 uM, GSK1016790A 35 nM, IL-4 300 nM, KCl 50 mM). A change of >10% above baseline was considered a response.

Table 1. Calcium imaging

Genotype	CA	AITC	Histamine	Capsaicin	IL-4
WT	23.99 (149/621)	21.9 (136/621)	5.8 (36/621)	7.6 (47/621)	15.8% (35/222)
TRPV1KO	39.9* (131/328)	37.2 (122/328)	8.8 (29/328)	0 (0/328)	40.3% (65/161)
TRPA1KO	0$ (0/326)	1.5 (5/326)	1.5 (5/326)	28.8 (94/326)	0% (0/326)
TRPV4KO	11.5# (39/340)	n.t.	5 (17/340)	3.5 (12/340)	13.5% (23/170)

Table 1. Percentages of DRG cells from WT and KO mice excited by various chemicals. n.t.: not tested. Parentheses: numbers of responsive cells/ tested. *, $, #: Significantly different from WT ($p<0.05$, chi square test). Note that no TRPA1KO cells responded to CA and few to AITC, no TRPV1KO cells responded to capsaicin, and very few TRPV4KO cells (1.5%, 5/340) responded to the TRPV4 agonist GSK 1016790A. *Increase in CA sensitivity in TRPV1KO may reflect developmental compensentory upregulation of TRPA1 in cells lacking TRPV1.

Fig. 3. DRG cell responses to CA

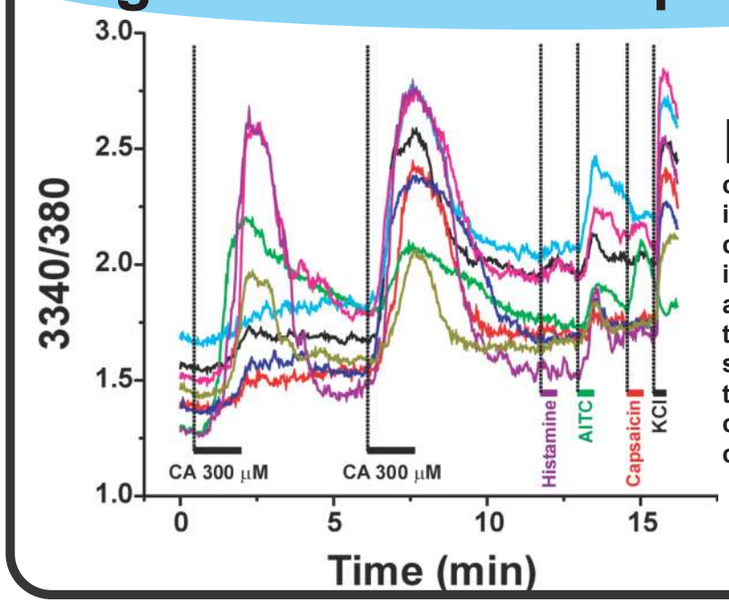

Fig. 3. Examples of WT DRG cell responses to CA. Dashed lines indicate time of application of each chemical. Here, 7/8 cells showed increased response to second application of CA (CA2) compared to first (CA1). Overall, there was a significant sensitization of responses to CA2 (see Fig. 4, blue bars). Many cells also responded to AITC and capsaicin.

Fig. 1. Behavior: cheek model

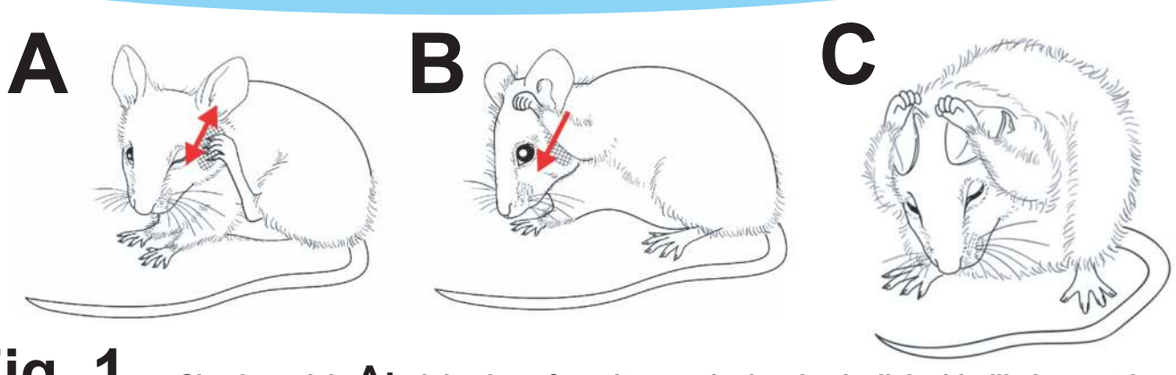

Fig. 1. Cheek model. **A:** injection of pruritogens in the cheek elicits hindlimb scratch bouts directed to the injection site. . **B:** Injection of algogens into the cheek elicits brief forepaw wipes across the injected cheek. **C**: facial groming. Shimada & LaMotte, Pain 139:681-7, 2008.

Fig. 4. Sensitization of DRG cell responses to CA is enhanced by IL-4

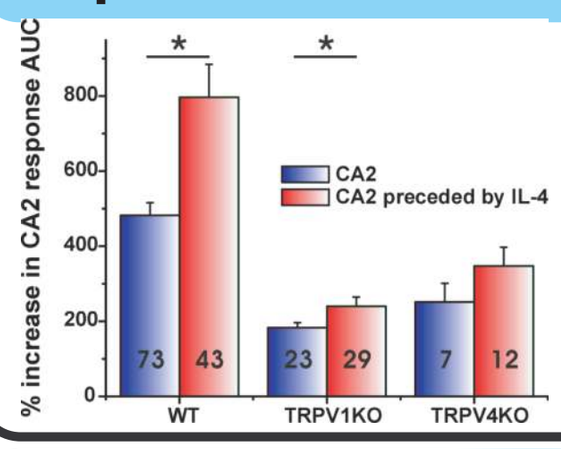

Fig. 4. Bars represent mean area under the curve (AUC) of DRG cell responses to a second application of CA (CA2), relative to the normalized response to an initial application of CA (CA1) 4 minutes earlier. Numbers represent the sample size. Responses to CA2 were significantly greater than responses to CA1 for all genotypes ($p<0.001$, paired t-test). Blue bars: response to CA2 following CA1. Red bars: response to CA2, preceded 1 min earlier by application of IL-4. *: $p<0.05$, unpaired t-test. Error bars: SEM.

Fig. 2. Reduced CA-evoked scratching in TRPV1 & TRPV4 KOs.

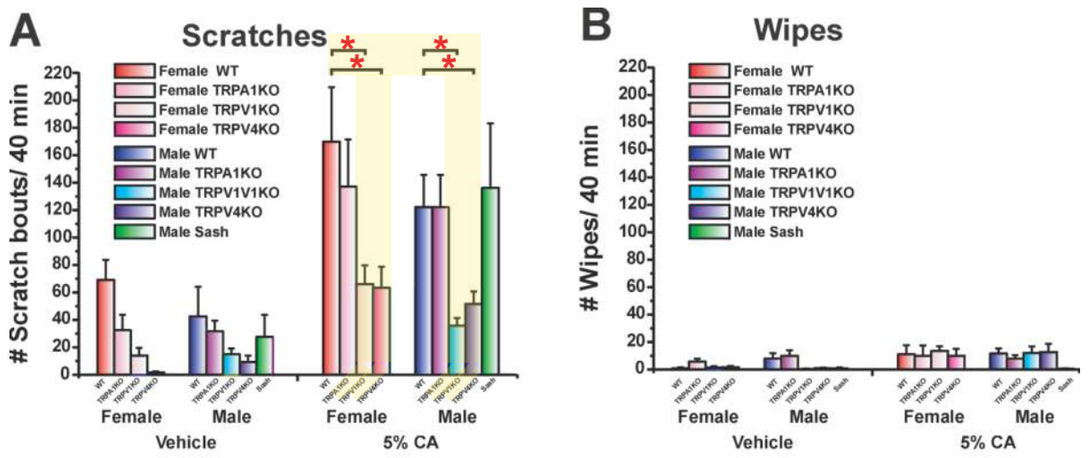

Fig. 2. Scratching elicited by intradermal injection of CA, but not wiping or grooming, was reduced in TRPV1 and TRPV4 KOs. Note that CA-evoked scratching was not reduced in TRPA1 KOs or in male Sash (mast cell deficient) mice.

A: Female (red) and male (blue) scratch bouts. * :significantly different from WT ($p<0.05$, unpaired t-test, n= 5-7/group).

B: wipes. No significant differences among sex or genotype.

C: grooming bouts. No significant differences among sex or genotype.

Fig. 5. DRG cell responses to IL-4

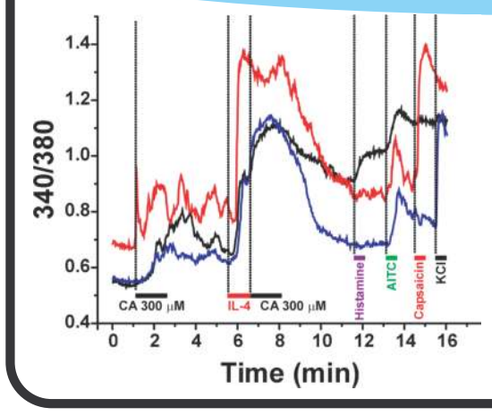

Fig. 5. CA is a contact sensitizer that triggers activation of Th2 cells that release cytokines. IL-4 excites DRG cells[3]. We reasoned that IL-4 might mediate CA-evoked itch via release of IL-4 from immune cells to excite pruriceptors. Here we show examples of WT DRG cell responses to IL-4 (format as in Fig. 3). IL-4 further sensitized responses to CA2 (Fig. 4, red bars) in WTs and TRPV1KOs.

3: Oetjen et al., *Cell* 171:217-228, 2017.

CONCLUSIONS

1. CA evokes scratching in a TRPV1/TRPV4-dependent manner
2. No sex difference
3. CA excites fewer DRG cells from TRPV4KOs
4. CA sensitizes DRG cell responses to subsequent CA
5. IL-4 requires TRPA1 to excite DRG cells and sensitizes responses to CA

CA-evoked itch is independent of TRPA1

ACKNOWLEDGMENTS

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