Responses of TRPV1 Knockout Mice to Trigeminal Irritants in Two Different Behavioral Assays

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Introduction

The trigeminal nerve is composed of polymodal neurons which innervate the nasal cavity, nasopharynx, oral cavity and cornea.[1] Although trigeminal nociceptive fibers are stimulated by a wide variety of chemical irritants, the mechanism of stimulation for many compounds is unclear. One of the best understood compounds is capsaicin which activiates TRPV1 channels.[2] In the present study two different behavioral assays were used to determine whether TRPV1 knockout mice detect trigeminal irritants with the same efficiency as wildtype (C57Bl/6J) mice.

Methods

Animals: Female mice were purchased from Jackson Labs (Bar Harbor, ME). 16 wildtype mice and 12 TRPV1-/- mice were used in the following experiments. During breaks between experiments, mice were housed in groups of 4, allowed food and water *ad libitum* and were maintained on a 12 hour light cycle. All animals were examined in both behavioral assays.

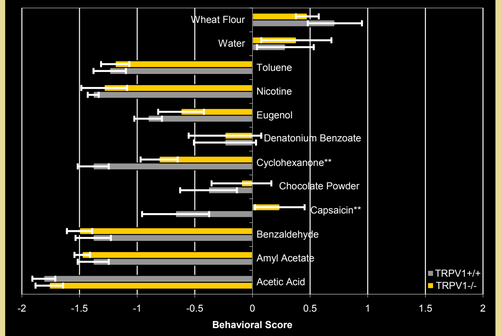
Cotton Swab Test: For liquid stimuli, cotton applicators were saturated with the compound before presentation to the animal. For powdered stimuli, the applicator was saturated with distilled water then placed in the compound so that it would adhere to the cotton. The stimulus presentation was alternated between rewarding (e.g. wheat flour) and aversive (e.g. capsaicin) compounds so animals would not associate the cotton applicator with noxious stimuli. Each animal was presented with each compound three times and their reaction to the stimulus was scored as follows:
-2 Trigeminal Reflex, -1 Aversive Response, 0 Neutral Response, +1 Favorable Response, +2 Feeding Response.

Cotton Swab Test

Diagram modified from Alimohammadi, 2004

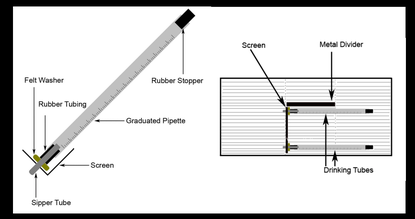
Behavioral Score

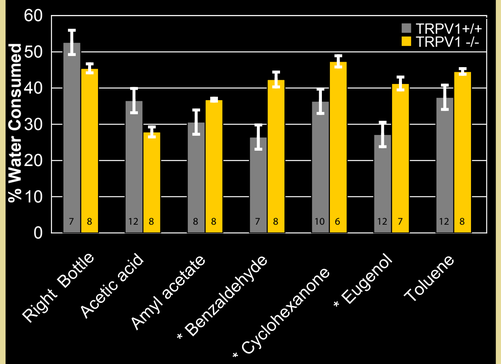
-2	Trigeminal Reflex:	Reflexive Withdrawal Movement
-1	Aversive Response:	Head Turn or Rejection Movement
0	Neutral Response:	No Response or Lack of Interest
+1	Favorable Response:	Sniffing & Investigation
+2	Feeding Response:	Attempted Feeding Behavior



TRPV1 knockout mice were significantly less averse to capsaicin and cyclohexanone than wildtype mice in the cotton swab test. Each bar represents mean ± SEM of 7 mice.
* p<0.05 ** p<0.01 ***p<0.001 by one-tailed t-test.

Free Choice Drinking Test





TRPV1-/- mice were significantly less averse to benzaldehyde, cyclohexanone and eugenol than wildtype mice in the free choice test. Each bar represents mean ± SEM. n for each group is displayed on bar. * p<0.05 **p<0.01 ***p<0.001 by one-tailed t-test.

Methods (continued)

Cotton Swab Test (continued): An individual's average score for each compound was used to produce a group average.[3,4] Independent, one tailed t-tests were run to detect differences between the responses of wildtype and TRPV1-/- mice.

Free Choice Test:Felt washers were placed over the sipper tube of two water bottles and protected with a wire screen. Each washer was soaked in either the irritant of interest or distilled water. Individual mice were housed with these tubes and water consumption was measured over 24 hrs. After the initial recording period, the washers were refreshed. The bottle's locations were switched to control for side basis and water consumption was measured over another 24 hrs. The values from individuals on the two days were summed and normalized to the total amount of water consumed. An independent, one tailed t-test was used to detect differences between the amount of water consumed by wildtype and TRPV1-/- mice.

Discussion

The results indicate cyclohexanone and eugenol stimulate TRPV1 which is consistent with previous work.[5,6] Additionally, they suggest benzaldehyde stimulates TRPV1. Since differences in responses to benzaldehyde were only detected with the free choice drinking assay, that assay may be more sensitive than the acute behavioral test. The two bottle assay can be used to screen for the ability to detect irritants.

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3 Alimohammadi, H. Trigeminal nerve-mediated nasal chemesthesis: chemosensory mechanisms of the Vth cranial nerve. PhD dissertation, Wake Forest University, 2004.
4 Alimohammadi, H & WL Silver. 2004. . Patterns of Variation in the Behavioral Responses of Rats to Irritants After Neonatal Capsaicin Treatment. AChemS XXVI, Sarasota, Fl, April 21-25, 2004.
5 Silver WL, TR Clapp, LM Stone & SC Kinnamon. 2006. TRPV1 Receptors and Nasal Trigeminal Chemesthesis. Chem Senses 31: 807-812.
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