Genetic Identification of C fibres that detect massage-like stroking of hairy skin in vivo

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Background:

Nociception

- Nociceptors: Sensory neurons that respond to painful stimuli.
  - Can respond to thermal, mechanical, or chemical stimuli.
  - Axons of Nociceptors can fall under two categories: C fibers or Aδ fibers. We'll focus on C fibers.

- C fibers are thin, unmyelinated axon fibers that are involved in nociception.
  - When a painful stimulus is detected, a signal is carried toward the spinal cord via an afferent neuron. The signal crosses the midline of the spinal cord and is transmitted upward to the brain.
  - Sensation of touch does not cross the midline and is transmitted upward on the same side of the spinal cord where it entered.

Receptors and Neurotransmitters

- Signals are transmitted from neuron to neuron by neurotransmitters.
- Neurotransmitters bind with receptors on the post-synaptic cell and cause a change in the post-synaptic membrane potential.
- Receptors are ionotropic or metabotropic.
- The MRPRB4+ and MRGPRD+ receptors are metabotropic.
- Different neurons express different receptors.

Methods

- Two types of mice were used:
  - Mrgprb4−/−;tdTomato−2A−cre mice
  - MrgprD−/−;EGFP−cre mice
- Each type was genetically targeted with an adenovirus-associated virus (AAV) expressing GCaMP3.0
Creation of mrgb4 knockout mice

The adenovirus showed high specificity and efficiency

Brush/pinch stimulus delivery

- Due to problems measuring the neural potentials electronically, neural activity was measured via 2-photon microscopy using a laminectomy.
- Responses were elicited using stroking or pinching stimuli.
- The mouse was anesthetized, held in position, and either stroked or pinched while an infrared microscope monitored neural activity in the spinal cord.

GCaMP3.0

- GCaMP3.0 is a fluorescence protein created by fusion of GFP and Calmodulin.
- During depolarization, voltage-gated calcium channels open and calcium enters the cell. Calmodulin is one protein that binds calcium.
- When the GCaMP3.0 complex is not bound to calcium, its conformation is such that it is quenched by the surrounding solvent.
- When calcium is bound, a conformational change occurs, allowing the GFP unit to absorb radiation in the infrared region and emit light in the visible region. This forms the basis of fluorescence.
Two Photon Excitation Microscopy

- Developed by Winfried Denk at Cornell University in the early 1990’s.
- Type of fluorescence microscopy that allows high resolution imaging of living tissue.
- Beam of infrared light is directed at specimen. When the fluorophore absorbs two infrared photons simultaneously, it is elevated to its excited state and emits one photon of lower energy.
- Emitted photon is typically in the visible light spectrum, allowing visualization of protein activity.

Methods

- Applied KCl directly to the exposed spinal cord and measured fluorescence response.
- Applied Methylene ATP to peripheral regions via injection.
- Injected capsaicin in mice genetically engineered to express the TRPV1 receptor.
- Demonstrated that the preparation was suitable to detect calcium transients from both central and peripheral stimulus.
- Fluorescence was quantified by measuring ΔF/F.

Chemical Stimulation of MRGPRD+ and MRGPRB4+

MRGPRD+ response to central administration of KCl.

MRGPRB4+ response to central administration of MeATP administered peripherally.
Methods

- After successfully showing a change in fluorescence from chemical stimuli, mechanical stimuli was tested.
- Hindpaw was stimulated with either a brush or pinched with tweezers.
- ΔF/F was measured in each type of mouse in response to each type of stimulus.

Methods

- Tested to see if mice developed a behavioral preference for activation of MRGPRB4+ neurons.
- Juvenile mice were injected with an AAV that encoded the hM3(Gq-coupled) DREADD.
- DREADD = Designer Receptors Exclusively Activated by Designer Drugs.
- DREADD's used in conjugation with a specific pharmacological agent can trigger depolarization in the membrane in which the DREADD exists.
- Clozapine-N-oxide (CNO) was used to activate the DREADD and was utilized in a chamber preference test.

Methods

- Mice were conditioned for 4 days before the test was run.
- Trained to prefer one chamber over another.
- Test to see if activation of MRGPRB4+ neurons can increase the amount of time mice spend in the non-preferred chamber.
- Test results showed that mice showed preference for the CNO chamber.

Preference Results

- Mice that initially did not prefer I.N.P. spent significantly more time in the non-preferred chamber once CNO was added.
- Mice that initially preferred I.P. spent significantly less time in the chamber once saline was placed in it.
Conclusions

- MRGPRB4 is similar to the MRGPRD receptor, which detects pinching stimuli.
- Its nerve-nets resemble receptive fields of C-tactile afferents in humans.
- This receptor innervates hairy skin.
- Its hypothesized function is detection of pleasant stroking stimuli associated with grooming.
- Unlike similar receptors, MRGPRB4 receptors could not be activated ex vivo.

- This is the first study to conclusively show that the pleasant sensation produced by petting is controlled by a specific neuron:
- This is significant because the positively reinforcing stroking sensation is integral to mammalian grooming and social interactions.
- The isolation of the MRGPRB4+ receptor will surely spur further research into mammalian social grooming behaviors.

Questions

- 1. Why would the evolution of receptors specialized for detecting hair-stroking stimuli be important for the survival of social mammals?
- 2. Why might the MRGPRB4+ neurons have been difficult to activate in isolated skin nerve preparations?
- 3. How did the researchers test to see whether the mice found the activation of the MRGPRB4+ pathway pleasurable?
- 4. What is so significant about the axons in the MRGPRB4+ pathway? (hint: pain)