

The role of Na_v1.7 and methylglyoxal-mediated activation of TRPA1 in itch and hypoalgesia in a murine model of type 1 diabetes

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Supplementary Methods

Supplementary Figures 1-2

Supplementary Methods

Behavioral testing

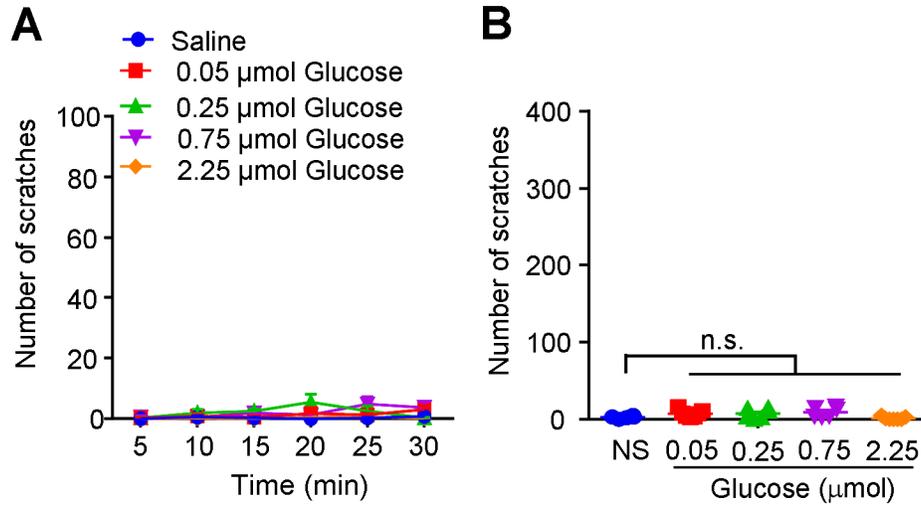
Neck model of itch: Mice were shaved at the nape of the neck more than 2 day prior to experiments as described previously. On the day of behavioral testing, mice were individually placed in small plastic chambers (10 × 10 × 12.5 cm) on an elevated metal mesh floor and allowed at least 30 min for habituation. Under brief anesthesia of isoflurane, mice were given an intradermal injection of 50 µl of agents via a 26 G needle into the nape of the neck. Immediately after the injection, mice were returned to their chambers and were videotaped from a side angle for 30 min (Sony HDR-CX610). The video was subsequently played back offline and scratching behavior was quantified by observers blind to the treatments or genotypes of animals. A scratch was counted when a mouse lifted hind paw to scratch the shaved region and returned the paw to the floor or to the mouth for licking.

Cheek model of itch: To distinguish itch and pain behavior, we used the cheek model by injection of chemicals into the cheek of mice. Mice were shaved on cheeks (approx. 5 × 8 mm area) at least 2 day before the experiment. On the day of experiment, mice were intradermally injected of 20 µl of agents via a 26G needle into the cheek under brief anesthesia with isoflurane. Immediately after the injection, mice were returned to their chambers and were video recorded for 30 min. The video was played back and the number of wipes and scratches were quantified by observers blind to the treatments. One wipe was defined when mouse unilaterally wipes the injected site with the forelimb, which was not part of grooming behavior. One scratch was defined as a lifting of the hind paw toward the injection site on the cheek and then returning the paw to the floor or to the mouth.

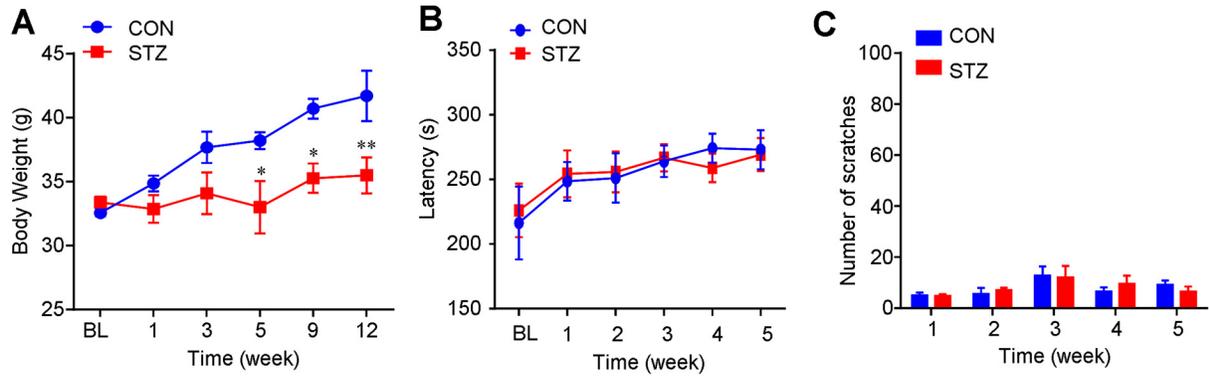
Alloknesis assay: Alloknesis after acute itch and chronic itch was evaluated as previous reports. For testing alloknesis after acute itch, a von Frey filament (0.7 mN) was applied to the skin area 5 mm outside the injection site and 30 min after the MGO injection. A scratch bout directed to the site of mechanical stimulation was considered as a positive response. The alloknesis score was determined by calculating the total number of scratching reaction elicited by ten mechanical stimuli. For testing alloknesis in diabetic model, von Frey stimuli were applied at the nape of the neck area to elicit scratching response at 1, 3, and 5 weeks after injection of streptozotocin.

Tail immersion test: Tail immersion test was employed to assess heat pain sensitivity in mice. Briefly, the terminal 3 cm of a mouse's tail was immersed in hot water bath at 52 °C and the latency of tail flick was recorded with a cutoff time of 10 seconds to avoid potential tissue injury. Behavioral tests were videotaped from a side angle and behavioral tests were done by observers blind to the treatments or animal genotypes.

Supplementary data Figures 1-2 and legends



Supplementary Figure 1. (A-B) Time course (A) and total number (B) of scratching bouts within 30 min induced by intradermal (i.d.) injection of glucose (0.05–2.25 μmol) into the nape of neck of mice ($n = 5-6$ for each group, one-way ANOVA followed by post hoc Dunnett's test). All data are expressed by means \pm SEM. NS, normal saline.



Supplementary Figure 2. (A) Body weight increased slowly than control mice ($n = 8-10$, $*p < 0.05$, $**p < 0.01$ compared with control mice, two-way repeated-measured ANOVA). (B) Rota-rod test showed comparable motor function between control and diabetic mice ($n = 8-10$, two-way repeated-measured ANOVA) (C) Diabetic mice did not show obvious spontaneous scratching behavior ($n = 8-10$, two-way repeated-measured ANOVA). All data are expressed by means \pm SEM.