Supporting Information

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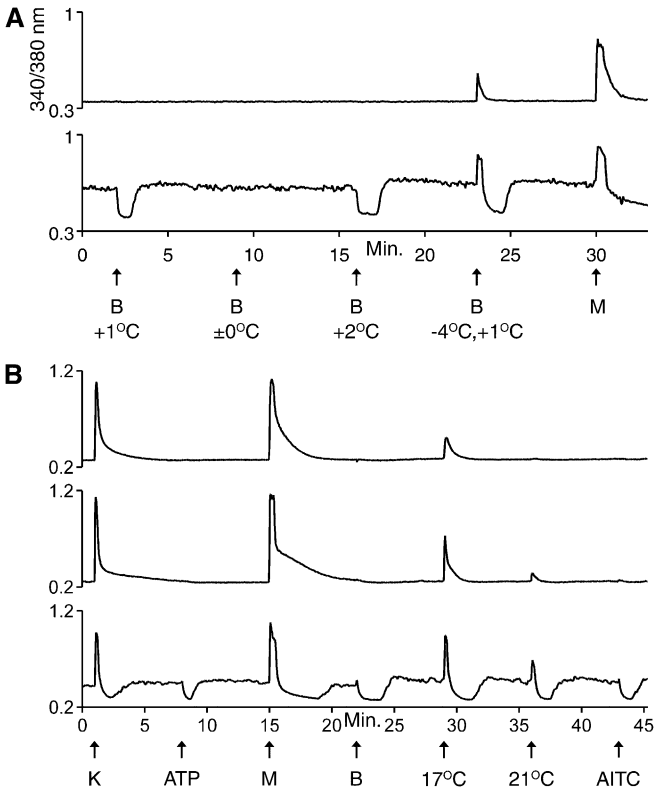

Fig. S1. Evidence for threshold differences between cold thermosensors. (*A*) The bottom trace is from a low-threshold cold thermosensor. The top trace is from a high-threshold cold thermosensor. Bath temperature at minute 0 was ~23 °C. Changes in bath temperature were monitored with a thermocoupler. Each replacement of static bath solution is designated by letter B, with an arrow indicating the time point at which the bath solution was replaced. Changes in bath temperature shown below each B indicate the temperature change from that recorded just before replacement of bath solution. At minute 2, replacement of the room-temperature bath solution transiently increased bath temperature by 1 °C (+1°C), resulting in a transient downward deflection of the $[Ca^{2+}]_i$ baseline, followed by evaporative cooling and return of $[Ca^{2+}]_i$ to the previous baseline. At minute 9, the replacement bath solution was taken from an adjacent well that was allowed to precool by evaporative cooling. The bath replacement with this solution did not cause a change in the bath temperature or any alteration of the $[Ca^{2+}]_i$ baseline. The third bath replacement, at minute 16, was done with a bath solution elevated to 2 °C above the prior static bath temperature. At minute 23, the static bath solution was replaced with solution precooled to 4 °C lower than the static bath solution, causing an upward deflection of $[Ca^{2+}]_i$, immediately followed by washing with room-temperature bath solution that caused a transient temperature increase of 1 °C over the prior static bath temperature. (*B*) The bottom trace is from a low-threshold cold thermosensor, which responded to application of 17 °C and 21 °C bath solution. The top two traces are from high-threshold cold thermosensors that responded to 17 °C bath solution. One did not respond to 21 °C bath solution, and the other responded only slightly. Notably, all low-threshold cold thermosensors responded to 21 °C bath solution, whereas approximately half of the high-threshold cold thermosensors responded very weakly.

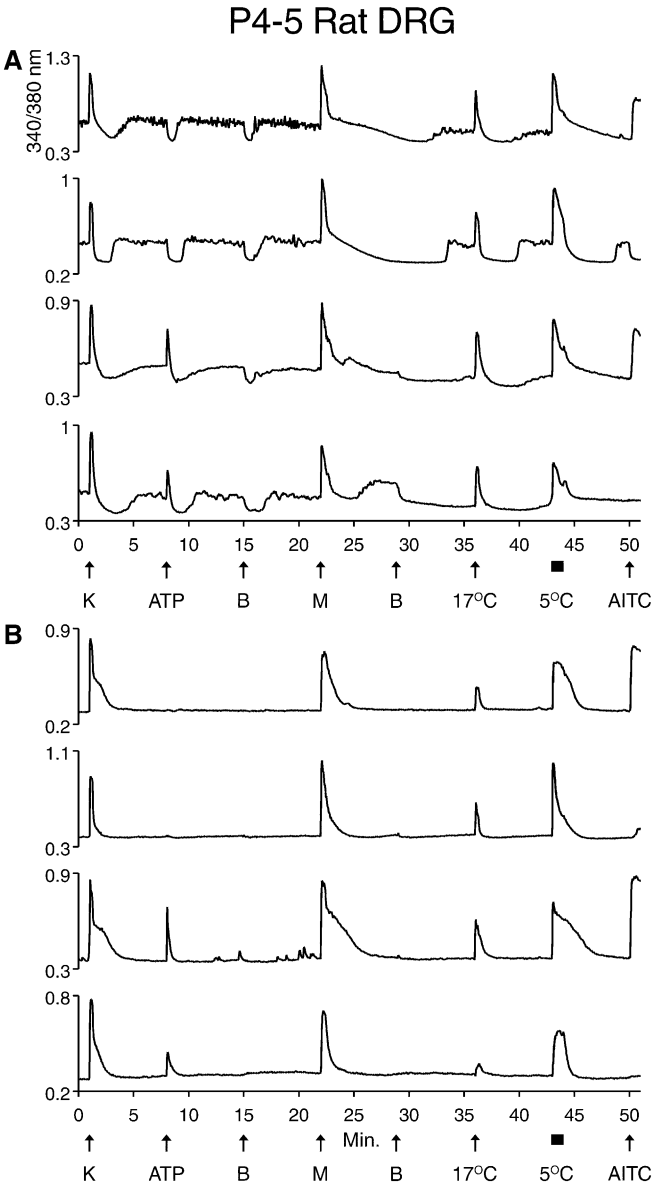

Fig. S2. ATP sensitivity of cold thermosensors from postnatal day (P)4–5 rat dorsal root ganglia (DRG). Shown are traces from cold thermosensors of P4–5 rat DRG for comparison with traces from cold thermosensors of P4–5 mouse DRG neurons shown in Fig. 3. (*A*) Four traces from rat low-threshold cold thermosensors. Top trace is from a neuron that responded to 100 μM allyl isothiocyanate (AITC), but not to 20 μM ATP. The second trace is from a neuron that did not respond to either ATP or AITC. The third trace is from a neuron that responded to both ATP and AITC. The bottom trace is from a neuron that responded to ATP, but not AITC. (*B*) Four traces from rat high-threshold cold thermosensors. The top trace is from a neuron that responded to AITC, but not ATP. The second trace is from a neuron that did not respond to either ATP or AITC (or possibly had a very weak response to AITC). The third trace is from a neuron that responded to both ATP and AITC. The bottom trace is from a neuron that responded to ATP, but not AITC.

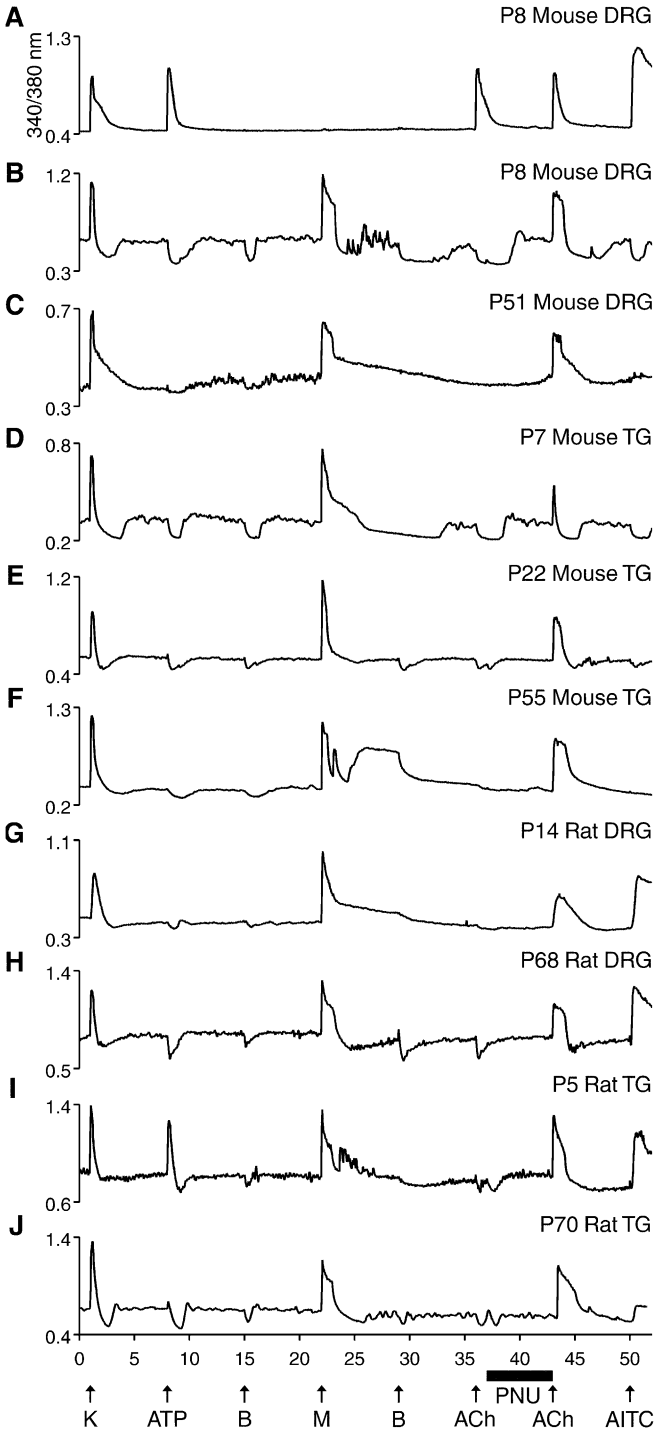

Fig. S3. Consistency of α7 nicotinic acetylcholine (ACh) receptors (nAChRs) responses (and not other nAChR subtypes) across a subset of cold thermosensors from mouse and rat DRG and trigeminal ganglia (TG) neurons. The same experimental protocol shown in Fig. 5*A* was used here. (*A*) This trace (same as in Fig. 5*A*) is shown as an example of a neuron that responded to 1 mM ACh before application of PNU-120596 (PNU). (*B–J*) Other traces from low-threshold cold thermosensors from mouse and rat DRG and TG cultures across a broad age range. Although not all of the low-threshold cold thermosensors responded to ACh in the presence of PNU, none ever responded to ACh in the absence of PNU, suggesting that this neuronal subclass only expresses α7 nAChRs and not other nAChR subtypes. High-threshold cold thermosensors also only responded to ACh in the presence of PNU (not shown).

Table S1. Constellations of receptors and ion channels expressed within the predominant variants of mouse cold-thermosensor neurons

Cell-specific constellations	Neonatal mice	Mature mice
Low threshold		
TRPM8 channel	+	+
Low K_V1.1/1.2 channels		
ATP receptor	+/–	–
TRPA1 channel	–	–
α7 nAChR	+/–	+/–
Predominantly Ca_V1 channels		*
Predominantly TTX-S Na_V channels		*
High threshold		
TRPM8 channel	+	+
High K_V1.1/1.2 channels		
ATP receptor	+/–	–
TRPA1 channel	–	–
α7 nAChR	+/–	+/–

+, Functional expression of the specified receptor or ion channel was detected; –, functional expression of the specified receptor or ion channel was not detected; +/–, mixed population of neurons that either functionally expressed or did not functionally express the specified receptor or ion channel; *, determined in a previous study (1). Notably, not all variants are included here. For example, functional expression of TRPA1 channels was detected in <5% of mouse cold thermosensors. Rat cold-thermosensor variants were essentially the same as mice, with the exception that >65% of rat cold thermosensors functionally expressed TRPA1 channels. Expression of K_V1.1/1.2 channels was not assessed in rat neurons, but expression patterns of ATP receptors and α7 nAChRs were similar to those in mouse.

1. Teichert RW, et al. (2012) Characterization of two neuronal subclasses through constellation pharmacology. *Proc Natl Acad Sci USA* 109(31):12758–12763.