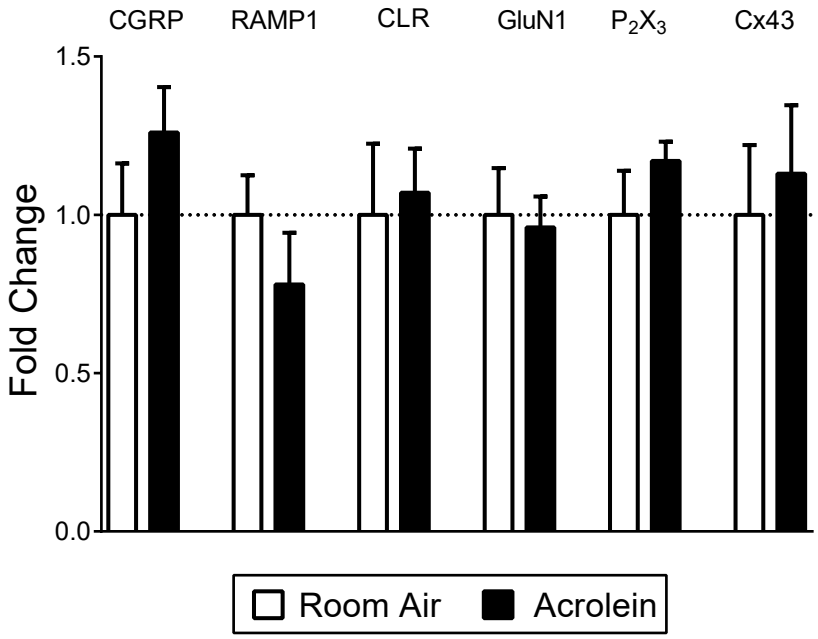
Supplemental Data

Supplemental Table 1. Real-Time qPCR Primers

Name	Accession #	Primer Sequence (5'-3')
GluN1	NM_017010	F:ATG GCT TCT GCA TAG ACC R:GTT GTT TAC CCG CTC CTG
P2X3	NM_001270621	F:AAG AAG GGG CTG CTA TTT CTG C R:AGG CAT GCA AGG GGT AAA G
CGRP	NM_001033956	F:AAG TTC TCC CCT TTC CTG T R:GGT GGG CAC AAA GTT GTC CT
RAMP1	NM_031645	F:ACT GGG GAA AGA CCA TAG GGA R:AGT CAT GAG CAG TGT GAC CGT A
CLR	NM_012717	F:CTG CAA GGT GTC CCA GTT CA R:CAG GCA GGA AGC AGA GGA AA
Cx43	NM_012567	F:TGG GGG AAA GGC GTG AG R:CTG CTG GCT CTG CTG GAA GGT

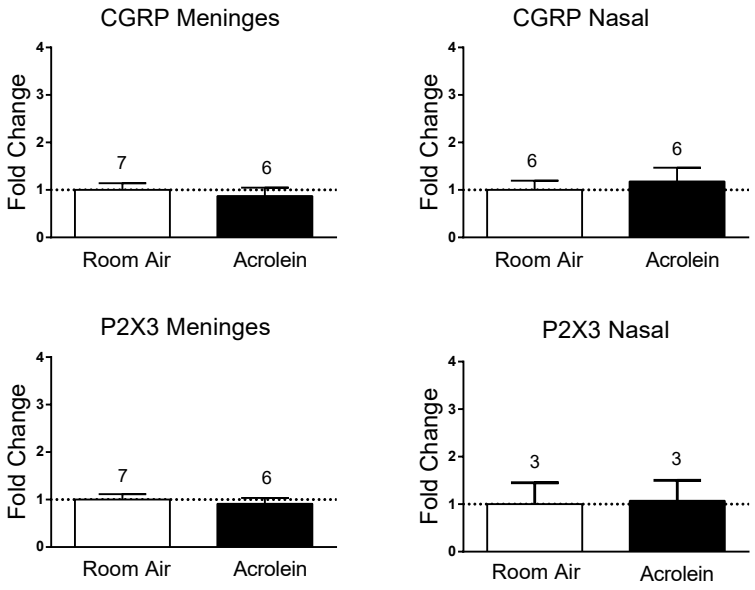
Supplemental Table 1. Primers were designed using IDT software (IDT, Skokie, IL) and verified by BLAST search. Primer pairs were used for RT-qPCR as described in methods for analysis of whole ganglion or laser dissection isolated cell populations.



Supplemental Figure 1. Fold changes in expression levels of signaling molecules in whole trigeminal ganglia following acrolein exposure. Relative expression levels of CGRP, RAMP1, CLR, GluN1, P_2X_3 and Cx43 do not differ in the trigeminal ganglia of rats exposed to acrolein vs room air. Each sample's value is normalized to β-actin values using the $\Delta\Delta C_T$ method and averaged across groups. Values are represented as mean ± S.E.M. N = 5 – 9 animals per group.

We compared mRNA levels of CGRP, its receptor subunit components RAMP1 and CLR, the glutamate receptor NR1, the purinergic receptor P_2X_3 and the gap junction hemichannel Cx43 in whole trigeminal ganglia following acrolein or room air exposure. As determined by RT-PCR, mRNA levels of CGRP (1.26 ± 0.43, n = 9), RAMP1 (0.78 ± 0.49, n = 9) and CLR (1.07 ± 0.34, n = 6) were not changed in acrolein-exposed animals relative to room air-exposed animals (1.0 ± 0.46, n = 8), (1.0 ± 0.33, n = 7) and (1.0 ± 0.55, n = 6) respectively. Likewise, message levels of GluN1 (0.96 ± 0.24, n = 6), P_2X_3 (1.17 ± 0.15, n = 6) and Cx43 (1.13 ± 0.53, n = 6) were not

changed in acrolein-exposed animals relative to room air-exposed animals (1.0 ± 0.33, $n = 5$), (1.0 ± 0.34, $n = 6$) and (1.0 ± 0.54, $n = 6$) respectively.



Supplemental Figure 2. Fold changes in expression levels of CGRP and P2X3 mRNA in meningeal and nasal afferent populations of trigeminal ganglia neurons following acrolein exposure. No change in expression of CGRP mRNA was observed in either nasal or meningeal labeled trigeminal neurons following acrolein exposure compared with room air-exposed animals. Likewise, no change in P2X3 mRNA levels was observed in either meningeal or nasal afferent samples following acrolein exposure. Each sample's value is normalized to β-actin values using the $\Delta\Delta C_T$ method and averaged across groups. Values are represented as mean ± S.E.M. Number of animals per group are indicated. * $P < 0.05$ compared to mRNA expression change in room air-exposed animals.

CGRP mRNA levels were not changed in acrolein-exposed animals relative to room air exposed animals in either the meninges cell sample (0.87 ± 0.44, ($n = 6$) vs 1.0 ± 0.35, $n = 7$) or the nasal cell sample (1.18 ± 0.71, ($n = 6$) vs 1.0 ± 0.48, $n = 6$) respectively. Likewise, no differences were observed in P_2X_3 mRNA levels in acrolein-exposed animals relative to room air exposed

animals in either the meninges cell sample (0.91 ±0.31, (n = 6) vs 1.0 ± 0.30, n = 7) or the nasal cell sample (1.07 ± 0.75, (n = 3) vs 1.0 ± 0.78, n = 3) respectively.