

Nociceptor Neurons Control Pollution-Mediated Neutrophilic Asthma

Jo-Chiao Wang, Amelia Kulle, Theo Crosson, Amin Reza Nikpoor, Surbhi Gupta, Anais Roger, Moutih Rafei, Ajitha Thanabalasuriar, Sebastien Talbot 

Department of Pharmacology and Physiology, University de Montreal, Montreal, Canada • Department of Microbiology and Immunology, McGill University, Montreal, Canada • Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Canada • Department of Pharmacology and Therapeutics, McGill University, Montreal, Canada • Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

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This **important** work shows that fine particulate matter exposure to the lungs led to nociceptor-dependent neutrophilic inflammation. Likely macrophage-neuronal crosstalk, via release of artemin from macrophages and activation of Gfra3 on the JNC neuron, potentiated the response. The data **convincingly** strengthens links between pollutants, immune and neural interactions.

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Abstract

The immune and sensory nervous systems, having evolved in parallel, communicate through shared receptors and transmitters to maintain homeostasis and respond to both external and internal disruptions. Although neural responses often confer protective benefits, they can also exacerbate inflammation during allergic reactions such as asthma. In our study, we modeled pollution-exacerbated asthma by exposing mice to ambient PM_{2.5} particles alongside ovalbumin. Compared to exposure to ovalbumin alone, this co-exposure significantly increased the numbers of neutrophils and $\gamma\delta$ T cells in bronchoalveolar lavage fluid. We found that silencing nociceptor neurons at the peak of inflammation using intranasal QX-314 or ablating TRPV1-expressing neurons reduced lung neutrophil accumulation. Live *in vivo* intravital imaging confirmed that neuronal ablation reduced neutrophil numbers and increased their net displacement capacity. In neurons isolated from mice with pollution-exacerbated asthma, the chemical-sensing TRPA1 channel exhibited heightened sensitivity to its cognate ligand. Elevated levels of artemin were detected in the bronchoalveolar lavage fluid of pollution-exposed mice but returned to baseline in mice with ablated nociceptor neurons. Alveolar macrophages expressing the pollution-sensing aryl hydrocarbon receptor were identified as a putative source of artemin following exposure to PM_{2.5}. This molecule enhanced TRPA1 responsiveness and, in turn, drove nociceptor-mediated neutrophil recruitment, revealing a novel mechanism by which lung-innervating neurons respond to air pollution in the context of allergy. Overall, our findings suggest that targeting artemin-driven pathways could provide a therapeutic strategy for controlling

neutrophilic airway inflammation in asthma, a clinical condition typically refractory to treatment.

Introduction

Organisms have evolved sophisticated fail-safe systems to preserve homeostasis, integrating threat detection, reflex responses, and tailored immune mechanisms¹. These systems are jointly orchestrated by the immune and sensory nervous systems, which are designed to sense external threats and internal disturbances. Both systems rely on shared metabolic pathways and signaling molecules—including receptors, cytokines, and neuropeptides². This common molecular framework supports constant interactions between these systems, which play pivotal roles in monitoring barrier tissues (e.g., skin³, lung^{4,5}, and gut⁶), activating anticipatory immune responses⁷, and managing pathologies such as allergies^{8,9}, infections¹⁰, and malignancies^{11–14}.

In the context of lung disease, vasoactive intestinal peptide (**VIP**), released by pulmonary sensory neurons following IL-5 stimulation, fosters allergic inflammation by acting on CD4⁺ T cells and innate lymphoid type 2 cells (**ILC2s**). This action elevates T helper type 2 (**T_H2**) cytokines, which are central drivers of asthmatic responses^{15,16}. Expanding on these insights, our work shows that vagal nociceptor neurons contribute to airway hyperresponsiveness^{1,17}, mucus metaplasia^{18,19}, and the detection of immunoglobulins E and G^{20,21}. These neurons also mediate antibody class switching in B cells²² and promote IgG production²³. More recently, we discovered that a subset of nociceptors is reprogrammed by the asthma-associated cytokine IL-13, rendering them susceptible to the inhibitory influence of neuropeptide Y (**NPY**) secreted by lung-innervating sympathetic neurons²⁴.

Wildfire frequency and severity increased by 2.2-fold within the last 20 years²⁵. Between 2008 and 2018 in California, wildfire-attributable PM_{2.5} exposure caused an estimated ~53,000 premature deaths²⁶. Combined with urban pollution, these events have driven escalating levels of particulate matter (PM_{2.5}), shifting asthma phenotypes from more responsive T helper 2 (T_H2) / eosinophilic types to treatment-resistant neutrophilic and mixed T_H17/T_H1 variants^{27–32}. Thus, recent estimates suggest that neutrophilic asthma accounts for 15–25% of all asthma cases, with about half of these resistant to standard therapies³³.

To investigate whether neuronal silencing can mitigate neutrophilic airway inflammation, we used an innovative asthma model which combines ovalbumin with fine particulate matter (**FPM**). We further explored the molecular mechanisms underlying heightened neuronal sensitivity in this setting, identifying artemin—produced by alveolar macrophages via the pollution-sensing aryl hydrocarbon receptor (**AhR**)—as a critical mediator of this hypersensitivity.

Results

Heightened nociceptor sensitivity in pollution-exacerbated asthma

Prof. Ya-Jen Chang's group developed a novel method to model pollution-exacerbated asthma and investigate the impact of fine particulate matter (**FPM**) on airway inflammation³⁴. Their work demonstrates that FPM exposure induces airway hyperreactivity and neutrophilic inflammation, promotes T_H1 and T_H17 immune responses, and increases epithelial cell apoptosis rates³⁴. Notably, γδ T cells significantly contribute to both the inflammation and airway hyperreactivity phenotypes by producing IL-17A³⁴. Building on this foundation, we explored whether lung-

innervating nociceptor neurons become sensitized during such asthma exacerbations. To test this, male and female C57BL/6 mice (6–10 weeks old) were sensitized with an intraperitoneal injection of ovalbumin (OVA; 200 µg/dose) and aluminum hydroxide (1 mg/dose) emulsion on days 0 and 7, followed by intranasal challenges of OVA (50 µg/dose), with or without FPM (20 µg/dose), on days 14–16. On day 17, lung-innervating jugular-nodose-complex (JNC) neurons were harvested, cultured for 24 hours, and loaded with the calcium indicator Fura-2AM (Fig. 1A). Stimulation with the TRPA1 agonist AITC (10–100 µM) and the pan-neuronal activator KCl (40 mM) revealed increased responsiveness in neurons from mice exposed to both FPM and OVA, compared to OVA-only-exposed mice, whose sensitivity was similar to that of wild-type controls (Fig. 1B–C).

Transcriptomic reprogramming of TRPV1⁺ neurons

Recent data indicate that the asthma-associated cytokines IL-4 and IL-13 can reprogram lung-innervating nociceptor neurons to adopt a pro-allergic phenotype²⁴. To investigate whether pollution-exacerbated asthma similarly reprograms these neurons, we used TRPV1^{cre}::tdTomato^{fl/wt} mice subjected to our standard OVA protocol, with or without FPM. We isolated and dissociated TRPV1⁺ JNC neurons, enriched them by excluding satellite glial and immune cells, and then purified them by FACS-sorting followed by RNA sequencing. Our analysis revealed that several differentially expressed genes (DEGs) were upregulated in the OVA-FPM group compared to naive mice (*Lifr*, *Oprm1*) (Fig. 2A–B), in OVA-FPM compared to OVA alone (*Oprm1*, *Nefh*, *P2ry1*, *Prkcb*, *Gabra1*, *Kcnv1*) (Fig. 2C–D), and in OVA alone compared to naive controls (*Cntn1*, *Piezo1*, *Npy1r*, *Kcna1*) (Fig. 2E–F). Collectively, these results suggest that pollution-exacerbated asthma both transcriptomically and functionally reprograms lung-innervating nociceptor neurons (Supplementary Table 1).

Silencing nociceptor neurons reduces inflammation

To determine whether silencing nociceptor neurons affects pollution-exacerbated asthma, we adapted a previously established neuron-blocking strategy originally developed for pain and itch neurons. This method uses nonselective ion channels (TRPA1 and TRPV1) as a drug entry port for QX-314, a charged form of lidocaine. During inflammation, QX-314 enters sensory fibers, blocking sodium currents and producing a targeted, long-lasting (>9 hours) electrical blockade of nociceptors without impairing immune cell function^{35–38}. Intranasal administration of QX-314 has already been shown to reverse allergic airway inflammation, coughing, mucus metaplasia, and hyperreactivity^{16,36,39–41}.

In our model, an intranasal dose of QX-314 administered at the peak of inflammation (day 16, 5 nmol, 50 µL) significantly reduced neutrophil counts in bronchoalveolar lavage fluid (BALF) of OVA-FPM-exposed mice, restoring them to levels typically seen in asthmatic (OVA-only) mice (Fig. 3A–B). To validate these findings, we used mice genetically engineered to either retain (TRPV1^{wt}::DTA^{fl/wt}; denoted as TRPV1^{WT}) or lack (TRPV1^{cre}::DTA^{fl/wt}; denoted as TRPV1^{DTA}) TRPV1-expressing nociceptors^{11,42,43}. Compared with controls, TRPV1^{DTA} mice had markedly fewer BALF neutrophils and lung γδ T cells (Fig. 3C–E), underscoring the critical role nociceptor neurons play in shaping the inflammatory and immune responses in pollution-exacerbated asthma.

Impact on myeloid cell motility

Building on these observations, we used intravital microscopy^{44,45} to understand how pollution-exacerbated asthma and lung innervation influence the motility of alveolar macrophages (AMs) and neutrophils. Although the total number of AMs (labelled by i.n. PKH26) remained unchanged (Fig. 4A–B), their net displacement was lower in nociceptor-ablated (Na_v1.8^{cre}::DTA^{fl/wt}; denoted as Na_v1.8^{DTA}) mice than in littermate controls (Na_v1.8^{wt}::DTA^{fl/wt};

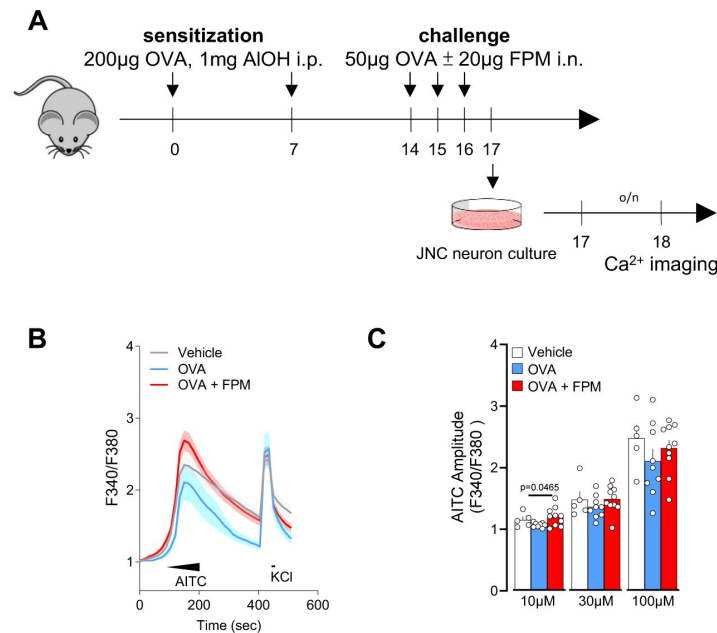


Figure 1.

Air pollution exacerbates nociceptor neuronal activity

(A–C) Male and female C57BL/6 mice (6–10 weeks old) were sensitized via intraperitoneal injection with an emulsion of ovalbumin (OVA; 200 µg/dose) and aluminum hydroxide (1 mg/dose) on days 0 and 7. On days 14–16, mice were challenged intranasally with OVA (50 µg/dose), either alone or in combination with fine particulate matter (FPM; 20 µg/dose). Bronchoalveolar lavage fluid was collected, and jugular-nodose complex neurons were cultured on day 17 for 24 hours before being loaded with the calcium indicator Fura-2AM. Cells were sequentially stimulated with the TRPA1 agonist AITC (successively to 10 µM at 60–90 seconds, 30 µM at 90–120 seconds, 100 µM at 120–150 seconds) and then with KCl (40 mM at 420–435 seconds). Calcium flux was continuously monitored throughout the experiment. The amplitude of AITC responses was measured by calculating the ratio of peak F340/F380 fluorescence after stimulation to the baseline F340/F380 fluorescence measured 30 seconds prior to stimulation. Data are plots as the per dish average of AITC and KCl responsive neurons and show that AITC (10 µM) responses was higher in JNC neurons from OVA-FPM exposed mice when compared to vehicle or OVA alone (**C**). Data are presented as means ± SEM (**B–C**). *N* are as follows: **B**: *n* = 35 neurons (control group), 19 neurons (OVA group), and 38 neurons (OVA + FPM group), **C**: *n* = 5 dishes totalling 35 neurons (control group), 8 dishes totalling 42 neurons (OVA group), and 10 dishes totalling 76 neurons (OVA + FPM group). *P*-values were determined by nested one-way ANOVA with post-hoc Bonferroni's. *P*-values are shown in the figure.

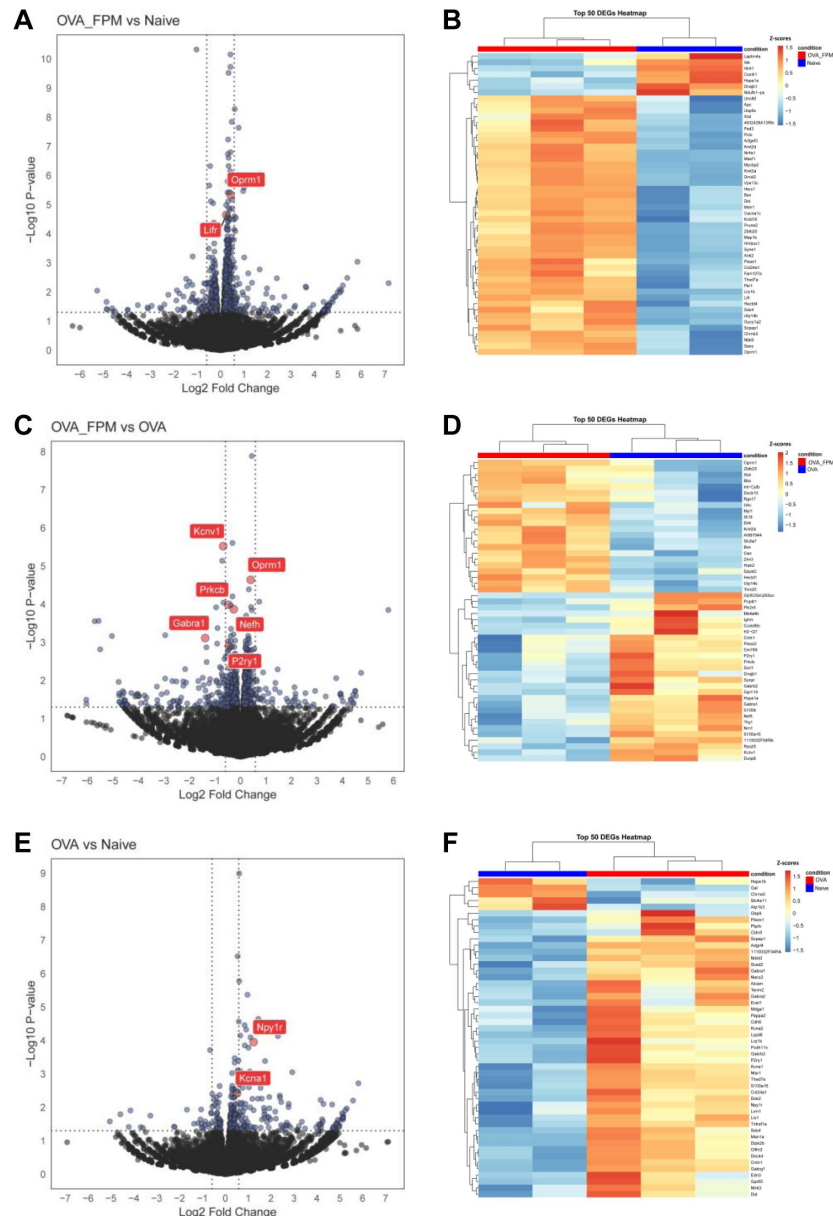


Figure 2.

Air pollution reprograms the transcriptome of nociceptor neurons

(A–F) Naïve male and female TRPV1^{cre::tdTomato}^{fl/wt} mice (6–10 weeks old) underwent either a pollution-exacerbated asthma protocol, the classic OVA protocol, or remained naïve. On day 17 (peak inflammation), jugular-nodose-complex (JNC) neurons were harvested and dissociated, and TRPV1⁺ neurons (tdTomato⁺) were sorted via FACS to remove stromal cells and non-peptidergic neurons. RNA was then isolated for sequencing.

Volcano plots (A, C, E) and heatmaps (B, D, F) show differentially expressed genes (DEGs) for three comparisons: OVA + FPM vs. naïve (A–B), OVA + FPM vs. OVA alone (C–D), and OVA alone vs. naïve (E–F). Notable genes with increased expression include *Lifr* and *Oprm3* in OVA + FPM vs. naïve, *Oprm1*, *Nefh*, *P2ry1*, *Prkcb*, *Gabra1*, and *Kcnv1* in OVA + FPM vs. OVA, and *Npy1r* and *Kcna1* in OVA alone vs. naïve.

Data are presented either as volcano plots (A, C, E), showing the log2 fold change of TPM between groups along with the corresponding $-\log_{10}$ p-values from DESeq2 analysis, or as heatmaps (B, D, F), showing the z-scores of rlog-transformed normalized counts. The experimental groups were naïve (n=2; A–B, E–F), OVA (n=3; C–F), and OVA-FPM (n=3; A–D). P-values were determined by DESeq2 (A, C, E) and are indicated in the figure.

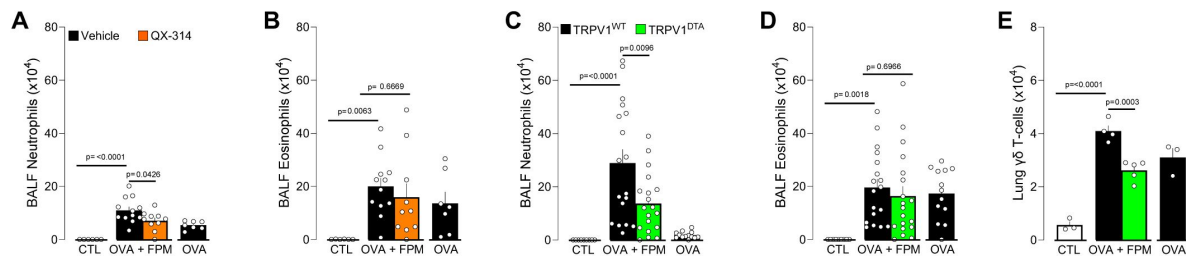


Figure 3.

Nociceptor neurons control pollution-exacerbated asthma

(A–B) Male and female C57BL/6 mice (6–10 weeks old) were sensitized intraperitoneally with ovalbumin (OVA; 200 µg/dose in 200 µL) and aluminum hydroxide (1 mg/dose in 200 µL) on days 0 and 7. On days 14–16, mice were challenged intranasally with OVA (50 µg/dose in 50 µL) alone or with fine particulate matter (FPM; 20 µg/dose in 50 µL). On day 16, 30 minutes after the final challenge, mice received intranasal QX-314 (5 nmol/dose in 50 µL). Bronchoalveolar lavage fluid (BALF) was collected on day 17 and analyzed by flow cytometry. Compared with naïve or OVA-exposed mice, those co-challenged with OVA + FPM showed increased BALF neutrophils **(A)**. QX-314 treatment normalized these levels, while BALF eosinophil levels remained comparable **(B)**.

(C–E) Male and female littermate control (TRPV1^{WT}) and nociceptor-ablated (TRPV1^{DTA}) mice (6–10 weeks old) were sensitized and challenged under the same OVA ± FPM protocol (days 0, 7, and 14–16). BALF or lungs were collected on day 17 and assessed by flow cytometry. Compared with naïve or OVA-exposed mice, OVA + FPM co-challenged mice exhibited higher BALF neutrophils **(C)** and lung γδ T cells **(E)**. Nociceptor ablation protected against these increases **(C, E)**, while BALF eosinophil levels remained comparable **(D)**.

Data are shown as mean ± SEM **(A–E)**. Experiments were replicated twice, and animals pooled **(A–E)**. *N* are as follows: **A–B**: control (*n*=6), OVA (*n*=7), OVA-FPM (*n*=12), OVA-FPM+QX-314 (*n*=10), **C–D**: TRPV1^{WT} + control (*n*=9), TRPV1^{WT} + OVA (*n*=13), TRPV1^{WT} + OVA-FPM (*n*=18), TRPV1^{DTA} + OVA-FPM (*n*=19), **E**: TRPV1^{WT} + control (*n*=3), TRPV1^{WT} + OVA (*n*=3), TRPV1^{WT} + OVA-FPM (*n*=4), TRPV1^{DTA} + OVA-FPM (*n*=5). *P*-values were determined by a one-way ANOVA with post-hoc Tukey's **(A–E)**. *P*-values are shown in the figure.

denoted as Na_v1.8^{DTA}) mice than in littermate controls (Na_v1.8^{wt}::DTA^{fl/wt}; denoted as Na_v1.8^{WT}; **Fig. 4C-F**, **Supplementary Video 1**). In contrast, neutrophil accumulation (labelled by i.v. αLy6G) was observed in pollution-exposed, asthmatic littermate controls, but was absent in nociceptor-ablated mice (**Fig. 4G-H**). These data are consistent with our BALF flow cytometry data (**Fig. 3A, 3C**). Furthermore, total neutrophil displacement was higher in Na_v1.8^{DTA} mice (**Fig. 4I-L**, **Supplementary Video 2**), without any bias toward a specific behavior subtype (e.g. adherent, crawling, patrolling, or tethering; **Fig. 4L**). Taken together, these data suggest that nociceptor neurons modulate AM motility and neutrophil accumulation while limiting the motility of recruited neutrophils.

Elevated cytokines and artemin under pollution-exacerbated asthma

To further explore the role of nociceptor neurons in airway inflammation, we performed an unbiased multiplex cytokine array. This revealed elevated levels of the asthma-promoting cytokine IL-4 and TNFα, with TNFα levels returning to baseline following nociceptor ablation (**Fig. 5A**). In addition, targeted ELISA showed increased artemin levels, which returned to normal in the absence of nociceptor neurons (**Fig. 5B**). Artemin, a member of the glial cell line-derived neurotrophic factor (GDNF) family, is pivotal for the development and function of sympathetic⁴⁶ and sensory⁴⁷ neurons through its interaction with the GFRα3-RET receptor complex. Activation of this receptor supports neuronal survival and growth during embryogenesis and drives neuronal hyperactivity under inflammatory conditions^{48–50}.

Mechanistic insights: artemin–nociceptor axis

To determine which vagal neuron subsets express the artemin receptor GFRα3-RET, we performed an in-silico analysis of single-cell RNA sequencing data^{51,52} and found that *Gfra3* is expressed by several nociceptor subtypes (**Fig. 5C–D**), including JG3 (OSM-R-expressing), JG4 (TRPA1/SSTR2-expressing), and JG6 (TRPM8-expressing). Concurrently, ImmGen data⁵³ indicated robust *Artn* expression in macrophages, with *Ahr* levels slightly lower than those in ILC2s and eosinophils (**SF. 1**). We then conducted an in-silico analysis of data from Karlsson et al.⁵⁴ (**SF. 2A**), Uhlén et al.⁵⁵ (**SF. 2B**) and Abdulla et al.⁵⁶ (**SF. 2C**), showing that *Ahr* is also expressed in human lung, that AHR protein is detected in human lung macrophages, and that *Artn* and *Ahr* are co-expressed in lung macrophages, respectively. Guided by these findings, we isolated alveolar macrophages from naive C57BL/6 mice and exposed them to fine particulate matter (FPM), observing a time-dependent increase in *Artn* transcripts (**Fig. 5E–G**). These results highlight key cellular interactions involving FPM, Ahr and artemin in the context of lung inflammation.

A recent study⁴⁹ linked artemin overexpression to increased mRNA levels of several nociceptor markers (*Gfra3*, *TrkA*, *Trpv1*, *Trpa1*), accompanied by heightened thermal sensitivity. Consistent with this, our experiments in JNC neurons from OVA-FPM co-exposed mice revealed amplified calcium responses to the TRPA1 agonist AITC (**Fig. 1B–C**). Follow-up tests in naive C57BL/6 mice showed elevated AITC responsiveness in artemin-exposed JNC neurons compared to vehicle-treated controls (**Fig. 5H–J**). Collectively, these data suggest that alveolar macrophages, through pollutant-driven production of artemin, sensitize nociceptor neurons and thereby potentiate allergic airway inflammation. This pathway underscores a critical link between environmental pollutants and the neurogenic exacerbation of allergic responses (**SF. 3**).

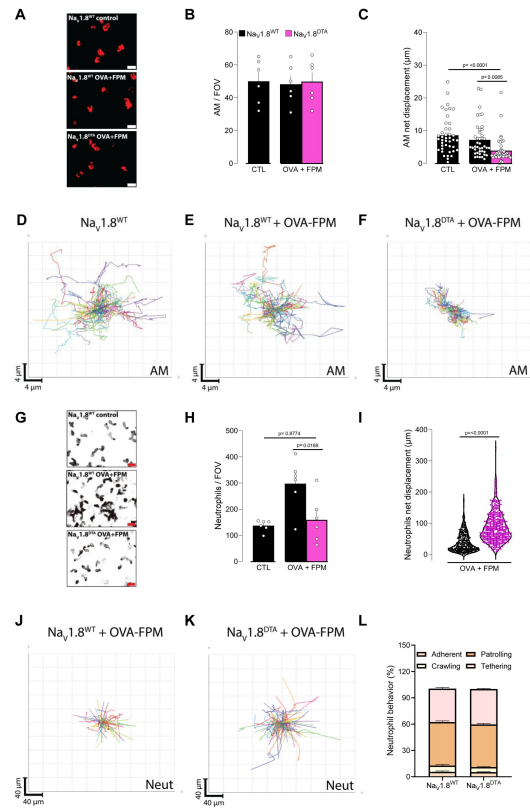


Figure 4.

Vagal sensory neurons gatekeep alveolar macrophage motility and neutrophil numbers

(A–D) Male and female littermate control (*NaV1.8*^{WT}::DTA^{fl/wt} denoted as *NaV1.8*^{WT}) and nociceptor-ablated (*NaV1.8*^{Cre}::DTA^{fl/wt} denoted as *NaV1.8*^{DTA}) mice (6–10 weeks old) were sensitized via intraperitoneal injection with an emulsion of ovalbumin (OVA; 200 μ g/dose) and aluminum hydroxide (1 mg/dose) on days 0 and 7. Phagocytes were labeled by intranasal injection of PKH26 Red Fluorescent Cell Linker Kit (25 pmol/dose) on day 10. Mice were then challenged intranasally with OVA (50 μ g/dose) alone or in combination with fine particulate matter (FPM; 20 μ g/dose) on days 14–16, and images were acquired on day 17. (A) Representative maximum-intensity projection of alveolar macrophages (AMs, red). Scale bar: 20 μ m. (B) Quantification of AM numbers per field of view (FOV). (C) Net displacement of AMs over 1 hour. (D–F) Representative tracks of individual AMs (each color represents a single AM) over 1 hour. While the AM numbers (A–B) were not impacted, their net displacement (C–F) was reduced in OVA-FPM-exposed *NaV1.8*^{DTA} mice.

(G–L) Male and female littermate control (*NaV1.8*^{WT}::DTA^{fl/wt} denoted as *NaV1.8*^{WT}) and nociceptor-ablated (*NaV1.8*^{Cre}::DTA^{fl/wt} denoted as *NaV1.8*^{DTA}) mice (6–10 weeks old) were sensitized via intraperitoneal injection with an emulsion of ovalbumin (OVA; 200 μ g/dose) and aluminum hydroxide (1 mg/dose) on days 0 and 7. Mice were then challenged intranasally with OVA (50 μ g/dose) alone or in combination with fine particulate matter (FPM; 20 μ g/dose) on days 14–16, and images were acquired on day 17. Immediately prior to perform the intravital imaging, we administered an intravenous Ly6G antibody to label neutrophils. (G) Representative maximum-intensity projection of neutrophils (black). Scale bar: 20 μ m. (H) Quantification of neutrophil numbers per FOV. (I) The total displacement of neutrophils over 20 minutes. (J–K) Representative tracks of individual neutrophils (each color represents a single neutrophil) over 20 minutes. (L) Frequency of neutrophil behaviors per FOV (adherent, crawling, patrolling, or tethering). Data show that OVA-FPM-exposed littermate control mice present an increase in neutrophil numbers per field of view (G–H), an effect absent in *NaV1.8*^{DTA}. Interestingly, OVA-FPM-exposed *NaV1.8*^{DTA} mice show an increase in neutrophil net displacement (I–K), an effect irrespective to one of the specific behaviors tested (L).

Data are presented as representative image (A, G; scale bar: 20 μ m), mean $\pm$ SEM (B, C, H), spider plot (D–F, J–K), violin plot showing median (I), and stacked bar graph showing mean $\pm$ SEM (L). N are as follows: B: *NaV1.8*^{WT} + control (n=6), *NaV1.8*^{WT} + OVA-FPM (n=6), *NaV1.8*^{DTA} + OVA-FPM (n=6), C: *NaV1.8*^{WT} + control (n=42), *NaV1.8*^{WT} + OVA-FPM (n=42), *NaV1.8*^{DTA} + OVA-FPM (n=42), H: *NaV1.8*^{WT} + control (n=6), *NaV1.8*^{WT} + OVA-FPM (n=6), *NaV1.8*^{DTA} + OVA-FPM (n=6), I: *NaV1.8*^{WT} + OVA-FPM (n=385), *NaV1.8*^{DTA} + OVA-FPM (n=469), J: *NaV1.8*^{WT} + OVA-FPM (n=10), *NaV1.8*^{DTA} + OVA-FPM (n=11). P-values were determined by a one-way ANOVA with post-hoc Tukey's (B, C, H) or unpaired Student T-test (I, L). P-values are shown in the figure.

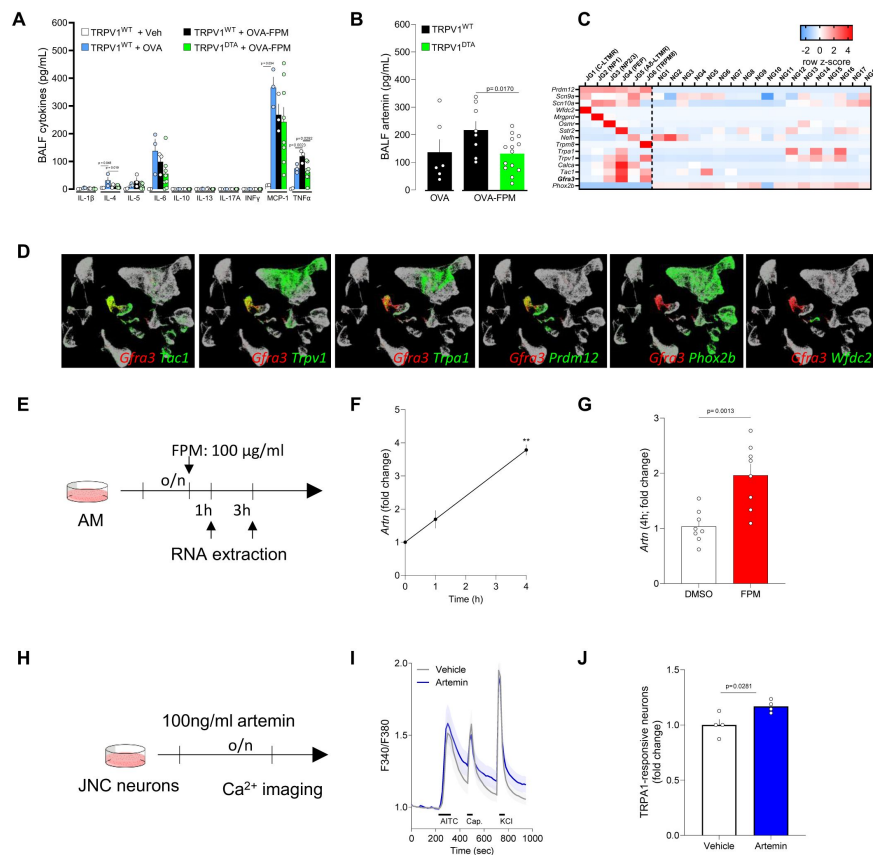


Figure 5.

Artemin sensitizes TRPA1 activity in vagal sensory neurons

(A-B) Male and female littermate control (TRPV1^{WT}) and nociceptor-ablated (TRPV1^{DTA}) mice (6–10 weeks old) were sensitized and challenged under the same OVA ± FPM protocol (days 0, 7, and 14–16). BALF was collected on day 17 and assessed by multiplex array and ELISA. Compared with naïve or OVA-alone groups, OVA + FPM co-challenged mice exhibited levels of TNFα, and artemin. Notably, ablating nociceptors prevented these increases.

(C) In-silico analysis of the GSE124312 dataset⁵¹. The heatmap displays transcript expression levels for the pan neural-crest lineage transcription factor (*Prdm12*), voltage-gated sodium channels (*Scn9a*, *Scn10a*), jugular subset markers (*Wfdc2*, *Mrgprd*, *Osmr*, *Sstr2*, *Nefh*, *Trpm8*), peptidergic neuron markers (*Trpa1*, *Trpv1*, *Calca*, *Tac1*, *Gfra3*), and the pan placodal lineage marker (*Phox2b*). *Gfra3* expression is enriched in the peptidergic neuron cluster labeled JG4. Experimental details and cell clustering are described by Kupari et al.⁵¹.

(D) In-silico analysis of GSE192987⁵² showing co-expression of *Gfra3* with *Trpa1* and other inflammatory markers. Data are visualized as row z-scores in a heatmap or via UMAPs (TPPT > 1). Experimental details and cell clustering are described by Zhao et al.⁵².

(E-G) Alveolar macrophages (3 × 10⁵ cells/well) from naïve male and female C57BL/6 mice were cultured overnight and then stimulated with vehicle (DMSO) or FPM (100 µg/mL). RNA was extracted 1- and 4-hours post-stimulation and *Artn* expression was assessed using qPCR. FPM exposure increased *Artn* transcript levels at both 1 and 4 hours (**F**, **G**).

(H-J) Naïve mice jugular-nodose-complex neurons were harvested, pooled, and cultured overnight with either vehicle or artemin (100 ng/mL). Cells were sequentially stimulated with AITC (TRPA1 agonist; 300 µM at 240–270 seconds), capsaicin (TRPV1 agonist; 300 nM at 320–335 seconds), and KCl (40 mM at 720–735 seconds). The percentage of AITC-responsive neurons (among all KCl-responsive cells) was normalized to vehicle-treated controls for each batch of experiments. Artemin-treated neurons showed increased responsiveness to AITC, while responses to capsaicin and KCl were unchanged (**I-J**).

Data are presented as means ± SEM (**A-B**, **F-G**, **J**), heatmap displaying the z-score of DESeq2 normalized counts (**C**), tSNE plots (**D**), schematics (**E**, **H**), means ± 95% CI of maximum Fura-2AM (F/F₀) fluorescence (**I**). *N* are as follows: **A**: TRPV1^{WT} + control (*n*=2), TRPV1^{WT} + OVA (*n*=3) TRPV1^{WT} + OVA-FPM (*n*=3), TRPV1^{DTA} + OVA-FPM (*n*=8), **B**: TRPV1^{WT} + OVA (*n*=6) TRPV1^{WT} + OVA-FPM (*n*=8), TRPV1^{DTA} + OVA-FPM (*n*=14), **F**: *n*=2/time point, **G**: *n*=8/group, **I**: vehicle (*n*=107 neurons), Artemin (*n*=122 neurons); **J**: *n*=4/group. *P*-values were determined by a one-way ANOVA with post-hoc Tukey's (**A**, **B**) or unpaired Student *T*-test (**G**, **J**). *P*-values are shown in the figure.

Discussion

Our research, in line with other studies, indicates that nociceptor neurons promote regulatory immunity in the context of bacterial⁵⁷, viral⁵⁸, or fungal⁵⁹ infections, as well as malignancies¹¹. To our knowledge, however, the impact of neuro-immunity on neutrophilic asthma is less understood. Using a model of pollution-exacerbated airway inflammation³⁴, we found that ablation or silencing of nociceptor neurons prevented the induction of neutrophilic airway inflammation, revealing a potentially novel therapeutic avenue for treating refractory asthma. This result parallels our earlier findings that nociceptor neuron silencing via charged blockers of voltage-gated sodium channels^{16,22,35}, or calcium channels³⁶ can halt eosinophilic airway inflammation.

During bacterial infections, Pinho-Ribeiro et al. demonstrated that nociceptor neurons inhibit neutrophil influx and antimicrobial activity by releasing the neuropeptide CGRP⁶⁰. In addition, Ugolini and colleagues showed that ablating Na_v1.8⁺ nociceptor neurons during HSV-1 infection increases skin neutrophil infiltration, cytokine production, and lesion severity⁵⁸. Building on these findings, we used intravital imaging to study neuro-immune interactions in our model, we observed that the total number of alveolar macrophages remained unchanged in OVA-exposed mice lacking nociceptor neurons, but their displacement was significantly reduced. In contrast, pollution-exposed asthmatic mice lacking nociceptor neurons exhibited a marked decrease in neutrophil recruitment and a corresponding increase in their displacement. These shifts in cellularity occurred regardless of whether nociceptor neurons were genetically ablated (TRPV1⁺ or Na_v1.8⁺ populations) or pharmacologically silenced with QX-314, as confirmed by flow cytometry. Although individual neutrophil behaviors—such as adherence, crawling, patrolling, and tethering—did not change, neuronal ablation effectively “removed the brakes” on neutrophil motility. This likely led to reduced neutrophil accumulation in the lung and overall dampening of lung inflammation in pollution-exacerbated asthma. These findings are consistent with the pioneering work by Baral et al., who showed that vagal TRPV1⁺ afferents suppress neutrophil recruitment and modulate lung γδ T cell numbers via CGRP signaling, thus supporting essential antibacterial clearance⁴. Related studies in influenza-infected mice revealed that vagal nociceptor deficiency significantly alters the lung immune landscape—expanding neutrophils and monocyte-derived macrophages⁵—and transcriptional analyses further indicated disrupted interferon signaling as well as imbalances among neutrophil subpopulations in nociceptor-ablated mice. Collectively, these results highlight the bidirectional crosstalk between recruited and tissue-resident phagocytes and nociceptors in air-pollution-exacerbated allergic airway inflammation.

The aryl hydrocarbon receptor (AhR) is a crucial modulator of inflammation, as seen in psoriasis models, where its activation reduces inflammation, whereas its absence exacerbates disease^{61,62}. Besides being expressed in alveolar macrophages, AhR is also present in ILC2s, eosinophils, and neurons. A recent preprint suggests AhR has a dual role in neural protection and axon regeneration: AhR activation in dorsal root ganglion neurons inhibits axon growth⁶³. At the same time, its deletion lessens inflammation and stress signaling, driving pro-regenerative pathways⁶³. Additional work indicates that AhR regulates the gut-brain axis⁶⁴. Although we have not directly examined AhR in nociceptor neurons, our findings suggest that neurons, much like alveolar macrophages, may sense fine particulate matter via AhR. This possibility could explain the direct neuronal transcriptomic reprogramming we observed after pollutant exposure. Ongoing studies aim to probe this hypothesis using nociceptor-specific AhR knockout models.

A growing body of evidence indicates that airway-innervating sensory neurons undergo significant transcriptomic alterations in neutrophilic asthma. Although some lung innervation arises from the dorsal root ganglia (DRG)—especially for nociception in the upper thoracic segments (T1–T5)—the majority (70–80%, sometimes up to 90%) of afferent fibers originate from

the JNC^{65,66}. Because the JNC mediates critical mechanosensory and chemosensory functions, our analysis focuses on this primary vagal pathway. While single-cell RNA sequencing has revealed that JNC neurons are highly heterogeneous and control breathing and tracheal reflexes^{67–72}, the molecular changes that occur during chronic inflammation remain poorly characterized.

Using an Nav1.8 reporter mice and intranasal retrograde tracing, we identified a subset of nociceptors innervating the lungs that are reprogrammed in allergic airway inflammation and by the asthma-driving cytokine IL-13²⁴. Our transcriptomic data using sorted TRPV1⁺ neurons confirm the upregulation of *Npy1r* during allergic airway inflammation²⁴ and highlight the upregulation of *Cacna1c*⁷³ and *Dmx12*⁷⁴ during pollution-exacerbated asthma, suggesting heightened vesicle trafficking and changes in synaptic release dynamics that may influence neuronal sensitivity and plasticity in asthmatic conditions. Other genes such as *Oprm1*⁷⁵ (mu-opioid receptor) and *Hint1*⁷⁶ (an opioid signaling modulator) suggest altered pain or irritant sensation in the lung, potentially shaping cough reflexes or bronchospasm. Changes in cytoskeletal regulators, including *Map1b*⁷⁷ and *Apc*⁷⁸, indicate that JNC axons may remodel their structure and synaptic architecture, potentially altering how airway signals are relayed to central circuits. *Lifr*^{79–82} (Leukemia inhibitory factor receptor alpha), which supports neuron survival and differentiation, may also be important in inflammatory or injury contexts in the lung.

Pathway analysis reveals gene clusters involved in synaptic structure, cytoskeletal organization, and epigenetic regulation. Enrichment in terms such as “synapse”, “presynaptic active zone,” and “postsynaptic density” indicates that both neurotransmitter release and postsynaptic excitability are being recalibrated, potentially amplifying neuro-immune feedback loops and leading to maladaptive reflexes like excessive bronchoconstriction or persistent cough in neutrophilic asthma. In parallel, pathways related to “cellular component organization,” “negative regulation of inclusion body assembly,” and “protein quality control” show that neurons may upregulate protective or adaptive mechanisms in response to chronic airway inflammation. These transcriptomic alterations point to a dynamic neuro-immune crosstalk in the inflamed lung, with neurons adapting their excitability and structural integrity in response to cytokines, environmental pollutants, and inflammatory cells, potentially exacerbating or perpetuating the pathophysiology of neutrophilic asthma.

Alveolar macrophages act as a critical early warning system in the lung⁴⁵, detecting pollutants such as FPM and initiating protective reflexes by secreting artemin. This cytokine activates and sensitizes nociceptor neurons—particularly TRPA1-expressing fibers—to noxious stimuli. Single-cell RNA sequencing identified a subset of TRPA1⁺, SSTR2⁺ neurons that express the glial cell line-derived neurotrophic factor receptor GFRα3 and are thereby sensitized by artemin. This finding aligns with earlier data showing that keratinocyte-derived TSLP can sensitize skin-innervating nociceptor neurons, promoting itch and atopic dermatitis⁸³. In the lungs, germline TRPA1 knockout reduces allergic airway inflammation^{84,85}, and clinical data from Genentech suggest that TRPA1 agonists are elevated in asthmatic human airways, contributing to both inflammation and hyperreactivity. GDC-0334, a selective TRPA1 antagonist they develop, mitigates airway inflammation, cough, and allergic responses in preclinical models and reduces pain and itch in humans⁸⁶. These findings strongly support the central role of TRPA1 sensitization and nociceptor activation in driving asthma pathology.

Our prior work showed that nodose ganglion neurons, which are highly TRPA1⁺, exhibit heightened thermal sensitivity and outgrowth responses to brain-derived neurotrophic factor (BDNF) but do not respond to nerve growth factor (NGF)⁴³. Here, we demonstrate that vagal nociceptors express GFRα3 and respond to artemin, which sensitizes TRPA1 channels and promotes subsequent airway inflammation. Indeed, jugular-nodose-complex (JNC) neurons undergo substantial transcriptomic reprogramming when exposed to OVA plus FPM, mirroring the effects of pro-asthmatic cytokines. Parallel data from other disease models support this link

between inflammation-induced sensitization and elevated artemin. For example, increased *Artn* and EGR1 levels in atopic dermatitis correlate with enhanced nerve density and scratching, both of which are attenuated in EGR1-deficient mice⁸⁷. Similarly, artemin overexpression boosts sensory neuron thermal responses, underscoring its importance in driving neuronal outgrowth and sensitivity in atopic conditions⁴⁹. These observations raise the possibility that blocking GFRα3 may offer a novel strategy to prevent maladaptive nociceptor involvement in pollution-exacerbated asthma.

Our previous work showed that vagal nociceptor neurons can detect allergen-IgE immune complexes and respond by releasing substance P (SP) and vasoactive intestinal peptide (VIP), but not CGRP²⁰. These findings were corroborated by analyzing bronchoalveolar lavage fluid (BALF) from asthmatic mice²⁴. Moreover, we demonstrated that SP drives mucus metaplasia in asthmatic lungs¹⁸ and influences antibody class switching in B cells²², a result that other groups have replicated^{23,39,88,89}. We also observed that TRPV1⁺ nociceptor ablation impairs antigen trafficking to lymph nodes and reduces IgG production^{23,90,91}, possibly decreasing the severity or onset of allergic responses. Emerging work highlights the varied immunomodulatory roles of different neuropeptides. For instance, CGRP impairs dendritic cell migration in psoriasis^{9,92}, whereas SP enhances dendritic cell migration to lymph nodes in atopic dermatitis⁸. VIP and neuromedin U (NMU) boost pro-asthmatic cytokines from lung ILC2s^{16,93,94–96}, while CGRP can have similar^{97,98} or opposing effects⁹⁹. In lung infections, CGRP reduces neutrophil and γδ T cell infiltration and protects against *Staphylococcus aureus* pneumonia^{4,100}. Conversely, CGRP exacerbates psoriasis by inducing dendritic cells to release IL-23, which activates IL-17-producing γδ T cells, intensifying inflammation⁵⁹. Likewise, during *C. albicans* infection, sensory neurons release CGRP that stimulates IL-23 production in CD301b⁺ dendritic cells, triggering T_H17 and γδ T cell responses (marked by IL-17A and IL-22), enhancing host defense⁷. In our model, nociceptor neuron ablation diminishes γδ T cell activation, which we posit is chiefly driven by SP/VIP, given the absence of heightened CGRP release in our asthma models^{16,22}. Future work will investigate whether neurons directly regulate γδ T cells and neutrophils through these or other neuropeptides.

Previous reports show that TRPV1⁺ neuron activation elicits a local type 17 immune response that enhances host defense against *Candida albicans*^{59,101} and *Staphylococcus aureus*⁷. While our mixed OVA-FPM was expected to induce a strong T_H17/T_H2 component, we did not observe an increase in IL-17A in our cytokine bead array. Similarly, increases in classic asthma-driving cytokines were modest (IL-4, IL-5) or absent (IL-13). One possible explanation is that these experiments were conducted in C57BL/6 mice, rather than BALB/c mice³⁴. We also noted an increase in TNFα and artemin, both of which were reduced by nociceptor neuron silencing. While we found that TNFα can reprogram nociceptor neurons²⁴, we propose that this TNFα may originate from activated neutrophils^{102,103}, consistent with the reduced TNFα and neutrophil activation seen in neuron-ablated mice. It is worth noting that the PM_{2.5} used in this study—Standard Reference Material (SRM) 2786—was obtained from an air intake system in the Czech Republic and that we cannot rule out that it may contain trace levels of LPS that could contribute to this neutrophil–TNFα hypothesis. Our rationale for choosing SRM2786 was that it is commercially available and represents a broad spectrum of ambient air pollutants, in contrast to more specialized sources such as diesel exhaust particles. Future studies will need to validate these findings using wildfire-collected particles¹⁰⁴, LPS-free air intake particles, and replication in BALB/c mice.

In summary, our study highlights how alveolar macrophages, via AhR-dependent pollutant detection and artemin secretion, sensitize TRPA1⁺ nociceptor neurons, thereby amplifying neutrophilic asthma. We propose several potential therapeutic strategies to curb neutrophilic airway inflammation: (i) targeting AhR signaling in alveolar macrophages, (ii) blocking Artemin's interaction with GFRα3, (iii) using TRPA1 antagonists such as GDC-0334, and (iv) silencing

nociceptor neurons with charged lidocaine derivatives. Collectively, these approaches offer new directions to manage the increasingly prevalent yet treatment-resistant, neutrophilic variant of asthma.

Materials and methods

Animals

All procedures involving animals adhered to the guidelines of the Canadian Council on Animal Care (CCAC), McGill University (xxx) and the Queen's University Animal Care Committee (UACC, protocol 2384). Mice were housed in individually ventilated cages with free access to water and food under 12-hour light cycles.

Parental strains C57BL/6 (# 000664), DTA^{fl/fl} (# 010527, # 009669), tdTomato^{fl/fl} (# 007914), TRPV1^{cre/cre} (# 017769) and Na_v1.8^{cre/cre} (# 036564) were purchased from The Jackson Laboratory. Male and female mice were bred in-house and used at 6–12 weeks of age. Crosses were performed to generate the following genotypes: Na_v1.8^{cre/wt}::DTA^{fl/wt}, TRPV1^{cre/wt}::DTA^{fl/wt}, TRPV1^{cre/wt}::tdTomato^{fl/wt}, and littermate control, namely: Na_v1.8^{wt/wt}::DTA^{fl/wt}, TRPV1^{wt/wt}::DTA^{fl/wt}. Male and female progeny mice were used between 8 and 16 weeks of age.

Ovalbumin Model of Allergic Airway Inflammation

On days 0 and 7, mice were sensitized by intraperitoneal (i.p.) injections of 200 µL of a solution containing 1 mg/mL grade V ovalbumin (OVA; Sigma, #A5503) and 5 mg/mL aluminum hydroxide (Sigma, #239186) in phosphate-buffered saline (PBS, ThermoFisher, #10010023). On days 14–16, mice were anesthetized with isoflurane (2.5%; CDMV, #108737) and intranasally instilled daily with 50 µg OVA in 50 µL PBS with or without fine particulate matter (FPM; 20 µg/dose; NIST 2786). Control mice were sensitized but did not undergo challenges. Unless otherwise indicated, mice were sacrificed on day 17.

Neuron silencing

In some experiments, QX-314 (Tocris, #1014; 5 nmol in 50 µL) or PBS was administered intranasally on day 16 to control asthmatic mice (as detailed in the asthma protocol). The mice were euthanized on day 17, and bronchoalveolar lavage fluid, lung tissues, and the jugular-nodose complex were collected.

Bronchoalveolar lavage

Bronchoalveolar lavage fluid was harvested in anesthetized mice, following a tracheal incision, by lavaging twice with 1 ml of either PBS or FACS buffer (2% FBS and 1 mM EDTA in PBS) through a Surflo ETFE IV Catheter 20G × 1" (Terumo Medical Products, # SR-OX2025CA). The lavage fluid was centrifuged at 350 × g for 6.5 minutes, and the supernatant was collected for ELISA analysis. The cell pellet was resuspended, subjected to RBC lysis (Cytek, # TNB-4300-L100 or Gibco, # A1049201), and stained for surface markers before flow cytometry.

Flow cytometry

Single-cell suspensions derived from bronchoalveolar lavage fluid or lung samples were stained with Ghost Dye Violet 510 (Cytek, # 13-0870-T100) and appropriate antibody cocktails in PBS. Cells were incubated at 4°C for 30 minutes, then fixed with 10% neutral buffered formalin (Sigma Aldrich, # HT501128) at room temperature for 15 minutes before data acquisition. To assess eosinophil and neutrophil infiltration in BALF, cells were stained with fluorochrome-conjugated antibodies against CD45 (clone: 30-F11), CD90.2 (clone: 53-2.1), CD11b (clone: M1/70), CD11c (clone: N418), Ly6C (clone: HK1.4), Ly6G (clone: 1A8), and Siglec-F (clone: 1RNM44N). For γδ T cell analysis

in lung tissue, staining included CD45 (clone: 30-F11), TCR $\gamma\delta$ (clone: GL3), CD90.2 (clone: 53-2.1), and lineage markers TCR β (clone: H57-597), CD19 (clone: 1D3/CD19), NK1.1 (clone: PK136), CD11b (clone: M1/70), CD11c (clone: N418), F4/80 (clone: BM8), and Fc ϵ RI α (clone: MAR-1), obtained from Biolegend or Thermo Fisher Scientific. Data were acquired using a BD FACS Canto II system.

Lung tissue harvesting

After diaphragm incision and transcardial perfusion with 10 ml of PBS, lung tissues were dissected, minced with razor blades, and either placed in TRIzol™ Reagent (Invitrogen, # 15596026) for RNA extraction or transferred into a digestion buffer consisting of 1.6 mg/ml collagenase type 4 (Worthington LS004189) and 100 μ g/ml DNase I (Roche, # 11284932001) in supplemented DMEM¹⁰⁷. The tissues were digested for 45 minutes at 37°C with mechanical dissociation through 18-gauge needles after 30 minutes, followed by filtration through a 70 μ m nylon mesh and RBC lysis. Cells were resuspended in FACS buffer for flow cytometry or fluorescence-activated cell sorting or in FBS-supplemented DMEM for in vitro stimulation in 96-well plates at 37°C with 5% CO₂, after which supernatants were collected.

Alveolar macrophage culture

Alveolar macrophages were obtained from the BALF of naïve mice, where they represented approximately 95% of the recovered cells. After centrifugation and RBC lysis, these cells were seeded at 3×10^5 per well in 96-well plates containing DMEM (Gibco 11965092) supplemented with 1 mM sodium pyruvate (Gibco, # 11360070), 2 mM GlutaMAX (Gibco, # 35050061), 100 U/mL penicillin and 100 μ g/mL streptomycin (Corning, # 30-002-CI), 10 mM HEPES (Gibco, # 15630080), and 10% FB Essence (VWR 10805-184), and cultured overnight. They were then stimulated with 100 μ g/ml FPM (NIST, # 2786) for 1–4 hours, followed by RNA extraction for quantitative PCR analysis.

Neuron culture

The jugular-nodose complex (JNC) was collected from anesthetized mice following exsanguination and placed in a digestion buffer containing 1 mg/ml (325 U/ml) collagenase type 4 (Worthington, # LS004189), 2 mg/ml (1.8 U/ml) Dispase II (Sigma, # 04942078001), and 250 μ g/ml (735.25 U/ml) DNase I (Roche, # 11284932001) prepared in supplemented DMEM without FB Essence. This mixture was incubated at 37°C for 60 minutes to ensure enzymatic digestion, followed by mechanical dissociation through progressive pipetting with tips of decreasing diameter and final passage through a 25-gauge needle. The cell suspension underwent density gradient centrifugation at $200 \times g$ for 20 minutes at low acceleration and deceleration, layering 150 mg/ml bovine serum albumin (BSA; Hyclone, # SH30574.02) in PBS to separate the cells. The bottom fraction was collected, RBC lysed and seeded onto glass-bottom dishes (Abidi, # 81218) coated with 50 μ g/ml laminin (Sigma, # L2020) and 100 μ g/ml poly-D-lysine (Sigma, # P6407). Cells were cultured overnight in Neurobasal-A medium (Gibco, # 10888022) supplemented with 1 mM sodium pyruvate (Gibco, # 11360070), 2 mM GlutaMAX™ (Gibco, # 35050061), 100 U/mL penicillin, 100 μ g/mL streptomycin (Corning, # 30-002-CI), 10 mM HEPES (Gibco, # 15630080), B-27 supplement (Gibco 17504-044), 50 ng/ml mouse nerve growth factor (NGF; Gibco, # 13257-019), 2 ng/ml mouse glial-derived neurotrophic factor (GDNF; Novus, # NBP2-61336), and cytosine- β -D-arabinofuranose (Thermo Scientific, # J6567106). In some experiments, 100 ng/ml artemin or 50 μ M HCl (as vehicle control) were added in vitro in place of NGF or GDNF. This culture system was subsequently used for calcium imaging.

Real-time quantitative PCR (qPCR)

qPCR was performed on stimulated alveolar macrophages that were lysed using TRIzol Reagent and stored at –80°C until RNA extraction. RNA from sorted cells was extracted using the PureLink RNA Micro Scale Kit (ThermoFisher, # 12183016). In contrast, RNA from lung tissues or lung cell suspensions was extracted with the E.Z.N.A.® Total RNA Kit I (Omega Bio-tek, # R6834). Extraction

procedures followed the manufacturers' instructions, including phenol-chloroform purification and mixing with an equal volume of isopropanol. Complementary DNA (cDNA) was synthesized using the SuperScript VILO Master Mix (Invitrogen, # 11755050), with 1–2 µg of RNA as the template in each reaction. Quantitative PCR was carried out with PowerUp SYBR Green Master Mix (Applied Biosystems, # A25742), using 50–100 ng of cDNA and 200 nM of each primer. The reactions were run on either a Mic qPCR Cycler (Bio Molecular Systems) or a CFX Opus Real-Time PCR System (Bio-Rad Laboratories). The primer pair for *Artn* was Forward: 5'-TGATCCACTTGAGCTTCGGG-3' and Reverse: 5'-CTCCATACCAAAGGGGTCCTG-3'.

Calcium imaging recording

Cultured neurons were loaded with 5 µM fura-2 AM (Cayman Chemical Company, # 34993) and incubated at 37°C for 40 minutes. After incubation, the cells were washed four times with standard external solution (SES; Boston BioProducts, # C-3030F) and maintained in this solution during imaging. The Fura-2 signals were recorded and used for downstream analyses. Agonists, diluted in SES, were delivered using a ValveLink 8.2 system (AutomateScientific) equipped with 250 µm Perfusion Pencil® tips (Automate Scientific) and controlled by Macro Recorder (Barbells Media, Germany). Between drug injections, SES flow was maintained to ensure a complete washout of each agonist. Imaging for Fura-2 experiments was conducted with a NIKON ECLIPSE Ti2 Inverted Microscope using an S Plan Fluor ELWD 20X objective lens to optimize UV light transmission. Images were captured every 3 or 4 seconds using sCMOS cameras such as PCO.Edge 4.2 LT (Excelitas Technologies), Prime 95B (Teledyne Photometrics), or Orca Flash 4.0 v2 (Hamamatsu Photonics). Regions of interest were manually delineated in NIS-Elements (Nikon), and the F340/F380 ratios were exported to Excel (Microsoft 365) for further analysis¹⁰⁸. The data were compressed by calculating a maximum value every 15 seconds for all subsequent evaluations.

Intravital microscopy

6–10 weeks old male and female littermate control ($\text{Na}_v1.8^{\text{wt}}::\text{DTA}^{\text{fl/wt}}$ denoted as $\text{Na}_v1.8^{\text{WT}}$) and nociceptor-ablated ($\text{Na}_v1.8^{\text{cre}}::\text{DTA}^{\text{fl/wt}}$ denoted as $\text{Na}_v1.8^{\text{DTA}}$) mice were sensitized via intraperitoneal injection of an emulsion containing ovalbumin (OVA; 200 µg/dose) and aluminum hydroxide (1 mg/dose) on days 0 and 7. On day 10, phagocytes were labeled by intranasal injection of PKH26 Red Fluorescent Cell Linker Kit (Sigma, # PKH26PCL) at 25 pmol/dose in Diluent B. Mice were then challenged intranasally with OVA (50 µg/dose) alone or in combination with fine particulate matter (FPM; 20 µg/dose) on days 14–16. Alveolar macrophage intravital imaging was performed on day 17 and is presented as a 1-hour time-lapse video.

Lung images were acquired using a Nikon CSU-X1 multichannel spinning-disk confocal upright microscope with a protocol adapted from previously published methods^{44,45}. Mice were anesthetized with 10 mg/kg xylazine hydrochloride and 200 mg/kg ketamine hydrochloride, and body temperature was maintained at 37°C with a heating pad (World Precision Instruments). The right jugular vein was cannulated for additional anesthetic as needed and for injecting anti-mouse Ly6G Alexa Fluor 647 (BioLegend, clone: 1A8, # 127610) to label neutrophils. After exposing the trachea and inserting a catheter connected to a small rodent ventilator (Harvard Apparatus), the mouse was placed in a right lateral decubitus position. A small incision was made between ribs 4 and 5 to create an opening of about 1.5 cm, and an intercostal lung window was carefully fitted and stabilized by a vacuum of roughly 20 mmHg. Time-lapse images were acquired without delay using a 20X water-immersion objective (numerical aperture 1).

Images are presented as maximum-intensity projections of z-stacks. Alveolar macrophage movement was tracked for over 1 hour, and neutrophil behavior was recorded for 20 minutes. Cell displacement was quantified using the ICY software's manual tracking plugin. Neutrophil behavior was classified into adherent, tethering, crawling, or patrolling using Imaris (Oxford Instruments) spot tracking. Track Duration and Track Speed Mean were used to define each behavior per field of view (FOV). Tethering was determined by a Track Duration under 150 seconds for cells that

rapidly entered the FOV before arresting and exiting again. Adherent cells remained immobile for more than 150 seconds, with a Track Speed Mean of $\leq 0.03 \mu\text{m/s}$. Crawling cells were motile with steady movement that persisted for at least half of the video's duration; these had a Track Speed Mean $> 0.03 \mu\text{m/s}$ and a Track Duration > 600 seconds. Patrolling cells shared the rapid entry observed with tethering cells but, instead of briefly arresting continued crawling and exited the FOV, showing a Track Speed Mean $> 0.03 \mu\text{m/s}$ and a Track Duration > 150 seconds but < 600 seconds.

Bulk RNA sequencing

RNA-seq was performed on JNCs isolated from TRPV1^{cre::tdTomato}^{fl/wt} mice subjected to OVA sensitization and challenge, as previously described. tdTomato⁺ cells were sorted using a BD FACSAria cell sorter²⁴, and total RNA was extracted following established protocols. Library preparation was carried out at the Institut de Recherche en Cancérologie et en Immunologie (IRIC) of the Université de Montréal. RNA quality was assessed using an Agilent Bioanalyzer, ensuring a minimum RNA Integrity Number (RIN) of 7.5. Libraries were prepared using a poly(A)-enrichment, single-stranded RNA-seq strategy (KapaBiosystems, KAPA RNA Hyperprep Kit, #KR1352) and sequenced on an Illumina NextSeq500 platform with 75-cycle single-end reads.

Basecalling was performed using Illumina RTA 2.4.11, and demultiplexing was conducted with bcl2fastq 2.20, allowing for one mismatch in the index. Trimmomatic was used to remove adapter sequences and low-quality bases from the 3' end of each read. The remaining high-quality reads were aligned to the GRCm38 mouse genome using STAR v2.5.11, which also generated gene-level read counts. Differential expression analysis was performed using DESeq2 on these read counts, normalized by the DESeq2 pipeline. Log2 fold changes and $-\log_{10}$ p-values were calculated from the normalized data, and genes were considered differentially expressed if their adjusted p-value (false discovery rate, FDR) was below 0.05. Further data analysis and visualization were conducted in RStudio.

Enzyme-linked immunosorbent assay (ELISA)

ELISA was used to measure artemin levels in BALF (R&D Systems, # DY1085-05) following the manufacturer's instructions. Inflammatory cytokines in BALF were detected with a Cytometric Bead Array Flex Set from BD Biosciences: Master Buffer Set (# 558266), IL-1 β (# 560232), IL-4 (# 558298), IL-5 (# 558302), IL-6 (# 558301), IL-10 (# 558300), IL-13 (# 558349), IL-17A (# 560283), IFN γ (# 558296), MCP-1 (# 558342), and TNF (# 558299), also used according to the manufacturer's guidelines.

In-silico analysis of mouse immune cells expression profile using the Immgen database

Using the publicly available Immgen database⁵³, we proceed to an in-silico analysis of RNA-sequencing data (DESeq2 data) of various mouse immune cells. As per Immgen protocol, RNA-sequencing reads were aligned to the mouse genome GENCODE GRCm38/mm10 primary assembly (GenBank assembly accession GCA_000001635.2) and gene annotations vM16 with STAR 2.5.4a. The ribosomal RNA gene annotations were removed from general transfer format file. The gene-level quantification was calculated by featureCounts. Raw reads count tables were normalized by median of ratios method with DESeq2 package from Bioconductor and then converted to GCT and CLS format. Samples with less than 1 million uniquely mapped reads were automatically excluded from normalization. Experimental details are defined in www.immgen.org/Protocols/ImmGenULI_RNAseq_methods.pdf.

In silico analysis of RNA-Seq data

In silico analysis of RNA-Seq data involved extracting information from Kupari et al.'s supplementary materials (GSE124312)⁵¹, with clusters based on that publication's designations. Additional data from Zhao et al., (GSE192987)⁵² were reanalyzed using R and plotted with UMAP to visualize the co-expression of relevant genes. Bulk JNC sequencing datasets were analyzed with DESeq2.

In-silico analysis of lung cancer patients' tumor expression profile using single-cell RNA sequencing

We performed an *in-silico* analysis of single-cell RNA-sequencing data from mouse lung tumor-infiltrating CD45⁺ cells in non-small cell lung cancer (NSCLC) models (GSE127465)¹⁰⁹, accessed via the publicly available Broad Institute Single-Cell Portal. The expression levels of *Ahr*, *Artn*, *Calca*, *Vip*, *Tac1*, and *Trpa1* were plotted within CD45⁺ myeloid cell populations using RStudio. Additionally, we conducted a Spearman correlation analysis to explore relationships among these genes. Gene expression data are presented as normalized counts per ten thousand. We extracted the relevant expression values and used RStudio to generate dot plots for individual cells in each myeloid population, as well as to perform correlation analyses of the selected genes. Experimental and clustering details can be found at Zilionis et al.,¹⁰⁹

In-silico analysis of human lung-tissue gene expression using the Human Protein Atlas (HPA) Single Cell Type Atlas

We retrieved the lung dataset generated by Karlsson et al.¹⁰⁶ from the HPA Single Cell Type Atlas, which pools single-cell RNA-sequencing reads into annotated clusters and reports expression as per-gene z-scores after median-of-ratios normalization. Using R (v4.3.2), we downloaded the cluster-level expression matrix, isolated all clusters and extracted transcript values for *Gfra3*, *Ahr*, *Artn*, *Calca*, *Tac1*, *Vip* and *Trpa1*. The positive z-scores observed in the "lung" clusters confirm that *Ahr* mRNA is expressed in patient lung tissue. Because the Karlsson pipeline equalizes library depth before z-score transformation, these values permit direct comparison of *Ahr* abundance to the whole-body baseline captured by the same atlas. Additional, experimental and clustering details can be found at Karlsson et al.,¹⁰⁶

In-silico analysis of AHR protein abundance in human lung macrophages using Human Protein Atlas immunohistochemistry data

We retrieved the antibody-based tissue microarray summary from Uhlén et al.⁵⁵ via the Human Protein Atlas pathology portal, which lists per-tissue immunoreactivity for each antibody as categorical scores ("not detected," "low," "medium," "high"). From the raw TSV file, we report protein expression (*Ahr*, *Artn*, *Gfra3*, *Calca*, *Vip*, *Tac1*) across cell types and tissue types. Because the HPA pipeline reports these scores after internal normalization of DAB signal across replicate tissue cores, the categorical value can be compared directly across tissues without further scaling. This processed result confirms constitutive AHR protein presence in lung macrophages in situ. Additional, experimental and clustering details can be found at Uhlén et al.⁵⁵

In-silico co-expression analysis of AHR and ARTN in human lung macrophages using the CZ CELLxGENE Discover single-cell atlas

We accessed the lung single-cell RNA-seq data curated by Abdulla et al.⁵⁶ through the CZ CELLxGENE Discover platform and downloaded the cell-level expression matrix together with standardized cell-type annotations. Raw UMI counts were normalized to ln (CPTT + 1) values

during ingestion into the atlas, a transformation that equalizes library depth and stabilizes variance while preserving the full gene–cell count matrix for downstream analyses. Additional, experimental and clustering details can be found at Abdulla et al. [56](#).

G-Profiler and Go-term

The top 50 differentially expressed genes (based on DESeq p-values) from each comparison were submitted to the web-based tool g:Profiler [110](#) for enrichment analysis (g:GOST). The resulting pathway enrichments—derived from multiple databases, including Gene Ontologies (GO; covering Molecular Function, Biological Process, and Cellular Component sub-ontologies) [111](#), the Kyoto Encyclopedia of Genes and Genomes (KEGG) [112](#), Reactome (REAC) [113](#), WikiPathways (WP) [114](#), TRANSFAC (TF) [115](#), miRTarBase (MIRNA), the Comprehensive Resource of Mammalian Protein Complexes (CORUM) [116](#), and the Human Phenotype Ontology (HP) [117](#)—are listed in Supplementary Table 1.

Data availability

Bulk RNA-sequencing raw and processed data have been deposited in the NCBI's gene expression omnibus (GSE298583). Processed data can also be accessed in the supplementary Table 1. Additional information and raw data are available from the lead contact upon reasonable request.

Statistics

P values ≤ 0.05 were considered statistically significant. One-way ANOVA, and Student's t-tests were conducted using GraphPad Prism, while DESeq2 and Seurat analyses, including their statistical tests, were performed in RStudio.

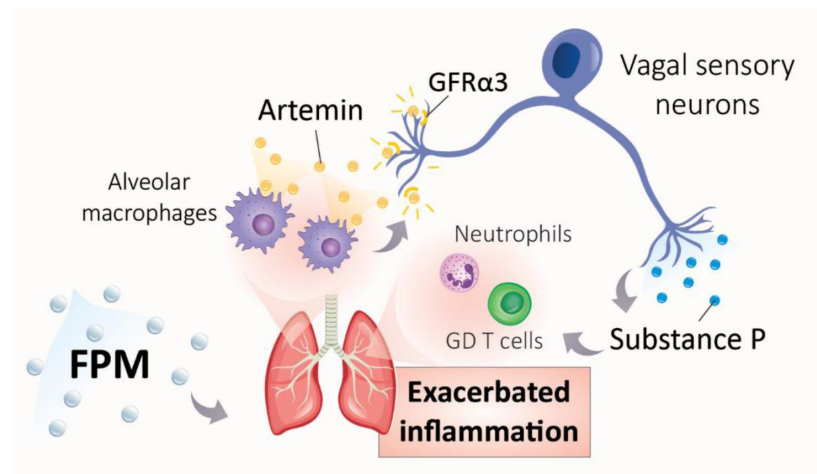
Replicates

The number of replicates (n) for each experiment is specified in the figure legends and represents the number of animals for in vivo data. For in vitro experiments, replicates may be culture wells or dishes, animals, fields of view during microscopy, or individual neurons in calcium imaging. All experiments included different preparations from distinct animals to ensure biological reproducibility.

Exclusion

One replicate in the naïve mice group of the RNA-sequencing analysis (Figure 2) was excluded because the PCA indicated it as an outlier. No other data were excluded from the study.

Supplementary figures



Supplementary Figure 3.

Schematic of nociceptor involvement in pollution-exacerbated allergic asthma

In our study, mice were exposed to PM₂₅ particles and ovalbumin (OVA) to model pollution-exacerbated asthma. Compared to mice exposed to OVA alone, co-exposure to PM₂₅ and OVA significantly increased bronchoalveolar lavage fluid (BALF) neutrophils and lung $\gamma\delta$ T cells levels. To counteract this heightened airway inflammation, we administered intranasal QX-314—a charged lidocaine derivative—at the peak of inflammation, effectively normalizing BALF neutrophil levels. Ablation of TRPV1⁺ nociceptor neurons produced a similar effect.

Further analysis with calcium imaging revealed that neurons from the jugular-nodose complex in pollution-exposed asthmatic mice were more sensitive via their TRPA1 channels. Levels of TNF α and the growth factor artemin were also elevated in the BALF of these mice, returning to normal following nociceptor ablation.

We identified alveolar macrophages as the source of artemin, which they secrete upon sensing fine particulate matter (FPM) through aryl hydrocarbon receptors. Artemin, in turn, heightened TRPA1 responsiveness to its agonist (mustard oil), thereby exacerbating airway inflammation. Our findings suggest that silencing nociceptor neurons can disrupt this pathway, offering a novel therapeutic approach to mitigate neutrophilic airway inflammation driven by pollution.

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Additional files

Supplementary Video 1. Intravital recording of alveolar macrophage motility. 6–10 weeks old male and female littermate control ($Na_v1.8^{wt::DTA^{fl/wt}}$ denoted as $Na_v1.8^{WT}$) and nociceptor-ablated ($Na_v1.8^{cre::DTA^{fl/wt}}$ denoted as $Na_v1.8^{DTA}$) mice were sensitized via intraperitoneal injection of an emulsion containing ovalbumin (OVA; 200 μ g/dose) and aluminum hydroxide (1 mg/dose) on days 0 and 7. On day 10, phagocytes were labeled by intranasal injection of PKH26 (25 pmol/dose). Mice were then challenged intranasally with OVA (50 μ g/dose) alone or in combination with fine particulate matter (FPM; 20 μ g/dose) on days 14–16. Alveolar macrophage intravital imaging was performed on day 17 and is presented as a 1-hour time-lapse video. [🔗](#)

Supplementary Video 2. Intravital recording of neutrophil motility. Male and female littermate control ($Na_v1.8^{wt::DTA^{fl/wt}}$ denoted as $Na_v1.8^{WT}$) and nociceptor-ablated ($Na_v1.8^{cre::DTA^{fl/wt}}$ denoted as $Na_v1.8^{DTA}$) mice (6–10 weeks old) were sensitized via an intraperitoneal injection of an ovalbumin (OVA; 200 μ g/dose) and aluminum hydroxide (1 mg/dose) emulsion on days 0 and 7. On days 14–16, mice were challenged intranasally with OVA (50 μ g/dose) alone or in combination with fine particulate matter (FPM; 20 μ g/dose). Immediately before intravital imaging on day 17, an intravenous Ly6G antibody was administered to label neutrophils. The resulting recording is presented as a 20-minute time-lapse video. [🔗](#)

Supplementary Table 1. Differentially expressed genes and pathway analysis of vagal nociceptors in pollution-exacerbated asthma. Naïve 6–10 weeks male and female $TRPV1^{cre::tdTomato^{fl/wt}}$ mice were either subjected to a pollution-exacerbated asthma protocol, to the classic ovalbumin (OVA) protocol or remained naïve. On day 17, jugular-nodose complex (JNC) neurons were harvested and dissociated, and $TRPV1^+$ ($tdTomato^+$) neurons were FACS-purified to remove stromal and non-peptidergic cells before being processed for RNA sequencing. The different tabs show the DESeq2 identified and analyzed using the web-based tool *g:Profiler* for each of these conditions. Other tabs show GO terms enriched in each condition. [🔗](#)

References

- 1 Talbot S., Foster S. L., Woolf C. J. (2016) **Neuroimmunity: Physiology and Pathology** *Annu Rev Immunol* **34**:421–447 <https://doi.org/10.1146/annurev-immunol-041015-055340> | [Google Scholar](#)
- 2 Foster S. L., Talbot S., Woolf C. J. (2015) **CNS injury: IL-33 sounds the alarm** *Immunity* **42**:403–405 <https://doi.org/10.1016/j.immuni.2015.02.019> | [Google Scholar](#)
- 3 Michoud F., et al. (2021) **Epineural optogenetic activation of nociceptors initiates and amplifies inflammation** *Nature biotechnology* **39**:179–185 [Google Scholar](#)
- 4 Baral P., et al. (2018) **Nociceptor sensory neurons suppress neutrophil and gammadelta T cell responses in bacterial lung infections and lethal pneumonia** *Nat Med* **24**:417–426 <https://doi.org/10.1038/nm.4501> | [Google Scholar](#)
- 5 Yang D., et al. (2024) **Vagal TRPV1 (+) sensory neurons regulate myeloid cell dynamics and protect against influenza virus infection** *bioRxiv* <https://doi.org/10.1101/2024.08.21.609013> | [Google Scholar](#)
- 6 Lai N. Y., et al. (2020) **Gut-innervating nociceptor neurons regulate Peyer’s patch microfold cells and SFB levels to mediate Salmonella host defense** *Cell* **180**:33–49 [Google Scholar](#)
- 7 Cohen J. A., et al. (2019) **Cutaneous TRPV1+ neurons trigger protective innate type 17 anticipatory immunity** *Cell* **178**:919–932 [Google Scholar](#)
- 8 Perner C., et al. (2020) **Substance P release by sensory neurons triggers dendritic cell migration and initiates the type-2 immune response to allergens** *Immunity* **53**:1063–1077 [Google Scholar](#)
- 9 Hanč P., et al. (2023) **Multimodal control of dendritic cell functions by nociceptors** *Science* **379**:eabm5658 [Google Scholar](#)
- 10 Chiu I. M., et al. (2013) **Bacteria activate sensory neurons that modulate pain and inflammation** *Nature* **501**:52–57 [Google Scholar](#)
- 11 Balood M., et al. (2022) **Nociceptor neurons affect cancer immunosurveillance** *Nature* **611**:405–412 [Google Scholar](#)
- 12 Restaino A. C., et al. (2023) **Functional neuronal circuits promote disease progression in cancer** *Science Advances* **9**:eade4443 [Google Scholar](#)
13. Barr J., et al. (2024) **Tumor-infiltrating nerves functionally alter brain circuits and modulate behavior in a mouse model of head-and-neck cancer** *bioRxiv* [Google Scholar](#)
- 14 Amit M., et al. (2024) **Next Directions in the Neuroscience of Cancers Arising outside the CNS** *Cancer Discovery* **14**:669–673 [Google Scholar](#)
- 15 Foster S. L., Seehus C. R., Woolf C. J., Talbot S (2017) **Sense and immunity: context-dependent neuro-immune interplay** *Frontiers in immunology* **8**:1463 [Google Scholar](#)

- 16 Talbot S., et al. (2015) **Silencing nociceptor neurons reduces allergic airway inflammation** *Neuron* **87**:341–354 [Google Scholar](#)
- 17 Tränkner D., Hahne N., Sugino K., Hoon M. A., Zuker C (2014) **Population of sensory neurons essential for asthmatic hyperreactivity of inflamed airways** *Proceedings of the National Academy of Sciences* **111**:11515–11520 [Google Scholar](#)
- 18 Talbot S., et al. (2020) **Vagal sensory neurons drive mucous cell metaplasia** *Journal of Allergy and Clinical Immunology* **145**:1693–1696 [Google Scholar](#)
- 19 Yang D., et al. (2022) **Nociceptor neurons direct goblet cells via a CGRP-RAMP1 axis to drive mucus production and gut barrier protection** *Cell* **185**:4190–4205 [Google Scholar](#)
- 20 Crosson T., et al. (2021) **FcεR1-expressing nociceptors trigger allergic airway inflammation** *Journal of Allergy and Clinical Immunology* **147**:2330–2342 [Google Scholar](#)
- 21 Bersellini Farinotti A., et al. (2019) **Cartilage-binding antibodies induce pain through immune complex-mediated activation of neurons** *Journal of Experimental Medicine* **216**:1904–1924 [Google Scholar](#)
22. Mathur S., et al. (2021) **Nociceptor neurons promote IgE class switch in B cells** *JCI insight* **6** [Google Scholar](#)
- 23 Tynan A., et al. (2024) **TRPV1 nociceptors are required to optimize antigen-specific primary antibody responses to novel antigens** *Bioelectronic Medicine* **10** [Google Scholar](#)
- 24 Crosson T., et al. (2024) **Cytokines reprogram airway sensory neurons in asthma** *Cell Rep* **43** <https://doi.org/10.1016/j.celrep.2024.115045> | [Google Scholar](#)
- 25 Cunningham C. X., Williamson G. J., Bowman D (2024) **Increasing frequency and intensity of the most extreme wildfires on Earth** *Nat Ecol Evol* **8**:1420–1425 <https://doi.org/10.1038/s41559-024-02452-2> | [Google Scholar](#)
- 26 Connolly R., et al. (2024) **Mortality attributable to PM2. 5 from wildland fires in California from 2008 to 2018** *Science Advances* **10**:ead11252 [Google Scholar](#)
- 27 Ray A., Kolls J. K (2017) **Neutrophilic inflammation in asthma and association with disease severity** *Trends in immunology* **38**:942–954 [Google Scholar](#)
- 28 Baan E. J., et al. (2022) **Characterization of Asthma by Age of Onset: A Multi-Database Cohort Study** *J Allergy Clin Immunol Pract* **10**:1825–1834 <https://doi.org/10.1016/j.jaip.2022.03.019> | [Google Scholar](#)
- 29 de Nijs S. B., Venekamp L. N., Bel E. H (2013) **Adult-onset asthma: is it really different?** *Eur Respir Rev* **22**:44–52 <https://doi.org/10.1183/09059180.00007112> | [Google Scholar](#)
- 30 Gianniou N., et al. (2018) **Acute effects of smoke exposure on airway and systemic inflammation in forest firefighters** *J Asthma Allergy* **11**:81–88 <https://doi.org/10.2147/JAA.S136417> | [Google Scholar](#)

- 31 Noah T. L., Worden C. P., Rebuli M. E., Jaspers I (2023) **The Effects of Wildfire Smoke on Asthma and Allergy** *Curr Allergy Asthma Rep* **23**:375–387 <https://doi.org/10.1007/s11882-023-01090-1> | [Google Scholar](#)
- 32 Wilgus M. L., Merchant M (2024) **Clearing the Air: Understanding the Impact of Wildfire Smoke on Asthma and COPD** *Healthcare (Basel)* **12** <https://doi.org/10.3390/healthcare12030307> | [Google Scholar](#)
- 33 Jatakanon A., et al. (1999) **Neutrophilic inflammation in severe persistent asthma** *American journal of respiratory and critical care medicine* **160**:1532–1539 [Google Scholar](#)
- 34 Thio C. L., et al. (2022) **Identification of a PD-L1+Tim-1+ iNKT subset that protects against fine particulate matter-induced airway inflammation** *JCI Insight* **7** <https://doi.org/10.1172/jci.insight.164157> | [Google Scholar](#)
- 35 Tochitsky I., et al. (2021) **Inhibition of inflammatory pain and cough by a novel charged sodium channel blocker** *Br J Pharmacol* **178**:3905–3923 <https://doi.org/10.1111/bph.15531> | [Google Scholar](#)
- 36 Lee S., et al. (2019) **Novel charged sodium and calcium channel inhibitor active against neurogenic inflammation** *eLife* **8** <https://doi.org/10.7554/eLife.48118> | [Google Scholar](#)
- 37 Binshtok A. M., Bean B. P., Woolf C. J (2007) **Inhibition of nociceptors by TRPV1-mediated entry of impermeant sodium channel blockers** *Nature* **449**:607–610 <https://doi.org/10.1038/nature06191> | [Google Scholar](#)
- 38 Roversi K., et al. (2022) **Nanophotonics Enable Targeted Photothermal Silencing of Nociceptor Neurons** *Small* **18**:e2103364 <https://doi.org/10.1002/smll.202103364> | [Google Scholar](#)
- 39 Aguilar D., et al. (2024) **Sensory neurons regulate stimulus-dependent humoral immunity in mouse models of bacterial infection and asthma** *Nat Commun* **15**:8914 <https://doi.org/10.1038/s41467-024-53269-3> | [Google Scholar](#)
- 40 Ito K., et al. (2024) **Comorbid functional dyspepsia reflects IL-33-mediated airway neuronal dysfunction in asthma** *J Allergy Clin Immunol* **154**:1422–1433 <https://doi.org/10.1016/j.jaci.2024.06.008> | [Google Scholar](#)
- 41 Verzele N. A. J., et al. (2024) **Evidence for vagal sensory neural involvement in influenza pathogenesis and disease** *PLoS Pathog* **20**:e1011635 <https://doi.org/10.1371/journal.ppat.1011635> | [Google Scholar](#)
- 42 Wang J.-C., Crosson T., Talbot S. (2022) **Asthma: Methods and Protocols** Springer pp. 297–314 [Google Scholar](#)
- 43 Crosson T., Talbot S (2022) **Anatomical differences in nociceptor neurons sensitivity** *Bioelectron Med* **8** <https://doi.org/10.1186/s42234-022-00088-w> | [Google Scholar](#)
- 44 Kulle A., et al. (2024) **Alveolar macrophage function is impaired following inhalation of berry e-cigarette vapor** *Proc Natl Acad Sci U S A* **121**:e2406294121 <https://doi.org/10.1073/pnas.2406294121> | [Google Scholar](#)
- 45 Neupane A. S., et al. (2020) **Patrolling Alveolar Macrophages Conceal Bacteria from the Immune System to Maintain Homeostasis** *Cell* **183**:110–125 <https://doi.org/10.1016/j.cell.2020.06.008> | [Google Scholar](#)

.2020.08.020 | [Google Scholar](#)

- 46 Honma Y., et al. (2002) **Artemin is a vascular-derived neurotrophic factor for developing sympathetic neurons** *Neuron* **35**:267–282 [https://doi.org/10.1016/s0896-6273\(02\)00774-2](https://doi.org/10.1016/s0896-6273(02)00774-2) | [Google Scholar](#)
- 47 Ilieva M., et al. (2019) **Artemin and an Artemin-Derived Peptide, Artefin, Induce Neuronal Survival, and Differentiation Through Ret and NCAM** *Front Mol Neurosci* **12**:47 <https://doi.org/10.3389/fnmol.2019.00047> | [Google Scholar](#)
- 48 Lippoldt E. K., Elmes R. R., McCoy D. D., Knowlton W. M., McKemy D. D (2013) **Artemin, a Glial Cell Line-Derived Neurotrophic Factor Family Member, Induces TRPM8-Dependent Cold Pain** *J Neurosci* **33**:12543–12552 <https://doi.org/10.1523/jneurosci.5765-12.2013> | [Google Scholar](#)
- 49 Elitt C. M., et al. (2006) **Artemin overexpression in skin enhances expression of TRPV1 and TRPA1 in cutaneous sensory neurons and leads to behavioral sensitivity to heat and cold** *J Neurosci* **26**:8578–8587 <https://doi.org/10.1523/JNEUROSCI.2185-06.2006> | [Google Scholar](#)
- 50 Elitt C. M., Malin S. A., Koerber H. R., Davis B. M., Albers K. M (2008) **Overexpression of artemin in the tongue increases expression of TRPV1 and TRPA1 in trigeminal afferents and causes oral sensitivity to capsaicin and mustard oil** *Brain Res* **1230**:80–90 <https://doi.org/10.1016/j.brainres.2008.06.119> | [Google Scholar](#)
- 51 Kupari J., Haring M., Agirre E., Castelo-Branco G., Ernfors P (2019) **An Atlas of Vagal Sensory Neurons and Their Molecular Specialization** *Cell Rep* **27**:2508–2523 <https://doi.org/10.1016/j.celrep.2019.04.096> | [Google Scholar](#)
- 52 Zhao Q., et al. (2022) **A multidimensional coding architecture of the vagal interoceptive system** *Nature* **603**:878–884 <https://doi.org/10.1038/s41586-022-04515-5> | [Google Scholar](#)
- 53 The Immunological Genome Project (2020) **ImmGen at 15** *Nat Immunol* **21**:700–703 <https://doi.org/10.1038/s41590-020-0687-4> | [Google Scholar](#)
- 54 Karlsson M., et al. (2021) **A singleB—[cell type transcriptomics map of human tissues** *Science advances* **7**:eabh2169 [Google Scholar](#)
- 55 Uhlén M., et al. (2005) **A human protein atlas for normal and cancer tissues based on antibody proteomics** *Mol Cell Proteomics* **4**:1920–1932 <https://doi.org/10.1074/mcp.M500279-MCP200> | [Google Scholar](#)
- 56 Biology C. S.-C., et al. (2023) **CZ CELLxGENE Discover: A single-cell data platform for scalable exploration, analysis and modeling of aggregated data** *bioRxiv* [Google Scholar](#)
- 57 Pinho-Ribeiro F. A., et al. (2023) **Bacteria hijack a meningeal neuroimmune axis to facilitate brain invasion** *Nature* **615**:472–481 <https://doi.org/10.1038/s41586-023-05753-x> | [Google Scholar](#)
- 58 Filtjens J., et al. (2021) **Nociceptive sensory neurons promote CD8 T cell responses to HSV-1 infection** *Nat Commun* **12**:2936 <https://doi.org/10.1038/s41467-021-22841-6> | [Google Scholar](#)
- 59 Kashem S. W., et al. (2015) **Nociceptive Sensory Fibers Drive Interleukin-23 Production from CD301b+ Dermal Dendritic Cells and Drive Protective Cutaneous Immunity** *Immunity* **43**:515–526 <https://doi.org/10.1016/j.immuni.2015.08.016> | [Google Scholar](#)

- 60 Pinho-Ribeiro F. A., et al. (2018) **Blocking Neuronal Signaling to Immune Cells Treats Streptococcal Invasive Infection** *Cell* **173**:1083–1097 <https://doi.org/10.1016/j.cell.2018.04.006> | Google Scholar
- 61 Bissonnette R., Stein Gold L., Rubenstein D. S., Tallman A. M., Armstrong A (2021) **Tapinarof in the treatment of psoriasis: A review of the unique mechanism of action of a novel therapeutic aryl hydrocarbon receptor-modulating agent** *J Am Acad Dermatol* **84**:1059–1067 <https://doi.org/10.1016/j.jaad.2020.10.085> | Google Scholar
- 62 Di Meglio P., et al. (2014) **Activation of the aryl hydrocarbon receptor dampens the severity of inflammatory skin conditions** *Immunity* **40**:989–1001 <https://doi.org/10.1016/j.immuni.2014.04.019> | Google Scholar
- 63 Halawani D., et al. (2024) **Aryl hydrocarbon receptor restricts axon regeneration of DRG neurons in response to injury** *bioRxiv* <https://doi.org/10.1101/2023.11.04.565649> | Google Scholar
- 64 Barroso A., Mahler J. V., Fonseca-Castro P. H., Quintana F. J (2021) **The aryl hydrocarbon receptor and the gut-brain axis** *Cell Mol Immunol* **18**:259–268 <https://doi.org/10.1038/s41423-020-00585-5> | Google Scholar
- 65 Kim S. H., et al. (2022) **Mapping of the Sensory Innervation of the Mouse Lung by Specific Vagal and Dorsal Root Ganglion Neuronal Subsets** *eNeuro* **9** <https://doi.org/10.1523/ENEURO.0026-22.2022> | Google Scholar
- 66 McGovern A. E., et al. (2015) **Evidence for multiple sensory circuits in the brain arising from the respiratory system: an anterograde viral tract tracing study in rodents** *Brain Struct Funct* **220**:3683–3699 <https://doi.org/10.1007/s00429-014-0883-9> | Google Scholar
- 67 Prescott S. L., Umans B. D., Williams E. K., Brust R. D., Liberles S. D (2020) **An Airway Protection Program Revealed by Sweeping Genetic Control of Vagal Afferents** *Cell* **181**:574–589 <https://doi.org/10.1016/j.cell.2020.03.004> | Google Scholar
- 68 Nonomura K., et al. (2017) **Piezo2 senses airway stretch and mediates lung inflation-induced apnoea** *Nature* **541**:176–181 <https://doi.org/10.1038/nature20793> | Google Scholar
- 69 Chang R. B., Storchlic D. E., Williams E. K., Umans B. D., Liberles S. D (2015) **Vagal Sensory Neuron Subtypes that Differentially Control Breathing** *Cell* **161**:622–633 <https://doi.org/10.1016/j.cell.2015.03.022> | Google Scholar
- 70 Usoskin D., et al. (2015) **Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing** *Nat Neurosci* **18**:145–153 <https://doi.org/10.1038/nn.3881> | Google Scholar
- 71 Schappe M. S., et al. (2024) **A vagal reflex evoked by airway closure** *Nature* **627**:830–838 <https://doi.org/10.1038/s41586-024-07144-2> | Google Scholar
- 72 Bin N. R., et al. (2023) **An airway-to-brain sensory pathway mediates influenza-induced sickness** *Nature* **615**:660–667 <https://doi.org/10.1038/s41586-023-05796-0> | Google Scholar
- 73 Isensee J., et al. (2021) **Depolarization induces nociceptor sensitization by CaV1.2-mediated PKA-II activation** *J Cell Biol* **220** <https://doi.org/10.1083/jcb.202002083> | Google Scholar

- 74 Costain G., et al. (2019) **Rare copy number variations affecting the synaptic gene DMXL2 in neurodevelopmental disorders** *J Neurodev Disord* **11** <https://doi.org/10.1186/s11689-019-9263-3> | Google Scholar
- 75 Fillingim R. B., et al. (2005) **The A118G single nucleotide polymorphism of the mu-opioid receptor gene (OPRM1) is associated with pressure pain sensitivity in humans** *J Pain* **6**:159–167 <https://doi.org/10.1016/j.jpain.2004.11.008> | Google Scholar
- 76 Garzon J., et al. (2015) **HINT1 protein: a new therapeutic target to enhance opioid antinociception and block mechanical allodynia** *Neuropharmacology* **89**:412–423 <https://doi.org/10.1016/j.neuropharm.2014.10.022> | Google Scholar
- 77 Guo Y., et al. (2023) **Elevated levels of FMRP-target MAP1B impair human and mouse neuronal development and mouse social behaviors via autophagy pathway** *Nat Commun* **14**:3801 <https://doi.org/10.1038/s41467-023-39337-0> | Google Scholar
- 78 Duraikannu A., Martinez J. A., Chandrasekhar A., Zochodne D. W (2018) **Expression and Manipulation of the APC-beta-Catenin Pathway During Peripheral Neuron Regeneration** *Sci Rep* **8** <https://doi.org/10.1038/s41598-018-31167-1> | Google Scholar
- 79 Davis S. M., Pennypacker K. R (2018) **The role of the leukemia inhibitory factor receptor in neuroprotective signaling** *Pharmacol Ther* **183**:50–57 <https://doi.org/10.1016/j.pharmthera.2017.08.008> | Google Scholar
- 80 Jain A., et al. (2024) **Nociceptor-immune interactomes reveal insult-specific immune signatures of pain** *Nat Immunol* **25**:1296–1305 <https://doi.org/10.1038/s41590-024-01857-2> | Google Scholar
- 81 Thakur M., et al. (2014) **Defining the nociceptor transcriptome** *Front Mol Neurosci* **7** <https://doi.org/10.3389/fnmol.2014.00087> | Google Scholar
- 82 Wang J. C., et al. (2024) **Basophils Activate Pruriceptor-Like Vagal Sensory Neurons** *bioRxiv* <https://doi.org/10.1101/2024.06.11.598517> | Google Scholar
- 83 Wilson S. R., et al. (2013) **The epithelial cell-derived atopic dermatitis cytokine TSLP activates neurons to induce itch** *Cell* **155**:285–295 <https://doi.org/10.1016/j.cell.2013.08.057> | Google Scholar
- 84 Caceres A. I., et al. (2009) **A sensory neuronal ion channel essential for airway inflammation and hyperreactivity in asthma** *Proc Natl Acad Sci U S A* **106**:9099–9104 <https://doi.org/10.1073/pnas.0900591106> | Google Scholar
- 85 Bessac B. F., et al. (2009) **Transient receptor potential ankyrin 1 antagonists block the noxious effects of toxic industrial isocyanates and tear gases** *FASEB J* **23**:1102–1114 <https://doi.org/10.1096/fj.08-117812> | Google Scholar
- 86 Balestrini A., et al. (2021) **A TRPA1 inhibitor suppresses neurogenic inflammation and airway contraction for asthma treatment** *J Exp Med* **218** <https://doi.org/10.1084/jem.20201637> | Google Scholar
- 87 Yeo H., et al. (2024) **The EGR1-Artemin Axis in Keratinocytes Enhances the Innervation of Epidermal Sensory Neurons during Skin Inflammation Induced by House Dust Mite Extract from Dermatophagoidesfarinae** *J Invest Dermatol* **144**:1817–1828 <https://doi.org/10.1016/j.jid.2024.01.017> | Google Scholar

- 88 Helme R. D., Eglezos A., Dandie G. W., Andrews P. V., Boyd R. L (1987) **The effect of substance P on the regional lymph node antibody response to antigenic stimulation in capsaicin-pretreated rats** *J Immunol* **139**:3470–3473 [Google Scholar](#)
- 89 Sponchiado M., et al. (2021) **Overexpression of Substance P in pig airways increases MUC5AC through an NF-kbeta pathway** *Physiol Rep* **9**:e14749 <https://doi.org/10.14814/phy2.14749> | [Google Scholar](#)
- 90 Hanes W. M., et al. (2016) **Neuronal Circuits Modulate Antigen Flow Through Lymph Nodes** *Bioelectron Med* **3**:18–28 <https://doi.org/10.15424/bioelectronmed.2016.00001> | [Google Scholar](#)
- 91 Huang S., et al. (2021) **Lymph nodes are innervated by a unique population of sensory neurons with immunomodulatory potential** *Cell* **184**:441–459 <https://doi.org/10.1016/j.cell.2020.11.028> | [Google Scholar](#)
- 92 Riol-Blanco L., et al. (2014) **Nociceptive sensory neurons drive interleukin-23-mediated psoriasiform skin inflammation** *Nature* **510**:157–161 <https://doi.org/10.1038/nature13199> | [Google Scholar](#)
- 93 Klose C. S. N., et al. (2017) **The neuropeptide neuromedin U stimulates innate lymphoid cells and type 2 inflammation** *Nature* **549**:282–286 <https://doi.org/10.1038/nature23676> | [Google Scholar](#)
- 94 Cardoso V., et al. (2017) **Neuronal regulation of type 2 innate lymphoid cells via neuromedin U** *Nature* **549**:277–281 <https://doi.org/10.1038/nature23469> | [Google Scholar](#)
- 95 Wallrapp A., et al. (2017) **The neuropeptide NMU amplifies ILC2-driven allergic lung inflammation** *Nature* **549**:351–356 <https://doi.org/10.1038/nature24029> | [Google Scholar](#)
- 96 Nussbaum J. C., et al. (2013) **Type 2 innate lymphoid cells control eosinophil homeostasis** *Nature* **502**:245–248 <https://doi.org/10.1038/nature12526> | [Google Scholar](#)
- 97 Sui P., et al. (2018) **Pulmonary neuroendocrine cells amplify allergic asthma responses** *Science* **360** <https://doi.org/10.1126/science.aan8546> | [Google Scholar](#)
- 98 Ike E., Kawano T., Takahashi K., Miyasaka T., Takahashi T (2023) **Calcitonin Gene-Related peptide receptor antagonist suppresses allergic asthma responses via downregulation of group 2 innate lymphoid cells in mice** *Int Immunopharmacol* **122** <https://doi.org/10.1016/j.intimp.2023.110608> | [Google Scholar](#)
- 99 Wallrapp A., et al. (2019) **Calcitonin Gene-Related Peptide Negatively Regulates Alarmin-Driven Type 2 Innate Lymphoid Cell Responses** *Immunity* **51**:709–723 <https://doi.org/10.1016/j.immuni.2019.09.005> | [Google Scholar](#)
- 100 Huang C., et al. (2023) **TRPV1(+) neurons alter Staphylococcus aureus skin infection outcomes by affecting macrophage polarization and neutrophil recruitment** *BMC Immunol* **24** <https://doi.org/10.1186/s12865-023-00584-x> | [Google Scholar](#)
- 101 Cohen J. A., et al. (2019) **Cutaneous TRPV1(+) Neurons Trigger Protective Innate Type 17 Anticipatory Immunity** *Cell* **178**:919–932 <https://doi.org/10.1016/j.cell.2019.06.022> | [Google Scholar](#)
- 102 Giambelluca M. S., et al. (2014) **TNF-alpha expression in neutrophils and its regulation by glycogen synthase kinase-3: a potentiating role for lithium** *FASEB J* **28**:3679–3690 <https://doi.org/10.1096/faseb.201400367>

doi.org/10.1096/fj.14-251900 | [Google Scholar](#)

- 103 Feiken E., Romer J., Eriksen J., Lund L. R (1995) **Neutrophils express tumor necrosis factor-alpha during mouse skin wound healing** *J Invest Dermatol* **105**:120–123 <https://doi.org/10.1111/1523-1747.ep12313429> | [Google Scholar](#)
- 104 Kobziar L. N., et al. (2022) **Wildland fire smoke alters the composition, diversity, and potential atmospheric function of microbial life in the aerobiome** *ISME Commun* **2** <https://doi.org/10.1038/s43705-022-00089-5> | [Google Scholar](#)
- 105 Tavares-Ferreira D., et al. (2022) **Spatial transcriptomics of dorsal root ganglia identifies molecular signatures of human nociceptors** *Sci Transl Med* **14**:eabj8186 <https://doi.org/10.1126/scitranslmed.abj8186> | [Google Scholar](#)
- 106 Karlsson M., et al. (2021) **A single-cell type transcriptomics map of human tissues** *Science advances* **7**:eabh2169 [Google Scholar](#)
- 107 Talbot S., et al. (2011) **Cigarette smoke-induced kinin B1 receptor promotes NADPH oxidase activity in cultured human alveolar epithelial cells** *Peptides* **32**:1447–1456 <https://doi.org/10.1016/j.peptides.2011.05.005> | [Google Scholar](#)
- 108 Pereira P. J., et al. (2015) **GRPR/PI3Kgamma: Partners in Central Transmission of Itch** *J Neurosci* **35**:16272–16281 <https://doi.org/10.1523/JNEUROSCI.2310-15.2015> | [Google Scholar](#)
- 109 Zilionis R., et al. (2019) **Single-Cell Transcriptomics of Human and Mouse Lung Cancers Reveals Conserved Myeloid Populations across Individuals and Species** *Immunity* **50**:1317–1334 <https://doi.org/10.1016/j.immuni.2019.03.009> | [Google Scholar](#)
- 110 Kolberg L., et al. (2023) **g: Profiler-interoperable web service for functional enrichment analysis and gene identifier mapping (2023 update)** *Nucleic acids research* **51**:W207–W212 [Google Scholar](#)
- 111 Ashburner M., et al. (2000) **Gene ontology: tool for the unification of biology** *Nature genetics* **25**:25–29 [Google Scholar](#)
- 112 Kanehisa M., Furumichi M., Sato Y., Kawashima M., Ishiguro-Watanabe M (2023) **KEGG for taxonomy-based analysis of pathways and genomes** *Nucleic acids research* **51**:D587–D592 [Google Scholar](#)
- 113 Gillespie M., et al. (2022) **The reactome pathway knowledgebase 2022** *Nucleic acids research* **50**:D687–D692 [Google Scholar](#)
- 114 Martens M., et al. (2021) **WikiPathways: connecting communities** *Nucleic acids research* **49**:D613–D621 [Google Scholar](#)
- 115 Wingender E (2008) **The TRANSFAC project as an example of framework technology that supports the analysis of genomic regulation** *Briefings in bioinformatics* **9**:326–332 [Google Scholar](#)
- 116 Tsitsiridis G., et al. (2023) **CORUM: the comprehensive resource of mammalian protein complexes5—[2022** *Nucleic acids research* **51**:D539–D545 [Google Scholar](#)
- 117 Köhler S., et al. (2021) **The human phenotype ontology in 2021** *Nucleic acids research* **49**:D1207–D1217 [Google Scholar](#)

Author information

Jo-Chiao Wang

Department of Pharmacology and Physiology, University de Montreal, Montreal, Canada

Amelia Kulle

Department of Microbiology and Immunology, McGill University, Montreal, Canada

Theo Crosson

Department of Pharmacology and Physiology, University de Montreal, Montreal, Canada

Amin Reza Nikpoor

Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Canada

Surbhi Gupta

Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Canada

Anais Roger

Department of Pharmacology and Physiology, University de Montreal, Montreal, Canada,
Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Canada

Moutih Rafei

Department of Pharmacology and Physiology, University de Montreal, Montreal, Canada

Ajitha Thanabalasuriar

Department of Microbiology and Immunology, McGill University, Montreal, Canada,
Department of Pharmacology and Therapeutics, McGill University, Montreal, Canada

Sebastien Talbot

Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Canada,
Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden
ORCID iD: [0000-0001-9932-7174](https://orcid.org/0000-0001-9932-7174)

sebas.talbot@gmail.com; sebastien.talbot@queensu.ca; sebastien.talbot@ki.se

Editors

Reviewing Editor

Anurag Agrawal

Ashoka University, Sonapat, India

Senior Editor

Satyajit Rath

Indian Institute of Science Education and Research (IISER), Pune, India

Reviewer #1 (Public review):

Summary:

In the presented study, the authors aim to explore the role of nociceptors in the fine particulate matter (FPM) mediated Asthma phenotype, using rodent models of allergic airway inflammation. This manuscript builds on previous studies, and identify transcriptomic reprogramming and an increased sensitivity of the jugular nodose complex (JNC) neurons, one of the major sensory ganglion for the airways, on exposure to FPM along with Ova during the challenge phase. The authors then use OX-314 a selectively permeable form of lidocaine, and TRPV1 knockouts to demonstrate that nociceptor blocking can reduce airway inflammation in their experimental setup.

The authors further identify the presence of Gfra3 on the JNC neurons, a receptor for the protein Artemin, and demonstrate their sensitivity to Artemin as a ligand. They further show that alveolar macrophages release Artemin on exposure to FPM.

Strengths:

The study builds on results available from multiple previous works, and presents important results which allow insights into the mixed phenotypes of Asthma seen clinically. In addition, by identifying the role of nociceptors, they identify potential therapeutic targets which bear high translational potential.

Weaknesses:

While the results presented in the study are highly relevant, there is a need for further mechanistic dissection to allow better inferences. Currently, certain results seem associative. Also, certain visualisations and experimental protocols presented in the manuscript need careful assessment and interpretation.

While Asthma is a chronic disease, the presented results are particularly important to explore Asthma exacerbations in response to acute exposure to air pollutants. This is relevant in today's age of increasing air pollution and increasing global travel.

Comments on revisions:

Thank you for addressing the suggestions. No further comments.

<https://doi.org/10.7554/eLife.101988.2.sa3>

Reviewer #2 (Public review):

Summary:

The authors sought to investigate the role of nociceptor neurons in the pathogenesis of pollution-mediated neutrophilic asthma. The authors overall achieved the aim of demonstrating that nociceptor neurons are important to the pathogenesis of pollution-exacerbated asthma. Their results support their conclusions overall, although there are ways the study findings can be strengthened. This work further evaluates how nociceptor neurons contribute to asthma pathogenesis important for consideration while proposing treatment strategies for under treated asthma endotypes.

Strengths:

The authors utilize TRPV1 ablated mice to confirm the effects of intranasally administered QX-314 utilized to block sodium currents.

Use of intravital microscopy to track alveolar macrophage and neutrophil motility in their model

The authors demonstrate that via artemin, which is upregulated in alveolar macrophages in response to pollution, sensitizes JNC neurons thereby increasing their responsiveness to pollution. Ablation or inactivity of nociceptor neurons prevented the pollution induced increase in inflammation.

Weaknesses:

While neutrophilic, unclear of the endotype of asthma represented by the model

Comments on revisions:

The authors have addressed or commented on all concerns.

<https://doi.org/10.7554/eLife.101988.2.sa2>

Reviewer #3 (Public review):

Asthma is a complex disease that includes endogenous epithelial, immune and neural components that respond to environmental stimuli. Small airborne particles with diameters in the range of 2.5 micrometers or less, so-called PM2.5, are thought to contribute to some forms of asthma. These forms of asthma may have neutrophils, eosinophils and macrophages in bronchoalveolar lavage. Here, Wang and colleagues build on a recent model that incorporated PM2.5 which was found to have a neutrophilic component. Wang altered the model to provide an extra kick via the incorporation of ovalbumin. The major strength of this work is that silencing TRPV1-expressing neurons either pharmacologically or genetically, modulated inflammation and the motility of neutrophils. By examining bronchoalveolar lavage fluid, they found not only that levels of a number of cytokines were increased, but also that artemin, a protein that supports neuronal development and function, was elevated, which did not occur in nociceptor- ablated mice. Their data strengthens links between pollutants, immune and neural interactions.

Comments on revisions:

The manuscript has been revised extensively, including the addition of new experiments, such as intravital microscopy. Did the comments from the reviewers, manifest by additional experiments and modifying how some of the data was presented, result in any changes in the hypotheses or the interpretation of such?

<https://doi.org/10.7554/eLife.101988.2.sa1>

Author response:

The following is the authors' response to the original reviews.

Reviewer #1 (Public review):

In the presented study, the authors aim to explore the role of nociceptors in the fine particulate matter (FPM) mediated Asthma phenotype, using rodent models of allergic airway inflammation. This manuscript builds on previous studies and identify transcriptomic reprogramming and an increased sensitivity of the jugular nodose complex (JNC) neurons, one of the major sensory ganglia for the airways, on exposure to FPM along with Ova during the challenge phase. The authors then use OX-314 a selectively permeable form of lidocaine, and TRPV1 knockouts to demonstrate that nociceptor blocking can reduce airway inflammation in their experimental setup. The

authors further identify the presence of Gfra3 on the JNC neurons, a receptor for the protein Artemin, and demonstrate their sensitivity to Artemin as a ligand. They further show that alveolar macrophages release Artemin on exposure to FPM.

We thank the reviewer for their valuable comments, which have significantly enhanced the quality of our manuscript. A point-by-point rebuttal is provided below.

Strength

The study builds on results available from multiple previous work and presents important results which allow insights into the mixed phenotypes of Asthma seen clinically. In addition, by identifying the role of nociceptors, they identify potential therapeutic targets which bear high translational potential.

Weakness

While the results presented in the study are highly relevant, there is a need for further mechanistic dissection to allow better inferences. Currently certain results seem associative. Also, certain visualisations and experimental protocols presented in the manuscript need careful assessment and interpretation. While Asthma is a chronic disease, the presented results are particularly important to explore Asthma exacerbations in response to acute exposure to air pollutants. This is relevant in today's age of increasing air pollution and increasing global travel.

Major

The JNC is a major group of neurons responsible for receiving sensory inputs from the airways. However, the DRG also contains nociceptors and is known to receive afference from the upper airways. An explanation of why the study was restricted to the JNC would be important.

We acknowledge that some afferents to the upper airways do arise from the DRG, specifically in the upper thoracic segments (T1–T5). We have added a statement in the text to note this subset of nociceptive and spinally mediated pathways. However, the preponderance of evidence indicates that the majority of airway and lung afferents (70–80%, sometimes up to 90%) originate from the jugular–nodose complex (JNC). Given this large imbalance—and because our study focuses on the mechanosensory, and chemosensory functions mediated primarily by the JNC—we restricted our analysis to this main vagal pathway. By contrast, DRG innervation, though functionally important for nociception and irritation-related reflexes, accounts for a smaller yet significant (~20–30%) fraction of the total afferent pool. The referenced tracing studies[1,2] support this distribution and are cited to clarify our rationale for emphasizing the JNC in our work.

Similarly, the role of the Artemin in the study remains associative. The study results present that Artemin sensitize nociceptors to lead to an increased inflammatory response (Supplementary Figure 2), however, both upstream and downstream evidence for this inference needs to be dissected further. For instance, the evidence for the role of Artemin in the model comes from ex vivo experiments with alveolar macrophages, but not in the experimental model created. Blocking or activation experiments could be performed, along with investigating the change in the total number of nociceptors with Artemin exposure. Similarly, the downstream effects of the potential Artemin-mediated JNC stimulation should be explored in the context of this experimental setup. A detailed dissection of the mechanisms is important. Additionally, it is also important to discuss the hypothesis leading to the selection of Artemin as a target, which currently seems arbitrary.

Our data show that exogenous i) OVA-FPM exposed AM secrete Artemin and that ii) recombinant Artemin can sensitize nociceptors, potentially heightening the inflammatory response. As suggested, we agree that more upstream and downstream evidence is needed for definitive mechanistic insight. In response, we have expanded our experiments to include intravital microscopy, which demonstrates impaired motility of alveolar macrophages and neutrophils in nociceptor-ablated mice, suggesting a bidirectional crosstalk between AMs and nociceptor neurons.

In future studies, we will perform blocking or activation studies to clarify Artemin's in vivo effects and confirm its role in modulating airway nociceptors. We also recognize the importance of examining whether Artemin exposure alters the phenotype of these neurons and lung innervation density. As recommended, we plan targeted interventions (e.g., Artemin-neutralizing antibodies or overexpression strategies) to delineate the mechanisms by which Artemin-mediated nociceptor stimulation influences the local inflammatory environment.

We have expanded our discussion to clarify that Artemin is a recognized growth factor known to sensitize certain sensory neurons, including those responsive to tissue injury and inflammation. This literature-based rationale guided our hypothesis that Artemin might increase nociceptor reactivity in the lung and thereby influence alveolar macrophage function. By combining ex vivo and intravital approaches, we have begun to map these interactions but agree that further in vivo studies are necessary to confirm causality, dissect signal transduction pathways, and fully validate Artemin's contributions to AM–nociceptor crosstalk. We have revised our manuscript accordingly to highlight these limitations.

A deeper exploration of the inflammatory parameters could be performed. The multiplex analysis of the cytokine analysis shows a reduction in certain cytokines like IL-6 and MCP (figure 3F), which needs to be discussed. Additionally, investigating the change in proportions of the different immune cell populations is important, which currently restricts the eosinophil and neutrophil counts in the BAL. This is also important as the study builds on work from Prof. Chang's group, which also identified the expansion of an invariant iNKT cell population by FPM, regulatory in nature. Adding data on airway hyperresponsiveness, if possible, would be a welcome addition, considering Asthma as the disease context.

We thank the reviewer for highlighting the need for a more comprehensive exploration of inflammatory parameters. To address these concerns:

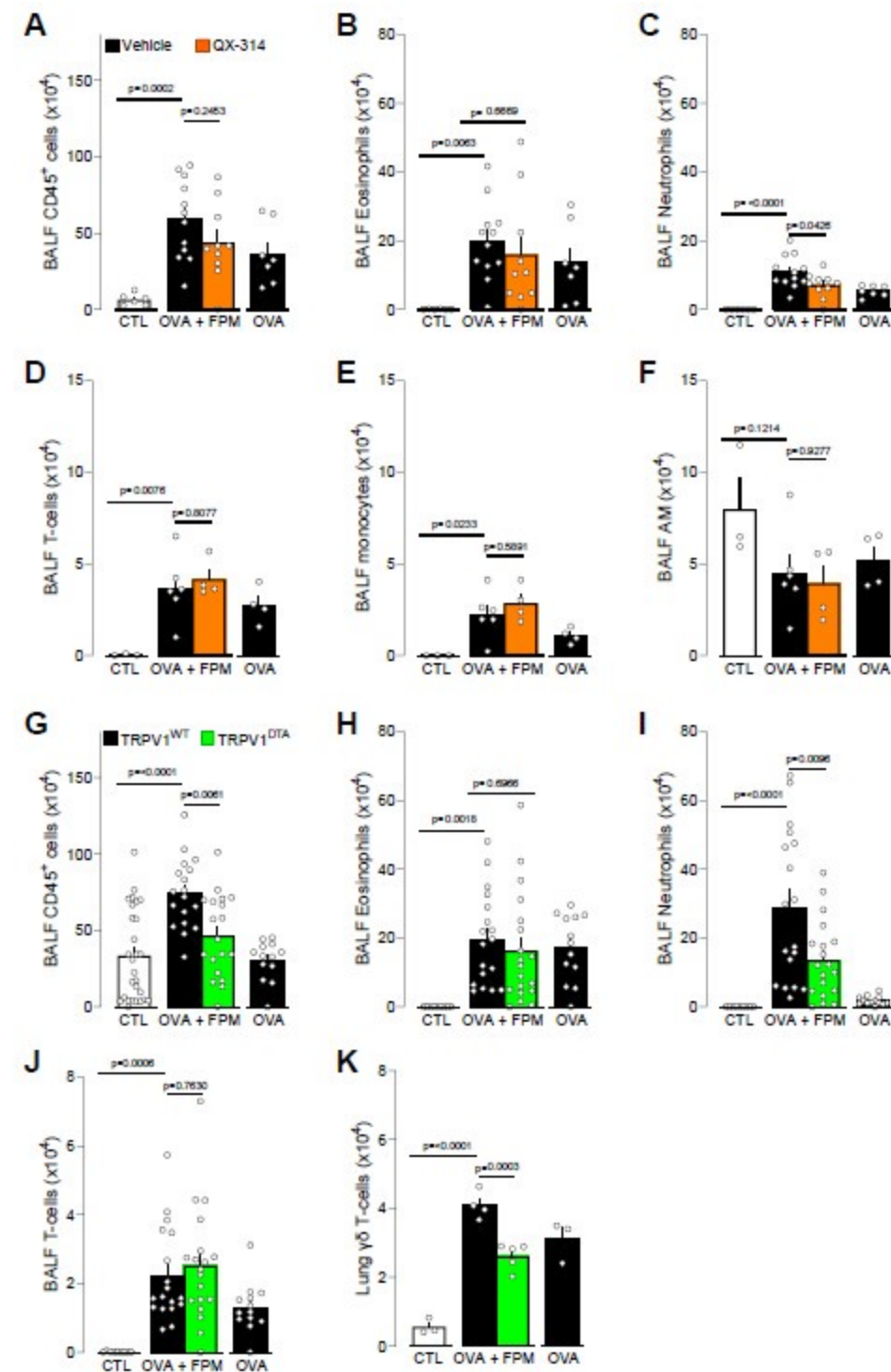
- (1) Cytokine Analysis: We re-ran all statistical analyses, including the CBA and ELISA assays, and confirmed that TNF α and Artemin are the only differentially expressed cytokines across experimental groups. We have expanded the Discussion to emphasize TNF α 's role in this context.
- (2) Immune Cell Profiling in BALF: Our data show that co-exposure with FPM exacerbates CD45⁺ cells, eosinophil, neutrophil, T-cells and monocyte infiltration. Notably, CD45⁺ cells and neutrophils were the only population reduced under nociceptor neuron loss-of-function conditions (QX314–treated or TRPV1-DTA mice, Author response image 1).

Of note, we also confirmed these data using intravital imaging and in a second line of nociceptor ablated mice (NaV1.8DTA). We are aware of Prof. Chang's work suggesting expansion of an invariant iNKT cell population this population in future

- (3) Airway Hyperresponsiveness (AHR): We recognize that adding AHR data would strengthen the asthma-related context. Unfortunately, we are not currently equipped to

perform AHR measurements, but we intend to include this in future experiments to provide a more complete assessment of airway function.

Author response image 1.



The authors could revisit the data presented in terms of visualization. For instance, the pooled data presented in some of the figures is probably leading to a wide variation which makes interpretation more difficult. Presenting data separately for each experimental replicate might help the reader. This is also important considering the

possible variation seen between experiments (for instance, in Figure 3A and 3C and 3B and 3D, the neutrophil and eosinophil panels for the same groups seem to have an almost 2-fold difference.). Similarly, in the cytokine analysis, the authors have used a common axis for depicting all cytokine values which leads to difficulties in interpretation (Figure 3F). Analysis of the RNA seq results and the DEGs could be revisited to include pathway analysis etc (Figure 2), and the supplementary information could include detailed lists of the major target genes.

To address this query, we have completely reformatted all graphs and included both gene lists and lists of enriched pathways for all three comparisons in Supplementary Table 1. We also confirmed our flow cytometry analysis functionally by performing intravital imaging.

The authors should also consider citing the previous experimental setup used for some particular protocols. For instance, the use of the specified protocol for OVA in a C57 background needs to be justified, as there are various protocols reported in the literature. Additionally, doses used in some experiments seem arbitrary (The FPM and Artemin exposure in Figure 4). Depicting the dose-response curve or citing previous literature for the same would be important. Similarly, different sample sizes seen in experiments should be explained, whether they are due to mortality, failure to exhibit phenotypes, or due to technical failures. The RNA seq experiment mentions only 2 biological replicates in one of the groups which should be addressed either by increasing the sample size or by replicating the experiment. Moreover, nested comparisons in experiments performed for Figure 1 need to be performed. Neurons isolated from each mouse should be maintained and analysed separately to retain biological replicates to better represent the heterogeneity.

We appreciate the request for clarity regarding the experimental protocols and sample sizes:

OVA Model in C57BL/6 Mice: We adapted a previously published OVA protocol in C57BL/6 mice[3-5] (PMID: 39661516), which uses two doses of sensitization to compensate for the lower Th2 response compared to BALB/c[6]. We increased the dose of OVA (100 µg) because our initial experiments produced low eosinophil infiltration. Although this dosage is on the higher side, some studies have noted local IFN γ induction in C57BL/6 mice; however, we did not detect IFN γ in our setup.

FPM and Artemin Doses: We did not perform a full dose-response assay for FPM and Artemin but used 100 ng/mL as reported in prior literature, where TRPA1 and TRPV1 mRNA were upregulated after 18 hours of incubation[7]. This reference has been added for clarity.

Sample Sizes and Exclusions: One control mouse was excluded from the RNA-seq experiment because a parallel PCA analysis indicated it was an outlier. This was the only exclusion in the study, and this have been indicated in the method section of the article.

Nested Comparisons and Biological Replicates: We reanalyzed the relevant data with a nested one-way ANOVA and updated the figures accordingly. Neurons isolated from each mouse were first averaged to preserve biological replicates and capture potential heterogeneity; and data was analysed on the per mouse averages.

The manuscript should be more detailed regarding the statistics employed. Currently, there is a section mentioned in the methods section, but details of corrections employed and specific stats for specific experiments should be described. There are also some minor grammatical errors and incomplete sentences in the manuscript which should be corrected. The authors should also consider a more expansive literature review in the introduction/discussion sections.

We have updated the figure legends and methods to include more detailed information on the specific statistical tests used for each experiment. In addition, we have fixed minor grammatical errors and incomplete sentences throughout the manuscript. Finally, we have expanded our Introduction and Discussion to include additional references and a broader literature context.

Reviewer #2 (Public review):

The authors sought to investigate the role of nociceptor neurons in the pathogenesis of pollution-mediated neutrophilic asthma.

We thank the reviewer for their valuable comments, which have significantly enhanced the quality of our manuscript. A point-by-point rebuttal is provided below.

Strength

The authors utilize TRPV1 ablated mice to confirm effects of intranasally administered QX-314 utilized to block sodium currents. The authors demonstrate that via artemin, which is upregulated in alveolar macrophages in response to pollution, sensitizes JNC neurons thereby increasing their responsiveness to pollution. Ablation or inactivity of nociceptor neurons prevented the pollution induced increase in inflammation.

Weakness

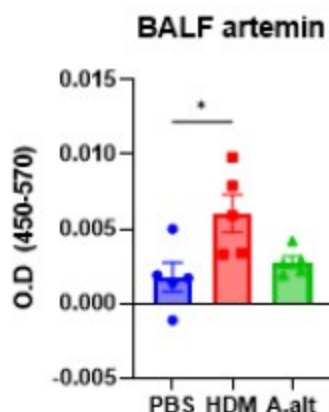
While neutrophilic, the model used doesn't appear to truly recapitulate a Th2/Th17 phenotype. No IL-17A is visible/evident in the BALF fluid within the model. (Figure 3F). Unclear of the relevance of the RNAseq dataset, none of the identified DEGs were evaluated in the context of mechanism. The authors overall achieved the aim of demonstrating that nociceptor neurons are important to the pathogenesis of pollution-exacerbated asthma. Their results support their conclusions overall, although there are ways the study findings can be strengthened. This work further evaluates how nociceptor neurons contribute to asthma pathogenesis important for consideration while proposing treatment strategies for undertreated asthma endotypes.

Major

Utilizing a different model, one using house dust mite or alternaria alternata or similar that is able to induce a true Th2/th17 type response that is also more translatable to humans for confirmation.

We appreciate the suggestion to use additional allergen models. In a pilot study, we did observe increased Artemin in the BALF of house dust mite-treated mice, although the levels were low under our current dosing schedule (20 µg/dose daily from Day 0–4 and Day 7–9, with sacrifice on Day 10; Author response image 2). Conversely, using an Alternaria alternata model at 100 µg/dose daily from Day 0–2 (sacrificed on Day 3) did not yield a detectable increase in Artemin. We suspect these findings may reflect the specific dose and timing used. We plan to refine our protocols (e.g., longer exposures or higher doses) for HDM and/or Alternaria to better model a Th2/Th17 response and further validate our observations in a setting more translatable to human asthma.

Author response image 2.



Additional analysis, maybe pathway analysis on the RNAseq dataset presented in Figure 2. Unclear how these genes are relevant/how they affect functionality. At present it is acceptable to say they are transcriptionally reprogrammed, but no protein evaluation is provided which would get more at function, however, the authors do show some functional data in Figure 1, so maybe this could somehow be discussed/related to Figure 2.

We have expanded our RNA-seq analysis to include gene lists and enriched pathways for all three comparisons in Supplementary Table 1. We have also revised our discussion to align these transcriptomic changes with the functional data shown in Figure 1. While we have not yet performed protein-level validation for all identified genes, the patterns observed in our RNA-seq dataset suggest pathways potentially tied to nociceptor activation and the downstream inflammatory response. We plan to conduct targeted protein analyses in future studies to further substantiate these findings.

Histology and localization of neutrophils/nociceptor neurons/alveolar macrophages would enhance the study findings.

We appreciate the reviewer's suggestion to include histological data showing the distribution of neutrophils, nociceptor neurons, and alveolar macrophages. While we have not yet performed detailed histological staining of these cell types, we have added live in-vivo intravital microscopy data (Figure 4) that illustrate impaired AM and neutrophil motility in nociceptor-ablated mice. We plan to include additional histological analyses in future studies to further localize these cells in the lung tissue.

Minor:

The first 3 figures are small and hard to read.

We have enlarged Figures 1 and 3 in the revised manuscript to improve readability. We have also added the corresponding gene lists and enriched pathways to Supplementary Table 1 for clarity.

The figures are mislabeled in the text. Figure 2 is discussed twice in two different contexts; the second mention is supposed to be labeled as Figure 2.

We corrected the mislabeled figures in the text, ensuring that each figure is referenced accurately.

Figure 4 isn't cited in the text. I think it is supposed to be referenced in the paragraph before the discussion starts and is currently labeled as Figure 1.

We have updated the text to properly cite Figure 4 in the relevant paragraph before the Discussion begins, rather than labeling it as Figure 1.

Notating which statistical analysis was used with each figure/subfigure would be beneficial. Also, it's important to notate if the data was analyzed for multiple comparisons.

We have revised each figure/subfigure legend to specify the statistical tests used, including information on whether corrections for multiple comparisons were applied. This provides a clearer understanding of how each dataset was analyzed.

Reviewer #3 (Public review):

Asthma is a complex disease that includes endogenous epithelial, immune, and neural components that respond awkwardly to environmental stimuli. Small airborne particles with diameters in the range of 2.5 micrometers or less, so-called PM2.5, are generally thought to contribute to some forms of asthma. These forms of asthma may have increased numbers of neutrophils and/or eosinophils present in bronchoalveolar lavage fluid and are difficult to treat effectively as they tend to be poorly responsive to steroids. Here, Wang and colleagues build on a recent model that incorporated PM2.5 which was found to have a neutrophilic component. Wang altered the model to provide an extra kick via the incorporation of ovalbumin. Building on their prior expertise linking nociceptors and inflammation, they find that silencing TRPV1-expressing neurons either pharmacologically or genetically, abrogated inflammation and the accumulation of neutrophils. By examining bronchoalveolar lavage fluid, they found not only that levels of the number of cytokines were increased, but also that artemin, a protein that supports neuronal development and function, was elevated, which did not occur in nociceptor-ablated mice. They also found that alveolar macrophages exposed to PM2.5 particles had increased artemin transcription, suggesting a further link between pollutants, and immune and neural interactions.

We thank the reviewer for their valuable comments, which have significantly enhanced the quality of our manuscript. A point-by-point rebuttal is provided below.

Weakness

There are substantial caveats that must be attached to the suggestions by the authors that targeting nociceptors might provide an approach to the treatment of neutrophilic airway inflammation in pollution-driven asthma in general and wildfire-associated respiratory problems in particular.

These caveats include the uncertainty of the relevance of the conventional source of PM2.5, to pollution and asthma. According to the National Institute of Standards and Technology (NIST), the standard reference material (SRM) 2786 is a mix obtained from an air intake system in the Czech Republic. It is not clear exactly what is in the mix, and a recent bioRxiv preprint, <https://www.biorxiv.org/content/10.1101/2023.08.18.553903v3.full.pdf> reveals the presence of endotoxin. Care should thus be taken in interpreting data using particulate matter. Regarding wildfires, there is data that indicates that such

exposure is toxic to macrophages. What impact might that then have on the production of cytokines, and artemin, in humans?

We recognize the potential limitations of using SRM2786 (obtained from a Czech air-intake system) as a model for realworld PM2.5 exposure. Our rationale for choosing SRM2786 is that it is commercially available and represents a broad spectrum of ambient air pollutants, in contrast to more specialized sources like diesel exhaust particles. However, we acknowledge in the discussion the presence of endotoxin in SRM2786, as suggested by recent reports, and agree that this may influence immune responses and should be considered when interpreting our data.

Regarding wildfire-associated exposure, we are aware that certain components of wildfire smoke can be toxic to macrophages. We do not think this play a significant role in the current study design as number of AMs, as determined by flow cytometry and intravital microscopy, are similar when comparing OVA-exposed mice to OVA-FPM exposed animals. Thus, these results rule out significant AM toxicity by FPM.

Ultimately, while our findings suggest that modulating nociceptor activity may reduce neutrophilic inflammation, we emphasize that additional research—including different PM2.5 sources, validation of endotoxin levels, and in vivo confirmation in human-relevant models—is necessary before drawing definitive conclusions about treating pollutiondriven asthma or wildfire-induced respiratory problems.

The Introductory paragraph implies links between wildfire events, particular exposure, and neutrophilic asthma. I am not aware of such a link having been established, in which case the paragraph needs revision. In the paragraph that begins with 'Urban pollution', it is suggested that eosinophilic asthma is treatment responsive in comparison to the neutrophilic form. That may not be the case, and they may often these cellular components may occur together. In much of the manuscript, there is a mismatch between the text and the figure numbers. For example, in the Results, Figure 2 should be Figure 3 some of the time, and Figure 3 is actually Figure 4, while the reference to Figure 1F-H is Figure 4H. Please check carefully.

(a) Introduction Paragraph and Wildfire–Neutrophilic Asthma Link

We add references to the introduction to support the link between wildfire, respiratory symptoms and the link to neutrophilic asthma [8-12].

(b) Distinction Between Eosinophilic and Neutrophilic Asthma

We recognize that eosinophilic and neutrophilic airway infiltrates can co-occur in the same individual and that treatment responsiveness can vary considerably. Our intention was to note that conventional asthma therapies (e.g., inhaled corticosteroids) are generally more effective for eosinophilic-driven disease than for neutrophilic phenotypes, but we agree that these inflammatory endotypes often overlap in clinical practice. We have revised the text in the “Urban pollution” section to acknowledge this complexity and to clarify that inflammatory cell populations in asthma are not always discrete.

Figure Numbering and Text–Figure Mismatch

We sincerely apologize for the confusion caused by mismatched figure labels and references in the Results section. We have carefully reviewed and corrected all figure references throughout the manuscript to ensure accuracy.

References

- (1) Kim, S. H. et al. Mapping of the Sensory Innervation of the Mouse Lung by Specific Vagal and Dorsal Root Ganglion Neuronal Subsets. *eNeuro* 9 (2022). <https://doi.org/10.1523/ENEURO.0026-22.2022>
- (2) McGovern, A. E. et al. Evidence for multiple sensory circuits in the brain arising from the respiratory system: an anterograde viral tract tracing study in rodents. *Brain Struct Funct* 220, 3683-3699 (2015). <https://doi.org/10.1007/s00429-014-0883-9>
- (3) Shen, C.-C., Wang, C.-C., Liao, M.-H. & Jan, T.-R. A single exposure to iron oxide nanoparticles attenuates antigen-specific antibody production and T-cell reactivity in ovalbumin-sensitized BALB/c mice. *International journal of nanomedicine*, 1229-1235 (2011).
- (4) Delayre-Orthez, C., De Blay, F., Frossard, N. & Pons, F. Dose-dependent effects of endotoxins on allergen sensitization and challenge in the mouse. *Clinical & Experimental Allergy* 34, 1789-1795 (2004).
- (5) Morokata, T., Ishikawa, J. & Yamada, T. Antigen dose defines T helper 1 and T helper 2 responses in the lungs of C57BL/6 and BALB/c mice independently of splenic responses. *Immunology letters* 72, 119-126 (2000).
- (6) Li, L., Hua, L., He, Y. & Bao, Y. Differential effects of formaldehyde exposure on airway inflammation and bronchial hyperresponsiveness in BALB/c and C57BL/6 mice. *PLoS One* 12, e0179231 (2017).
- (7) Ikeda-Miyagawa, Y. et al. Peripherally increased artemin is a key regulator of TRPA1/V1 expression in primary afferent neurons. *Molecular pain* 11, s12990-12015-10004-12997 (2015).
- (8) Baan, E. J. et al. Characterization of Asthma by Age of Onset: A Multi-Database Cohort Study. *J Allergy Clin Immunol Pract* 10, 1825-1834 e1828 (2022). <https://doi.org/10.1016/j.jaip.2022.03.019>
- (9) de Nijs, S. B., Venekamp, L. N. & Bel, E. H. Adult-onset asthma: is it really different? *Eur Respir Rev* 22, 44-52 (2013). <https://doi.org/10.1183/09059180.00007112>
- (10) Gianniou, N. et al. Acute effects of smoke exposure on airway and systemic inflammation in forest firefighters. *J Asthma Allergy* 11, 81-88 (2018). <https://doi.org/10.2147/JAA.S136417>
- (11) Noah, T. L., Worden, C. P., Rebuli, M. E. & Jaspers, I. The Effects of Wildfire Smoke on Asthma and Allergy. *Curr Allergy Asthma Rep* 23, 375-387 (2023). <https://doi.org/10.1007/s11882-023-01090-1>
- (12) Wilgus, M. L. & Merchant, M. Clearing the Air: Understanding the Impact of Wildfire Smoke on Asthma and COPD. *Healthcare (Basel)* 12 (2024). <https://doi.org/10.3390/healthcare12030307>

<https://doi.org/10.7554/eLife.101988.2.sa0>