

Extended allergen exposure in asthmatic monkeys induces neuroplasticity in nucleus tractus solitarius

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Background: Extended exposure to allergen exacerbates asthma symptoms, in part via complex interactions between inflammatory cells and mediators. One consequence of these interactions is the triggering of local and central nervous system (CNS) neuronal activity that might further exacerbate the asthmalike symptoms by causing bronchoconstriction, mucous secretion, increased microvascular leak, and cough. One CNS region that might be particularly important is the caudomedial nucleus tractus solitarius (NTS). NTS neurons not only integrate primary afferent inputs from lung sensory nerve fibers but also have direct exposure to inhaled allergens and allergen-induced blood-borne inflammatory mediators via a deficient blood-brain barrier. Given the capacity of CNS neurons to undergo plasticity, allergen-induced changes in NTS neuronal properties could contribute to the exaggerated respiratory responses to extended allergen exposure.

Objective: In a recently developed rhesus monkey model of allergic asthma, we tested the hypothesis that extended exposure to allergen increases the intrinsic excitability of NTS neurons.

Methods: Three adult monkeys were sensitized and then repeatedly exposed to aerosols of house dust mite allergen; 4 monkeys served as controls. Whole-cell current-clamp recordings were made to measure 3 indices of excitability: resting membrane potential, input resistance, and number of action potentials evoked by current injections.

Results: Extended allergen exposure depolarized the resting membrane potential by 14% and increased the number of action potentials evoked by current injections (5-fold).

Conclusion: The finding that NTS neurons in a primate model of allergic asthma undergo intrinsic increases in excitability suggests that CNS mechanisms might contribute to the exaggerated symptoms in asthmatic individuals exposed to allergen. (*J Allergy Clin Immunol* 2001;108:557-62.)

Key words: Asthma, vagus nerve, solitary nucleus

Abbreviations used

CNS: Central nervous system
HDMA: House dust mite allergen
NTS: Nucleus tractus solitarius
RAR: Rapidly adapting receptor

It is well established that extended repeated exposures to allergen exacerbates asthma symptoms^{1,2} and that allergen-induced release of various local inflammatory mediators plays an important role in these asthma exacerbations.^{3,4} However, what might have been underappreciated until now is that the inflammatory mediators released during allergen exposure also trigger increases in local and central nervous system (CNS) neuronal activity that might further exacerbate the asthmalike symptoms.⁴ Stimulation of 2 types of primary sensory nerves innervating the lungs—the vagal nonmyelinated lung C fibers and the thinly myelinated A δ rapidly adapting receptors (RARs)—initiates powerful CNS reflex responses resembling asthmalike symptoms: bronchoconstriction, mucous secretion, increased microvascular leak, and cough.^{5,6} Electrophysiologic recordings of the impulse activity of the first-order vagal lung C fibers^{7,8} and RARs^{9,10} have provided direct evidence that acute exposure to allergen or to specific mediators released during allergen exposure not only stimulates these primary sensory nerves in the vagus but also sensitizes them to other stimulants.

The first-order vagal sensory nerves make their first synapses in the CNS on second-order neurons in the nucleus tractus solitarius (NTS). These NTS neurons provide the initial and perhaps pivotal regulatory moment in the lung CNS reflex pathways. First, these neurons process the primary peripheral sensory information from the lung nerve fibers to ultimately encode and transform the inputs into reflex outputs to the lungs, airways, and respiratory muscles.¹¹ Second, NTS neurons receiving primary sensory afferent signals over the vagus nerve have the capacity to integrate the peripheral sensory information with convergent signals from other brain regions, including the adjacent area postrema, the most caudal of the circumventricular organs.¹²⁻¹⁶ By virtue of its lack of a blood brain barrier and its prominent axonal projections to the NTS, the area postrema provides an anatomical pathway whereby mediators released during allergen exposure can affect NTS neurons. In this regard,

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we have previously shown that stimulation of area postrema neurons facilitates NTS neuronal processing of vagal afferent inputs, essentially amplifying the output of the NTS neurons to sensory signals.¹³ Finally, it is now appreciated that the caudomedial NTS, where the lung sensory fibers terminate, also lacks a complete blood brain barrier and features local complexes of fenestrated capillaries and perivascular spaces that afford the NTS neurons direct exposure to blood-borne inflammatory mediators.¹⁷ Pertinently, it has recently been documented that NTS neurons contribute to immune responses independent of vagal afferent projections.^{18–20} The introduction of allergen into the lungs and airways thus has the capacity to influence NTS neurons not only over sensory neural pathways but also via convergent inputs from other brain regions and via direct humoral mechanisms.

Beyond the allergen-associated acute increases in the firing activity of the primary lung sensory nerves, allergic inflammation associated with acute allergen exposure also causes phenotypic changes in the cell bodies of the lung fibers. These changes include an increase in the number of mRNA-encoding tachykinins,²¹ de novo substance P expression in A δ vagal afferent fiber (which includes RAR) cell bodies,²² and increases in neuronal membrane excitability, including depolarization of the membrane potential and blockade of an anomalous rectifier.⁷ The extent to which such changes contribute to the exacerbated asthmalike symptoms will turn on the behavior of neurons in the NTS. Although no studies have heretofore reported phenotypic changes in neurochemical or electrophysiologic properties of NTS neurons after chronic allergen exposure, there is evidence of allergen-induced neuroplasticity in other CNS regions. Electroencephalographic recordings have shown that hypothalamic and cortical activity are markedly increased after allergen sensitization in the rat,²³ perhaps providing a neural basis to help explain some of the subtle deficits in cognitive processing reported to occur with allergen exposure in human beings.²⁴ Given the extensive capacity of CNS neurons to undergo neuroplasticity,^{25–28} persistent changes in the NTS neuronal properties might become particularly important in the exaggerated airway and respiratory responses to extended repeated allergen exposures.

Our group recently developed a rhesus monkey model of allergic asthma.²⁹ The monkeys exhibited many of the features of allergic asthma, meeting the following 8 criteria: (1) a positive skin test result to house dust mite allergen (HDMA); (2) airway obstruction with inhalation of HDMA; (3) shedding of airway epithelium; (4) an abundance of immune cells (including eosinophils) in the bronchoalveolar lavage; (5) airway hyperresponsiveness to histamine; (6) airway mucous cell hyperplasia and epithelial hypertrophy; (7) thickening of the airway basement membrane zone; and (8) accumulation of eosinophils in the airway walls.²⁹ This model has allowed us to undertake studies in an allergic asthmatic animal that is morphologically and immunologically similar to the human being to test the hypothesis that

extended repeated exposure to allergen increases the intrinsic excitability of NTS neurons. Experiments were performed on brainstem slices taken in the NTS and tractus solitarius containing the vagal primary sensory afferent fibers conveyed. The brainstem slices were taken from allergic asthmatic and field control monkeys. We studied neurons in the caudomedial NTS—specifically, in the NTS region 0 to 1.5 mm caudal to the obex and medial to the tractus, where the majority of lung sensory nerve fibers synapse.¹¹ Whole-cell current-clamp recordings were used to measure 2 indices of neuronal excitability: the resting membrane potential and the number of action potentials discharged by the neuron in response to depolarizing current injections. We also measured the input resistance to determine whether changes in resting membrane conductances were associated with the changes in membrane potential.

METHODS

All protocols were approved by the Institutional Animal Care and Use Committee in compliance with the Animal Welfare Act and Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Allergic asthmatic monkey model

Seven rhesus monkeys (*Macaca mulatta*; 4.93 to 6.95 kg; 3 to 5 years old at the time of necropsy; both sexes) were studied. Four monkeys served as controls; the other 3 underwent sensitization and repeated exposures to aerosols of HDMA (*Dermaphgyoides farinae*, Greer), developing characteristics of asthma, as described previously.²⁹

Brainstem slice preparation

The monkeys in the HDMA groups were studied approximately 18 hours after the last HDMA exposure. They were sedated with Telazol (8 mg/kg, delivered intramuscularly) and killed with overdose of sodium pentobarbital. After decapitation, the brain was rapidly exposed and submerged in ice-cold high-sucrose artificial cerebrospinal fluid. Transverse brainstem coronal slices (250 μ m thick) were cut and prepared for recording, as described previously.³⁰ All experiments were performed at 33° to 34°C.

Whole-cell current-clamp recording

Each slice was viewed through use of a fixed-stage upright microscope equipped with infrared differential interference contrast for visualizing the neurons. Whole-cell recordings in NTS neurons were obtained through use of KCl solution-filled glass electrodes and an Axoclamp 1D patch-clamp amplifier (Axon Instruments).³¹ Whole-cell voltages were filtered at 2 kHz, digitized at 10 kHz, and stored in a DX/D computer. The seal resistance was always >1 G Ω , the series resistance was <30 M Ω , and the pipette resistance was 2.5–4.5 M Ω . Data were analyzed through use of pClamp6 software (Axon Instruments).

All caudomedial NTS neurons that had stable resting membrane potentials and were synaptically activated by stimulating the tractus solitarius were included in the study. The resting membrane potential was measured immediately after the whole-cell configuration. The voltage was then current-clamped at –60 mV; all neurons were thus studied at the same membrane potential. Steady-state input resistance was determined by injecting hyperpolarizing currents (10 to 100 pA; 200-ms duration pulses) and measuring the resultant steady-state change in membrane potential. Neuronal spiking in

response to nonspecific excitation was tested by intracellularly injecting brief (2-second) depolarizing current pulses (20 to 100 pA) and measuring all of the following: (1) the peak frequency of the evoked spikes, defined as the peak frequency in the first 200 ms; (2) total number of spikes evoked; (3) spike frequency adaptation, defined as the ratio of the mean spiking frequency in the last 500 ms to the peak spiking frequency in the first 200 ms; and (4) latency to the first spike evoked.

Data analysis

Data were expressed as means $\pm$ SEMs. Significance was $P < .05$. An unpaired t test³² was used to compare the membrane potential and input resistance in HDMA-exposed and control monkeys. To determine the HDMA effect on the spiking responses to the depolarizing current injections, we used a 2-way ANOVA with exposure (HDMA vs control) as the between factor and injected current as the within factor. We compared the peak frequency and total number of evoked spikes, spike frequency adaptation, and latency to first evoked spike.

RESULTS

Data were obtained from 20 neurons, 9 of them being neurons from 3 field control monkeys and the other 11 being neurons from 2 HDMA-exposed monkeys. No data were obtained from 1 monkey in each group.

Fig 1, A shows a photomicrograph of an NTS slice with a bipolar stimulating electrode placed in the tractus solitarius and the patch pipette in the caudal NTS medial to the tractus. The whole-cell configuration for recording whole-cell voltages is shown in Fig 1, B. An example of a tractus solitarius-evoked action potential from the neuron is shown in Fig 1, C. All neurons studied were in this caudomedial region of the NTS, from obex to 1.5 mm caudal to the obex and medial to the tractus solitarius, and all were activated with short-latency tractus-evoked excitatory postsynaptic potentials (2.52 ± 0.30 ms) or action potentials (3.09 ± 0.62 ms). The onset latencies of the evoked responses were not different in the 2 groups ($P > .05$).

The effect of extended repeated allergen exposures on the resting membrane potential is shown in Fig 2. Neurons from the HDMA-exposed monkeys had a significantly more depolarized resting membrane potential (-52.8 ± 1.5 mV vs -45.7 ± 2.5 mV for control vs HDMA; $P = .033$).

Fig 3 illustrates the increased neuronal excitability to depolarizing current injections in the HDMA-exposed monkeys. As shown in the example (Fig 3, A) and in the group data (Fig 3, B and C), when the membrane potential was depolarized from a holding potential of -60 mV with current injections of increasing amplitudes, the total number of spikes (Fig 3, B) and the peak frequency (Fig 3, C) were greater in neurons from the HDMA-exposed monkeys than in those from the field controls.

There was no effect of HDMA exposure on the incidence or extent of spike frequency adaptation. Three neurons from the HDMA-exposed group and 2 neurons from the field control group discharged action potentials in the first 200 ms and then adapted completely. Four neurons from each group adapted more slowly; in those neurons the ratio of the mean spiking frequency in the last 500 ms to the peak spiking frequency in the first 200 ms was not

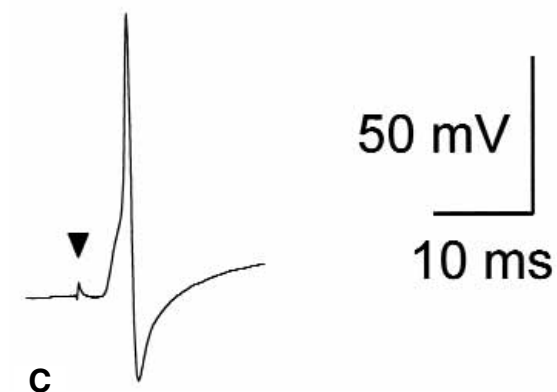
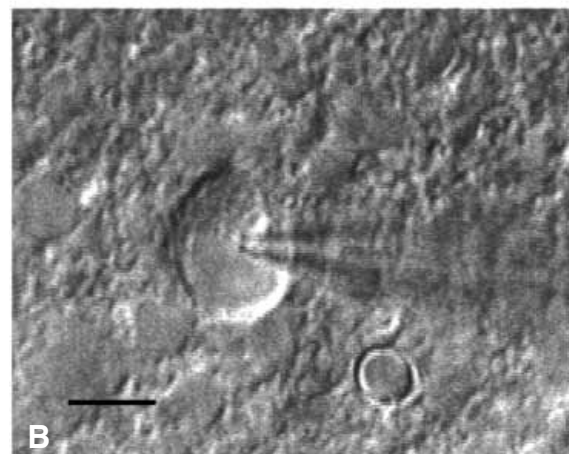
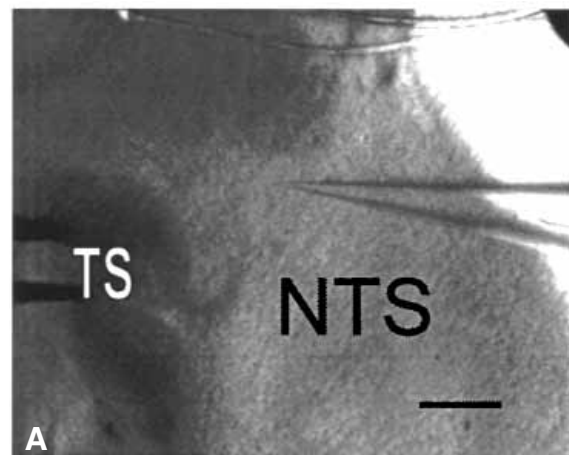


FIG 1. A, Photograph shows an NTS slice with stimulating electrode in the tractus solitarius (TS) and a recording pipette in the caudomedial NTS. Bar, 100 μ m. B, Photograph shows the whole-cell formation in a caudomedial NTS neuron. Bar, 10 μ m. C, Example of TS-evoked action potential from the neuron shown in Fig 1, B ($\blacktriangledown$, stimulus artifact).

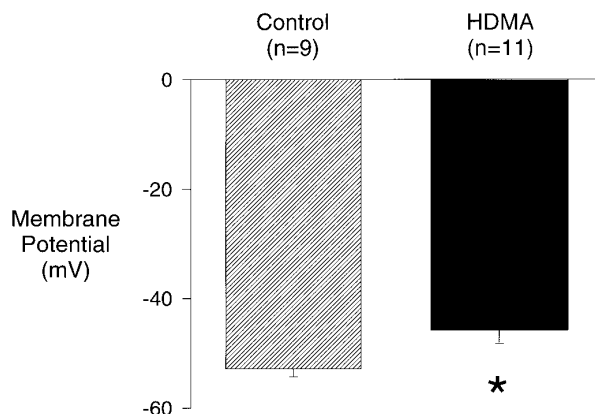


FIG 2. Group data show membrane potential of neurons from control and HDMA-exposed monkeys. NTS neurons from the allergen-exposed monkeys had a significantly more depolarized resting membrane potential.

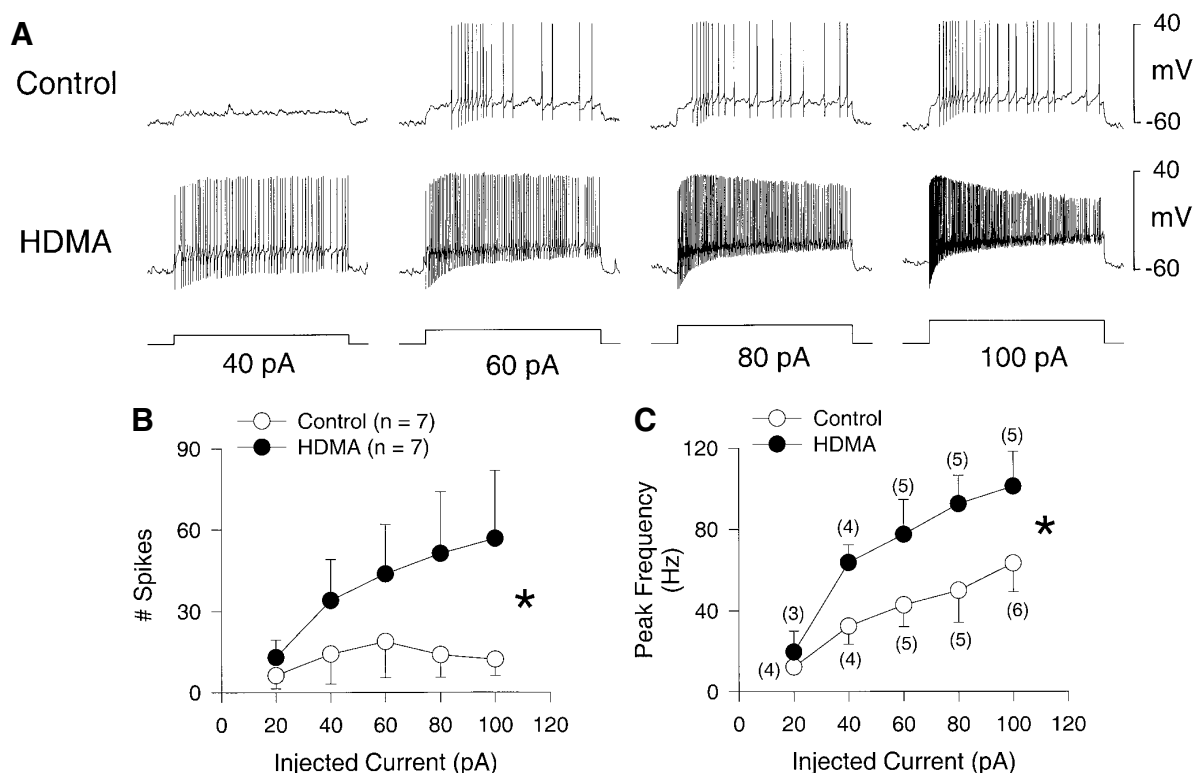


FIG 3. Neuronal response to intracellular depolarizing current injections. **A**, Responses to current injections in neurons from a control monkey (*top panels*) and an allergen-exposed monkey (*bottom panels*). **B**, Group data show the total number of spikes evoked by depolarizing current injections. As the amplitude of the current injection was increased, the difference in the number of evoked spikes was greater in the allergen-exposed monkeys than in the control monkeys ($P = .19$ for exposure [control vs HDMA]; $P = .002$ for current; $P = .048$ for interaction between current and exposure). **C**, Group data show that the peak spiking frequency was greater in the allergen-exposed monkeys ($P = .0017$ for exposure [control vs HDMA]; $P = .001$ for current; $P = .82$ for interaction between current and exposure). Numerals in parentheses indicate numbers of neurons.

different in the 2 groups ($P = .16$, exposure [control vs HDMA]). One neuron from the control group did not discharge action potentials to current injections. HDMA exposure also did not affect the latency to the first evoked action potential ($P = .48$, exposure [control vs HDMA]).

Although there was a trend toward a decrease in the input resistance in the NTS neurons from the HDMA-exposed monkeys, the difference did not reach statistical significance ($473 \pm 115 \text{ M}\Omega$ vs $234 \pm 49 \text{ M}\Omega$ for control vs HDMA; $P = .076$).

DISCUSSION

The major finding of this work was that extended repeated exposures to allergen in a primate model of allergic asthma resulted in an increased excitability of NTS neurons, as evidenced by a more depolarized resting membrane potential and an increased spiking response to intracellular injections of depolarizing currents. Although there was a trend toward a decrease in the membrane input resistance, the difference did not reach statistical significance. The NTS neurons were located in the caudomedial NTS, where vagal afferent fibers originating from the lungs and airways terminate,³³ and they were activated with short latencies to stimulation of the tractus solitarius, suggesting that they were part of autonomic—including pulmonary—afferent pathways.

To our knowledge, this is the first study to demonstrate that extended exposure to allergen causes changes in intrinsic membrane properties of neurons in the NTS. The chronically more depolarized membrane potentials suggest that in the resting condition the NTS neurons are closer to the threshold voltage for discharging action potentials than are neurons from field control monkeys. Thus subthreshold sensory inputs, which might have no effect in control monkeys, could evoke postsynaptic spikes and hence a neuronal output in HDMA-exposed monkeys. In addition, suprathreshold inputs might evoke more action potentials in the postsynaptic neurons from allergen-exposed monkeys, thereby amplifying the neuronal output at these synapses. The increased peak number of the spikes evoked by depolarizing current injections suggests a heightened responsiveness to rapid changes in the membrane potential; moreover, in terms of the total spikes evoked, as the magnitude of the depolarizing current was increased, the effects of HDMA exposure became more prominent, suggesting that acute robust volleys of afferent traffic will evoke a disproportionately larger effect on neurons from allergen-exposed monkeys. Although there was a tendency for the input resistance to be smaller in the allergen-exposed monkeys, the difference did not reach statistical significance. Interestingly, in an investigation of the effect of acute allergen exposure on the properties of nodose ganglion cells, Undem et al⁷ found a more depolarized resting membrane potential, though with various changes in input resistance, including decreases, increases, and no change.

The precise mechanisms linking prolonged allergen exposure to the increase in the intrinsic excitability of the NTS neurons were not addressed in this study; however, there is a parallel to the increased excitability of spinal neurons during prolonged neuropathic or inflammatory pain. The neural mechanisms of chronic pain have been characterized by Woolf and Doubell^{34,35} as an increase in the intrinsic postsynaptic excitability of spinal neurons that might be triggered in part by the combination of (1) an induced novel input from peripheral A β afferent fibers, (2) an exaggerated input from the peripheral nociceptive C fibers, (3) local changes in inhibitory and excitatory synaptic inputs, and (4) phenotypic changes in the

postsynaptic neurons themselves.³⁵ With regard to the current study, previous findings that exposure to allergen and inflammatory mediators excites and sensitizes the primary lung C fibers and A δ RAR fibers,⁷ coupled with findings that allergen exposure induces phenotypic changes in the cell bodies of the lung sensory fibers,²¹ point to the possibility that a change in the frequency, magnitude, or pattern of lung sensory inputs to the NTS triggers the postsynaptic neuronal changes—a possibility buttressed by previous findings that NTS neurons have the capacity to undergo fine structural plasticity during prolonged excitatory input from baroreceptor afferent nerve fibers.²⁸ On the other hand, the changes in the postsynaptic neurons could be mediated independently of the sensory neural pathways through changes in local synaptic inputs from other brain regions^{12,13} or via changes induced by prolonged direct access to allergen or allergen-induced mediators.¹⁷

Regardless of the underlying mechanism, the ability of these NTS neurons to undergo plasticity in their membrane properties will shape the extent to which the CNS lung sensory reflexes contribute to the asthmalike responses to extended allergen exposure. Of direct relevance to the capacity of NTS neurons to undergo functional plasticity is the ability of NTS neurons to undergo fine structural plasticity, as has previously been demonstrated during prolonged excitatory input from baroreceptor afferent nerve fibers.²⁸

There are some limitations to this study. Because of the expense of developing the model and the need to coordinate these neurophysiologic studies with a number of studies on various other aspects of the allergic asthmatic monkeys, the sample size was small. Despite the small sample size, by using whole-cell recordings in brainstem slices, we were able to detect statistically significant differences in 2 of 3 intrinsic membrane properties of neurons taken from the field control and allergic asthmatic monkeys. In addition, though all recordings were made in the same discrete NTS region where lung sensory afferent fibers terminate and though all neurons were shown to receive peripheral afferent input via the tractus solitarius, we could not unequivocally identify the neurons as part of the lung RAR or C fiber pathway in the slice preparation. However, given localization of lung sensory afferent fiber synapses in this NTS region, it seems reasonable to assume that most of the neurons studied have some relationship to lung reflexes.

In summary, these findings demonstrate in a primate model of allergic asthma the ability of a CNS region containing neurons that process lung sensory signals to undergo marked increases in excitability with extended allergen exposure. Resolving the extent of the contribution of CNS neuronal mechanisms in allergic asthma might help in the development of novel therapeutic strategies that can prevent the functional plasticity in the central network, thereby minimizing the respiratory symptoms of allergen exposure in people with asthma.

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REFERENCES

- Delfino RJ, Coate BD, Zeiger RS, Seltzer JM, Street DH, Koutrakis P. Daily asthma severity in relation to personal ozone exposure and outdoor fungal spores. *Am J Respir Crit Care Med* 1996;154:633-41.
- Arshad SH, Hamilton RG, Adkinson NF Jr. Repeated aerosol exposure to small doses of allergen: a model for chronic allergic asthma. *Am J Respir Crit Care Med* 1998;157:1900-6.
- Lemanske RF. Inflammatory events in asthma: an expanding equation. *J Allergy Clin Immunol* 2000;105(6 Pt 2):S633-S636.
- Undem BJ, Kajekar R, Hunter DD, Myers AC. Neural integration and allergic disease. *J Allergy Clin Immunol* 2000;106(5 Suppl):S213-S220.
- Coleridge JCG, Coleridge HM. Afferent vagal C-fibre innervation of the lungs and airways and its functional significance. *Rev Physiol Biochem Pharmacol* 1984;99:1-109.
- Mills JE, Widdicombe JG. Activity of lung irritant receptors in pulmonary microembolism, anaphylaxis and drug induced bronchoconstriction. *J Physiol* 1969;203:337-57.
- Undem BJ, Hubbard W, Weinreich D. Immunologically-induced neuromodulation of guinea pig nodose ganglion neurons. *J Auton Nerv Syst* 1998;44:7-34.
- Coleridge HM, Coleridge JCG, Ginzel KH, Banzett RB, Morrison MA. Stimulation of 'irritant' receptors and afferent C-fibres in the lungs by prostaglandins. *Nature* 1976;264:451-3.
- Berggren DR, Myers DL, Mohrman M. Activity of rapidly-adapting receptors to histamine and antigen challenge before and after sodium cromoglycate. *Arch Int Pharmacodyn* 1985;273:88-99.
- Riccio MM, Myers AC, Undem BJ. Immunomodulation of afferent neurons in guinea-pig isolated airway. *J Physiol (Lond)* 1996;491:499-509.
- Loewy AD. Central autonomic pathways. In: Loewy AD, Spyer KM, editors. *Central regulation of autonomic function*. New York: Oxford University Press; 1990. p. 88-103.
- van der KD, Koda LY. Organization of the projections of a circumventricular organ: the area postrema in the rat. *J Comp Neurol* 1983;219:328-38.
- Bonham AC, Hasser EM. Area postrema and aortic or vagal afferents converge to excite cells in nucleus tractus solitarius. *Am J Physiol* 1993;264(5 Pt 2):H1674-H1685.
- Aylwin ML, Horowitz JM, Bonham AC. Non-NMDA and NMDA receptors in the synaptic pathway between area postrema and nucleus tractus solitarius. *Am J Physiol* 1998;275(4 Pt 2):H1236-H1246.
- Chen C-Y, Bonham AC. Non-NMDA and NMDA receptors transmit area postrema input to aortic baroreceptor neurons in nucleus tractus solitarius. *Am J Physiol* 1998;275(Heart Circ. Physiol.):H1695-H1706.
- Chen CY, Bonham AC. Non-NMDA and NMDA receptors transmit area postrema input to aortic baroreceptor neurons in NTS. *Am J Physiol* 1998;275(5 Pt 2):H1695-H1706.
- Gross PM, Wall KM, Pang JJ, Shaver SW, Wainman DS. Microvascular specializations promoting rapid interstitial solute dispersion in nucleus tractus solitarius. *Am J Physiol* 1990;259(6 Pt 2):R1131-R1138.
- Gordon FJ. Effect of nucleus tractus solitarius lesions on fever produced by interleukin-1beta. *Auton Neurosci* 2000;85:102-10.
- Goehler LE, Gaykema RP, Hansen MK, Anderson K, Maier SF, Watkins LR. Vagal immune-to-brain communication: a visceral chemosensory pathway. *Auton Neurosci* 2000;85:49-59.
- Dantzer R, Konsman JP, Bluthé RM, Kelley KW. Neural and humoral pathways of communication from the immune system to the brain: parallel or convergent? *Auton Neurosci* 2000;85:60-5.
- Fischer A, McGregor GP, Saria A, Philippin B, Kummer W. Induction of tachykinin gene and peptide expression in guinea pig nodose primary afferent neurons by allergic airway inflammation. *J Clin Invest* 1996;98:2284-91.
- Undem BJ, Hunter DD, Liu M, Haak-Frendscho M, Oakragly A, Fischer A. Allergen-induced sensory neuroplasticity in airways. *Int Arch Allergy Immunol* 1999;118:150-3.
- Thakur IS. Change in the electroencephalographic and g-aminobutyric acid transaminase and succinic semialdehyde dehydrogenase in the allergen induced rat brain. *Biochem Int* 1988;16:235-43.
- Marshall PS, O'Hara C, Steinberg P. Effects of seasonal allergic rhinitis on selected cognitive abilities. *Ann Allergy Asthma Immunol* 2000;84:403-10.
- Nicoll RA, Malenka RC. Contrasting properties of two forms of long-term potentiation in the hippocampus. *Nature* 1995;377:115-8.
- Paulsen O, Sejnowski TJ. Natural patterns of activity and long-term synaptic plasticity. *Curr Opin Neurobiol* 2000;10:172-9.
- Bower AJ. Plasticity in the adult and neonatal central nervous system. *Br J Neurosurg* 1990;4:253-64.
- Chan RK, Peto CA, Sawchenko PE. Fine structure and plasticity of barosensitive neurons in the nucleus of solitary tract. *J Comp Neurol* 2000;422:338-51.
- Schelegle ES, Gershwin LJ, Miller LA, Fanucci MV, Van Winkle LS, Gerriets JP, et al. Allergic asthma induced in rhesus monkeys using house dust mite (*Dermatophagoides farinae*). *Am J Pathol* 2001;158:333-41.
- Chen C-Y, Horowitz JM, Bonham AC. A presynaptic mechanism contributes to depression of autonomic signal transmission in NTS. *Am J Physiol* 1999;277:H1350-H1360.
- Aylwin ML, Horowitz JM, Bonham AC. Non-NMDA and NMDA receptors in the synaptic pathway between area postrema and nucleus tractus solitarius. *Am J Physiol* 1998;275:H1236-H1246.
- Ott L. *An introduction to statistical methods and data analysis*. North Scituate (MA): Duxbury Press; 1977.
- Bonham AC, Joad JP. Neurons in commissural nucleus tractus solitarii required for full expression of the pulmonary C fibre reflex in rat. *J Physiol (Lond)* 1991;441:95-112.
- Woolf CJ. Evidence for a central component of post-injury pain hypersensitivity. *Nature* 1983;306:686-8.
- Woolf CJ, Doubell TP. The pathophysiology of chronic pain: increased sensitivity to low threshold Ab-fibre inputs. *Curr Opin Neurobiol* 1994;4:525-34.