
Alteration of brain activation patterns in nonallergic rhinitis patients using functional magnetic resonance imaging before and after treatment with intranasal azelastine

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Background: Although nonallergic rhinitis (NAR) patients tend to be more sensitive to chemical/olfactory stimuli, a suprathreshold olfactory response or the presence of specific olfactory receptor genes do not explain why their symptoms are triggered by such exposures.

Objective: To investigate differential neurogenic responses to azelastine in NAR patients, using functional magnetic resonance imaging (fMRI) in response to specific olfactory triggers.

Methods: A longitudinal study design on 12 subjects with a physician diagnosis of NAR previously demonstrated to be clinically responsive to intranasal azelastine (Astelin) was performed. Subjects underwent fMRI during exposure to unpleasant (hickory smoke) and pleasant (vanilla) odorants while off and then on azelastine for 2 weeks. The olfactory fMRI paradigm consisted of a visually triggered sniff every 21 seconds with synchronized delivery of a 4 second pulse of odorant. Each odorant was presented 18 times over 4–6-minute fMRI runs. Continuous fresh air was presented to wash out each odorant after presentation.

Results: Nonallergic rhinitis patients exhibited increased blood flow to several regions of the brain in response to both pleasant and unpleasant odorants, specifically in odor-sensitive regions, while off intranasal azelastine. Treatment with intranasal azelastine significantly attenuated blood flow to regions of the brain relevant to either olfactory sensation or sensory processing in response to these odorants compared with fresh air.

Conclusion: The general reduction compared with increase in brain activation in NAR patients on versus off azelastine suggests that a possible effect of this medication may be reduction of brain responses to odorants.

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INTRODUCTION

Nonallergic rhinitis (NAR) is a chronic rhinitis condition in which patients present with upper respiratory symptoms such as nasal congestion, postnasal drainage, and rhinorrhea triggered by a spectrum of odorants (eg, smoke, perfumes/potpourris) and environmental weather conditions (eg, temperature and barometric pressure changes) and are skin test negative to seasonal and perennial aeroallergens.^{1,2} Treatment

response also may be an important phenotypic marker for NAR. Specific Food and Drug Administration-approved therapies for NAR, such as intranasal azelastine, are particularly effective in alleviating congestion and postnasal drainage symptoms in these patients, even though the mechanisms of action for this effect remain elusive.^{3–6}

Although NAR patients are more sensitive to chemical/olfactory stimuli, a suprathreshold olfactory response has not adequately explained why their symptoms are triggered by these exposures.⁷ Studies thus far have not definitively established a clear immunologic or nonimmunologic mechanism involved for NAR.^{8,9} Many investigators have suggested that autonomic dysfunction secondary to release of neuropeptides (eg, substance P and neurokinin A, found in the vasculature walls of veins and arteries in the nasal mucosa) and nitric oxide are contributory factors to the pathogenesis of NAR.⁸ Nonallergic rhinitis patients also might have an abnormality in their olfactory transduction pathway, which could lead to pathological changes in the olfactory mucosa.¹⁰ Previously, certain olfactory receptor genes were demonstrated to be overexpressed in patients with NAR.¹¹ More recently, transient receptor potential ion channels, which play a major role in pain, have been speculated to play a central role in the physiologic responses exhibited by NAR patients in response

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to a spectrum of chemicals, temperatures, and physical stimuli.¹² This observation is supported by studies that found intranasal capsaicin, which activates the transient response potential vanilloid1 receptor, is effective in reducing symptoms in NAR patients.^{13,14}

Neuroimaging, coupled to a flow dilution olfactometer that is compatible with functional magnetic resonance imaging (fMRI), has been used previously to demonstrate that the emotional potency of odor-invoked memory is correlated with specific activation of the amygdala.¹⁵ Simple sniffing activates the piriform cortex,¹⁶ whereas sniffing plus odor perception activates the piriform cortex, entorhinal and orbitofrontal cortex, the hippocampus, thalamus, caudate nucleus, and insula.¹⁷ Patterns of brain activation differ depending on the olfactory task performed during brain imaging procedures.^{15,17} In this study, fMRI coupled with olfactory stimulation (ie, exposure to specific odorants known to trigger their symptoms, nontrigger odorants, or clear air) was used to determine the differential neuroimaging responses in NAR patients while off and on intranasal azelastine sequentially for 2 weeks.

METHODS

Study design

A longitudinal study design outlined in Table 1 was conducted on 12 patients with NAR.

Subject population. All patients signed an informed consent approved by the local University Institutional Review Board before participation in any study procedures. Patients enrolled were between the ages of 21 and 55 years and were required to have a physician diagnosis of NAR that included negative skin prick testing, negative nasal eosinophilia, symptoms in response to olfactory/chemical stimuli with an irritant index scale greater than 24, and symptoms induced by specific odorants tested in this study.¹ Eligible patients were also required to have significantly reduced symptoms on intranasal azelastine (Astelin) based on their clinical history and a rhinitis symptom score that categorized symptom severity on a scale of 1 to 10. Patients with allergic or mixed rhinitis, positive skin prick testing, or who were pregnant, taking concomitant medications (saline washes, nasal corticosteroids, oral antihistamines, or leukotriene-modifying agents, anticholinergic agents, oral or topical decongestants, oral corticosteroids, intranasal cromolyn sodium), or who had structural abnormalities of the nasal cavity or sinuses were excluded. Subject demographics are summarized in Table 2.

Table 1. Study Design for Azelastine-Responsive Nonallergic Rhinitis Patients

Screening visit	2 Weeks off medication	Visit 1	2 Weeks on medication	Visit 2
Odorant challenge		fMRI with olfactory stimuli		fMRI with olfactory stimuli

Table 2. Patient Demographics

Mean age (yrs)	Sex (F/M)	Race (W/B)	Mean Irritant Index Score (0–210 units)
48.5	10/2	11/1	66

Study visits. During the screening visit, subjects completed a University of Pennsylvania Smell Identification Test to assess their baseline olfactory function.¹⁸ They were then challenged to an unpleasant odorant (hickory smoke) and a pleasant odorant (vanilla) to confirm that these triggers differed in the degree of irritation using a flow dilution olfactometer (OLFACT; Osmic Enterprises, Inc, Cincinnati, Ohio).^{19,20} Positive respondents were required to have symptoms to both low and high hickory smoke concentrations. Once a patient qualified, they were washed off intranasal azelastine for 2 weeks to ensure that they were symptomatic at the time of their first fMRI scan (visit 1). Using a specially designed magnetic resonance imaging (MRI)-compatible olfactometer, patients were exposed to a random presentation order of an unpleasant smoke stimulus (Charsol H-3, Red Arrow, Manitowoc, Wisconsin), a pleasant vanilla stimulus (Vanilla Fragrance Oil, SaveOnScents, Brooklyn, New York), or non-odorized air while being scanned in the fMRI scanner. At the conclusion of the first fMRI visit, all subjects were restarted on intranasal azelastine, 2 puffs each nostril twice per day, and scheduled to return for a second fMRI scan in 14 ± 2 days. At the end of study, patients were instructed to resume medications they were taking before the onset of the study.

fMRI behavioral procedures. Subjects laid supine in the bore of the MRI system, wearing MRI-compatible goggles to deliver visual stimulation from a behavioral paradigm developed in E-Prime, version 1.1 (Psychology Software Tools, Inc, Sharpsburg, Pennsylvania) to control the flow of the experiment. Subjects were fitted with an MRI-compatible oxygen face mask coupled to a flow dilution olfactometer. To eliminate visual, mechanical, and sniffing artifacts, a timepoint-by-timepoint censoring technique that removed images with uncorrectable motion was used. Because this technique is sensitive to head movement during sniffing, timepoints during sniffing were removed from analysis to eliminate artifacts from excessive head motion. Furthermore, because such artifacts are equally present in all conditions, these effects were statistically able to be subtracted out.

Every 21 seconds, the subject was visually cued to inhale through the nose (ie, to sniff) normally, not in an exaggerated fashion. The paradigm consisted of a 5-second countdown (eg, prepare to sniff, 4, 3, 2, 1, SNIFF!) to allow precise timing of odor presentations. Concurrent with the instruction to sniff, an odor stimulus or nonodorized air was delivered from the olfactometer to the participant via the oxygen mask. During the odor presentation task, smoke, vanilla, or non-odorized air (blank) was presented in a predetermined pseudo-random order for 4 seconds. Subjects practiced sniffing outside the scanner, using an unattached oxygen mask to

acclimate themselves to the task instructions and timing. During intervals of no odorant, clean air was continuously flowing through the mask. The olfactometer was loaded with standard concentrations of smoke (mean = 2.43 ppm) and vanilla (mean = 254 ppb) and delivered in a 2-L airflow measured using a PhoCheck 5000 photoionization detector (Ionscience, Waterbury, Connecticut) calibrated to isobutylene.

Before acquisition of fMRI data, a high-resolution T1-weighted anatomical image was acquired (T1-weighted modified driven equilibrium fourier transform, modified driven time = 1.1 s, repeat time = 13 ms, echo time = 6 ms, field of view = $256 \times 192 \times 192$ mm, matrix $256 \times 192 \times 96$, flip angle = 20 degrees).²¹ A multi-echo reference scan was acquired to correct the fMRI data for ghost and geometric image distortions commonly found in the inferior frontal regions, where olfactory processing occurs.²² A single fMRI scanner run consisted of 6 presentations of each stimulus (smoke, vanilla, or blank). Each scanner run lasted 6 minutes and 36 seconds; 152 fMRI images were acquired (T2*-weighted gradient-echo echo-planar imaging, 30, 2-mm oblique axial slices with 1-mm gap, repeat time 3,000 msec, echo time 29 msec, field of view 208×208 mm, matrix 64×64 , flip angle = 75 degrees). Four fMRI scanner runs were conducted, yielding a total of 24 presentations of each stimulus and a total of 608 fMRI images. Raw MRI data were reconstructed with in-house software developed in IDL (www.itvvis.com). All subsequent MRI data preprocessing was conducted in Analysis of Functional NeuroImages (<http://afni.nimh.nih.gov>), including motion correction, spatial smoothing, normalization, and time point censoring. After data preprocessing, brain activation was calculated for each subject at each visit for the 3 sniff presentations, smoke, vanilla, and nonodorized air. These calculations were conducted at each point in the fMRI data, that is, voxel-by-voxel, and resulting activation images were used for statistical analyses.

Statistical analysis. Based on results by Herz et al, the minimum number of participants necessary to see a differential response between a potentially emotionally unpleasant odor (eg, smoke) and no odor would be 12, with a power of approximately 80% at a P -value of .05.¹⁵

The brain activity signals were examined for 2 primary effects. First, because event-related odor presentations have not been extensively used, odors were compared with nonodorized air on a voxel-by-voxel basis and with a region-of-interest (ROI) approach to present evidence that this event-related approach works. Data from both visits were combined, and the data from the nonodorized air signal was contrasted with the data from the smoke and vanilla presentations. Second, the off-vs-on azelastine medication effect was examined in 2 ways. First, the azelastine treatment effect combined vanilla and smoke responses at the first visit and then compared them with the combined vanilla and smoke response at the second visit, using a voxel-by-voxel approach. Second, brain responses to odors and nonodorized air were examined in those parts of the brain that responded reliably to

the task using the ROI method, which combines multiple voxel activity signals across a defined region of the image.

For the ROI method, a reliable response was defined as above-threshold activation in 5 of 10 activation maps that were created to qualitatively examine the data. These activation maps were calculated using a 1-sample, 2-sided t test to estimate the brain response to smoke, vanilla, nonodorized air, smoke + vanilla, or smoke + vanilla + nonodorized air both on and off azelastine. This approach was taken to test whether activation in response to odor presentation was different from baseline activity levels observed between trials. Thus, 10 activation maps were created to examine the fMRI activation patterns. If a voxel was active in at least 6 of these maps, it was deemed reliably active (Fig 1). The number 6 was chosen because it meant that the region responded to at least 2 conditions in which individual odors were presented (eg, smoke, vanilla, or nonodorized air). In the voxel-by-voxel analyses, areas of significant brain activation were determined by thresholding the contrast images at a P value of $P < .005$ and requiring at least 11 or more adjacent voxels to be active in a contiguous cluster. This standard multitiered thresholding approach provided a corrected P -value of 0.05 according to Monte Carlo simulations conducted in Analysis of Functional NeuroImages, which was conducted to determine what combination of P value threshold and cluster size threshold represented a corrected P value of .05. For the descriptive secondary analysis of odor selectivity that demonstrated bilateral piriform cortex activation, a more lenient P value threshold of .01 and a more stringent cluster size of 15 or more voxels was used. Both combinations of P values (.005 and .01) and cluster sizes (11 and 15, respectively) yielded corrected P values of .05 in accordance with these standard methods.^{23–25} For descriptive purposes and to verify the similarity of our findings with those of others, a secondary voxel-by-voxel comparison was conducted of odors vs nonodorized air, using a more lenient $P < .01$ but requiring 15 contiguous active voxels, which also yields a corrected P value of .05. A more lenient P value was selected to descriptively show that our paradigm activated the cortical olfactory system bilaterally. This comparison shows that the right hemisphere piriform cortex region also exhibits odor selectivity (Fig 1).

RESULTS

Initial olfactory function assessment of subjects to confirm their responsiveness to olfactorants showed that subjects rated the smoke stimulus almost 3 times as irritating as exposure to vanilla (5.9 for smoke vs 2.2 for vanilla rated on a 1–10 Likert category scale). The results of the screening University of Pennsylvania Smell Identification Test scores were considered to be within normal range and remained unchanged whether the subject was on (34.4) or off (34.8) intranasal azelastine, indicating that olfactory function was not altered by the drug.¹⁹

For the comparison between odors and nonodorized air, only the left piriform cortex was observed (Fig 1). This left

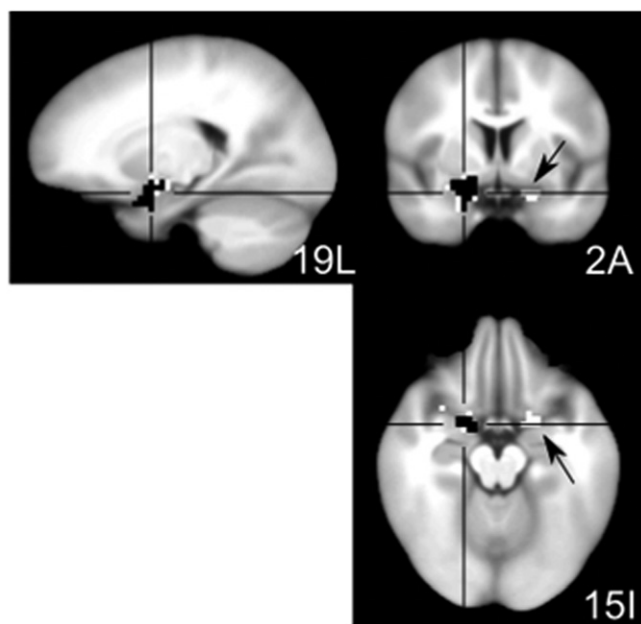


Figure 1. Brain activation for odors vs nonodorized air. Black cluster at cross-hairs shows the 1 site of significant activation ($P < .05$) in the left piriform cortex for odors compared with nonodorized air. The white cluster at arrows show a site of activation in the right piriform cortex. Although this region did not reach significance at our stricter threshold, it responded more to odors in our secondary analysis. As illustrated in the graph, both regions are more active for odors than nonodorized air but do not differ statistically before and after treatment.

hemisphere region and a homologous right hemisphere region that was observed in a secondary analysis (Fig 1) responded selectively to odors. Neither of these regions exhibited a significant treatment response ($P > .05$). Nonallergic rhinitis patients exhibited increased blood flow to several regions of the brain in response to sniffing smoke, vanilla, or nonodorized air both on and off intranasal azelastine (Fig 2). These regions activated reliably across visits and conditions but did

not exhibit changes in response to treatment. However, activation of these regions suggests that our task produced a repeatable pattern of brain response from visit to visit.

Treatment with intranasal azelastine significantly attenuated the sniffing response to smoke or vanilla in several regions while increasing the response in 2 regions ($P < .05$) illustrated in Figure 3. The 2 regions of increasing brain response to sniffing smoke or vanilla with treatment included an area spanning the head of the caudate nucleus bilaterally and olfactory cortex in the midline subgenual cingulate gyrus. In addition, a small region in the pons increased with treatment. Thirteen regions showed a decreasing brain response to sniffing smoke or vanilla with treatment ($P < .05$). These include 2 sites in the left uncus and 3 regions of the cerebellum (see clusters 7 and 12; Fig 3). Two additional sites in the right inferior frontal gyrus also exhibited a decrease in response to treatment (see clusters 9 and 11; Fig 3).

DISCUSSION

The current study was novel in that it used a high magnetic field scanner to examine the brain response to olfactory stimuli to identify differences in neural processing associated with odor detection before and after treatment with intranasal azelastine in NAR patients previously demonstrated to have a therapeutic response to this medication. Neuroimaging techniques have been previously used to study human brain responses to olfactory cues,^{26,27} including the neural basis of olfactory-based emotional memories.¹⁵ Odor perception elicits activation in the piriform, entorhinal and orbitofrontal cortex, amygdala, and hippocampus regions of the brain as well as the thalamus, caudate nucleus, and insula.²⁸ In this study, we observed that sniffing, whether nonodorized air, smoke, or vanilla, activated similar regions, including the insula bilaterally, thalamus, caudate nucleus, subthalamic nucleus, hypothalamus, and anterior cingulate cortex (Fig 2). These regions did not respond to treatment, suggesting that the effect of repeated task participation did not have a general impact on sniffing responses in the brain. One major difference between this and other studies is the testing of NAR patients, who may differ from healthy subjects in the way their brains respond to olfactory processes such as sniffing. Despite those potential differences, we observed greater piriform cortex activation for smoke and vanilla than for nonodorized air (Fig 1), similar to previous reports.^{17,22,27} The main hypothesis of this study was that treatment would alter the brain response to olfactory stimulants or irritants. To that end, the data from Figure 3 provide support for this hypothesis. We observed 2 regions that reacted to treatment with an increased response to sniffing smoke or vanilla and several regions that exhibited a decrease to sniffing smoke or vanilla, including the subgenual anterior cingulate region, which may process olfactory information.²⁹

This study has several potential limitations that require discussion. For all but 1 participant, the first fMRI visit took place off treatment and the second while on treatment. Thus, for most subjects there could be a confounder effect between experience with the fMRI process and treatment. However, this confounder

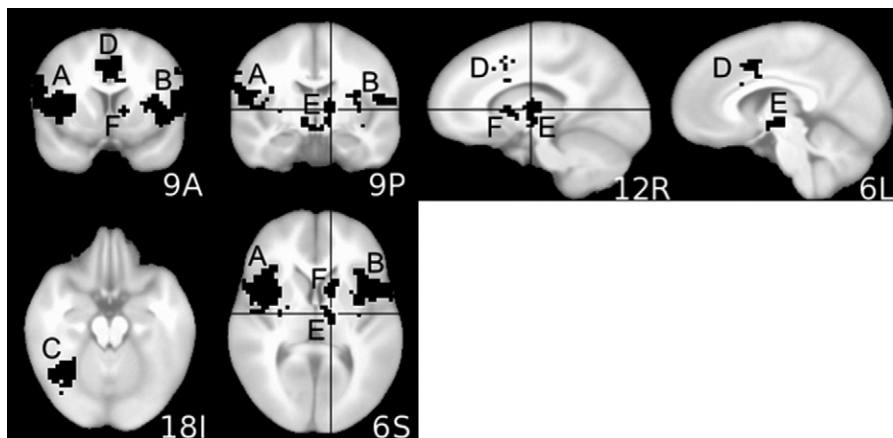


Figure 2. Brain regions that activate reliably across conditions. Regions exhibiting activation ($P < .05$) for sniffing odors or nonodorized air include the following: A. Left insula and operculum. B. Right insula and operculum. C. Left fusiform cortex and cerebellum. D. Bilateral anterior cingulate cortex. E. Left hypothalamus, right thalamus, and bilateral subthalamic nucleus. F. Right head of the caudate nucleus.

was mitigated in that sniffing representations did not change with each visit (Fig 2). We did not observe differences in responses to smoke and vanilla, nor was there a difference in treatment effects for these 2 different odorants. Possibly the event-related approach to fMRI taken by this study reduces our ability to discriminate brain responses to smoke vs vanilla and potentially different treatment effects. However, these results also could suggest that NAR patients do not necessarily always discriminate between odorant triggers, as commonly believed, or may have heterogeneous responses to irritant triggers, as has been reported. Therefore, classification of NAR patients solely based on response to a specific nonallergic trigger may not be entirely appropriate, because irritant trigger threshold responses vary from person to person. Finally, this proof-of-concept study was limited to NAR only patients, and each subject acted as their own control. Future studies that include allergic rhinitis and healthy individuals without chronic rhinitis symptoms would

need to be conducted to determine whether results from this study are unique to NAR.

Intranasal azelastine has been approved by the Food and Drug Administration for treatment of both seasonal allergic rhinitis and NAR.³⁰ It exerts its therapeutic effects through multiple mechanisms, including H_1 receptor antagonism, vagal nerve stimulation, and inhibition of leukotriene mediators and neuropeptides such as substance P.³⁰ However, the therapeutic benefits patients with NAR experience while taking azelastine have not been completely explained through these mechanistic pathways. In that subjects participating in this study were required to have demonstrated a significant treatment response to azelastine, these results support a potential neurogenic mechanism of this medication in treating NAR.

Assessing the neurogenic effects of medications on large groups of NAR patients using fMRI coupled with olfactometry may not be practical primarily because of cost. However, re-

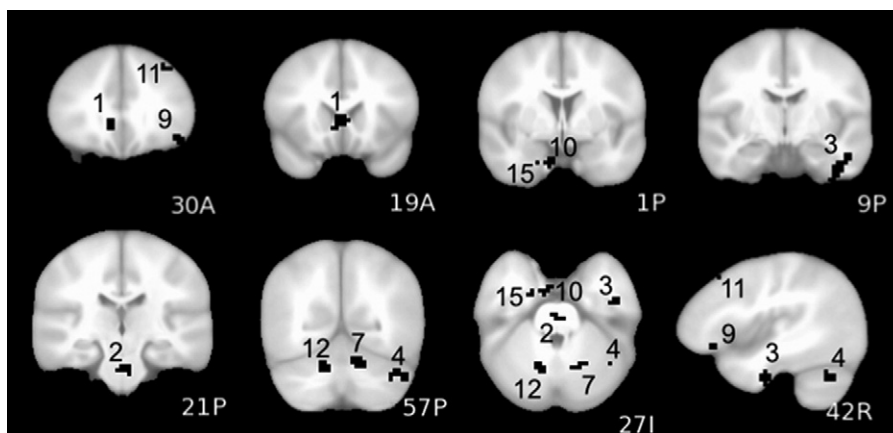


Figure 3. Brain regions that respond to azelastine treatment. Fifteen regions responded to intranasal azelastine treatment. Regions are numbered by size of the voxel cluster and notably include an increase signal ($P < .05$) in olfactory processing regions in the medial orbitofrontal cortex (1) and the pons (2) and decreased signals in the left uncus (10 and 15), inferior temporal gyrus (3), frontal regions (9, 11), and cerebellum (4, 7, and 12).

cently an NAR exposure chamber (Cetero Research, Inc, Toronto, Ontario, Canada) was developed that is capable of assessing upper respiratory symptoms using total nasal symptom scores and acoustic rhinometry in response to temperature and irritant exposures such as ozone, fragrances, and irritants in 6 NAR patients simultaneously.³¹ They were able to reproducibly identify NAR patients who were responders and nonresponders to specific triggers, making it feasible to further investigate putative mechanisms for NAR as well as to recruit well-characterized patients for clinical trials to assess medications designed for NAR.

In summary, this investigation has demonstrated that odorous air produces differential brain metabolic changes compared with clean air and that treatment with azelastine affected these differential, odor-related responses. Whether azelastine's treatment effect on brain metabolism relates conceptually to its symptomatic therapeutic effect requires further investigation. In addition, further work is needed to determine whether a differential brain metabolic change occurs in response to odorants that specifically are induced by trigeminal or nontrigeminal stimuli or whether this brain activation response is secondary to activation or inhibition of specific receptors on effector cells within the nose or brain.

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