

The neural regulation and impact of posterior nasal neurectomy on nasal ciliary motion in vivo in a murine allergic rhinitis model

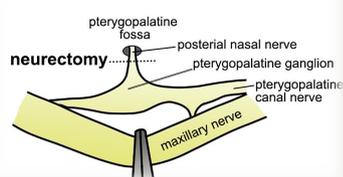
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Rhinology 62: 5, 631 - 640, 2024
<https://doi.org/10.4193/Rhin23.337>

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The neural regulation and impact of posterior nasal neurectomy on nasal ciliary motion in vivo in a murine allergic rhinitis model

Posterior nasal neurectomy on murine allergic rhinitis model



In vivo observation on nasal ciliary motion



Denervation effect on ciliary motion

- Nasal ciliary beat frequency decreased to 78%
- Diminished response towards allergen and histamine



Agent effects on ciliary motion in vivo

↑ stimulatory ↓ inhibitory

- | | |
|--|--|
| ● Ovalbumin (allergen) ↓ | ● Histamine ↑ → ↓ |
| ● Acetylcholine chloride (M receptor agonist) ↑ | ● Oxymetazoline hydrochloride (α receptor agonist) ↓ |
| ● Neostigmine bromide (cholinesterase inhibitor) ↑ | ● Isoprenaline hydrochloride (β receptor agonist) ↑ |

Pang C, Liu C, Yu N, et al. Rhinology 2024. <https://doi.org/10.4193/Rhin23.337>

RHINOLOGY
 Official Journal of the European and International Societies

Abstract

Background: Posterior nasal neurectomy (PNN) is a commonly employed surgical approach for the treatment of allergic rhinitis (AR). Due to its denervation effect on the nasal mucosa, PNN may potentially alter the motion and defensive capability of cilia. Previous research on the effects of neural regulation and denervation on cilia has been limited by the absence of a feasible in vivo evaluation method for assessing ciliary function. **Methodology:** Utilizing a new system developed by our team for visualizing and analyzing ciliary motion in vivo, we analysed ciliary beat frequency and distance in vivo and histomorphological changes in a murine PNN and AR model. Ovalbumin, histamine and neurotransmitters (acetylcholine chloride, α receptor agonist and β receptor agonist) were applied to investigate the responsiveness and neural regulation of the nasal mucosa. **Results:** Denervation resulting from PNN led to a reduction in nasal ciliary beat frequency (CBF) to 78% of the control, as well as diminished response towards allergens and histamine. Among neurotransmitters examined, α receptor agonists exhibited inhibitory effects on in vivo ciliary motion while acetylcholine and β receptor agonists demonstrated stimulatory effects. PNN did not affect the reactivity of in vivo cilia towards these neurotransmitters. **Conclusions:** PNN-induced denervation can reduce ciliary motion, potentially compromising the defensive capability of nasal mucosa. Neural regulation and the neurotransmitters involved have significant effect on ciliary motion.

Key words: nasal cilia, in vivo, posterior nasal neurectomy, allergic rhinitis, innervation

Introduction

Posterior nasal neurectomy (PNN) is a commonly performed surgical procedure for allergic rhinitis (AR) ⁽¹⁾. It aims to inhibit nasal gland secretion by blocking parasympathetic nerves and reduce nasal mucosa sensitivity by blocking sensory nerve fibers, thereby alleviating allergic symptoms such as runny nose, nasal itching, and sneezing ⁽²⁻⁴⁾. However, the underlying mechanism behind the recurrence of rhinitis symptoms in certain patients remains elusive.

Since the sympathetic, parasympathetic, and sensory nervous systems all participate in the regulation of ciliary function ^(5, 6), denervation of the nasal mucosa caused by PNN may affect ciliary motion and the defensive capability of AR patient nasal mucosa, further inducing recurrence of the rhinitis or other long-term symptoms. However, previous evaluation methods for ciliary function relied on ex vivo techniques and there is currently no feasible in vivo technique or animal model to evaluate cilia after PNN. Therefore, it remains unclear how denervation of the nasal mucosa following PNN affects ciliary structure and function.

The neural regulation of ciliary motion has received limited attention to date, primarily due to the previous reliance on ex vivo specimens lacking innervation, which hindered research on neural regulation. Our team has successfully developed an in vivo system for visualizing and analyzing ciliary motion directly on the nasal mucosa surface of living experimental animals. This approach preserves the physiological micro-environment of cilia in vivo, including innervation of the nasal mucosa ^(7, 8).

In this study, we aim to establish rat AR and PNN models, evaluate the histological and functional changes of in vivo nasal ciliated epithelium, and further explore neural regulation on ciliary motion in vivo to provide evidence for the treatment and prognosis of allergic rhinitis.

Materials and methods

Animals

Three-month-old adult male Sprague-Dawley rats, weighing between 200 and 300g, were selected from the Medical Animal Center of Chinese PLA General Hospital. The animals were housed in a temperature- and light-controlled room with automated systems. The ambient temperature was maintained at 25°C, and a simulated day-night cycle of 12 hours each was provided. Rats had ad libitum access to food and water throughout the study period. All animal procedures were conducted in accordance with the guidelines approved by the Ethics Committee of Chinese PLA General Hospital (Beijing, China).

Allergic rhinitis and PNN rat model protocol

The allergic rhinitis rat model was induced by ovalbumin (OVA) as depicted in Figure 1A. Intraperitoneal injections of 1000µg chicken ovalbumin and 20 mg aluminum hydroxide gel diluted

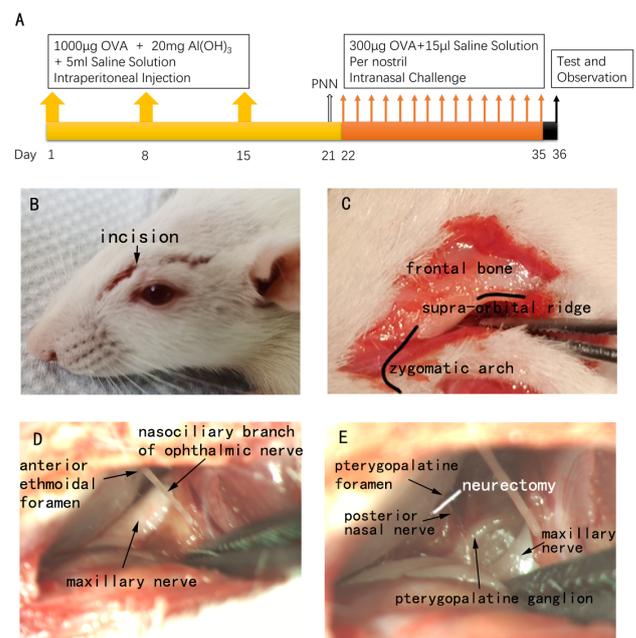


Figure 1. Ovalbumin (OVA) sensitization and posterior nasal neurectomy (PNN) protocol in rats. A: Ovalbumin (OVA) sensitization protocol. B-E: Details of PNN procedure. The posterior nasal nerve was cut before entering the pterygopalatine foramen.

in 5ml normal saline per body were administered on the 1st, 8th, and 15th days. Bilateral PNN surgery was performed on the 21st day, followed by daily intranasal challenges with 300µg OVA in 15µl normal saline per nostril from the 22nd to the 35th day. The control group received phosphate-buffered saline (PBS) instead of OVA.

PNN surgical approach was conducted as previously reported by Nishijima et al. ⁽¹⁾. Rats' bilateral posterior nasal nerves were cut at pterygopalatine foramen. (Figure 1B-E) Rats in which only the pterygopalatine foramen was exposed without neurectomy were used as controls. To mitigate the potential influence of unilateral PNN on the untreated side, a bilateral procedure was employed for all rats in the PNN-treated group instead of utilizing each rat as its own internal control through unilateral PNN. The animals were divided into the following four groups: 1) Control group: rats treated with PBS without PNN; 2) AR group: rats sensitized with OVA without PNN; 3) Control + PNN group: rats treated with PBS and subjected to PNN; 4) AR + PNN group: rats sensitized with OVA and subjected to PNN.

Behavioral assessment for AR phenotype was conducted by sneezing and nasal scratching counting during 20 minutes after the final intranasal challenge on 35th day. The tail blood was collected for serum histamine and IgE test on 36th day. Within one week after the finally 35th day, the baseline of ciliary motion in vivo was analyzed and OVA, histamine and neurotransmitter

reaction experiments were carried out. The nasal mucosa was taken for histological and immunohistochemical analysis. The specific treatment and analysis methods are described below.

PNN rat model

The PNN procedure was performed on the experimental animals on the 21st day following intraperitoneal injection of OVA and prior to nasal challenge. Before surgery, the experimental animals underwent a 3-hour water fast and were administered diluted pentobarbital sodium saline at a dosage of 60 mg/kg via intraperitoneal injection for anesthesia.

Following the surgical protocol established by a research team of the University of Tokyo⁽¹⁾, the surgical approach was conducted as follows: a 2 cm longitudinal incision was made along the superior orbital margin (Figure 1B). The skin and soft tissue was dissected from periorbital area (Figure 1C). Gently retract ocular contents laterally. The nasociliary branch of the ophthalmic nerve entering the anterior ethmoidal foramen were exposed and cut. By continued blunt dissection, the maxillary nerve was exposed in the pterygopalatine fossa (Figure 1D). The pterygopalatine ganglion could be identified bentrondially to maxillary nerve with the posterior nasal vidian nerve branch entering the pterygopalatine foramen. Cut the posterior nasal nerve as distally as possible to prevent damage to the pterygopalatine ganglion (Figure 1E).

Histomorphology and immunohistochemical staining

Within one week after the finally 35th day of AR modeling, the nasal septum mucosa of animals in the four groups (3 cases in each of control group, AR group, PNN group and AR + PNN group) was taken for hematoxylin-eosin staining (H&E staining) for histomorphological analysis. And the nasal septum mucosa of another 3 cases in each of the four groups was taken for immunohistochemical (IHC) staining. The antibodies used were as follows: sensory nerve marker SP: anti-substance P (anti-SP; dilution 1:1000; Abcam); parasympathetic nerve marker VIP: anti-vasoactive intestinal peptide (anti-VIP; dilution 1:1000; Abcam); sympathetic nerve marker NPY: anti-neuropeptide Y (anti-NPY; dilution 1:2000; Abcam); cholinergic nerve marker ChAT: anti-choline acetyltransferase (anti-ChAT; dilution 1:100; Abcam). The quantitative analysis of IHC was done using ImageJ software (v1.54). The slides were reviewed by a pathologist with experience over 10 years.

Analysis of ciliary motion in vivo

Ciliary motions in vivo of 5 rats in each of the four groups were observed using the method as reported by our team previously⁽⁷⁾. Preliminary experiments demonstrated comparable ciliary motion and response to PBS solution in septal, turbinate, and sinus mucosa of the same rats (n=5) (Figure S1). Therefore, we opted for observing the septal mucosa in subsequent inves-

tigations due to its flatness and optimal suitability under our experimental conditions. In vivo ciliary motion measurement in brief: rats' nasal septum mucosa was exposed after anesthesia. The ciliary motion wave could be directly observed by digital microscope (Keyence VHX6000, Japan), and the images were recorded by high-speed microscopic camera. The values of ciliary beat frequency (CBF) and ciliary beat distance (CBD) in vivo were accurately analyzed by ImageJ software by two observers blinded to the groups. The standardized data -- CBD ratio was used for the comparison of CBD, that is, the ratio of the measured CBD value to the basic CBD value.

Effect of OVA and histamine on ciliary motion in vivo after PNN surgery

To investigate the impact of PNN on the responsiveness of nasal mucosa cilia to OVA and histamine, in vivo observation was conducted on four groups. Firstly, the baseline ciliary motion in vivo was recorded. Subsequently, OVA solution (300µg OVA + 15µl PBS) or histamine solution (0.1 mol/L, 15µl), with PBS solution as control, were applied onto the surface of nasal mucosa to observe changes in ciliary motion. Prior to applying the test solutions, a saline-moistened cotton ball was used to occlude the posterior nostril to prolong the exposure time.

Effect of neurotransmitter on nasal ciliary motion in vivo

To investigate the impact of neural regulation on in vivo nasal ciliary motion in normal, AR and post-PNN groups, we administered specific receptor agonists and corresponding blockers to the nasal mucosa of rats. The M receptor agonist acetylcholine chloride solution (0.1mg/ml, 15µl), α receptor agonist oxymetazoline hydrochloride solution (0.5mg/ml, 15µl), β receptor agonist isoprenaline hydrochloride solution (0.25%, 15µl) was used for this purpose. We observed changes in ciliary motion for a duration of 60 minutes following nasal administration of these test drugs. Prior to administering the test solutions, a saline-moistened cotton ball was placed to block the posterior nostril, thereby prolonging the interaction time of the test solutions.

Statistical analysis

G-power software was used for the sample size calculation [Universität Düsseldorf: G*Power (hhu.de)]. The degree of freedom (E= [number of animals in all groups] – [number of groups]) of variance analysis was estimated, and the value of E should be between 10 and 20. Therefore, we chose a small but sufficient sample size of five in each of the four groups with an E=16 to avoid sacrificing too many animals. GraphPad Prism 8.5 software was used for plotting and statistical analysis. For symptom assessment, serum histamine and IgE levels, CBF and CBD data of cilia in vivo, one-way ANOVA was used for comparison between multiple groups, d Tukey's multiple comparisons test was used for comparison between any two groups. For various drug

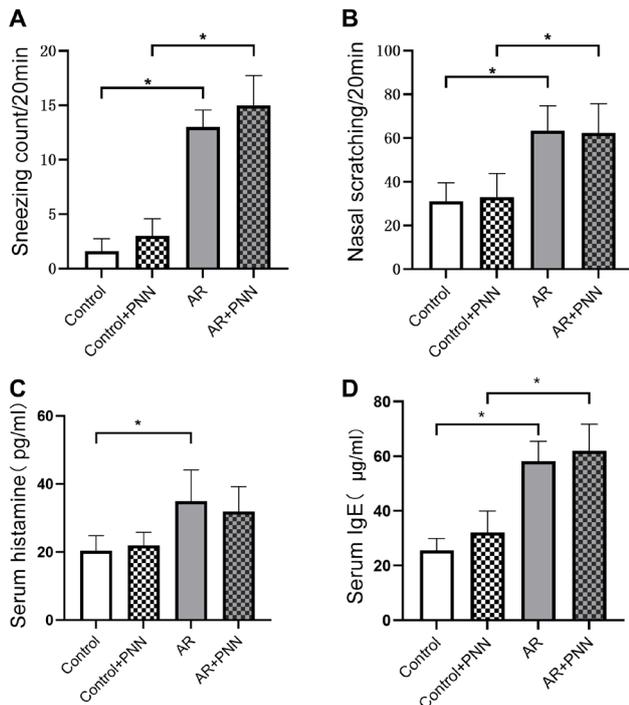


Figure 2. Sneezing count (A), scratching nose count (B), serum histamine (C) and IgE (D) in each group. *P < 0.05.

response experiments recorded over time, Mann-Whitney U test and Wilcoxon rank sum test were used for comparison among groups. Repeated ANOVA was used for multiple timepoint comparison. P-value less than 0.05 indicated that it was statistically significant.

Results

Symptom evaluation of AR and determination of serum histamine and IgE

On the 35th day of OVA or PBS sensitization, behavioral observations were conducted on the rats to assess allergic rhinitis symptoms. In comparison to the Control group, the AR group exhibited a significant increase in sneezing counts, nose scratching behavior, as well as serum histamine and IgE levels ($p < 0.05$). However, there was no significant difference observed in these parameters before and after PNN procedures (Control group vs. Control+ PNN group, $p = 0.99$; AR group vs. AR + PNN group, $p = 0.99$) (Figure 2). These findings indicate successful AR modeling but demonstrate that PNN did not significantly alleviate AR-related symptoms or affect serum histamine and IgE levels in rats.

Histopathological changes of nasal mucociliary epithelium after AR and PNN

In the Control group, HE stains showed that the nasal mucociliary epithelium exhibited normal morphology, with intact ciliary structure and abundant cilia. No significant alterations in ciliary

epithelium were observed following PNN in the Control + PNN group. Conversely, the AR group displayed evident damage to the nasal mucosal epithelium, characterized by cilia lodging, partial shedding, and fusion. However, PNN treatment did not significantly ameliorate cilia damage induced by AR in the AR + PNN group (Figure 3A).

Immunohistochemistry results demonstrated positive immunoreactivity for SP, VIP, NPY, and ChAT within the nasal mucosa of both control group and AR group, indicating intact innervation in pre-PNN nasal mucosa. Conversely, post-PNN groups (Control + PNN group and AR+PNN group) exhibited a notable reduction in SP, VIP, NPY, and ChAT immunoreactivity; suggesting that PNN led to decreased secretion of related neuropeptides and denervation of the nasal mucosa involving sympathetic, parasympathetic, and sensory nervous systems (Figure 3B-E).

Effect of PNN on ciliary motion of nasal mucosa in vivo

After PNN, the CBF and CBD of normal rats (Control + PNN group, $n = 5$) were reduced to 78% and 92% of the Control group ($n = 5$), respectively. The difference in CBF was statistically significant ($p = 0.02$). Furthermore, following PNN, there were no significant changes observed in the CBF and CBD of AR rats (AR + PNN group vs. AR group, each $n = 5$; $p = 0.927$ and 0.703) (Figure 4). These results indicate that PNN did not significantly improve ciliary motion function damage caused by AR while instead weakened normal nasal ciliary motion.

Effects of OVA, histamine, choline and epinephrine on ciliary motion

OVA response

The OVA challenge was performed on the nasal mucosa in vivo of the Control, AR, Control + PNN, and AR + PNN groups ($n = 5$ per group), with PBS solution used as the control ($n = 5$ per group). Following OVA challenge, both the Control group and Control + PNN group exhibited inhibited ciliary motion. In contrast, the ciliary motion in the AR + PNN group showed a gradual decrease after OVA challenge, while that of the AR group remained at its baseline level. These findings suggested that there was a tolerance to allergen inhibition (OVA) observed in AR cilia, while this is weakened by PNN (Figure 5A-D and Figure S2A-D).

Histamine response

To investigate the in vivo response of nasal cilia to histamine following PNN, histamine solution was applied to the nasal mucosa of four groups ($n = 5$ per group). Histamine initially stimulated and subsequently inhibited CBF during 60 minutes in control group, (Figure 5E) nevertheless, CBF in AR group remained at a high level after stimulated by histamine. Notably, the peak CBF of the control+PNN group (124% baseline) was lower than the control group (155% baseline) with statistical significance

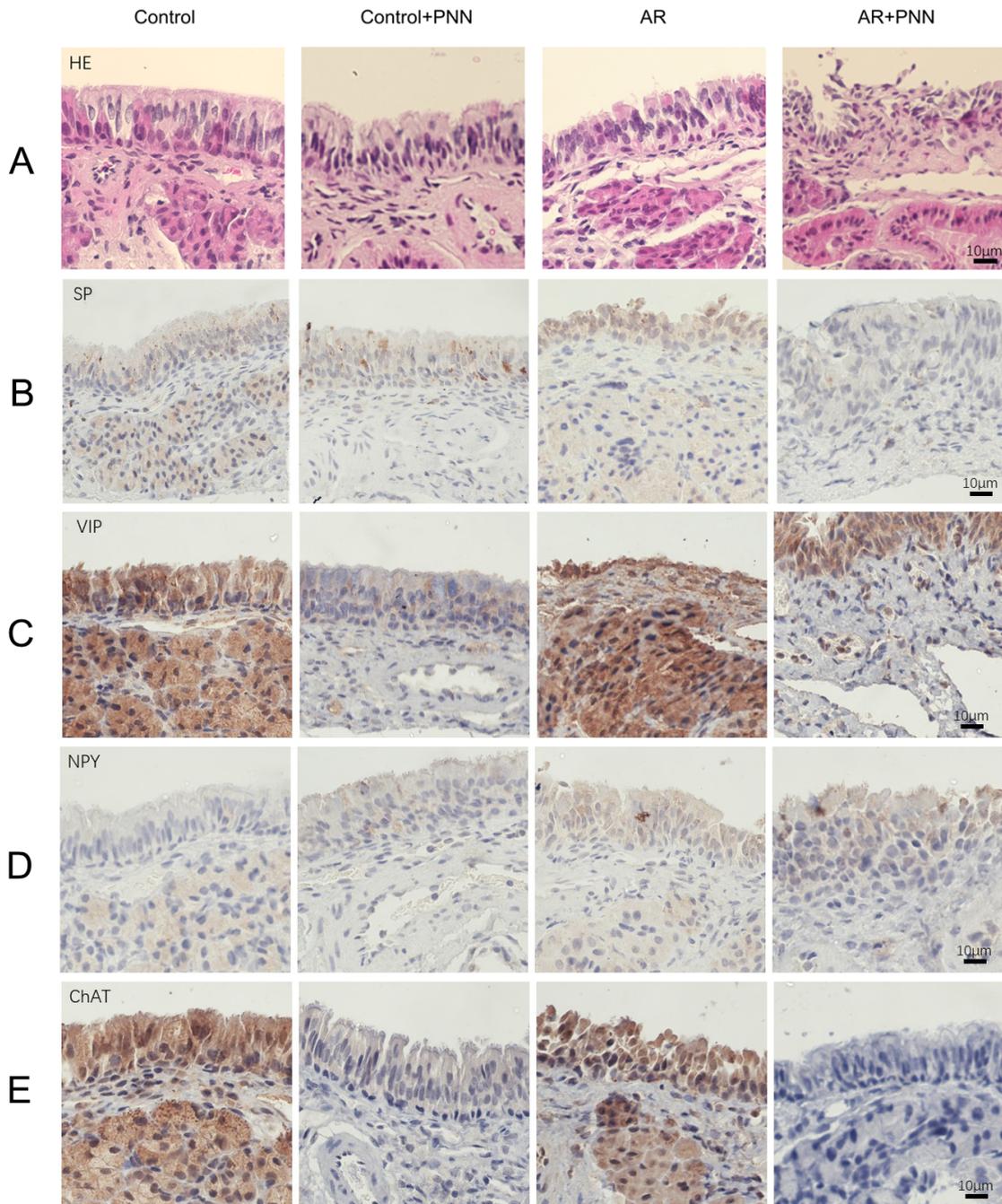


Figure 3. The results of Hematein-Eosin (HE) and immunohistochemistry staining in the four groups. A: Hematein-Eosin (HE) staining results. B-E: After posterior nasal neurectomy (PNN), the positive staining of sensory nerve markers (substance P, SP) (B), parasympathetic nerve markers (vasoactive intestinal peptide, VIP) (C), sympathetic nerve markers (neuropeptide Y, NPY) (D) and cholinergic nerve markers (choline acetyltransferase, ChAT) (E) decreased significantly. F-J: Quantitative analysis results of SP, VIP, NPY and ChAT in lamina propria. *: $p < 0.05$; **: $p < 0.01$.

($p < 0.05$). Additionally, CBD gradually declined across all four groups following histamine challenge (Figure S2E-H). These findings suggest that AR cilia demonstrate some extent of tolerance towards the inhibitory effect of histamine.

Choline response

As depicted in Figure 6A-D and Figure S3A-D, following the

administration of the choline agonist acetylcholine on the nasal mucosa in vivo, there was an increase in both CBF and CBD for all four groups ($n = 5$ per group), which subsequently returned to baseline levels. There were no significant differences observed among the four groups regarding the peak CBF at 10 minutes ($F = 1.697$, $p = 0.21$). Pretreatment with atropine, an M receptor blocker, antagonized acetylcholine-induced stimula-

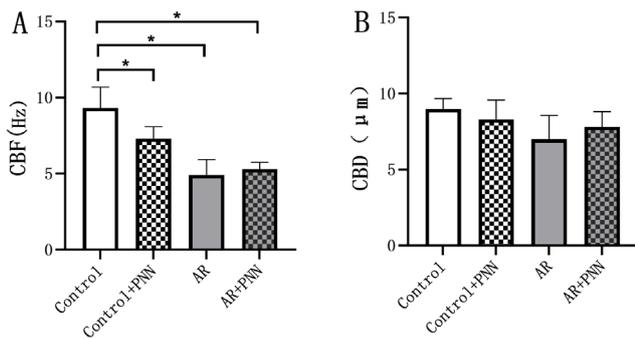


Figure 4. Effects of allergic rhinitis (AR) and posterior nasal neurectomy (PNN) on the baseline values of ciliary beat frequency (CBF) and ciliary beat distance (CBD) in vivo. A: CBF decreased significantly in AR and PNN group. B: CBD did not change significantly in the four groups. * $P < 0.05$.

tion of ciliary motion (Figure 6D and Figure S3D). This finding demonstrated that choline could enhance ciliary motion, and AR or PNN does not alter choline reactivity of cilia. Upon administration of neostigmine bromide, a cholinesterase inhibitor, an increase in both CBF and CBD was observed for non-surgery groups (Control group and AR group; $n = 5$ per group), whereas no significant increase was noted for post-PNN groups (Control + PNN group and AR + PNN group; $n = 5$ per group) (Figure 6B and Figure S3B). These results indicate that denervation of PNN inhibits choline synthesis leading to a potential reduction in basal level ciliary motion without affecting expression of choline receptors.

Epinephrine response

After the administration of α -epinephrine receptor agonist oxymetazoline, the CBF and CBD were inhibited in all four groups ($n = 5$ per group), as shown in Figure 6E and Figure S3E. This inhibition effect was weakened when an α -epinephrine receptor blocker phentolamine was administered, as depicted in Figure 6F and Figure S3F. Similarly, the administration of β -adrenergic receptor agonist isoproterenol ($n = 5$ per group) stimulated an increase in CBF and CBD (Figure 6G and S3G). This stimulation was antagonized by a β -adrenergic receptor blocker propranolol (Figure 6H and S3H). The response of nasal ciliary motion to these neurotransmitters was consistent across all four groups, suggesting that both AR disease status and denervation of PNN did not significantly alter the expression and reactivity of α and β receptors within the cilia epithelium.

A summary of the effects of all the agents in this study can be found in Table S1.

Discussion

This study is the first investigation into the impact of denervation induced by PNN procedures on ciliary motion, and further explored neural regulation of cilia. Potential mechanisms by

which PNN may impact ciliary motion include its potential to attenuate the presence of neural substances that exert inhibitory effects on cilia or mitigate AR-induced damage to ciliary structures^(2,9). On the other hand, the denervation of nasal mucosa may also destroy nerves with a positive regulatory effect on cilia function, thus limiting their motility. Previous ex vivo experiments have demonstrated that sympathetic or parasympathetic nerves can modulate ciliary motion and neurotransmitters such as choline and epinephrine play a role in regulating this process^(6,10,11). However, the impact of neural regulation and denervation induced by surgical procedures on in vivo ciliary motion remains unclear due to a lack of feasible techniques for evaluating ciliary motion and establishing surgical denervation models.

Previously, a research team from Tokyo University, Japan established a rat model for PNN and analyzed its effects on histology of the nasal mucosa as well as nasal secretion; however, they did not specifically analyze changes in the ciliated epithelium⁽¹⁾. The rat PNN model established by the University of Tokyo was confirmed to induce sensory, sympathetic, and parasympathetic denervation throughout most of the nasal mucosa, which is successfully reproduced in our experiment. A previous study used retrograde neuronal tracers to demonstrate that the rat nasal mucosa receives sympathetic fibers from the superior cervical ganglion, parasympathetic fibers from the pterygopalatine and otic ganglia, and sensory innervation from the trigeminal ganglion^(12,13). Additionally, Grote's study revealed that the posterior nasal nerve constitutes the main pathway through which post-ganglionic sympathetic and parasympathetic fibers approach the nose⁽¹⁴⁾. These data support our findings that PNN could denervate sensory, sympathetic, and parasympathetic nerves in the nasal mucosa. Although no significant changes were observed in animal behavior-based symptoms following PNN modeling – an aspect subject to stochasticity – it is important to note that our primary objective was to assess ciliary motion's response to neural regulation and PNN-induced denervation; a confirmation achieved through histological staining. It is worth mentioning that similar results were reported by the study conducted at the University of Tokyo where no symptomatic relief was observed after implementing this PNN model; however, they did find decreased nasal secretion which partially supports its clinical efficacy⁽¹⁾.

The visualization and analysis system of nasal cilia in vivo, established by our team, enables the investigation of changes in ciliary motion following denervation of nasal mucosa in live animals^(7,8). Remarkably, we discovered that PNN significantly reduced the baseline value of normal ciliary motion in vivo, indicating a comprehensive weakening effect on ciliary motion and the defensive capability of nasal mucosa. Previous studies have reported the effective inhibition of nasal secretion, local inflammatory cell infiltration, and cytokine secretion in AR

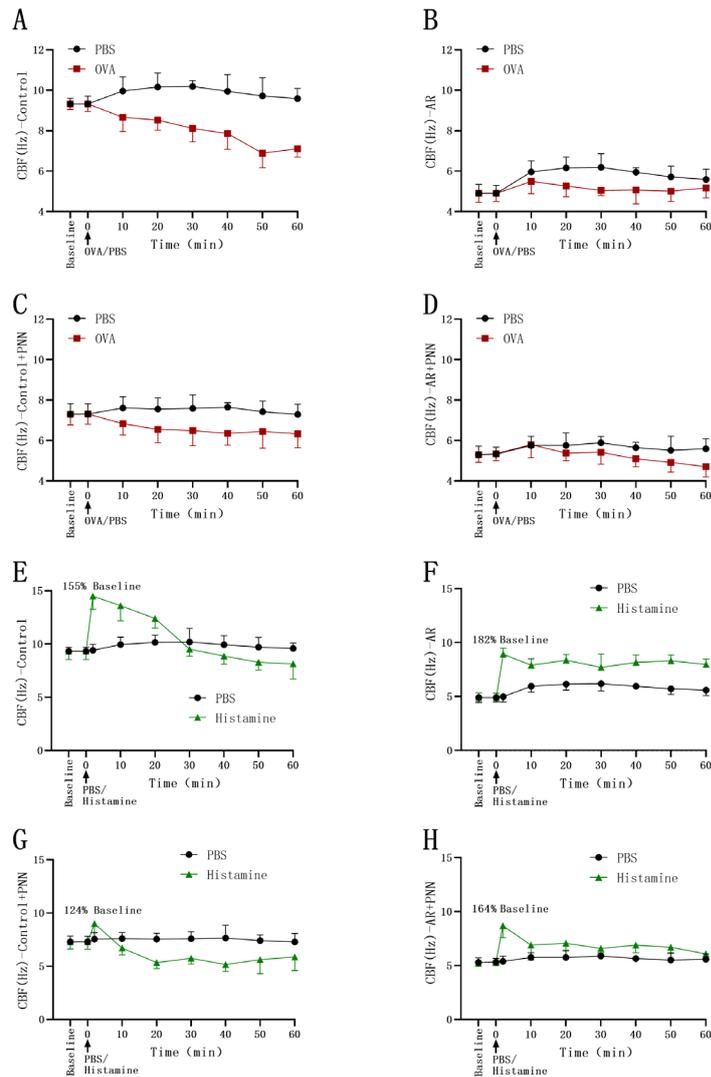


Figure 5. Ciliary beat frequency (CBF) response to ovalbumin (OVA) (A-D) and histamine solution (E-H) within 60 min in each group. A & C: CBF of normal cilia in vivo was inhibited by OVA solution. B & D: AR cilia in vivo was not inhibited by OVA. E & G: CBF of normal cilia in vivo increased first and then decreased upon histamine effect. F & H: AR cilia in vivo increased first but did not decrease upon histamine, showing a tolerance to histamine inhibition effect. **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$. (CBF at 2min and 60min compared with baseline).

patients treated with PNN⁽⁹⁾, while its impact on ciliary motion has never been studied. We initially hypothesized that PNN may mitigate AR-induced damage to cilia; however, no detrimental or beneficial effects were observed on the structure or motion of cilia treated with PNN. This may be attributed to the dual action of PNN: it can reduce damage caused by neurogenic inflammatory factors while also potentially decreasing stimulatory neurotransmitters for ciliary motion. These opposing effects appear to counterbalance each other. Furthermore, the sensitization process in AR rats leads to structural damage and impaired ciliary motion, which may render it less susceptible to further neural influences.

We observed that OVA exerts an inhibitory effect on non-sensitized ciliary motion, whereas histamine initially stimulates

and subsequently inhibits it. This finding diverges from previous ex vivo studies, where OVA challenge on specimens ex vivo led to an increase in CBF of nasal cilia^(15,16), and histamine had either inhibition⁽¹⁵⁾ or stimulation⁽¹⁷⁾ effects of ciliary motion. The disparity between our findings and those of ex vivo studies may be attributed to the absence of comprehensive regulatory factors in vivo, particularly neural regulation. The inhibitory effect of OVA on cilia of living non-sensitized animals may be due to inhibitory neural agents induced by OVA. Furthermore, we discovered that AR cilia in vivo exhibit tolerance towards the inhibitory effects of both OVA and histamine, maintaining their baseline level of motion. Neural regulation might also contribute to this phenomenon. Neuropeptides and other neural substances may be the underlying mechanism, aiding in sustaining

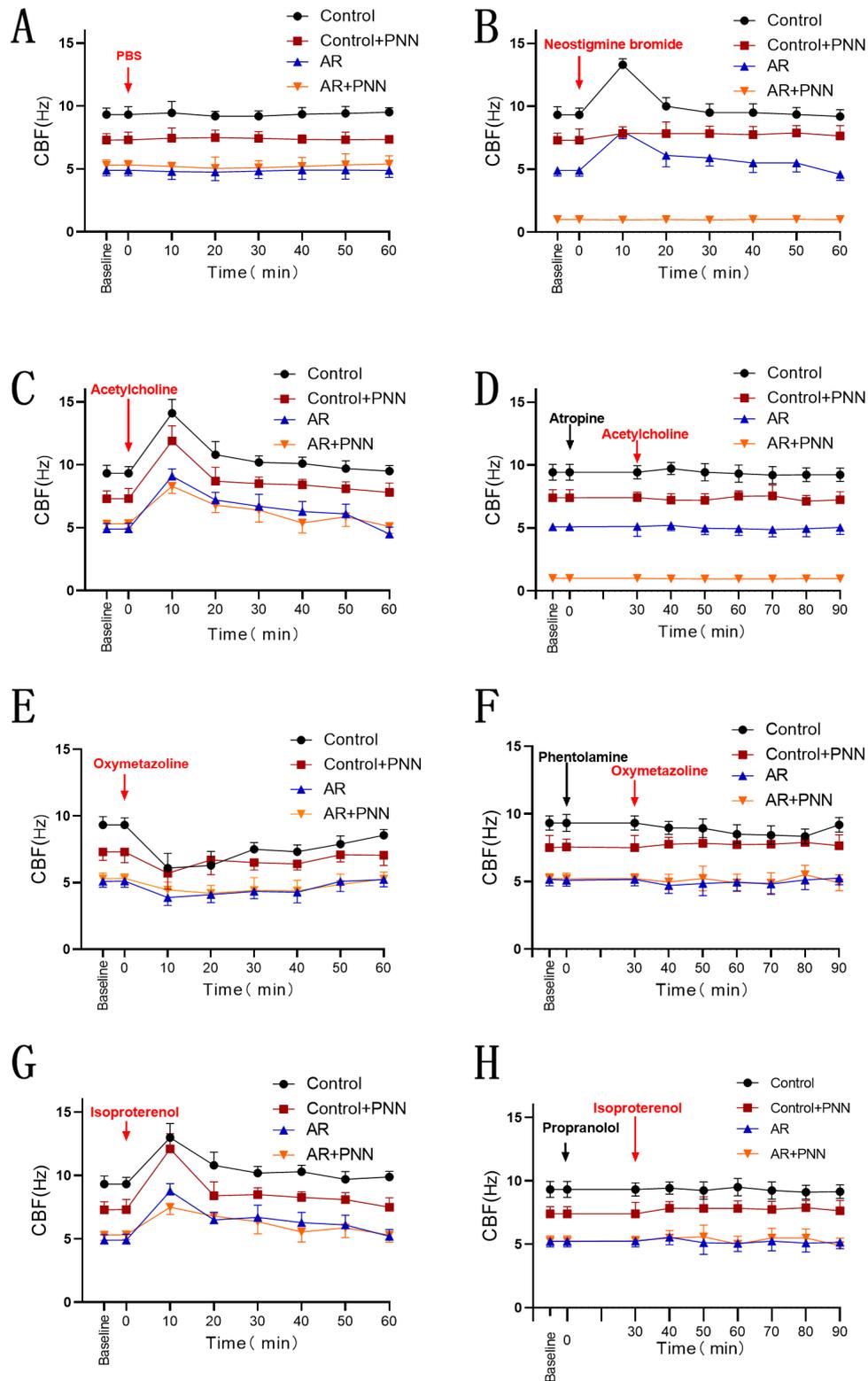


Figure 6. Ciliary beat frequency (CBF) response to choline (A-D), a receptor (E-G) and β receptor substances (H-J). A: PBS solution as control; B: Cholinesterase inhibitor, neostigmine bromide promoted CBF of non-PNN group (Control group & AR group), but post-PNN group (Control + PNN group & AR + PNN group) had no significant reaction to neostigmine bromide; C: Acetylcholine promoted CBF of the four groups. D: Pretreatment with atropine for 30 min could antagonize the effect of acetylcholine. E: a receptor agonist, Oxymetazoline inhibited CBF of four groups. F: Pretreatment with α -receptor antagonist, phentolamine for 30 min could antagonize this effect. G: β receptor agonist, isoprenaline promoted CBF of the four groups. H: β receptor antagonist, propranolol could antagonize promotion effect of isoprenaline.

ciliary motion under histamine influence. These results provided evidence for the notion that neural regulation plays a crucial role in the reactivity and tolerance of AR cilia towards allergens and histamine.

We have found that α receptor agonists exert an inhibitory effect on ciliary motion, while acetylcholine and β receptor agonists promote ciliary motion in vivo in rats. Consistent with our findings, a previous study by Ingels on isolated human nasal cilia cells demonstrated that parasympathetic agonists and β agonists positively stimulate CBF, whereas α receptor agonists have a negative regulatory effect⁽¹⁸⁾. Mercke measured the ciliary motion in vivo of rabbit maxillary sinus using the photoelectric method and found that choline, β_1 , β_2 , and $\alpha + \beta$ agonists accelerate ciliary motion, while α_1 and α_2 inhibit it, suggesting an overall positive effect of sympathetic and parasympathetic innervations on ciliary motion⁽⁵⁾. Cervin also measured the ciliary motion in vivo of experimental rabbits using the same photoelectric method. After injecting norepinephrine (mainly acting as an α -receptor agonist with little effect on β -receptors) into the maxillary artery, there was a 16% increase in the frequency of ciliary motion. However, this effect lasted only for approximately 20 seconds after injection before returning to its initial state within 3 minutes; furthermore, injections of either α or β blockers had no significant impact on these results⁽¹⁹⁾. Nonetheless, the photoelectric method used at that time had large measurement bias which made the results less reliable. The in vivo ciliary motion imaging analysis system used in this study has previously demonstrated high accuracy and repeatability^(7,8), enabling it to effectively reflect the state of cilia function in vivo, including innervation as investigated here. We observed the disappearance of sympathetic, parasympathetic, and cholinergic nerves in the nasal mucosa after PNN; however, there was no significant change in the responsiveness of ciliary motion to acetylcholine and epinephrine receptor agonists. Additionally, the stimulation effect of neostigmine bromide (a cholinesterase inhibitor) on ciliary motion diminished after PNN. These findings suggest that decreased release of acetylcholine by cholinergic nerve fibers may contribute to reduced ciliary motion following PNN without altering the expression or function of related receptors. This conclusion aligns with previous studies investigating the impact of PNN on nasal mucosal secretion and sensitivity, providing

further evidence for understanding how neurotransmitters regulate ciliary motion⁽¹⁾.

One limitation of this study is that the experimental animals were under anesthesia, potentially impacting the functional state of cilia in vivo. However, this method currently represents the closest physiological state of cilia in living animals. Another limitation arises from species disparity between experimental animals and humans regarding posterior nasal innervation and postoperative effects of PNN. Therefore, further studies are required to confirm the effect of PNN procedure on human nasal cilia.

Conclusion

In this study, the effects of PNN approach, allergen (OVA), histamine and various neurotransmitters on nasal ciliary motion in vivo were observed by our novel visualization system. Denervation induced by PNN weakened ciliary motion of normal nasal mucosa and diminished ciliary responsiveness to allergen and histamine. Among neurotransmitters, α receptor agonists exhibited an inhibitory effect, while acetylcholine and β receptor agonists exhibited stimulating effects on ciliary motion in vivo. These results indicated that innervation has an important regulatory effect on ciliary motion in vivo. Discomfort after PNN may be associated to weakened ciliary function. Furthermore, medications with stimulatory effects on ciliary motion such as acetylcholine and β receptor agonists may be considered to improve ciliary function, which merit further exploration.

Authorship contribution

LC and PL conceived and designed research. CP, CL, NY, WY and MX performed experiments and interpreted results of experiments. CP, CL and NY analyzed data and prepared figures. CP drafted paper. LC and PL edited and revised manuscript. All authors read and approved final version of manuscript.

Conflict of interest

The authors do not have any conflict of interest to declare.

Funding

This work is supported by National Key Research and Development Program of China (No.2022YFC2405503).

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Rhinology 62: 5, 631 - 640, 2024
<https://doi.org/10.4193/Rhin23.337>

Received for publication:

September 8, 2023

Accepted: July 1, 2024

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Associate Editor:

Basile Landis

This manuscript contains online supplementary material

SUPPLEMENTARY MATERIAL

Table S1. Summary of effects of agents on ciliary motion

Agents	Control	Control+PNN	AR	AR+PNN
Ovalbumin (allergen)	Inhibition	Inhibition	None	Inhibition
Histamine	First stimulation, then inhibition	First stimulation, then inhibition	Stimulation	Stimulation
Acetylcholine chloride (M receptor agonist)	Stimulation	Stimulation	Stimulation	Stimulation
Neostigmine bromide (cholinesterase inhibitor)	Stimulation	Stimulation	Stimulation	Stimulation
Oxymetazoline hydrochloride (α receptor agonist)	Inhibition	Inhibition	Inhibition	Inhibition
Isoprenaline hydrochloride (β receptor agonist)	Stimulation	Stimulation	Stimulation	Stimulation

Abbreviations: posterior nasal neurectomy, PNN; allergic rhinitis, AR.

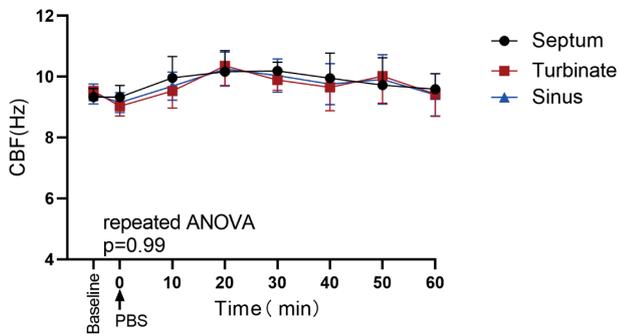


Figure S1. Pre-experiments showed similar ciliary motion baseline and response to PBS solution in septal, turbinate, and sinus mucosa of same rats (n=5).

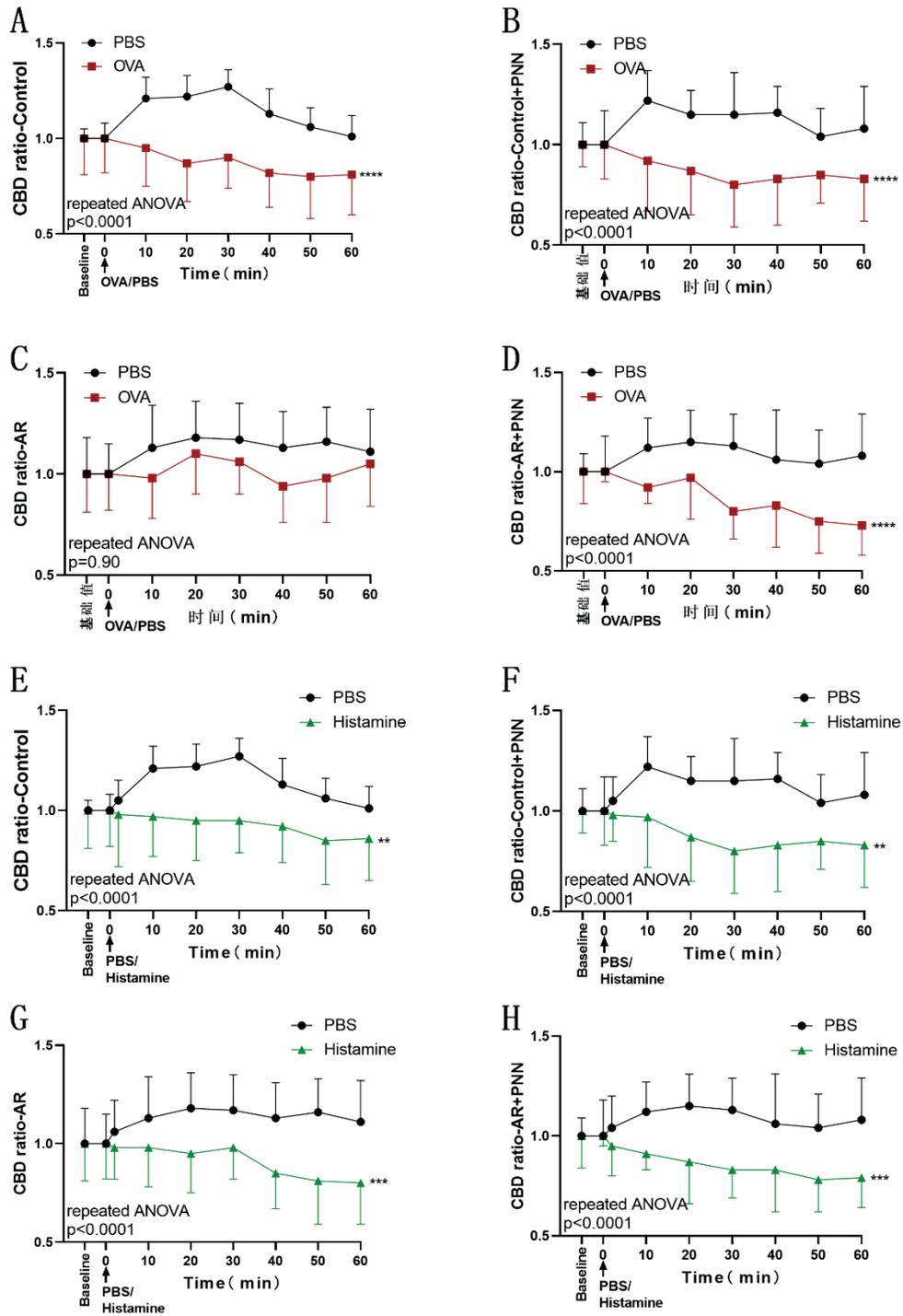


Figure S2. Ciliary beat distance (CBD) response to ovalbumin (OVA) (A-D) and histamine solution (E-H) within 60 min in each group. A & C: CBD of normal cilia in vivo was inhibited by OVA solution. B & D: AR cilia in vivo has a tolerance to OVA. E-H: Histamine has inhibitory effect on CBD of all groups within 60 min. Posterior nasal neurectomy (PNN) did not significantly change histamine response of each group. **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$. (CBD at 60min compared with baseline).

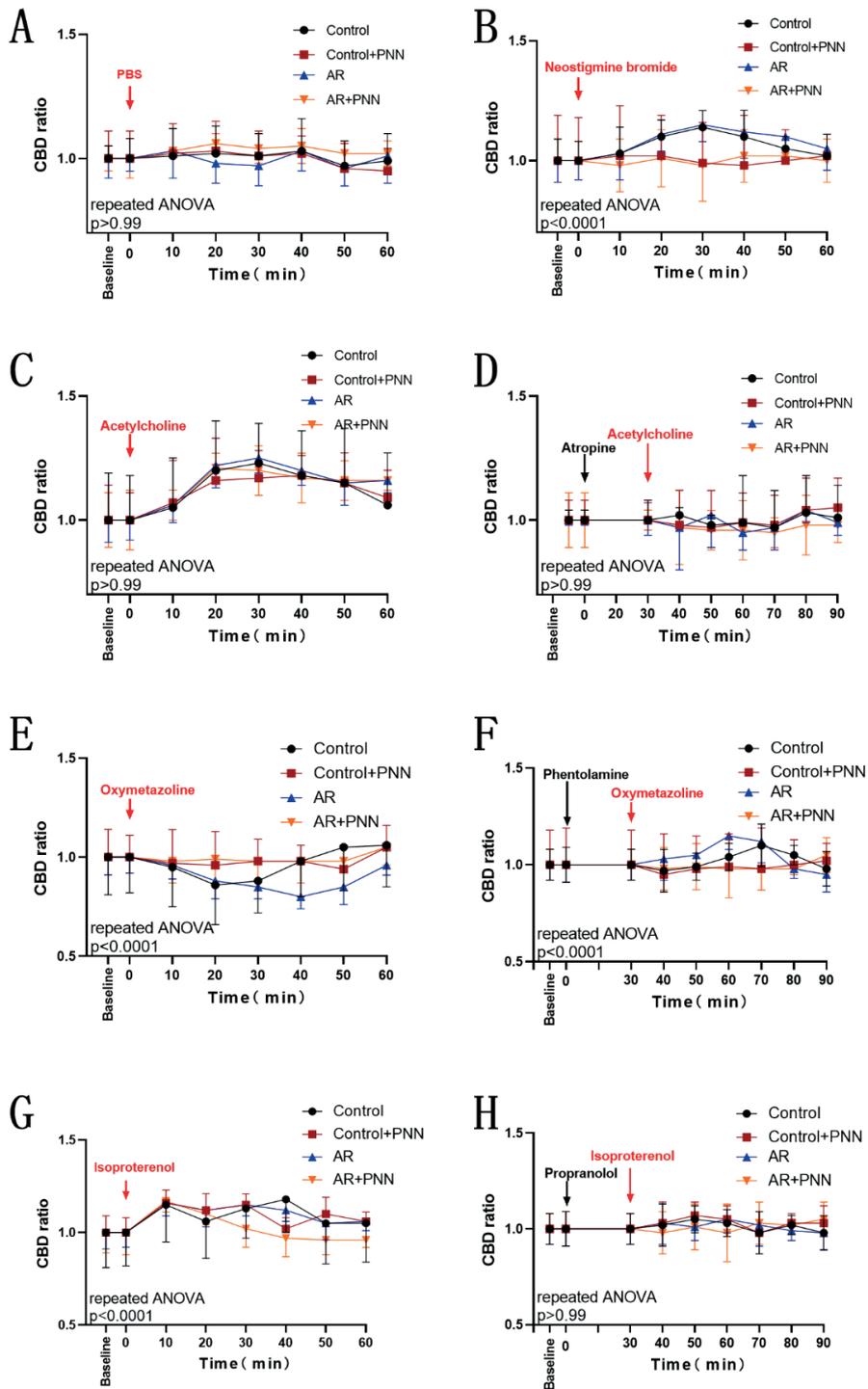


Figure S3. Ciliary beat distance (CBD) response to choline (A-D), α receptor (E-G) and β receptor substances (H-J). A: PBS solution as control; B: Cholinesterase inhibitor, neostigmine bromide promoted CBD of non-PNN group (Control group & AR group), but post-PNN group (Control + PNN group & AR + PNN group) had no significant reaction to neostigmine bromide; C: Acetylcholine promoted CBD of the four groups. D: Pretreatment with atropine for 30 min could antagonize the effect of acetylcholine. E: α-receptor agonist, Oxymetazoline inhibited CBD of the four groups. F: Pretreatment with α-receptor antagonist, phentolamine for 30 min could antagonize this effect. G: β-receptor agonist, isoproterenol promoted CBD of the four groups. H: β-receptor antagonist, propranolol could antagonize promotion effect of isoproterenol.