

# Allergen disrupts amygdala-respiration coupling

Kolsoum Dehdar<sup>a</sup>, Morteza Salimi<sup>a</sup>, Mohammad Reza Raoufy<sup>a,b,\*</sup>

<sup>a</sup> Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

<sup>b</sup> Institute for Brain Sciences and Cognition, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

## ARTICLE INFO

Edited by M. Dutschmann

### Keywords:

Allergic asthma  
Amygdala activity  
Respiration rhythm  
Amygdala-respiration coupling

## ABSTRACT

Allergic asthma affects both the respiratory function and central nervous system. Communication between the amygdala and respiratory control system is critical for regulating breathing function. To date, no study provides the effect of allergic inflammation on amygdala-respiration coupling. Here, we simultaneously recorded respiration and local field potentials of the amygdala during awake immobility in a rat model of allergic asthma. A decreased synchrony was found between amygdala and respiration in asthmatic rats. Allergen also reduced the modulatory effect of the respiration phase on amygdala power at delta, theta and gamma2 (80–120 Hz) frequencies. Moreover, in the animal model of allergic asthma, delta and theta oscillations strongly coordinate local gamma2 activity in the amygdala. These findings suggest that allergen can induce brain alterations and therefore shed light on future works to address how disruption of amygdala-respiration coupling contributes to respiratory dysfunction in allergic asthma.

## 1. Introduction

Asthma is a chronic inflammatory disease of the airways characterized by chest tightness, cough, wheezing and airway hyper-responsiveness (Bateman et al., 2008). Patients with asthma experience increased bronchoconstriction caused by various factors, including allergen exposure, viral infections, exercise, irritants, and psychosocial factors. An immune response is generally accepted to be a major mechanism in asthma pathogenesis. Moreover, the immune system in relation with the nervous system is shown to raise asthma symptoms. Peripheral cytokines can affect brain function via three key pathways during inflammation: 1) the direct entry of cytokine into the brain across the blood-brain barrier by a saturable transport mechanism; 2) infusion of cytokines into the brain through circumventricular organs, having incomplete blood-brain barrier; and 3) transmission of the inflammatory signal through the vagus nerve (Banks and Kastin, 1991; Blatteis et al., 1983; Ek et al., 1998; Quan and Banks, 2007). The central nervous system (CNS), in turn, communicates with immune systems through activation of the sympathetic nervous system, hypothalamic–pituitary–adrenal axis, and vagal nerve, exhibiting a broad and close-knit regulatory network (Borovikova et al., 2000; Webster et al., 2002). These findings point to the critical role of CNS in the progression of asthma symptoms.

Amygdala is a key substrate in the neural system necessary for

modulating immune and respiratory systems (Nobis et al., 2018; Stoeckel et al., 2018). There are bidirectional interactions between respiration and amygdala function. Animal studies provide evidence for the link between the amygdala and respiration, which is potentially mediated via axonal projection from the extended amygdala, comprising chiefly of the central amygdala (CeA) and the bed nucleus of the stria terminalis (BNST), to the brainstem (Avery et al., 2014; Oler et al., 2012). Importantly, this circuit is crucial for mediating breathing function (Dong and Swanson, 2006; Saha et al., 2000; Zhang et al., 1986). Electrical or pharmacological activation of the amygdala leads to an increase in respiratory rate (Applegate et al., 1983; Zhang et al., 2009). Besides, amygdala inhibition by microinjection of muscimol reduces tachypneic responses to brief alerting stimuli (Bondarenko et al., 2014). Consistent with observations in animals, electrical stimulation of the amygdala increased respiratory rate in an epileptic patient (Masaoka and Homma, 2004). Moreover, a lesion of the left amygdala results in the reduction of respiratory rate during anticipatory anxiety (Masaoka et al., 2003).

Conversely, it has been documented that breathing dysfunction or even apnea occurred when seizures spread to the amygdala and upon amygdala electrical stimulation (Dlouhy et al., 2015; Nobis et al., 2020, 2018). On the other hand, respiration itself affects amygdala activity through two possible routes. First, the nucleus tractus solitarius receive sensory information from respiratory systems through vagal afferents

\* Corresponding author at: Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, PO Box: 1411713116, Iran.

E-mail address: [raoufy@modares.ac.ir](mailto:raoufy@modares.ac.ir) (M.R. Raoufy).

<https://doi.org/10.1016/j.resp.2021.103835>

Received 27 August 2021; Received in revised form 25 November 2021; Accepted 27 December 2021

Available online 28 December 2021

1569-9048/© 2021 Elsevier B.V. All rights reserved.

and then sends direct projections to the amygdala (Berthoud and Neu-huber, 2000). Second, olfactory sensory neurons (OSNs), which are responsive to both chemical and mechanical (nasal airflow) stimuli, mediate the effect of nasal breathing on brain oscillations by axonal projection to various brain regions, including the amygdala (Inokuchi et al., 2017). A human study exhibited respiration-entrained oscillations in the amygdala phase-locked to nasal breathing (Zelano et al., 2016). Additionally, an animal study indicated that nasal respiration produces electrical activity in widespread brain regions such as the amygdala coupled to breathing rhythm (Tort et al., 2018).

These findings combine to suggest an amygdala-respiration-amygdala (ARA) loop; so that amygdala regulates breathing rhythm (Dlouhy et al., 2015; Nobis et al., 2018); conversely, respiratory rhythm is involved in modulating amygdala activities (Zelano et al., 2016). It appears that an inextricable link between respiration and the amygdala has an essential role in regulating breathing function (Dong and Swanson, 2006; Saha et al., 2000).

Allergic asthma is believed to cause structural and functional alterations in the amygdala. Asthma induces enhancement of c-Fos immunoreactivity and neuronal discharge in the amygdala (Chen et al., 2020; Costa-Pinto et al., 2005). Furthermore, allergen exposure increased microglia and astrocyte reactivity, accompanied by an elevation of activity in the amygdala (Dehdar et al., 2019). The respiratory pattern is also strongly disrupted due to allergic asthma (Raoufy et al., 2017). Human and animal experiments demonstrated that allergic inflammation reduces the self-similarity and complexity of respiratory rhythm (Eslami-Behrooz et al., 2018; Raoufy et al., 2016).

Therefore, we hypothesized that allergic inflammation impairs ARA loop resulting in perturbation of amygdala-respiration oscillations coupling. For this purpose, by using simultaneous recording of amygdala and respiration local field potentials (LFPs), we explored amygdala

activity and its coupling with respiration in an animal model of allergic asthma during awake immobility.

## 2. Material and methods

### 2.1. Animals

Twenty adults (4–5 weeks old) pathogen-free male Wistar rats weighing 80–100 g were purchased from the Pasteur Institute (Tehran, Iran). The animals were housed on a 12-h light-dark-cycle, with access to food and water. All procedures were in accordance with NIH Guidance for the Care and Use of Laboratory Animals (2011) and approved by the "Ethics Committee of Faculty of Medical Sciences, Tarbiat Modares University [IR.TMU.REC.1395.538]."

### 2.2. Experimental groups

The rats were randomly allocated to one of two groups: control or OVA. On days 0 and 7, both groups received two intraperitoneal (i.p.) injections of saline or OVA-Al(OH)<sub>3</sub> [1 mg OVA (Grade III; Sigma) and 100 mg Al(OH)<sub>3</sub>, in 1 mL saline] and 14 aerosol inhalations (for 30 min every two days from day 14 to day 40) of either saline or OVA solution (2 percent wt/vol) (Fig. 1A).

### 2.3. Assessment of allergic responses in the lung and brain

To confirm the asthma model's induction, after 48 h from the last aerosol exposure rats were anesthetized with urethane (1.6 g/kg, i.p.), and lungs were removed and fixed in formalin solution 10 %. Then five-micrometer sections were cut and stained with hematoxylin and eosin (H&E) to identify inflammatory cells that infiltrated the airway.

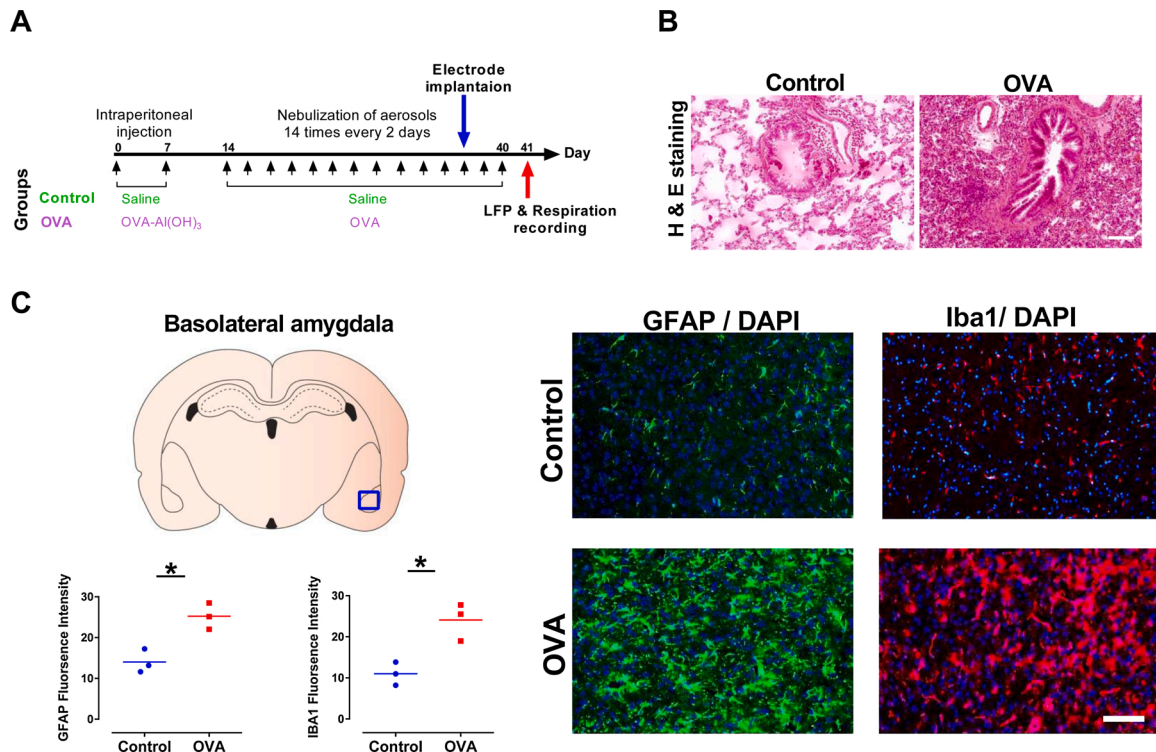


**Fig. 1. Allergen induces lung and brain inflammation.** (A) Represents the timeline of the study. The rats received 2 intraperitoneal injections of saline or OVA-Al (OH)<sub>3</sub> on day 0 and day 7, and 14 inhalation exposures (for 30 min, every 2 days, from day 14 to day 40) with aerosols of saline or OVA solution. Electrode implantation was performed on day 33. LFP and respiration were recorded on day 41. (B) Hematoxylin and eosin (H&E) staining of lung sections to identify peribronchial inflammatory cell infiltration (Scale bar = 50  $\mu$ m). (C) Schematic section of immune staining in the brain (upper left panel), Iba1 and GFAP immunoreactivity, in basolateral amygdala (right panel) as well as quantitative assessment on each test (lower left panels). Data were analyzed by *t*-test, *n* = 3 per group. \**p* < 0.05 compared to control. OVA, ovalbumin. OVA, ovalbumin; LFP, local field potential.

Immunofluorescence staining was performed as previously described (Dehdar et al., 2019). Rats were perfused transcardially with phosphate-buffered saline (PBS) followed by a 4% paraformaldehyde (PFA) solution. After perfusion, brains were removed, post-fixed with PFA overnight at 4 °C, and then transferred to 30 % sucrose for 48 h. Coronal sections of the amygdala (thickness: 8  $\mu$ m) were incubated overnight at 4 °C with a primary antibody for anti-Iba1 (1:100, SC-98468), anti-GFAP (1:300, Z0334). After washing with PBS, slides were incubated with secondary antibody (goat anti-rabbit AlexaFluor®594; 1:1000, A-11036, goat anti-rabbit AlexaFluor®488; 1:1000) at room temperature for 1 h. Cell nuclei were stained with DAPI (blue). Image documentation was done using fluorescence microscopy (Olympus BX51 TRF, USA). Four sections from each animal (3 animals/groups) were analyzed using ImageJ software (NIH, USA) for intensity quantification. GFAP and Iba-1 fluorescence intensity was assessed by measuring the mean gray value in BLA. Each value was normalized to the mean of tissue background intensity and presented as the percent of the saline group. Fluorescence intensity was analyzed using Image J software.

#### 2.4. Surgery

Animals were anesthetized with i.p. injections of ketamine-xylazine anesthesia (100 and 10 mg/kg). Then rats were placed on a stereotaxic

frame (Narishige, Japan), and body temperature was maintained at 37 °C by a heating pad. Before surgery, 0.5 mL of lidocaine chlorhydrate 2 % was subcutaneously injected in the incision site over the skull scalp for local anesthesia. After exposing the skull, stainless-steel recording electrodes (127  $\mu$ m in diameter, A.M. system Inc., USA) were implanted unilaterally into the basolateral amygdala (BLA) (AP: -2.5 mm, L: 5 mm, DV: -7.4 mm) (Paxinos and Watson, 2013). A stainless-steel screw was implanted at the right side of parietal bone as a reference (Fig. 2B).

#### 2.5. Electrophysiological and respiration recordings

Seven days after surgery (24 h after the last aerosol exposure), rats were placed in a whole-body plethysmograph (20 cm inner diameter, 20 cm height) (Fig. 2A), which was customized to allow simultaneous recordings of local field potentials (LFPs). The plethysmograph chamber was connected to a CO<sub>2</sub> sensor, and medical air was continuously delivered into the chamber at 4 L/min. For acclimatization, rats were placed to plethysmograph one hour per day for two days before recording. Spontaneous amygdala LFP and respiration were recorded simultaneously for 5 min, and 60 s periods of awake immobility were collected for signal processing. For this purpose, implanted electrodes were fixed to a socket and then connected to a miniature buffering head-stage with high-input impedance (BIODAC-A, TRITA Health Technology

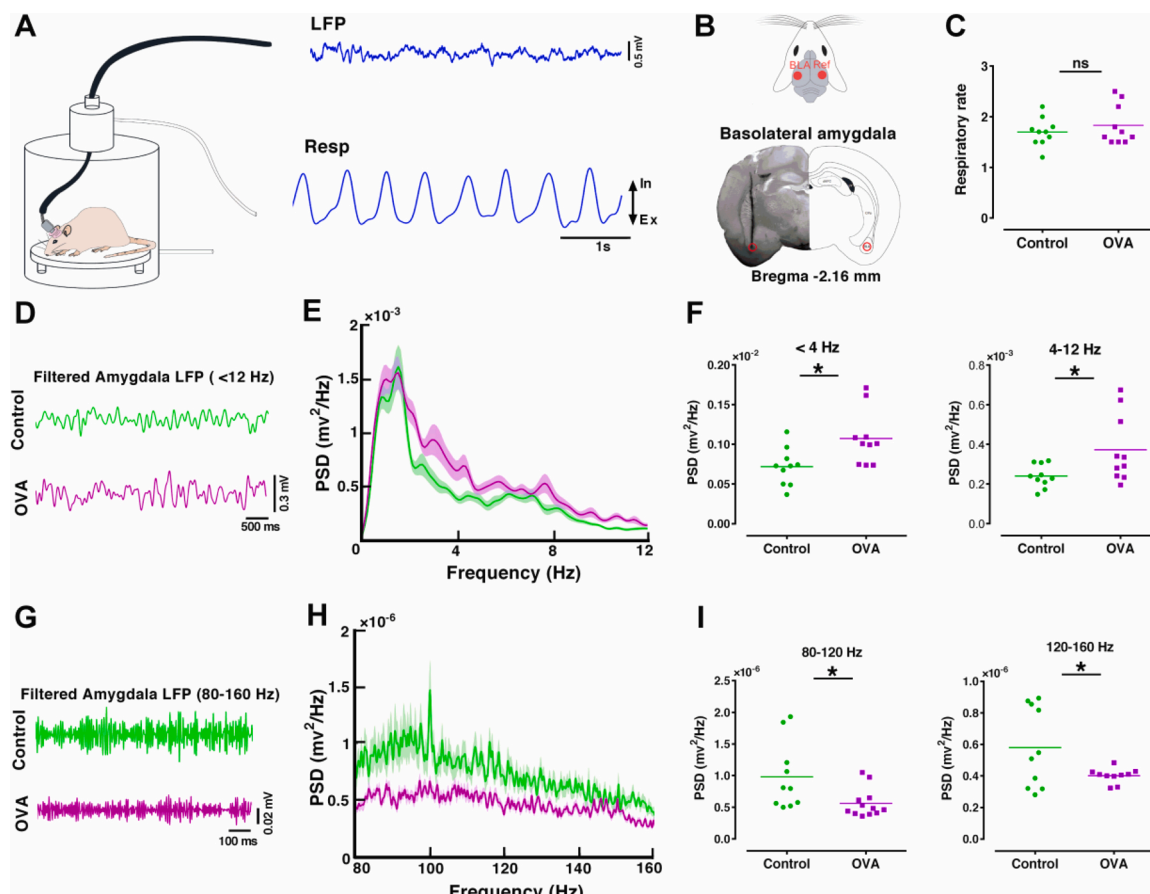


**Fig. 2. Allergen alters amygdala activity.** (A) Schematic representation of a plethysmograph recording of respiration (Resp) along with the amygdala LFP during awake immobility. (B) Schematic depiction of electrode implantation sites on rat skull (top panel), Histological verification of the electrode location in basolateral amygdala along to stereotaxic diagram adapted from Paxinos and Watson (2013) rat brain atlas (bottom panel). (C) OVA and Control groups indicated the same respiratory rate. (D) Representative example of filtered signal at delta (<4 Hz) and theta (4-12 Hz). (E) Averaged power spectra of oscillations in the amygdala at frequencies <12 Hz. (F) PSD of the amygdala increases at delta and theta in OVA group (G) Representative example of filtered signal at gamma (80-160 Hz). (H) Averaged gamma PSD of LFPs in the amygdala. (I) OVA exposure decreases PSD of gamma 2 (80-120 Hz) and gamma 3 (120-160 Hz) in the amygdala LFPs. Shaded area denote standard errors. Horizontal bars indicate mean values. Data were analyzed by *t*-test, *n* = 10 per group. \**p* < 0.05 compared to control. OVA, ovalbumin; PSD, power spectral density; LFP, local field potential.

Co., Tehran, Iran). From there, a cable was established connection to the main AC coupled amplifier (1000 amplification) and the recording system (BIODAC-ESR18622, TRITA Health Technology Co., Tehran, Iran). Spontaneous LFPs of amygdala and respiration were simultaneously recorded by two distinct channels digitized at 1 kHz and stored for offline analyses with custom-written MATLAB scripts (The Mathworks, Inc.). LFPs and respiration were obtained by low pass filtering < 500 Hz and bandpass filtering 0.5–10 Hz, respectively. The video tracker recorded the movement of animals to detect awake immobility periods. We have focused on immobile animals to exclude confounding factors, including behavioral variables (i.e., locomotor activity).

## 2.6. Histological verification

For confirming the electrode position, rats were deeply anesthetized with i.p. injection of urethane (1.6 g/Kg) and then transcardially perfused with PBS followed by 4% PFA solution. Afterwards, brains were carefully dissected and fixed with PFA 48 h. Fixed brains were sectioned at 50  $\mu$ m and then compared with matching slices in the Rat Brain Atlas of Paxinos and Watson (Paxinos and Watson, 2013). The accurate location of electrodes was verified by light microscopy (Fig. 2B). Animals with misplaced electrodes were excluded and replaced.

## 2.7. Spectral analysis and estimation of breathing rate

Respiratory frequency and power spectral density (PSD) of amygdala signals were computed using means of Welch's periodogram (6 s Hamming windows with 90 % overlap).

## 2.8. Cross-correlation analysis

Respiration and LFP raw signals were bandpass filtered in the respiratory frequencies range between 1 and 2 Hz. We computed cross-correlation for filtered signals by xcorr function, with the "coeff" option to normalize values between -1 and 1.

## 2.9. Phase-power coupling

We analyzed the coupling between the phase of respiration and the power of amygdala signals. The respiration phase was obtained by calculating the Hilbert transform phase of the respiration signals. Amygdala power of delta (<4 Hz), theta (4–12 Hz), gamma1 (30–80 Hz), gamma2 (80–120 Hz), and gamma3 (120–160 Hz) calculated using the spectrogram with a one-sample time step. Respiration phases were binned into the 120, each 3 degrees wide, and the average power of amygdala samples was calculated in each bin. Coupling strength was expressed as a mean resultant vector length (MRL), the average of power vectors in the respiration phase. Finally, a Gaussian kernel was applied only for making the picture more soothing and pleasant. The dimension of the standard deviation is the size of the same pixels. In the example figures, we applied the phase binning for the respiratory signals with 120 phase-angle bins and 3 degrees per bin. (Salimi et al., 2021; Tort et al., 2009). Amygdala local phase-power coupling was calculated using the similar framework which was described above.

## 2.10. Statistical analysis

GraphPad Prism (GraphPad Software, San Diego, CA) was used for statistical analysis. The normality of the data distribution within each parameter was assessed by the Kolmogorov-Smirnov test. Differences between control and OVA groups were assessed by *t*-test. We used *P*-values less than 0.05 to denote statistical significance.

## 3. Results

### 3.1. Allergen induces lung and brain inflammation

Examination of H&E stained lung tissue showed that allergen exposure caused a considerable increase of peribronchial inflammatory infiltrates in sensitized rats (Fig. 1B). To check the inflammatory status of the BLA, using immunostaining of GFAP-labeled for astrocytes cells and Iba-1-labeled for microglial cells were assessed. We observed that an Iba-1-labeled microglial and GFAP-labeled astrocytes cells were significantly augmented in the amygdala of the OVA group compared to control rats. In these conditions, the massive proliferation of microglial and reactivation of astrocytes cells result in intensity increased of Iba-1 and GFAP expression respectively ( $p < 0.05$ , Fig. 1C).

### 3.2. Allergen alters amygdala activity

We simultaneously recorded basolateral amygdala oscillations and respiratory activity of rats within the plethysmograph during awake immobility (Fig. 2A, B). No significant alteration was found between the respiratory rate of the Control and OVA groups (Fig. 2C). Spectral analysis of amygdala LFPs revealed a significant increase of delta (< 4 Hz) and theta (4–12 Hz) power of OVA group compared to Control group ( $F = 2.15$ ,  $p = 0.01$ ,  $F = 8.10$ ,  $p = 0.02$ , respectively; Fig. 2E, F). However, the power of gamma2 (80–120 Hz) and gamma3 (120–160 Hz) were significantly decreased in OVA group ( $F = 5.47$ ,  $p = 0.02$ ,  $F = 30.08$ ,  $p = 0.04$ , respectively; Fig. 2H, I).

### 3.3. Allergen reduced amygdala-respiration synchronization

To further explore the impact of allergen on the relationship between respiration and amygdala activity, we calculated cross-correlation by computing the mean correlation and lag time of respiration and amygdala LFPs. The mean correlation for the respiratory-amygdala filtered signals was significantly diminished in OVA group ( $F = 3.56$ ,  $p = 0.03$ , Fig. 3B, C). No significant lag time difference was found between Control and OVA groups.

### 3.4. Allergen disrupts phase-power coupling

Respiration is capable of modulating the brain oscillations such as theta and gamma (Ito et al., 2014; Yanovsky et al., 2014). We performed a phase-power coupling analysis to investigate whether allergen could influence respiration phase-amygdala activity coupling. This analysis revealed that respiration-delta (<4 Hz), -theta (4–12 Hz) and -gamma2 (80–120 Hz) phase-power coupling strength were significantly diminished in OVA group compared to Control group ( $F = 1.20$ ,  $p = 0.03$ ,  $F = 1.05$ ,  $p = 0.04$ ,  $F = 2.51$ ,  $p = 0.04$ , respectively, Fig. 4A–F). Moreover, local phase-power coupling analysis of amygdala exhibited that allergic inflammation enhanced the strength coupling of delta-gamma2 and theta-gamma2 ( $F = 2.03$ ,  $p = 0.009$ ,  $F = 4.23$ ,  $p = 0.03$ , respectively, Fig. 4G–I). There was no significant difference in delta/theta-gamma3 coupling in the amygdala between groups (data not shown).

## 4. Discussion

This study investigated the effect of allergen on amygdala-respiration interaction, which has not been previously described. Our findings exhibit that allergen leads to synchrony reduction between amygdala and respiration rhythms. Moreover, the modulation effect of the respiration phase on amygdala power at delta, theta, and gamma2 frequencies decreases in the animal model of allergic asthma. Allergen also increased local phase-power coupling between delta-gamma2 and theta-gamma2 in amygdala. Here, we provide new insight into the interaction between amygdala activity and respiration rhythm, which is

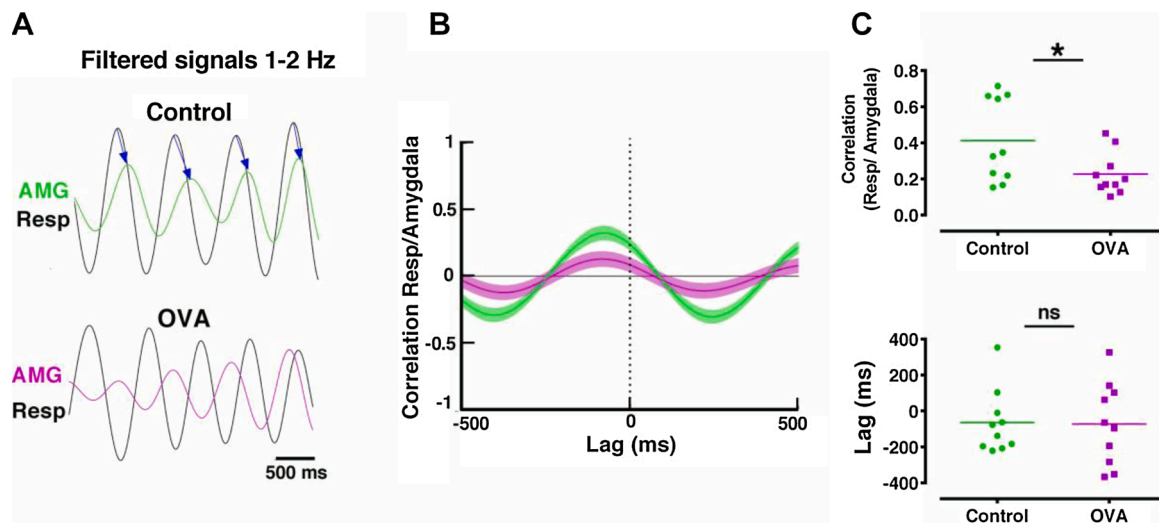


**Fig. 3.** Allergen drops synchrony between respiration and amygdala activity. (A) Examples of respiration and amygdala filtered signals (1–2 Hz) in the Control (upper) and OVA (lower). Arrows are drawn from the leading to the lagging. (B) Mean cross-correlation for simultaneously respiration and amygdala recording. (C) Maximum respiration-amygdala cross-correlation values. OVA exposure decreases correlation between respiration and amygdala oscillations. Shaded area denote standard errors. Horizontal bars indicate mean values. Data were analyzed by *t*-test, *n* = 10 per group. \**p* < 0.05 compared to control. OVA, ovalbumin; LFP, local field potential.

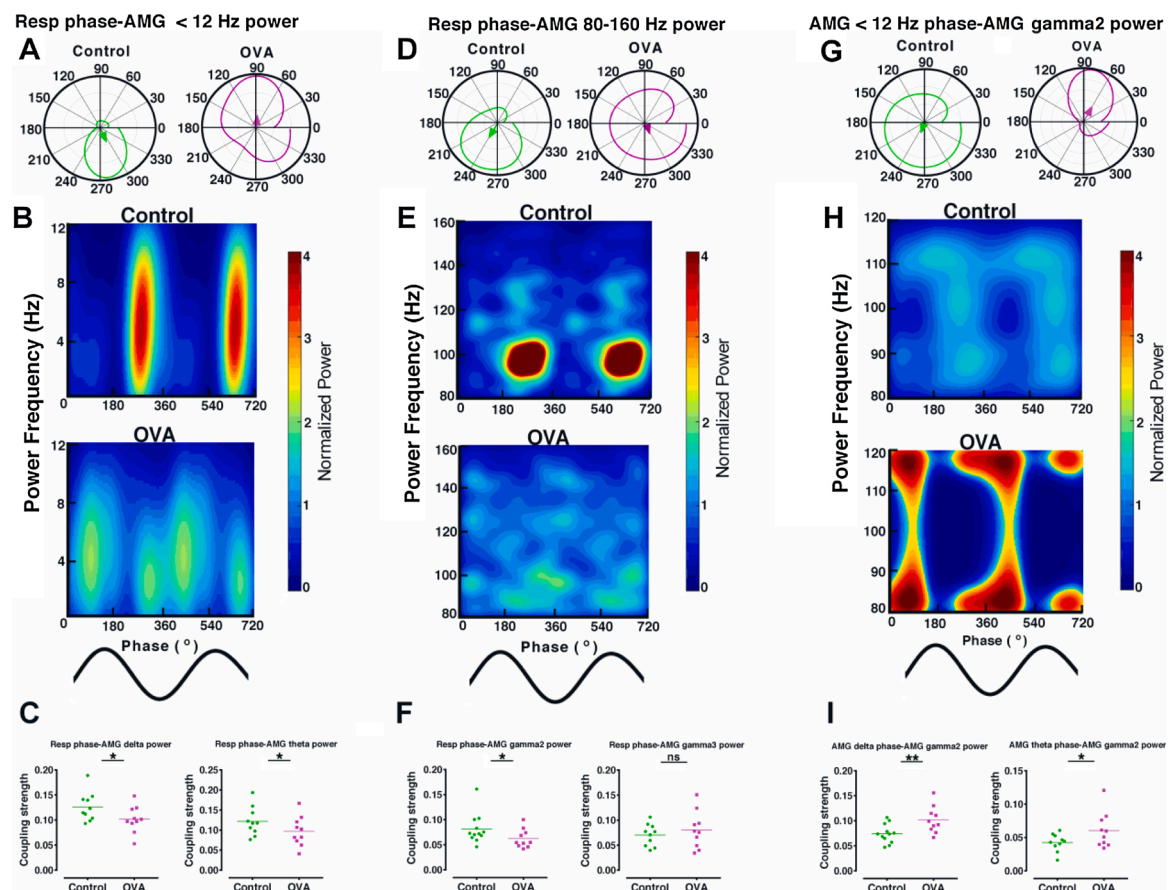


**Fig. 4.** Allergen disrupts phase-power coupling. (A, D) Circular distribution of <12 Hz and 80–160 Hz amygdala power for respiration phase (left: control and right: OVA). Green and purple arrows indicate mean resultant length. (B, E) Color maps depicting the relationship between respiration phase and amygdala power at <12 Hz and 80–160 Hz in representative control (top panel) and OVA (bottom panel) rat. (C, F) Asthmatic rats show lower phase-power coupling strength of respiration-delta, respiration-theta, respiration-gamma2. (H) Circular distribution of gamma2 power for <12 Hz phase in amygdala (left: control and right: OVA). (I) Color maps show gamma2 power as a function of <12 Hz phase in amygdala of representative control (top panel) and OVA (bottom panel) rat. (G) Asthmatic rats had greater local delta-gamma2 and theta-gamma2 coupling strength in amygdala. Horizontal bars indicate mean values. Data were analyzed by *t*-test, *n* = 10 per group. \**p* < 0.05, \*\**p* < 0.01 compared to control. OVA, ovalbumin; LFP, local field potential.

disrupted due to allergen.

Interaction between neural processing and body function provides a loop called brain-body-brain loop, which plays an important role in regulating various aspects of cognitive functions (Damasio, 2001; Ekman et al., 1983). For instance, an emotional stimulus is shown to trigger responses in some brain areas, including the amygdala, which can cause freezing and breathing changes; on the other side, breathing rhythm entrains olfactory bulb oscillations which in turn organizes neural firing in the medial prefrontal cortex, allowing to maintain the ongoing behaviour, perhaps through its connections with amygdala (Bagur et al., 2021). Regarding accumulated evidence from bidirectional amygdala-respiration interplay, we propose a loop between the breathing rhythm and activity of amygdala (ARA loop) necessary for regulating respiration and coordinating behavioural performance with breathing (Fig. 5). Our study demonstrates that allergen results in attenuation of amygdala-respiration synchrony and modulation effect of respiration phase on amygdala activity. These findings suggest that allergen via ARA loop disruption leads to respiratory dysfunction and disorganizes harmony between behaviour and breathing. Loop components have not been investigated in this study, and future works need to disentangle the contribution of each part. Nevertheless, a remaining question is how allergen disrupt amygdala-respiration interaction? It could be speculated via three possible ways. First, allergen has been documented to induce an inflammation process in the brain that drives changes in amygdala activity (Dehdar et al., 2019). Allergen is also associated with increased glial proliferation and inflammatory response in the respiratory control center leading to respiratory pattern alteration (Chen et al., 2001, 2008). Thus, it seems that allergen causes inflammatory reactions in both amygdala and respiratory control center and subsequently disturbs connection between these regions which in turn contributes to unsettled regulatory effect of amygdala on respiration. Second, allergen exposure also induces T-helper 2 cytokines release and mucocellular metaplasia in the nasal epithelium (Chen et al., 2008; Tonelli et al., 2009; Wagner et al., 2002). Olfactory sensory neurons send projections to the olfactory bulb, and then through the lateral and accessory olfactory tracts to the amygdala (Inokuchi et al., 2017). Hence, olfactory bulb neuronal changes driven by allergic inflammation can alter respiration-entrained brain rhythms and provide a way by which impair the modulatory effect of breathing rhythm on the amygdala function. Third, the vagus nerve has been shown to play a crucial role in immunomodulation (Goehler et al., 1997). Peripheral inflammatory mediators released by allergens activate vagal fibers, and in this way send a signal from the periphery to NTS, which is one of the

respiratory control centers (Mascarucci et al., 1998; Thayer et al., 2011). Therefore, given the anatomical connection between NTS and the amygdala, activity changes of the NTS in response to allergic inflammation can alter the interaction between the amygdala and the respiratory control center. Taken together, these findings suggest possible ways that allergen could contribute to disrupting ARA loop. It would be interesting to dissect the exact mechanisms underlying the ARA loop impairment due to allergic inflammation in future investigations.

Former studies have shown phase-locking of breathing cycles and brain oscillations, facilitating long-range information integration across the neural hubs (Ito et al., 2014; Lockmann et al., 2016). In support of this possibility, it has been exhibited that the respiration phase could modulate the amplitude of gamma-frequency oscillations in several brain regions (Líbano and Rubido, 2019; Rojas-Líbano et al., 2018; Yanovsky et al., 2014). Specifically, exclusive coupling of respiration phase with amplitude of gamma2 was found in the olfactory bulb, pre-limbic cortex, parietal cortex, and dorsal hippocampus during awake immobility. It indicates that brain uses distant slow rhythms, in addition to local slow oscillations to modulate fast oscillations for network communication (Zhong et al., 2017). Brain oscillations entrained by respiration are suggested as a temporal binding process necessary for cognitive functions, including attention, learning, and memory-related functions (Zelano et al., 2016). On the other hand, several studies have reported behavioural complications associated with allergen exposure, including cognitive deficits, memory decline, attention deficiency, anxiety, and depression (Feng et al., 2017; Salimi et al., 2019; Strine et al., 2008; Trikojat et al., 2017). In this context, our findings denote that amygdala power at delta, theta and gamma2 less depends on the phase of respiration in the rat model of allergic asthma. It appears that the impaired modulatory effect of the respiration phase on the amygdala power could be considered as a possible factor contributing to abnormal behaviour and neurological deficits due to allergic inflammation.

Of note, several animal studies have shown that anxiety-like behaviour is associated with enhancement of local low-fast frequencies coupling and reduced gamma activity in the amygdala (Stujenske et al., 2014). Furthermore, it has been demonstrated that allergic inflammation could induce anxiety and emotional reactivity (Costa-Pinto et al., 2005; Tonelli et al., 2009). However, the precise mechanisms underlying the observed anxiety in allergies remain unclear. In line with this context, we interestingly found that allergen increased local low-fast frequency coupling and decreased power at gamma2 oscillations in the amygdala. These results justify how allergic

### Amygdala-respiration-amygdala (ARA) loop

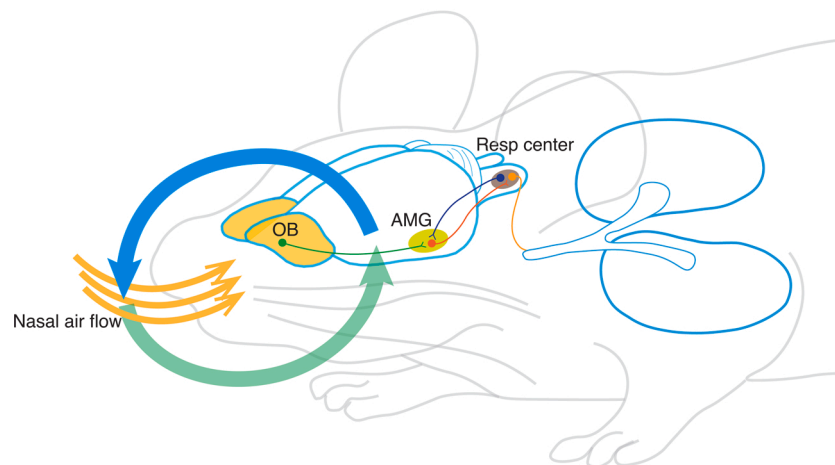


**Fig. 5. Proposed model of amygdala-respiration-amygdala loop.** Amygdala affects breathing function through axonal projection to respiratory control center; conversely, nasal respiration impacts amygdala activity by producing respiration-entrained rhythms. Vagal afferents also transmit sensory information from the respiratory system to the amygdala.

inflammation can induce anxiety-like behaviour.

Our findings open a new window into a deeper scientific understanding of the pathophysiology of allergic asthma. Notably, the current study is the early report that suggests asthma drives alteration in ARA loop. Although the effect of allergen on airways is well understood, little is known about its impact on brain function. Our findings emphasize the contribution of allergic inflammation in CNS neuronal activity. We should address some limitations of the current study. First, the reciprocal interaction of respiration with the amygdala may not be specific to this side of the brain may be widely observed in many other regions which can receive special attention in future studies. Next, despite local field potential being one of the main techniques that indicate the activity and connectivity of the neural circuit directly, but further methods including pharmacologic, optogenetic as well as spike activity recording are required. The present study could help develop multipronged therapeutic strategies to maintain brain structure and function, thereby minimizing the behavioural impairment in people with asthma. Our work motivates future studies to uncover underlining mechanisms that generate or mediate asthma-induced alteration of brain function.

## 5. Conclusion

In summary, we investigated the effect of allergen on amygdala-respiration coupling. Our principal finding is that allergen exposure in sensitized rats disrupts the dynamics of amygdala-respiration interaction. Current study could be considered as groundwork for future studies to evaluate contribution of the disrupted amygdala-respiration coupling in asthma symptom severity, in order to develop novel therapeutic approaches.

## Funding

This work was supported by funds from the Tarbiat Modares University (IG-39709) and from the Iran National Science Foundation (INSF, 96006064). The funders had no role in the study.

## Research ethics

All procedures were in accordance with NIH Guidance for the Care and Use of Laboratory Animals (2011) and approved by the "Ethics Committee of Faculty of Medical Sciences, Tarbiat Modares University [IR.TMU.REC.1395.538]."

## Data availability

Data will be made available on request.

All foci coordinates, activation probability maps, in addition to the supplemental information will be available on ANIMA: a data-sharing initiative for neuro-imaging meta-analyses: [anima.fz-juelich.de](http://anima.fz-juelich.de).

## Declaration of Competing Interest

The authors declare that they have no competing interests.

## Acknowledgement

We would like to thank Mr. Reza Vaziri for his technical assistance.

## References

- Applegate, C.D., Kapp, B.S., Underwood, M.D., McNall, C.L., 1983. Autonomic and somatomotor effects of amygdala central N. stimulation in awake rabbits. *Physiol. Behav.* 31, 353–360.
- Avery, S.N., Clauss, J.A., Winder, D.G., Woodward, N., Heckers, S., Urbano, J., 2014. NeuroImage BNST neurocircuitry in humans. *Neuroimage* 91, 311–323.
- Bagur, S., Lefort, J.M., Lacroix, M.M., de Lavilléon, G., Herry, C., Chouvaeff, M., Billand, C., Geoffroy, H., Benchenane, K., 2021. Breathing-driven prefrontal oscillations regulate maintenance of conditioned-fear evoked freezing independently of initiation. *Nat. Commun.* 12, 1–15.
- Banks, W.A., Kastin, A.J., 1991. Blood to brain transport of interleukin links the immune and central nervous systems. *Life Sci.* 48, 117–121.
- Bateman, E.D., Hurd, S.S., Barnes, P.J., Bousquet, J., Drazen, J.M., FitzGerald, M., Gibson, P., Ohta, K., O'Byrne, P., Pedersen, S.E., 2008. Global strategy for asthma management and prevention: GINA executive summary. *Eur. Respir. J.* 31, 143–178.
- Berthoud, H., Neuhuber, W.L., 2000. Functional and chemical anatomy of the afferent vagal system. *Auton. Neurosci. Basic Clin.* 85, 1–17.
- Blatteis, C.M., Bealer, S.L., Hunter, W.S., Llanos, J.-Q., Ahokas, R.A., Mashburn Jr, T.A., 1983. Suppression of fever after lesions of the anteroventral third ventricle in guinea pigs. *Brain Res. Bull.* 11, 519–526.
- Bondarenko, E., Hodgson, D.M., Nalivaiko, E., 2014. Amygdala mediates respiratory responses to sudden arousing stimuli and to restraint stress in rats. *Am. J. Physiol. Integr. Comp. Physiol.* 306, R951–R959.
- Borovikova, L.V., Ivanova, S., Zhang, M., Yang, H., Botchkina, G.I., Watkins, L.R., Wang, H., Abumrad, N., Eaton, J.W., Tracey, K.J., 2000. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 405, 458–462.
- Chen, C.-Y., Bonham, A.C., Schelegle, E.S., Gershwin, L.J., Plopper, C.G., Joad, J.P., 2001. Extended allergen exposure in asthmatic monkeys induces neuroplasticity in nucleus tractus solitarius. *J. Allergy Clin. Immunol.* 108, 557–562.
- Chen, S.-D., Wen, Z.-H., Chang, W.-K., Chan, K.-H., Tsou, M.-T., Sung, C.-S., Tang, G.-J., 2008. Acute effect of methylprednisolone on the brain in a rat model of allergic asthma. *Neurosci. Lett.* 440, 87–91.
- Chen, Z., Jian, N.L., Wang, X.Y., Dong, R., 2020. The amygdala via the paraventricular nucleus regulates asthma attack in rats. *CNS Neurosci. Ther.* 26, 730–740.
- Costa-Pinto, F.A., Basso, A.S., Britto, L.R.G., Malucelli, B.E., Russo, M., 2005. Avoidance behavior and neural correlates of allergen exposure in a murine model of asthma. *Brain Behav. Immun.* 19, 52–60.
- Damasio, A., 2001. The feeling of what happens. *Gift. Talent. Int.* 16, 83–83.
- Dehdar, K., Mahdoudost, S., Salimi, M., Gholami-Mahtaj, L., Nazari, M., Mohammadi, S., Dehghan, S., Jamaati, H., Khosrowabadi, R., Nasiraei-Moghaddam, A., Barkley, V., Javan, M., Mirnajafi-Zadeh, J., Sumiyoshi, A., Raoufy, M.R., 2019. Allergen-induced anxiety-like behavior is associated with disruption of medial prefrontal cortex - amygdala circuit. *Sci. Rep.* 9, 1–13.
- Dlouhy, B.J., Gehlbach, B.K., Kreple, C.J., Kawasaki, H., Oya, X., Buzza, C., Granner, M. A., Welsh, M.J., Howard, M.A., Wemmie, J.A., Richerson, X.G.B., 2015. Breathing inhibited when seizures spread to the amygdala and upon amygdala stimulation. *J. Neurosci.* 35, 10281–10289.
- Dong, H., Swanson, L.W., 2006. Projections from bed nuclei of the stria terminalis, dorsomedial nucleus: implications for cerebral hemisphere integration of neuroendocrine, autonomic, and drinking responses. *J. Comp. Neurol.* 494, 75–107.
- Ek, M., Kurosawa, M., Lundeberg, T., Ericsson, A., 1998. Activation of vagal afferents after intravenous injection of interleukin-1 $\beta$ : role of endogenous prostaglandins. *J. Neurosci.* 18, 9471–9479.
- Ekman, P., Levenson, R.W., Friesen, W.V., 1983. Autonomic nervous system activity distinguishes among emotions. *Science* 221, 1208–1210.
- Eslami-Behrooz, M., Javan, M., Raoufy, M.R., 2018. Effect of airway remodeling and hyperresponsiveness on complexity of breathing pattern in rat. *Respir. Physiol. Neurobiol.* 247, 65–70.
- Feng, B., Jin, H., Xiang, H., Li, B., Zheng, X., Chen, R., Shi, Y., Chen, S., Chen, B., 2017. Association of pediatric allergic rhinitis with the ratings of attention-deficit/hyperactivity disorder. *Am. J. Rhinol. Allergy* 31, 161–167.
- Goehler, L.E., Relton, J.K., Dripps, D., Kiechle, R., Tartaglia, N., Maier, S.F., Watkins, L. R., 1997. Vagal paranglia bind biotinylated interleukin-1 receptor antagonist: a possible mechanism for immune-to-brain communication. *Brain Res. Bull.* 43, 357–364.
- Inokuchi, K., Imamura, F., Takeuchi, H., Kim, R., Okuno, H., Nishizumi, H., Bito, H., Kikusui, T., Sakano, H., 2017. Nrp2 is sufficient to instruct circuit formation of mitral-cells to mediate odour-induced attractive social responses. *Nat. Commun.* 8, 1–13.
- Ito, J., Roy, S., Liu, Y., Cao, Y., Fletcher, M., Lu, L., Boughter, J.D., Gru, S., Heck, D.H., 2014. Whisker barrel cortex delta oscillations and gamma power in the awake mouse are linked to respiration. *Nat. Commun.* 5, 1–10.
- Líbano, D.R., Rubido, N., 2019. Nasal Respiration Entrain Neocortical Long - Range Gamma Coherence During Wakefulness, pp. 1–15.
- Lockmann, A.L.V., Laplagne, D.A., Leão, R.N., Tort, A.B.L., 2016. A respiration-coupled rhythm in the rat hippocampus independent of theta and slow oscillations. *J. Neurosci.* 36, 5338–5352.
- Masaoka, Y., Homma, I., 2004. Amygdala and emotional breathing in humans. *Post-Genomic Perspectives in Modeling and Control of Breathing*. Springer, pp. 9–14.
- Masaoka, Y., Hirasawa, K., Yamane, F., Hori, T., Homma, I., 2003. Effects of left amygdala lesions on respiration, skin conductance, heart rate, anxiety, and activity of the right amygdala during anticipation of negative stimulus. *Behav. Modif.* 27, 607–619.
- Mascarucci, P., Perego, C., Terrazzino, S., De Simoni, M.G., 1998. Glutamate release in the nucleus tractus solitarius induced by peripheral lipopolysaccharide and interleukin-1 $\beta$ . *Neuroscience* 86, 1285–1290.
- Nobis, W.P., Schuele, S., Templer, J.W., Zhou, G., Lane, G., Rosenow, J.M., Zelano, C., 2018. Amygdala-stimulation-induced apnea is attention and nasal-breathing dependent. *Ann. Neurol.* 83, 460–471.
- Nobis, W.P., González Otárola, K.A., Templer, J.W., Gerard, E.E., VanHaerents, S., Lane, G., Zhou, G., Rosenow, J.M., Zelano, C., Schuele, S., 2020. The effect of seizure spread to the amygdala on respiration and onset of ictal central apnea. *J. Neurosurg.* 132, 1313–1323.

- Oler, J.A., Birn, R.M., Patriat, R., Fox, A.S., Shelton, S.E., Burghy, C.A., Stodola, D.E., Essex, M.J., Davidson, R.J., Kalin, N.H., 2012. NeuroImage Evidence for coordinated functional activity within the extended amygdala of non-human and human primates. *Neuroimage* 61, 1059–1066.
- Paxinos, G., Watson, C., 2013. *The Rat Brain in Stereotaxic Coordinates: Hard Cover Edition*. Elsevier.
- Quan, N., Banks, W.A., 2007. Brain-immune communication pathways. *Brain Behav. Immun.* 21, 727–735.
- Raoufy, M.R., Ghafari, T., Darooei, R., Nazari, M., Mahdavian, S.A., Eslaminejad, A.R., Almasnia, M., Gharibzadeh, S., Mani, A.R., Hajizadeh, S., 2016. Classification of asthma based on nonlinear analysis of breathing pattern. *PLoS One* 11, 1–17.
- Raoufy, M.R., Ghafari, T., Mani, A.R., 2017. Complexity analysis of respiratory dynamics. *Am. J. Respir. Crit. Care Med.* 196, 247–248.
- Rojas-Libano, D., Del Solar, J.W., Aguilar-Rivera, M., Montefusco-Siegmund, R., Maldonado, P.E., 2018. Local cortical activity of distant brain areas can phase-lock to the olfactory bulb's respiratory rhythm in the freely behaving rat. *J. Neurophysiol.* 120, 960–972.
- Saha, S., Batten, T.F.C., Henderson, Z., 2000. A GABAergic projection from the central nucleus of the amygdala to the nucleus of the solitary tract: a combined anterograde tracing and electron microscopic immunohistochemical study. *Neuroscience* 99, 613–626.
- Salimi, M., Ghazvineh, S., Zare, M., Parsazadegan, T., 2019. Distraction of olfactory bulb-medial prefrontal cortex circuit may induce anxiety-like behavior in allergic rhinitis. *PLoS One* 1–14.
- Salimi, M., Ghazvineh, S., Nazari, M., Dehdar, K., Garousi, M., Mirnajafi-zadeh, J., Reza, M., 2021. Allergic rhinitis impairs working memory in association with drop of hippocampal – prefrontal coupling. *Brain Res.* 1758, 1–10.
- Stoeckel, M.C., Esser, R.W., Gamer, M., Von Leupoldt, A., 2018. Breathlessness amplifies amygdala responses during affective processing. *Psychophysiology* 13092, 1–12.
- Strine, T.W., Mokdad, A.H., Balluz, L.S., Berry, J.T., Gonzalez, O., 2008. Impact of depression and anxiety on quality of life, health behaviors, and asthma control among adults in the United States with asthma, 2006. *J. Asthma* 45, 123–133.
- Stujenske, J.M., Likhtik, E., Topiwala, M.A., Gordon, J.A., 2014. Fear and safety engage competing patterns of theta-gamma coupling in the basolateral amygdala. *Neuron* 83, 919–933.
- Thayer, J.F., Loerbroks, A., Sternberg, E.M., 2011. Respiratory Physiology & Neurobiology Inflammation and cardiorespiratory control: the role of the vagus nerve. *Respir. Physiol. Neurobiol.* 178, 387–394.
- Tonelli, L.H., Katz, M., Kovacsics, C.E., Gould, T.D., Joppy, B., Hoshino, A., Hoffman, G., Komarow, H., Postolache, T.T., 2009. Allergic rhinitis induces anxiety-like behavior and altered social interaction in rodents. *Brain Behav. Immun.* 23, 784–793.
- Tort, A.B.L., Komorowski, R.W., Manns, J.R., Kopell, N.J., Eichenbaum, H., 2009. Theta – gamma coupling increases during the learning of item – context associations. *Proc. Natl. Acad. Sci. U. S. A.* 106, 2–7.
- Tort, A.B.L., Ponsel, S., Jessberger, J., Yanovsky, Y., Brankač, J., Draguhn, A., 2018. Parallel detection of theta and respiration-coupled oscillations throughout the mouse brain. *Sci. Rep.* 8 (6432), 1–14.
- Trikojat, K., Buske-Kirschbaum, A., Plessow, F., Schmitt, J., Fischer, R., 2017. Memory and multitasking performance during acute allergic inflammation in seasonal allergic rhinitis. *Clin. Exp. Allergy* 47, 479–487.
- Wagner, J.G., Hotchkiss, J.A., Harkema, J.R., 2002. Enhancement of nasal inflammatory and epithelial responses after ozone and allergen coexposure in Brown Norway rats. *Toxicol. Sci.* 67, 284–294.
- Webster, J.I., Tonelli, L., Sternberg, E.M., 2002. Neuroendocrine regulation of immunity. *Annu. Rev. Immunol.* 20, 125–163.
- Yanovsky, Y., Ciatipis, M., Draguhn, A., Tort, A.B.L., Brankač, J., 2014. Slow oscillations in the mouse hippocampus entrained by nasal respiration. *J. Neurosci.* 34, 5949–5964.
- Zelano, C., Jiang, H., Zhou, G., Arora, N., Schuele, S., Rosenow, J., Gottfried, J.A., 2016. Nasal respiration entrains human limbic oscillations and modulates cognitive function. *J. Neurosci.* 36, 12448–12467.
- Zhang, J.X., Harper, R.M., Frysinger, R.C., 1986. Respiratory modulation of neuronal discharge in the central nucleus of the amygdala during sleep and waking states. *Exp. Neurol.* 91, 193–207.
- Zhang, W., Zhang, N., Sakurai, T., Kuwaki, T., 2009. Orexin neurons in the hypothalamus mediate cardiorespiratory responses induced by disinhibition of the amygdala and bed nucleus of the stria terminalis. *Brain Res.* 1262, 25–37.
- Zhong, W., Ciatipis, M., Wolfenstetter, T., Jessberger, J., Müller, C., Ponsel, S., Yanovsky, Y., Brankač, J., Tort, A.B.L., Draguhn, A., 2017. Selective entrainment of gamma subbands by different slow network oscillations. *Proc. Natl. Acad. Sci. U. S. A.* 114, 4519–4524.