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Identification of a Locus Coeruleus-amygdala Angiotensinergic Circuit: Implications for Stress-related Cardiovascular Diseases

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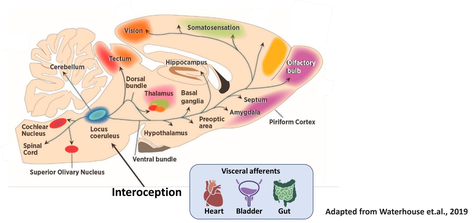
Background

- The locus coeruleus (LC) is a noradrenergic nucleus in the brain sensitive to afferent interoceptive signals. It responds to behavioral challenges by increasing noradrenaline release through ascending projections to the hypothalamus, thalamus, cortex, and amygdala.



- The brain renin-angiotensin system (RAS) plays a role in stress-related cardiovascular diseases, and previous research has identified angiotensin II (Ang II) and its type 1 receptor (AT_1R) in the LC.

Objectives

To gain a deeper understanding of the function of the AT_1R in the LC, particularly its involvement in transmitting interoceptive cardiovascular signals by regulating LC activity.

Methods

Animals:10-12-week-old adult male C57BL/6J mice, AT_1R-cre mice, tdTomato-Flox mice or AT_1R-flox mice were used for this study.

Virus: The AAV-DIO-mcherry virus was used for anterograde tracing, the AAV-DIO-hM4Di-mcherry virus was used for AT_1R^+ neuron inhibition, and AAV-DIO-hM3Dq-mcherry virus was used for AT_1R^+ neuron activation.

RNAscope: The RNAscope assay was performed according to the manufacture's instructions and images were analyzed via Zeiss spinning disk confocal microscope.

Immunostaining: Mice were perfused with 4% PFA, and brains were cut into 30 μm thickness free floating sections for antibody incubation. After staining, images were captured with the Zeiss spinning disk confocal microscope.

Surgery: Viruses were bilaterally injected into the LC of AT_1R-Cre or AT_1R-flox mice at 4.95 mm caudal, ±0.8mm lateral to bregma, and 4.4 mm below the skull surface with an UltraMicroPump III and microprocessor controller (World Precision Instruments, FL). A total volume of 400nl was injected at a rate of 100 nl/min.

Anxiety test: Mice were kept in home cage or placed into the restraint stress tubes for 30 min and then elevated plus maze (EPM) were used to test their anxiety level. The EPM tests were performed for 5 min.

Fig.1 Expression of *Agt* and AT_1R in LC

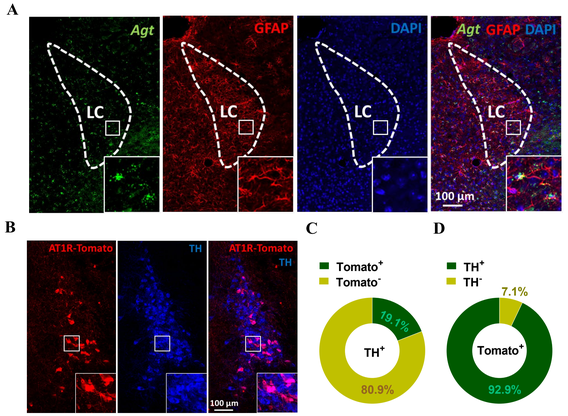


Fig. 1: AGT mRNA expressing astrocytes and AT_1R^+ norepinephrine neurons were found in the LC. (A) Representative images through the LC of the C57 mouse co-staining AGT mRNA and astrocyte marker GFAP. **(B)** Representative images through the LC of the AT_1R-tomato mice with co-staining against norepinephrine neuron marker TH. **(C)** Pie chart depicting the percentage value of TH^+ neurons that co-expressed AT1R-tomato. **(D)** Pie chart depicting the percentage value of AT_1R-tomato$^+$ neurons that co-expressed TH. *AGT,* angiotensinogen; *GFAP*, Glial fibrillary acidic protein; *TH*, Tyrosine Hydroxylase.

Fig.2 LC AT_1R^+ Neurons Project to Amygdala and Extended Amygdala Regions

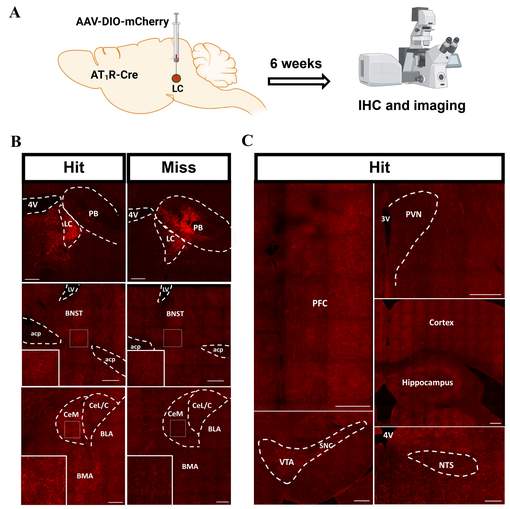


Fig. 2 AT_1R^+ neurons in the LC project to central/medial amygdala and BNST. (A) Experimental protocol for anterograde tracing. **(B)** AT_1R-cre induced mCherry signals were found in medial division of the central amygdala (CeM), basomedial amygdala (BMA) and bed nucleus of the stria terminalis (BNST). **(C)** Projections from LC AT_1R^+ neurons were not found in prefrontal cortex (PFC), paraventricular nucleus (PVN), cortex, hippocampus, ventral tegmental area (VTA) or Nucleus tractus solitarius (NTS). *DIO*, double-floxed inverse open reading frame; *BLA*, basolateral amygdala; *CeL*, lateral division of the central amygdala; *PB*, parabrachial nucleus. Scale bar: 200 μm.

Fig.3 AT_1R deletion from LC attenuates stress-induced anxiety

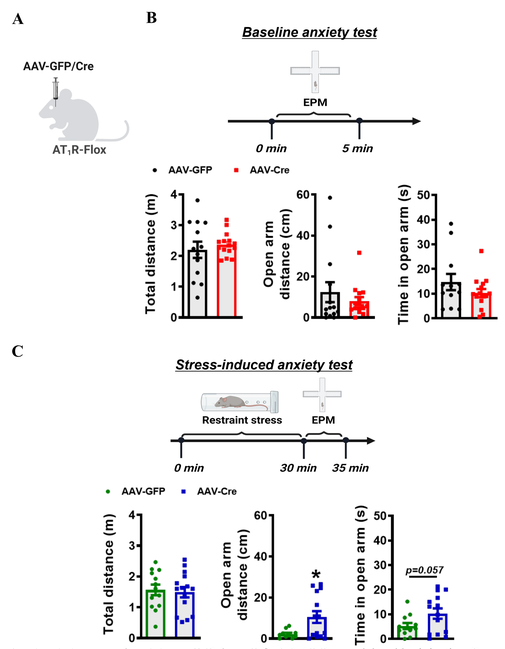


Fig. 3 Anxiety level changes after delete AT_1R from LC. (A) AT_1R was deleted by injecting Cre-expressing virus into the LC of the AT_1R-Flox mice. **(B)** AT_1R deletion from LC didn't affect mice's general anxiety in the elevated plus maze (EPM) test . **(C)** AT_1R deletion from LC attenuated the restraint stress-reduced anxiety in the EPM test.

Fig.4 Alteration in Baseline Anxiety through Chemogenetic Manipulation of LC- AT_1R^+ Neurons

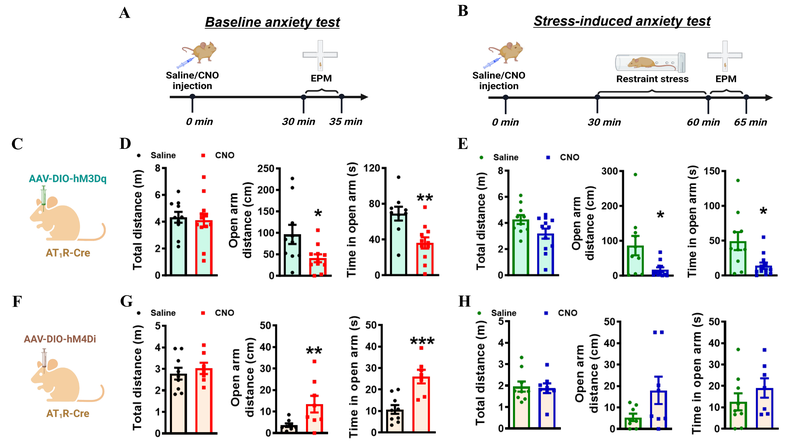


Fig. 4 Anxiety level changes after chemogenetic manipulation of the LC- AT_1R^+ neurons. (A-B) Experimental protocols to test baseline and stress-induced anxiety. **(C)** Cre-inducible excitatory DREADDs virus (AAV-DIO-hM3Dq) was injected into the LC of AT_1R-Cre mice. **(D-E)** Increased baseline and stress-induced anxiety levels after AT_1R^+ neuron activation. **(F)** Cre-inducible inhibitory DREADDs virus (AAV-DIO-hM4Di) was injected into the LC of AT_1R-Cre mice. **(G)** Decreased baseline anxiety level after AT_1R^+ neuron silencing. **(H)** Inhibition of LC AT_1R^+ neurons didn't affect mice's stress-induced anxiety. *CNO,* Clozapine-n-oxide; *hM3Dq,* human M3 muscarinic receptor; *hM4Di*, human M4 muscarinic receptor.

Fig.5 Decreased acoustic startle response after chemogenetic silencing of the LC- AT_1R^+ neurons

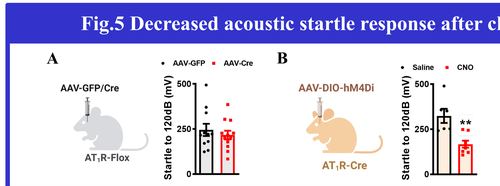


Fig. 5: Effects of LC AT_1R deletion and AT_1R^+ neurons silencing on acoustic startle response. (A) AT_1R deletion from LC did not affect mice's startle response. **(B)** Silencing LC AT_1R^+ neurons decreased mice's startle response

Fig.6 LC AT_1R in regulating stress hormone release

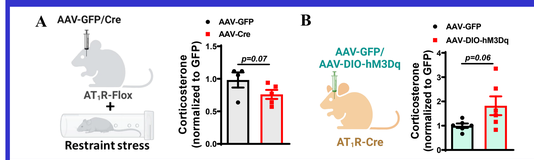
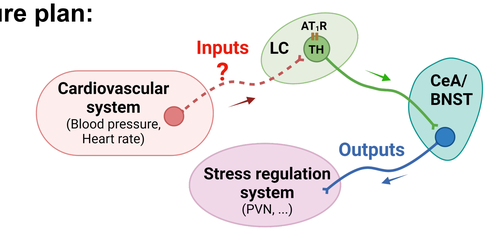


Fig. 6 Changes in plasma corticosterone level by LC AT_1R deletion or AT_1R^+ neurons activation. (A) In the restraint-stressed mice, plasma corticosterone level showed a trend of decrease after deleting AT_1R from LC. **(B)** Plasma corticosterone level had a trend of increase after activate LC AT_1R^+ neurons.

Summary and conclusion

- The angiotensin system component AGT and AT_1R were found in the LC (**Fig. 1**).
- LC AT_1R^+ neurons project to amygdala and extended amygdala regions (**Fig. 2**).
- AT_1R in LC plays critical roles in regulating anxiety and startle responses. **(Fig.3-6)**
- These data provide evidence for a novel angiotensinergic LC cell type and LC-CeA circuit. These studies have the potential to provide insights into how interoceptive brain-heart or heart-brain information is integrated and influenced by anxiety and stress-induced cardiovascular disorders.

Future plan:



Acknowledgements and Funding:

NIH National Heart, Lung, and Blood Institute

American Heart Association.