

Angiotensin II Signaling and Fear Extinction: Translational Evidence and Novel Receptor Targets

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There is an alarming paucity of new clinical studies for novel treatments for fear-based disorders and in particular post-traumatic stress disorder (PTSD). In the last 2 decades there has been a paradigm shift in exploring pharmacotherapeutics that are not simply static anxiolytics to be taken daily for symptom alleviation but that might instead act at key mechanisms of recovery like fear extinction. Such compounds may enhance natural extinction or be used specifically as adjunctive treatments for extinction-based therapies (e.g., exposure therapy). These treatments are targeted to signaling pathways that modulate fear expression and extinction circuits to facilitate rapid and long-lasting symptom remission (1). Multiple treatments that have been tested in animals and humans increase extinction learning and/or retention, including those that act at glutamatergic, glucocorticoid, oxytocin, dopamine, norepinephrine, and endocannabinoid receptor targets. Although some remain promising in translation to extinction-based therapy, limitations have arisen (e.g., potential enhancement of reconsolidation of fear memory, inconsistent effects, or potential side effects), underscoring the need to test alternative targets, in particular repurposed drugs that have good safety profiles in the trauma-exposed populations. Two studies in this issue of *Biological Psychiatry* (2,3) provide new evidence for angiotensin II (Ang-II) receptors 1 and 2 (AT₁R and AT₂R) as potential novel targets for this approach (4).

AT₁R and AT₂R are G protein-coupled receptors that are activated by Ang-II to modulate cardiovascular, inflammatory, and stress systems (5). AT₁R and AT₂R are located peripherally and in the central nervous system, including the fear network [i.e., the amygdala, the hippocampus, and the prefrontal cortex (PFC) (6)] (Figure 1). Ang-II signaling blockers, including angiotensin-converting enzyme inhibitors and the AT₁R antagonist losartan, are commonly prescribed for hypertension and are safe medications with few reported side effects. Previous epidemiological analyses identified an intriguing association between PTSD and angiotensin-converting enzyme inhibitors and losartan in patients with hypertension. Patients taking these medications had fewer PTSD symptoms than patients taking other hypertension medications, prompting an examination of Ang-II and AT₁R signaling contributions to PTSD-related functions (7,8). Given the diverse signaling capabilities of AT₁R, the mechanism for potential PTSD symptom amelioration was unclear. Preclinical studies in mice suggested that losartan associations with reduced PTSD symptoms may be via enhancement of fear extinction, with systemic losartan treatment enhancing extinction learning and retention

(9). Notably, losartan effects on extinction were observed even with long-term treatment, suggesting that tolerance does not develop to these proextinction effects. Later studies suggested that these effects could be through the blockade of AT₁R at corticotropin-releasing factor cell types, as AT₁R gene deletion in corticotropin-releasing factor cells mimics the extinction enhancement observed with losartan treatment (10). These studies also reported relative selectivity of AT₁R effects for fear extinction without affecting fear learning, suggesting that this receptor may be a compelling target for adjunctive exposure therapy because it can mitigate any potential “side effects” of treatment on reconsolidation or new fear associations during treatment. However, it was not known if these effects on extinction mechanisms translated to humans.

Zhou *et al.* (3) tested whether losartan improves extinction learning in humans and examined its effects on fear circuit function. In a randomized, placebo-controlled design, 70 healthy men received a single dose of losartan (50 mg) or a placebo after completing a fear conditioning task. After 90 minutes, participants completed extinction learning during skin conductance response and functional magnetic resonance imaging recording. Consistent with the findings in rodents, losartan treatment improved early extinction learning compared with placebo as measured by reduced skin conductance response and increased ventromedial PFC (vmPFC) activity during early extinction. Mediation analysis suggested that losartan effects on extinction of skin conductance response to the conditioned stimulus (CS+) was mediated by increased vmPFC activity. Losartan also increased functional connectivity between the vmPFC and the basolateral amygdala. These data support the translation of Ang-II signaling pathways in extinction across species and suggest that losartan treatment could be boosting vmPFC-amygdala circuit activity to enhance fear inhibition. Given that the treatment was systemic, however, the circuit mechanism, i.e., the critical node of AT₁R signaling blockade for extinction effects, is still unclear and should be addressed in further animal studies (see below). The next critical steps in humans are to determine if losartan treatment enhances long-term extinction retention and to subsequently test its efficacy to improve fear-based symptoms and response to exposure-based therapies.

Another important signaling pathway for the Ang-II system is the AT₂R, which can functionally oppose many AT₁R signaling effects (5,6). Like AT₁R, AT₂R is detected across multiple regions of the fear circuit; however, its contribution to learned fear processes had not yet been described. Yu *et al.* (2)

SEE CORRESPONDING ARTICLES ON PAGES 899 AND 910

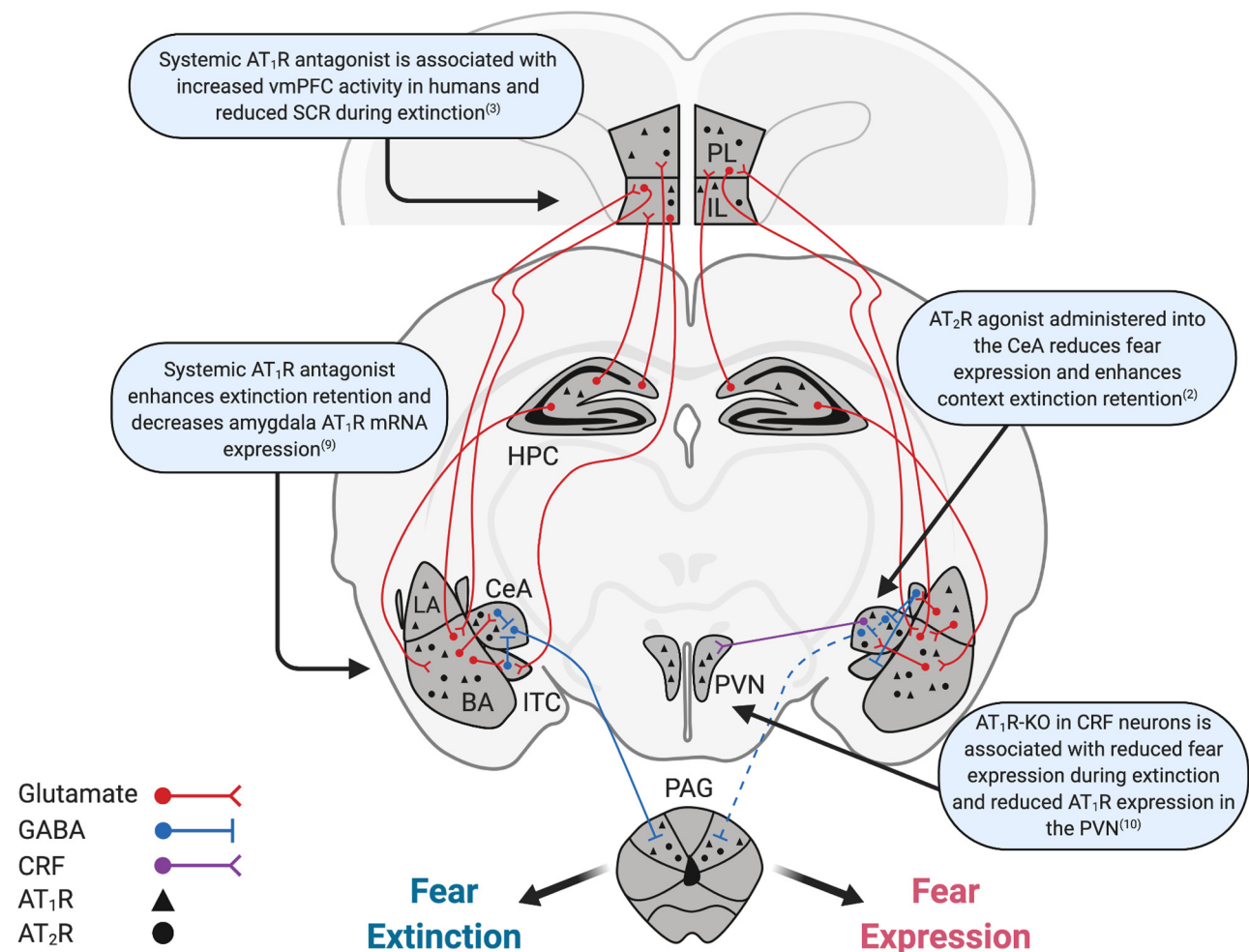


Figure 1. Established fear extinction and expression network with recent angiotensin II receptor 1 and 2 (AT₁R and AT₂R) effects on fear and extinction overlaid. AT₁R and AT₂R are reported across multiple nodes of the fear extinction network and could modulate the circuit at many potential points in the pathway (see main text for examples). BA, basolateral amygdala; CeA, central nucleus of the amygdala; CRF, corticotropin-releasing factor; GABA, gamma-aminobutyric acid; HPC, hippocampus; IL, infralimbic cortex; ITC, intercalated cells; KO, knockout; LA, lateral amygdala; mRNA, messenger RNA; PAG, periaqueductal gray; PL, prelimbic cortex; PVN, paraventricular nucleus. SCR, skin conductance response; vmPFC, ventromedial prefrontal cortex. Figure created using [BioRender.com](https://www.biorender.com) (San Francisco, CA).

fill this gap with their report on the molecular and functional profile of AT₂R signaling in the amygdala and its role in fear extinction. AT₂R agonist C21 infusions into the central nucleus of the amygdala (CeA) before extinction learning decreased freezing during extinction to both contextual and discrete fear cues. The decrease in freezing persisted 24 hours later for contextual cues, and corticosterone response to re-exposure to threat-related context was also diminished by C21 treatment, suggesting that AT₂R activation reduces both hormonal and behavioral responses to learned fear cues and facilitates extinction. Locomotor and anxiety-like behavior were unaffected by C21 treatment, indicating a relatively specific effect of AT₂R activation on learned fear behavior. To probe the potential mechanism for AT₂R to reduce learned fear, Yu *et al.* (2) examined the role of specific AT₂R cell types and projections using retrograde labeling, showing that AT₂R cells in the CeA include gamma-aminobutyric acidergic neurons that

project to the periaqueductal gray (PAG), a midbrain region controlling defensive responses to threat. These projections are a likely contributor to AT₂R signaling effects on learned fear. However, AT₂R is also detected in other regions of the fear circuit (Figure 1), indicating that the amygdala may not be the only player in fear circuit functions that could be modulated by AT₂R signaling. Collectively, these results provide important new insights regarding how Ang-II receptors beyond AT₁R modulate fear expression and extinction, opening the door for investigating the Ang-II signaling system more broadly in relation to regulating fear memories and as a target for fear disorders.

These studies prompt many more questions about the underlying circuit and signaling mechanisms through which ATRs have their functional effects on fear expression and extinction. AT₁R is expressed throughout the majority of the key nodes of the fear extinction circuit including the

amygdala, the PFC (e.g., infralimbic and prelimbic cortex), and the hippocampus, as well as downstream regions that regulate hormonal and behavioral output of learned fear responses (e.g., the paraventricular nucleus of the hypothalamus and the PAG). AT₂R is densely expressed in the amygdala and the PFC, and possibly the hippocampus (6). Therefore, AT₁R and AT₂R activation could be an important fine-tuning mechanism for learned fear expression and extinction across many of these connections. For example, the infralimbic cortex has bidirectional connections with the amygdala and an indirect inhibitory pathway to the medial division of the CeA via the intercalated cells (Figure 1). Therefore, AT₁R blockade or AT₂R activation in the infralimbic cortex may potentially facilitate inhibitory learning and the inhibition of fear expression via the amygdala, which is consistent with the effects of losartan on vmPFC activity reported by Zhou *et al.* (3). Another possibility is that Ang-II modulation of fear expression is via circuits downstream of the amygdala that control both hormonal and behavioral output of fear expression (e.g., paraventricular nucleus of the hypothalamus and the PAG), consistent with the findings of Yu *et al.* (2). The pharmacology of AT₁R and AT₂R is also fascinating and complex. AT₁R and AT₂R can recruit multiple heterotrimeric G proteins, and both are expressed at the cellular, mitochondrial, and nuclear membrane in neurons (6). AT₁R and AT₂R form heterodimers with each other, which reduces AT₁R signaling efficacy (6). Therefore, AT₂R signaling effects that functionally oppose AT₁R signaling on fear extinction could be via multiple possible mechanisms, including modulation of competing circuits (e.g., enhance CeA gamma-aminobutyric acidergic signaling to the PAG) and/or potentially through opposing signaling and/or heterodimerization if expressed in the same cell. Understanding the mechanisms underlying AT₁R and AT₂R opposing functional effects on fear expression and extinction is an exciting avenue for future research.

From a clinical perspective, the growing body of clinical and preclinical research on Ang-II modulation of extinction provides a mechanistic framework for characterizing the therapeutic benefit of Ang-II for fear-based disorders such as PTSD. A multisite randomized control trial of losartan as a stand-alone treatment for PTSD is currently underway and will be revealing with regard to determining the overall clinical efficacy of Ang-II modulation on PTSD symptoms (study details at <https://clinicaltrials.gov/ct2/show/NCT02709018>). However, this study does not directly examine if losartan treatment affects extinction phenotypes in these patients (7,8). The findings by Zhou *et al.* (3) and Yu *et al.* (2) of translatability and novel ATR targets support further examination of Ang-II modulators to modify extinction and as potential adjunctive treatments with exposure therapy. Addressing the remaining questions raised by these studies will be important for understanding the mechanisms that confer risk for fear-based disorders like PTSD, for optimizing interventions, and for clarifying the biological bases for the role of Ang-II in fear extinction enhancement.

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References

1. Lebois LAM, Seligowski AV, Wolff JD, Hill SB, Ressler KJ (2019): Augmentation of extinction and inhibitory learning in anxiety and trauma-related disorders. *Annu Rev Clin Psychol* 15:257–284.
2. Yu Z, Swiercz AP, Moshfegh CM, Hopkins L, Wiaderkiewicz J, Speth RC, *et al.* (2019): Angiotensin II type 2 receptor-expressing neurons in the central amygdala influence fear-related behavior. *Biol Psychiatry* 86:899–909.
3. Zhou F, Geng Y, Xin F, Li J, Feng P, Liu C, *et al.* (2019): Human extinction learning is accelerated by an angiotensin antagonist via ventromedial prefrontal cortex and its connections with basolateral amygdala. *Biol Psychiatry* 86:910–920.
4. Shekhar A (2014): Angiotensin type 1 receptor antagonists-A novel approach to augmenting posttraumatic stress disorder and phobia therapies? *Biol Psychiatry* 75:836–837.
5. Kamik SS, Unal H, Kemp JR, Tirupula KC, Eguchi S, Vanderheyden PML, Thomas WG (2015): International Union of Basic and Clinical Pharmacology. XCIX. Angiotensin receptors: Interpreters of pathophysiological angiotensinergic stimuli [corrected]. *Pharmacol Rev* 67:754–819.
6. Jackson L, Eldahshan W, Fagan SC, Ergul A (2018): Within the brain: The renin angiotensin system. *Int J Mol Sci* 19:E876.
7. Khoury NM, Marvar PJ, Gillespie CF, Wingo A, Schwartz A, Bradley B, *et al.* (2012): The renin-angiotensin pathway in posttraumatic stress disorder: Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers are associated with fewer traumatic stress symptoms. *J Clin Psychiatry* 73:849–855.
8. Nylocks KM, Michopoulos V, Rothbaum AO, Almlil L, Gillespie CF, Wingo A, *et al.* (2015): An angiotensin-converting enzyme (ACE) polymorphism may mitigate the effects of angiotensin-pathway medications on posttraumatic stress symptoms. *Am J Med Genet B Neuropsychiatr Genet* 168B:307–315.
9. Marvar PJ, Goodman J, Fuchs S, Choi DC, Banerjee S, Ressler KJ (2014): Angiotensin type 1 receptor inhibition enhances the extinction of fear memory. *Biol Psychiatry* 75:864–872.
10. Hurt RC, Garrett JC, Keifer OP Jr, Linares A, Couling L, Speth RC, *et al.* (2015): Angiotensin type 1a receptors on corticotropin-releasing factor neurons contribute to the expression of conditioned fear. *Genes Brain Behav* 14:526–533.