


## ALTERNATE FORMAT RESEARCH ARTICLE

# Novelty-like activation of locus coeruleus protects against deleterious human pretangle tau effects while stress-inducing activation worsens its effects

Tamunotonye Omoluabi<sup>1</sup> | Sarah E. Torraville<sup>1</sup> | Aida Maziar<sup>1</sup> | Abhinaba Ghosh<sup>1</sup> |  
 Kyron D. Power<sup>1</sup> | Camila Reinhardt<sup>1</sup> | Carolyn W. Harley<sup>2</sup> | Qi Yuan<sup>1</sup> 

<sup>1</sup> Division of Biomedical Sciences, Faculty of Medicine, Memorial University, St. John's, Newfoundland and Labrador, Canada

<sup>2</sup> Department of Psychology, Faculty of Science, Memorial University, St. John's, Newfoundland and Labrador, Canada

## Correspondence

Qi Yuan, Division of Biomedical Sciences, Faculty of Medicine, Memorial University, St. John's, NL A1B 3V6, Canada.  
 Email: [qi.yuan@med.mun.ca](mailto:qi.yuan@med.mun.ca)

Carolyn W. Harley is the co-senior author.

## Funding information

Canadian Institute of Health Research project grant, Grant/Award Number: #PJT-16124

## Abstract

The earliest abnormality associated with Alzheimer's disease (AD) is the presence of persistently phosphorylated pretangle tau in locus coeruleus (LC) neurons. LC neuron numbers and fiber density are positive predictors of cognition prior to death. Using an animal model of LC pretangle tau, we ask if LC activity patterns influence the sequelae of pretangle tau. We seeded LC neurons with a pretangle human tau gene. We provided daily novelty- or stress-associated optogenetic activation patterns to LC neurons for 6 weeks in mid-adulthood and, subsequently, probed cognitive and anatomical changes. Prior LC phasic stimulation prevented spatial and olfactory discrimination deficits and preserved LC axonal density. A stress-associated activation pattern increased indices of anxiety and depression, did not improve cognition, and worsened LC neuronal health. These results argue that variations in environmental experiences associated with differing LC activity patterns may account for individual susceptibility to development of AD in humans.

## KEYWORDS

locus coeruleus, olfactory discrimination, optogenetic stimulation, pretangle tau, spatial discrimination

## 1 | NARRATIVE

### 1.1 | Contextual background

The incidence of Alzheimer's disease (AD) rises sharply with age. However, there are people older than 100-years-old who remain mentally alert. Why are some people vulnerable to developing AD while others are more resilient? It appears that the locus coeruleus (LC), a brain-stem structure involved in arousal, learning, and stress responses, has a key role in AD initiation and evolution.

Braak et al. examined 2332 human brains by decades from 0 to 100 years of age.<sup>1</sup> They found the earliest abnormality related to AD was the presence of hyperphosphorylated tau in the noradrenergic LC neurons.<sup>1</sup> The first appearance of this abnormality occurs in the brains of young individuals as early as 10-years-old. The hyperphosphorylated post-mortem tau is soluble, in contrast to the later insoluble tau of tangles, and is therefore referred to as pretangle tau. Pretangle tau is produced throughout life and is later co-localized with insoluble tau tangles.<sup>1,2</sup> By age 30, pretangle tau in cortically projecting LC neurons is observed in >90% of the brains examined, arguing that

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2021 The Authors. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring* published by Wiley Periodicals, LLC on behalf of Alzheimer's Association

LC pretangle tau production is a part of human brain aging.<sup>1</sup> The co-occurrence of soluble and insoluble tau suggests that soluble pretangle tau production is a step in AD progression. In fact, some studies suggest that soluble pretangle tau is the more toxic species.<sup>3,4</sup> Despite the early appearance of pretangle tau in human brains, the symptomatic memory impairments of AD do not appear until Braak's later tangle stages III-IV. Memory impairment in aged cohorts are not universal. They appear in about 17% of the 75- 84-year-old age group and 32% of people older than 85.<sup>5</sup> Thus individual differences strongly modulate the likelihood of AD development.

To date, the mechanism underlying tau driven changes in the LC and related neuromodulatory cell groups is not well understood and no treatments to target tau pathology are available. The majority of sporadic AD likely results from intricate interactions between genes and environmental factors, where aging and lifestyle play important roles. Vulnerability to AD has been explained by a "cognitive reserve" hypothesis,<sup>6,7</sup> emphasizing environmental factors such as education, mental stimulation, and novelty in delaying AD pathogenesis. In a recent comprehensive longitudinal study,<sup>8</sup> high lifespan cognitive reserve indicators encompassing education, early and late-life cognitive and social activities, correlated with a reduced risk of mild cognitive impairment and progression to AD. The cognitive reserve theory could explain individual variability in developing AD, despite some degree of universal pathology, particularly pretangle tau.

Robertson<sup>6</sup> further developed the cognitive reserve hypothesis by proposing that the key elements of cognitive reserve, such as education, mental stimulation, and environmental novelty, all involve upregulation of the noradrenergic system, which declines with age. Protracted activation of noradrenergic system may, as Robertson postulated, reduce the risk of AD.

However, elevated LC activation can be detrimental as well. Stress, especially chronic stress, such as work-related stress, enduring childhood, and mid-age stress, has been associated with greater AD risk.<sup>9,10</sup> The LC noradrenergic system is critically involved in stress. Overactivity of the LC during sleep also interferes with memory consolidation.<sup>11</sup> The multifaceted roles of the LC are associated with differing LC firing patterns.<sup>12,13</sup> In rodents<sup>14,15</sup> and primates,<sup>16</sup> burst phasic firing is elicited by salient or novel stimuli, and crucially involved in optimizing task performance according to environmental contingencies.<sup>17</sup> The resultant NE release enhances signal processing in the cortex.<sup>13,18</sup> Tonic LC activity, on the other hand, is associated with disengagement from tasks and with a reduced signal-to-noise ratio in the brain.<sup>17</sup> We, and others, have established that the phasic firing of LC neurons that occurs with exposure to novelty, promotes learning and memory, whereas high tonic firing produces indices of stress in rodents.<sup>15,19-21</sup>

On the other hand, LC cell number and health themselves serve as a "neural reserve."<sup>22</sup> LC neuron number, health, and fiber density are significant predictors of cognitive impairment in studies of normal brain aging and AD,<sup>22-24</sup> suggesting that this nucleus is an essential cell group for understanding and remediating memory and learning deficits. Magnetic resonance imaging (MRI) of human LC is now possible. A loss of MRI signal contrast in the region of LC suggests impaired LC health in both humans<sup>25,26</sup> and animals.<sup>27</sup>

## RESEARCH IN CONTEXT

- 1. Systematic Review:** A PubMed search of human and animal studies on locus coeruleus (LC) and Alzheimer's disease (AD) reveals that LC health determines brain health in aging and development to AD. Upregulation of the LC noradrenergic system has been proposed by Robertson and others as an AD intervention. However, the effects of LC activation on behavior and cognition are firing-pattern specific. It is unknown how LC firing patterns influence LC health and AD progression.
- 2. Interpretation:** We show that a novelty-like phasic activity pattern protects against deleterious changes linked to pretangle tau. A stress-inducing tonic pattern worsens mood and neuronal health. These results refine Robertson's LC cognitive reserve hypothesis, suggesting that lifestyle patterns associated with differing LC activities may modify AD progression differently.
- 3. Future Directions:** Human interventions focusing on promoting LC phasic activity and neuronal health (deep brain stimulation, pharmacology, and novel activity) may hinder AD.

## HIGHLIGHTS

- Novelty-like locus coeruleus (LC) activation prevents pretangle tau impairments.
- Stress-inducing LC activation increases anxious and depressive behavior.
- LC health is affected by prior lifestyle-related activation patterns.
- These results suggest translational strategies for optimizing human brain health.

Is there any link between LC activity and LC health, two critical risk factors in AD? Do LC activity patterns matter? Here we hypothesize that patterns of LC activation during adult life are critical factors in brain aging and in the progression to AD.

The present experiments were designed to test the central question of whether patterns of LC activity associated with either novelty (phasic patterns) or highly stressful events (tonic patterns) would alter the health of the LC and associated cognitive functions. Specifically, we predicted that the deleterious effects of human soluble pretangle tau on LC neurons would be strongly modulated by patterns of LC activity that could be related to lifestyle factors, such as engagement with novelty or aversive stress.

We developed a rat model of human pretangle tau in LC neurons that captures the major features of the five stages of pretangle tau

appearance and spread in humans characterized by Braak.<sup>1,28</sup> We inserted a gene into rat LC neurons to produce human tau persistently phosphorylated on the 14 sites for proline-directed kinases,<sup>29</sup> known to be phosphorylated early when soluble tau produces deleterious changes.<sup>30</sup> The pretangle human tau produced appears initially as condensates at intracellular LC neuronal sites shown to be sites of pretangle condensates in human LC.<sup>29,31</sup> Subsequently, pretangle tau spreads along LC axons to other subcortical neuromodulatory cell groups that support learning and memory, as LC pretangles do in human brain.<sup>32</sup> In this pretangle tau model, the early cognitive impairments are impairments in learning difficult olfactory discriminations.<sup>29</sup> Smell impairments are also markers of aging human cognition.<sup>33</sup> The appearance of the olfactory deficit occurs along with a reduction of LC noradrenergic fibers in the olfactory cortex in rats.<sup>29</sup> A similar reduction of LC fibers has been reported with aging in humans.<sup>34,35</sup>

Here we again use gene-insertion techniques to achieve the expression of two genes in LC neurons, a gene to initiate human pretangle tau expression and another gene to initiate the production of blue light-sensitive excitatory ion channels. This permits us to modulate the firing patterns of LC neurons using fiber optics to deliver pulses of blue light in the vicinity of LC neurons. We ask if phasic LC activation associated with novelty and enhanced learning<sup>15,20</sup> can beneficially affect subsequent LC health and cognition. Conversely, we ask if the tonic stress-associated LC patterns<sup>20</sup> will worsen the outcomes associated with LC pretangle tau.

## 1.2 | Study conclusions and discussion

Our results indicate that novelty-related phasic LC activation can reverse the deleterious cognitive and anatomical effects of LC pretangle tau production. Exposure to 5 days per week of 20 minutes of phasic activation each day prevented the failure in difficult olfactory and spatial discrimination that normally develop in pretangle tau rats. They did not differ from controls of the same age. Prior novelty-related phasic activation also prevented the loss of LC fibers in brain areas implicated in olfactory and spatial discrimination performance. Thus selective LC activation patterns related to curiosity and novelty investigation prevent the deleterious effects of LC pretangle tau expression. Correlations between behavioral performance and LC fiber density in the relevant target areas support the hypothesis that maintaining LC axonal health and density is a key factor in supporting normal cognition. Phasic activated rats had comparable anxiety levels to the control rats without pretangle tau.

A tonic LC activation pattern, previously shown to produce anxiety and aversion, did not ameliorate the deleterious effects of LC pretangle tau on cognition or axonal density. Instead, it increased depression in subsequent testing, suggesting that prior stressful experiences can further worsen the outcomes associated with pretangle tau expression. An index of poor LC neuronal health, in particular, a marker of apoptosis, was elevated following the tonic LC activation exposure. This outcome directly supports the hypothesis that prior life stress worsens LC health.

Mood-related disorders such as depression have significant comorbidity with cognitive deficiencies such as AD. Twenty-five percent of individuals age 85 and older are reported to present both mood and cognition impairment.<sup>36</sup> Moreover, aging adults with depressive symptoms are found to be associated with a roughly 50% co-occurrence of AD.<sup>37</sup> However, the molecular mechanisms underpinning the link between depression and AD remain poorly understood. Individuals with elevated levels of tau are more likely to display depression symptoms.<sup>38,39</sup> Therefore, abnormal hyperphosphorylated tau is possibly a common underlying factor contributing to both AD and depression. However, in our model, the pretangle tau alone did not generate a depressive phenotype. On the other hand, depression has been associated with abnormal functioning of the LC noradrenergic system.<sup>40–42</sup> Here, a stress-eliciting tonic pattern, but not a phasic pattern, promoted later depressive behavior, suggesting that tonic activation-associated anxiety is detrimental to LC health and makes pretangle tau rats more prone to develop depression.

By contrast, phasic novelty-related LC activation enhanced macrophage markers associated with neuronal resilience, and with the disposal of defective proteins, consistent with phasic LC patterns' positive effects on LC axons and LC-related cognitive behaviors.

The spread of pretangle tau to other neuromodulatory groups was not altered by either pattern of activation, although there was considerable variability among animals and this aspect will require further study. Neuronal activity is associated with enhanced tau release *in vitro*<sup>43</sup> and *in vivo*.<sup>44</sup> It is surprising that pretangle tau spread is not accelerated by LC activation. It is possible that pretangle tau release is promptly cleared in the early stage. Measurement of acute release of pretangle tau in brain interstitial fluid following neuronal activation in this model may illuminate the kinetics of release and clearance of extracellular tau.<sup>44</sup> Nonetheless it suggests that LC neuronal health and LC-related cognitive health are separable from the mechanisms of pretangle tau spread.

As with all studies that use animal models, it is important to consider the limitations both in terms of our mechanistic understanding and in terms of translation to humans. The reasons for the profound differences in outcome of periods of exposure to phasic and tonic LC activation are not clear. We need to know more about the details of downstream structures, NE release, and receptor activation associated with LC patterns to begin to address this issue. Fortunately, the increasing availability of NE sensors<sup>45</sup> may make it possible to characterize these differences in the future.

Although outcomes with the pretangle used in this animal model are much worse when pretangle expression is initiated in older animals and which then include the loss of LC neurons,<sup>29</sup> we have not yet seen tau tangles themselves. This may relate to time course or other issues. Nonetheless the data from this model are consistent with the proposal that abnormal soluble tau may be the more destructive tau species over time.<sup>3,4</sup>

The animal model also may exaggerate the deficits associated with LC pretangle tau expression in that most LC neurons express pretangle tau, whereas in the human data, pretangle tau expression appears limited to a smaller ensemble of LC neurons,<sup>1</sup> likely having specific

projections to entorhinal cortex. The use of retrograde viral gene probes to infect only LC neurons projecting to specific targets such as entorhinal cortex would be valuable to better approximate the human observations.

The improvements in LC health observed here with optogenetic control of LC firing patterns are supportive of the idea that life experiences are key modulators on the effects of pretangle tau expression in LC. This idea could now be addressed by using differing life experience paradigms in the animal model and probing for the importance of the LC role in such life experience effects with pharmacological manipulations.

Although it is not possible to use the selective optogenetic approach described here in humans to promote LC health, there are other options. In particular, modafinil, an FDA-approved medication, has been shown to suppress tonic and enhance phasic LC activation,<sup>46</sup> and improve several cognitive functions.<sup>47</sup> Results from our study predicts that modafinil should be ideal for LC health promotion because it would produce a clear phasic signal relative to LC background activity. Converging ultrasound waves could also be used to nondestructively activate deeper brain structures such as LC using specific patterns.<sup>48</sup> This approach could also be used in parallel animal models for optimization. Finally, the use of LC MRI signals in both animals<sup>27</sup> and humans<sup>25,26</sup> to evaluate LC health provides yet another tool to test ideas and strategies to optimize brain health in human aging and prevent progression to AD.

## 2 | CONSOLIDATED RESULTS AND STUDY DESIGN

To introduce pretangle tau seeding in the LC and activate LC noradrenergic neurons, we used tyrosine hydroxylase (TH)-CRE rats injected with dual CRE-dependent AAVs in the LC, one containing a human pretangle tau that is pseudophosphorylated at 14 proline-enriched sites (E14),<sup>29</sup> and the other containing a light-sensitive channel rhodopsin 2 (ChR2). An AAV omitting ChR2 co-infused with the pretangle tau E14 was used in a sub-cohort of rats to control for light effects without activating neurons. Another control group was injected with the single control AAV without E14 (non-E14) in the LC. Rats injected with AAVs at 3 months of age were implanted with optical cannula in the LC 5 months later, followed by chronic daily light stimulations for 6 weeks. A phasic LC-activation pattern that has been shown to promote learning and positive emotion, and a tonic LC pattern that induces stress<sup>20</sup> were used for daily stimulations.

Batteries of behavioral tests started  $\approx$ 1 month following the termination of the light stimulation (9 to 10 months post AAV infusion). We used open field, marble burying, and the elevated plus maze to index the stress/anxiety levels of the rats. The tonic-stimulated E14 rats showed increased anxiety, with less rearing than the non-E14 group and more freezing in the open field than other groups. We and others have shown that acute LC tonic stimulations induce anxiety-like behavior.<sup>20</sup> The result here suggests that a period of LC tonic stimulation has enduring anxiogenic effects. Both tonic-stimulated and

light-control E14 rats had an increased number of marbles buried compared to non-E14 rats, indicating an elevated stress level in pretangle tau rats. However, the phasic stimulated E14 rats showed comparable performance in the open field and marble burying tests to that of the non-E14 rats, suggesting that phasic activation of the LC may have an anxiolytic effect. The stress-reducing effect of LC phasic activation is consistent with its association with positive emotions.<sup>20</sup> In contrast, tonic stimulation of LC is associated with negative emotion,<sup>20</sup> and here chronic tonic stimulation induced depressive behavior in E14 rats. In the sucrose consumption measurement, a test detecting anhedonia/depressive behavior, the tonic-stimulated E14 rats showed reduced sucrose water consumption over 24 hours.

Pretangle tau seeding in the LC impairs odor discrimination learning.<sup>29</sup> Here both spatial and olfactory discriminations were impaired in the E14 light control rats. Critical to our understanding of the roles of LC activity patterns in cognitive functions in AD, however, phasic LC stimulation rescued the ability of pretangle tau rats to remember and separate similar patterns in both the spatial and olfactory tasks. This was not paralleled with tonic LC stimulation. A period of novelty- and learning-promoting LC activation thus has lingering effects on improving cognition in the pretangle tau model.

We then conducted histology and immunohistochemistry (IHC) following behavior (12 months post AAV infusions) to examine the effects of LC stimulation patterns on LC health. LC cell counts in Nissl staining revealed no difference among groups, suggesting no LC cell loss at this stage. However, an apoptosis marker, active caspase-3 (AC-3), was significantly increased in the tonic stimulated E14 rats, suggesting a decline of health associated with tonic stimulation. On the other hand, using dopamine beta-hydroxylase (DBH) and norepinephrine transporter (NET) as markers for noradrenergic fibers, we observed decreased levels of fiber density in both the olfactory piriform cortex (PC) and hippocampal dentate gyrus (DG), two structures critical for olfactory and spatial pattern separations<sup>49-51</sup> by the pretangle tau seeding. The LC fiber degeneration was rescued by LC phasic stimulation. Tonic stimulation neither worsened nor improved axonal health compared to that in E14 light control rats.

LC fiber density and cell counts indicate cognitive health in humans and correlate closely with AD stages.<sup>22,51,52</sup> We examined the correlation of LC fiber density in the PC and DG with behavioral performance. We found significant positive correlations of LC fiber density with learning performance in rats in both the spatial discrimination and a difficult olfactory learning task that requires rats to associate a reward with an odor from a similar odor pair.

Next, we examined the levels of microglia/macrophages associated with LC activations using IHC and western blotting (WB). Microglia activation is associated with AD progression and has a double-sword role, with stage-dependent beneficial and detrimental effects.<sup>53,54</sup> Human and animal studies reveal that early stage microglia activation is protective, whereas in late stages of AD, microglial inflammatory activity increases while microglial clearance activity decreases.<sup>55</sup> Here, the total microglia level indexed by ionized calcium-binding adaptor molecule-1 (IBA-1) was not altered by either pretangle tau seeding or LC stimulations. However, cluster of differentiation 68 (CD-68),



amacrophage/mononuclear phagocyte marker, and triggering receptor expressed on myeloid cells-2 (TREM2), a microglia/macrophage marker, showed increased expression in the hippocampus with pretangle tau seeding. Phasic LC stimulation further increased the CD-68 and TREM2 levels. This implies that CD-68 and TREM2 mediated phagocytosis as a compensatory effort to rid the system of abnormal pretangle tau is further enhanced by LC phasic activation.

Finally, to understand how LC activation patterns may influence pretangle tau spread and the potential contribution of pretangle tau in subcortical and cortical cells to behavior, we used human tau (HT7 as a marker) co-localizing GFP antibody to index pretangle tau spread. GFP<sup>+</sup> cells appeared in several subcortical regions including serotonergic raphe nuclei, pontine tegmental nuclei that contain cholinergic neurons, and substantia nigra dopaminergic neurons. The patterns of spread and densities of GFP cells did not appear different among groups. However, small sample numbers were explored here. Sparse spread to the entorhinal cortex was also observed in a subset of brains and was not modulated by the LC stimulations. In addition, tau phosphorylation measured by AT8 was not observed outside of the brainstem, and largely did not overlap with GFP, suggesting that rat tau itself may have adopted the pretangle phosphorylation pattern.<sup>56</sup> Tau tangles, indexed by Thioflavin-S staining, were not observed in any brain regions.

### 3 | DETAILED METHODS AND RESULTS

#### 3.1 | Methods

##### 3.1.1 | Subjects

Offspring of both sexes from homozygous tyrosine hydroxylase (TH)-CRE male breeders (Sage Labs) and Sprague-Dawley female rats were used. Rats were kept in a standard 12-hour light-dark cycle except during surgeries and light stimulations, with food and water ad libitum. Experimental procedures were approved by the Institutional Animal Care Committee at Memorial University of Newfoundland and followed the Canadian Council's Guidelines on Animal Care and the National Institutes of Health (NIH) guidelines.

##### 3.1.2 | Experimental design and statistical analysis

Figure 1A provides a flowchart of the experiment. Human pretangle tau pseudophosphorylated at 14 proline-enriched sites (E14)<sup>29</sup> and light sensitive channels channel rhodopsin 2 (ChR2) were packaged in adeno-associated viral (AAV) vectors: AAV9-rEF1a-DIO-WPRE-htauE14-EGFP (1.3E<sup>+13</sup> vg/mL, Vivorek) and AAV9-rEF1a-DIO-WPRE-ChR2-mCherry (4.25E<sup>+13</sup> vg/mL, a gift from Deisseroth at Stanford). Control AAV was AAV9-rEF1a-DIO-WPRE-mCherry (1.5E<sup>+13</sup> vg/mL). Rats were injected with AAVs bilaterally in LC at 3-months-old and received LC optical cannulae implantation ≈5 months

later. Phasic (30 minutes daily) or tonic (20 minutes daily) LC stimulation was given in the home cage over 6 weeks (5 days/week), except in the non-E14 control group. Batteries of behavioral tests were conducted 9 to 11 months post-infusion, followed by histology, IHC and WB studies at 12 months post-infusion.

Rat were randomly assigned to five conditions: (1) E14 phasic (E14+ChR2; 10 Hz, 30 msec light pulses, 300 msec every 2 seconds)<sup>20</sup>; (2) E14 tonic (E14+ChR2; 25 Hz)<sup>20</sup>; (3) E14 phasic control (E14+mCherry; 10 Hz); (4) E14 tonic control (E14+mCherry; 25 Hz); (5) Non-E14 controls (mCherry without light stimulation). A subset of this group underwent optical cannulae implantations to control for potential LC damage.

One-way analyses of variance (ANOVAs) followed by post hoc tests were used for behavioral tests, histology/IHC studies, and WB experiments. Pearson correlations were used for the correlations of LC fiber density versus behavior.

##### 3.1.3 | Stereotaxic surgery

Infusions and optical cannulae implantations were conducted under isoflurane following procedures described recently.<sup>29</sup> LC coordinates were 11.8 to 12.2 mm posterior, 1.2 and 1.4 mm bilateral, and 6.3 mm ventral with respect to bregma. Each infusion contained 0.5 μL double AAVs (equal volume mixture) or a single control AAV, plus 0.2 μL fluorescent beads given at a rate of 0.5 μL/minute.

##### 3.1.4 | Behavioral experiments

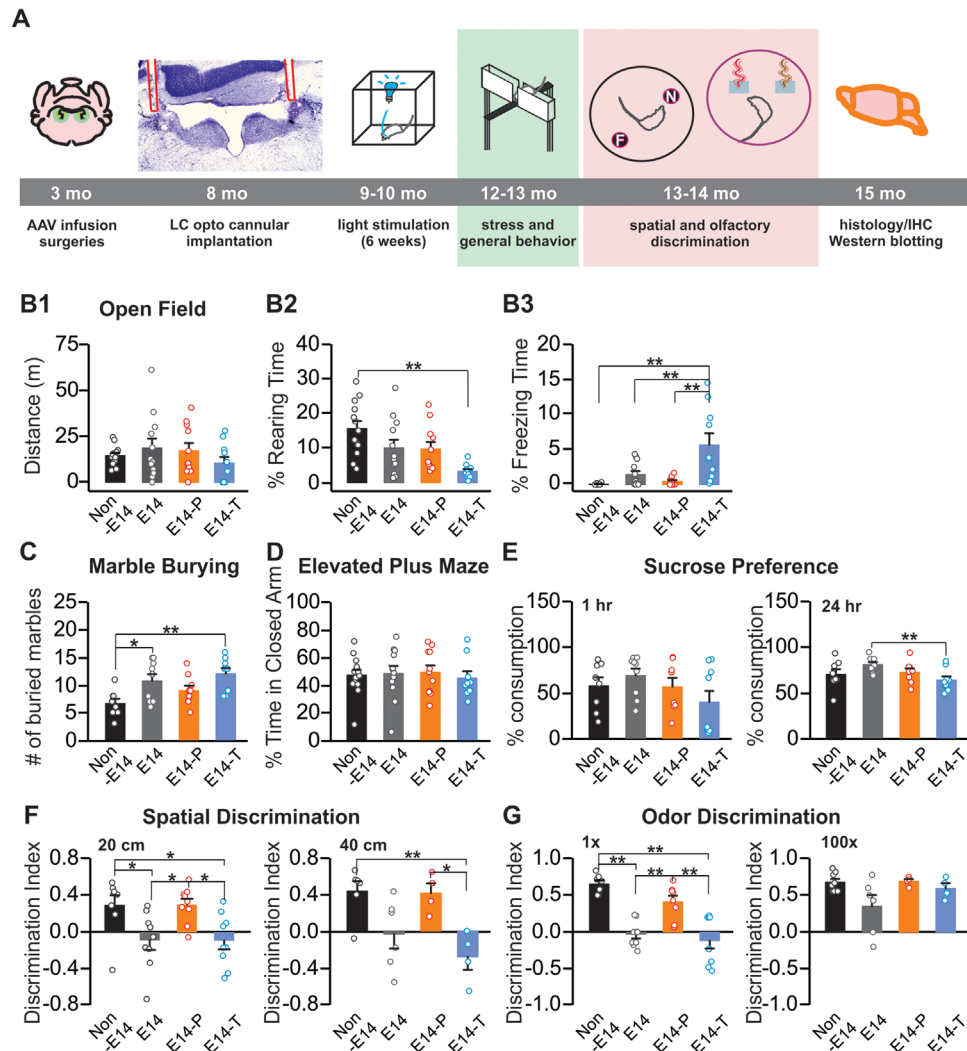
###### *LC light stimulation*

LC light stimulation was conducted in rats' home cages during the dark cycle in order to avoid any potential effect of sleep interruption induced by optogenetic activation during the light cycle. Blue light of 450 nm (90 mW) was supplied via a patch cord (400 μm/0.48 nA; Doric Lenses). Phasic (10 Hz) or tonic (25 Hz) stimulation was given 30 or 20 minutes daily for 6 weeks.

###### *Exploratory, anxiety, and depressive behavior*

Rats were given one 10-minute trial to explore an open field (60 × 60 × 40.5 cm<sup>3</sup>) and recorded with ANY-Maze software (Stoelting). Distance traveled, time spent rearing (including free and supported rearing), and time spent freezing were recorded and analyzed offline. Freezing was counted as no body movement except breathing. The rearing was defined as the two front paws being lifted above the ground.

Anxiety was measured by the open field behavior, a 5 minute Elevated Plus Maze trial (50 × 10 cm<sup>2</sup>/arm with an 11 × 11 cm<sup>2</sup> central platform, 38 cm walls on the closed arms), and a marble burying test (number of 16 marbles buried in 30 minutes). Depressive behavior was measured by 1 hour and 24 hours sucrose (0.75%) percentage consumption.



**FIGURE 1** Phasic locus coeruleus (LC) activation improves spatial and olfactory discrimination, whereas tonic LC stimulation induces anxiety and depression. (A) Schematics of experimental designs. (B1-B3) Open field test measuring mobility (distance, B1), exploratory activity (rearing, B2), and anxiety (freezing, B3). Non-E14:  $n = 13$ ; E14:  $n = 12$ ; E14-P (phasic):  $n = 11$ ; E14-T (tonic):  $n = 10$ . (C) Marble burying test. Non-E14:  $n = 8$ ; E14:  $n = 9$ ; E14-P:  $n = 9$ ; E14-T:  $n = 9$ . (D) Elevated plus maze test. Non-E14:  $n = 14$ ; E14:  $n = 12$ ; E14-P:  $n = 11$ ; E14-T:  $n = 9$ . (E) Sucrose water consumption test. Non-E14:  $n = 8$ ; E14:  $n = 9$ ; E14-P:  $n = 9$ ; E14-T:  $n = 9$ . (F) Spontaneous location recognition tests. Test in the left panel (20 cm inter-object distance; Non-E14:  $n = 8$ ; E14:  $n = 9$ ; E14-P:  $n = 9$ ; E14-T:  $n = 9$ ) is more challenging than that in the right panel (40 cm inter-object distance). (G) Similar odor discrimination tests. Non-E14:  $n = 7$ ; E14:  $n = 9$ ; E14-P:  $n = 8$ ; E14-T:  $n = 9$ . Test in the right panel used 100x concentrated odor pairs of those in the left panel. \* $P < .05$ ; \*\* $P < .01$

#### Spontaneous location recognition task

Rats were given 10 minutes in an open arena ( $60 \times 60 \times 40.5 \text{ cm}^3$ ) with three identical objects (1, 2, and 3) placed at specific positions. Objects 2 and 3 were placed either 20 or 40 cm apart. During testing (24 hours later), rats were placed in the same arena with two identical objects, one in the same position as Object 1 (a familiar location, F), the other midway between previous Objects 2 and 3 (a novel location, N). The discrimination ratio was the difference between time spent at the novel and familiar objects over the total time spent on both objects ( $t_{\text{novel}} - t_{\text{familiar}} / t_{\text{total}}$ ).<sup>57</sup>

#### Odor discrimination task

Discrimination of similar odors was tested using a perforated micro-centrifuge tube containing filter paper with  $60 \mu\text{L}$  of odorant or min-

eral oil. The first three trials used odorless mineral oil, the next three trials used odor 1 (O1, 1-heptanol, 0.001%), and the last trial used an odor mixture that had a similar smell to O1 (O2, 1-heptanol and 1-octanol in a 1:1 ratio, 0.001%). A subset of rats was re-tested rats with 100 times concentrated odors (0.1%). The tests were videotaped, and sniffing time within a 1 cm radius around the odor tube was measured offline. The discrimination index was the ratio of the sniffing time difference between the O2 and the third presentation of O1 to the total sniffing time ( $(t_{\text{O2}} - t_{\text{O1-3}}) / (t_{\text{O2}} + t_{\text{O1-3}})$ ).

For go-no-go odor discrimination learning, the data were acquired previously.<sup>29</sup> Briefly, water-deprived rats were trained to perform a similar odor discrimination with water as a reward, using the same odor pairs as in the odor discrimination test (rewarded odor: 1-heptanol, 0.001%; non-rewarded odor: 1-heptanol and 1-octanol in a 1:1 ratio,

0.001%). We plotted % correct response rates (average of the last three training blocks) against LC fiber density.

### 3.2 | Histology, IHC, and imaging

Brains were extracted following transcardiac perfusion and stored in 20% sucrose in 0.1 M phosphate-buffered saline (PBS). Twenty-five micrometers of coronal and horizontal sections cut by a cryostat (HM550, ThermoFisher) were mounted on chrome-gelatin coated slides, and 50  $\mu\text{m}$  sections at 150  $\mu\text{m}$  intervals were collected in PVP cryoprotectant (1% polyvinylpyrrolidone, 30% sucrose, 30% ethylene glycol in 0.1 M PBS) for free-floating IHC.

All histology and IHC followed established procedures.<sup>29</sup> Nissl staining was used for LC cell counts. Primary antibodies used included: AC-3 (AC-3559565, BD Biosciences, 1:400), DBH (MAB308, Millipore-Sigma, 1:2000), NET (MA5-24647, Invitrogen, 1:2000), Iba-1 (019-19741, Wako, 1:2000), CD68 (MCA341GA, BioRAD, 1:2000), human tau (HT7, MN1000, ThermoFisher, 1:1000), GFP (A-11122, ThermoFisher, 1:2000), tryptophan hydroxylase (TPH, ab52954, Abcam, 1:1000), tyrosine hydroxylase (TH, EMD Millipore, 1:2000), choline acetyltransferase (ChAT; Millipore, MAB305, 1:5000), and AT8 (MN1020, Invitrogen, 1:1000) in PBS with 0.2% Triton-X and 2% goat serum.

After primary antibody incubation at 4°C over 1 to 3 nights, tissue was washed and incubated with Alexa Fluor secondary antibodies (Invitrogen, 1:1000) at room temperature for 2 hours before coverslipping with Fluoroshield Mounting Medium with DAPI (Invitrogen). Non-fluorescent stained sections underwent a 90-minute incubation in 1% A+B (PK-6101, Avidin-Biotin-complex, Vector Laboratories). Sections were then washed before reacting in a DAB-tetrachloride solution (50 mL Tris buffer, 50 mL water, 50 mg DAB, 30  $\mu\text{L}$  hydrogen peroxide) for 5 to 20 minutes.

Bright-field and fluorescence microscopy used an EVOS M5000 imaging system (Thermo Fisher Scientific). Image analysis was conducted with ImageJ. For LC fiber density in the posterior piriform cortex (PC) and dentate gyrus (DG), images were background subtracted and converted into binary images. Fiber length was calculated using the DiameterJ plugin. LC neurons were identified as described by Garcia-Cabezas et al<sup>58</sup> and the number of neurons per unit area was calculated (Figure S1). AC3<sup>+</sup> cells were counted within the LC boundary defined by the fluorescence marker mCherry or GFP (Figure S1).

#### 3.2.1 | Western blotting

Hippocampal tissue was extracted after decapitation and stored frozen. Brain tissue processing followed established protocols.<sup>59</sup> Total protein concentration was quantified by standard BCA assay (Pierce). Equal amounts of protein (25  $\mu\text{g}/\mu\text{L}$ ) were separated by SDS-PAGE on 10% gels and were then transferred to nitrocellulose membranes (0.45  $\mu\text{m}$ ; Thermo Fisher Scientific). The membranes were blocked for 2 hours with 5% non-fat dry milk or BSA at room tempera-

ture, then incubated at 4°C overnight with the following antibodies: brain-derived neurotrophic factor (BDNF; ab108319; Abcam, 1:2000), TREM2 (AF1729, R&D Systems, 1:5000), interleukin 1 $\alpha$  (IL-1 $\alpha$ ; OT12F8, Novus Biologicals, 1:2000), human leukocyte antigen-DR isotype (HLA-DR; sc-53319, Santa Cruz Biotechnology, 1:5000), anti-phospho-Tau (AT8; MN1020, Invitrogen, 1:1000), and paired helical filaments (PHF; MN1060, Invitrogen, 1:1000). After washing, the membranes were incubated for 1.5 hours at room temperature, with either horseradish peroxidase-labeled anti-rabbit immunoglobulin G (IgG; 31460) or anti-mouse IgG (31430, Thermo Fisher Scientific, 1:10000). The protein bands were visualized using chemiluminescent substrate (34577, Thermo Fisher Scientific) and quantified with ImageJ. Each sample was normalized to Ponceau staining of total proteins.

### 3.3 | Results

#### 3.3.1 | Phasic LC stimulation promotes spatial and olfactory discrimination while tonic stimulation results in anxiety and depressive behavior

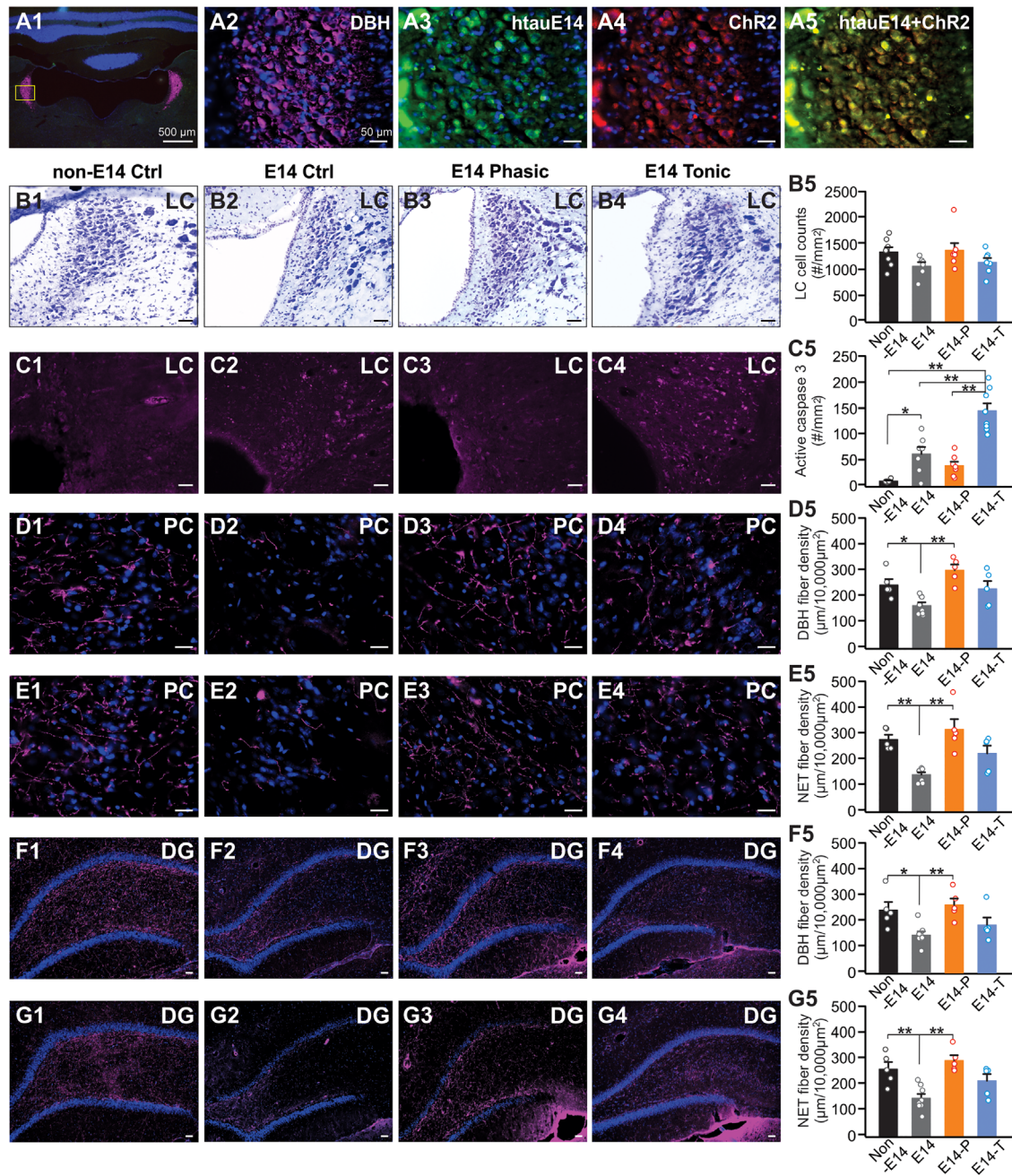
Light stimulation (10 to 30 Hz) generated  $\approx$ 8 to 15 Hz output in LC neurons in anesthetized rats<sup>20</sup> (also Figure S2). In awake rats, 10-Hz phasic patterns induced enhanced exploration and learning, whereas the 25-Hz tonic pattern produced stress and aversion.<sup>20</sup> Here we carried out chronic LC stimulation in the home cage at 6 to 7 months (Figure 1A), a time point when LC fiber degeneration and similar odor discrimination learning impairment occur.<sup>29</sup> We ask whether manipulating LC activity patterns at 6 to 7 months can alter these outcomes.

Tonic 25 Hz LC stimulation in the adult rats was accompanied by increased anxiety, as reflected in reduced rearing ( $F_{3,42} = 6.23$ ,  $P = .001$ ; Figure 1B2) and increased freezing ( $F_{3,42} = 10.43$ ,  $P < .001$ ; Figure 1B3) and more marble burying ( $F_{3,31} = 5.155$ ,  $P = .005$ ; Figure 1C). Anxiety levels are comparable in phasic E14 and non-E14 rats (Figure 1B and C). Performance in the Elevated Plus Maze did not differ among the groups ( $F_{3,42} = 0.133$ ,  $P = .940$ ; Figure 1D). Tonic stimulation increased vulnerability to depression compared with E14 light-control rats. Sucrose consumption in tonic-stimulation rats was reduced over 24 hours ( $F_{3,30} = 3.034$ ,  $P = .044$ ; Figure 1E). Pretangle tau E14 expression itself induced spatial ( $F_{3,31} = 5.582$ ,  $P = .004$  for 20 cm object distance;  $F_{3,16} = 6.25$ ,  $P = .005$  for 40 cm object distance; Figure 1F) and olfactory ( $F_{3,29} = 20.017$ ,  $P < .001$  for 1x odor concentration; Figure 1G) discrimination deficits. There was no improvement in difficult spatial and olfactory discrimination tasks with the tonic stimulation (Figure 1F and 1G).

In contrast, prior chronic phasic stimulation rescued both spatial (Figure 1F) and olfactory discrimination deficiencies (Figure 1G) in E14 rats. The phasic rats showed discrimination performance comparable to that of non-E14 controls.

The phasic and tonic light control groups for E14 showed no differences (Figures S3 and S4) and were combined into a single E14 control group.



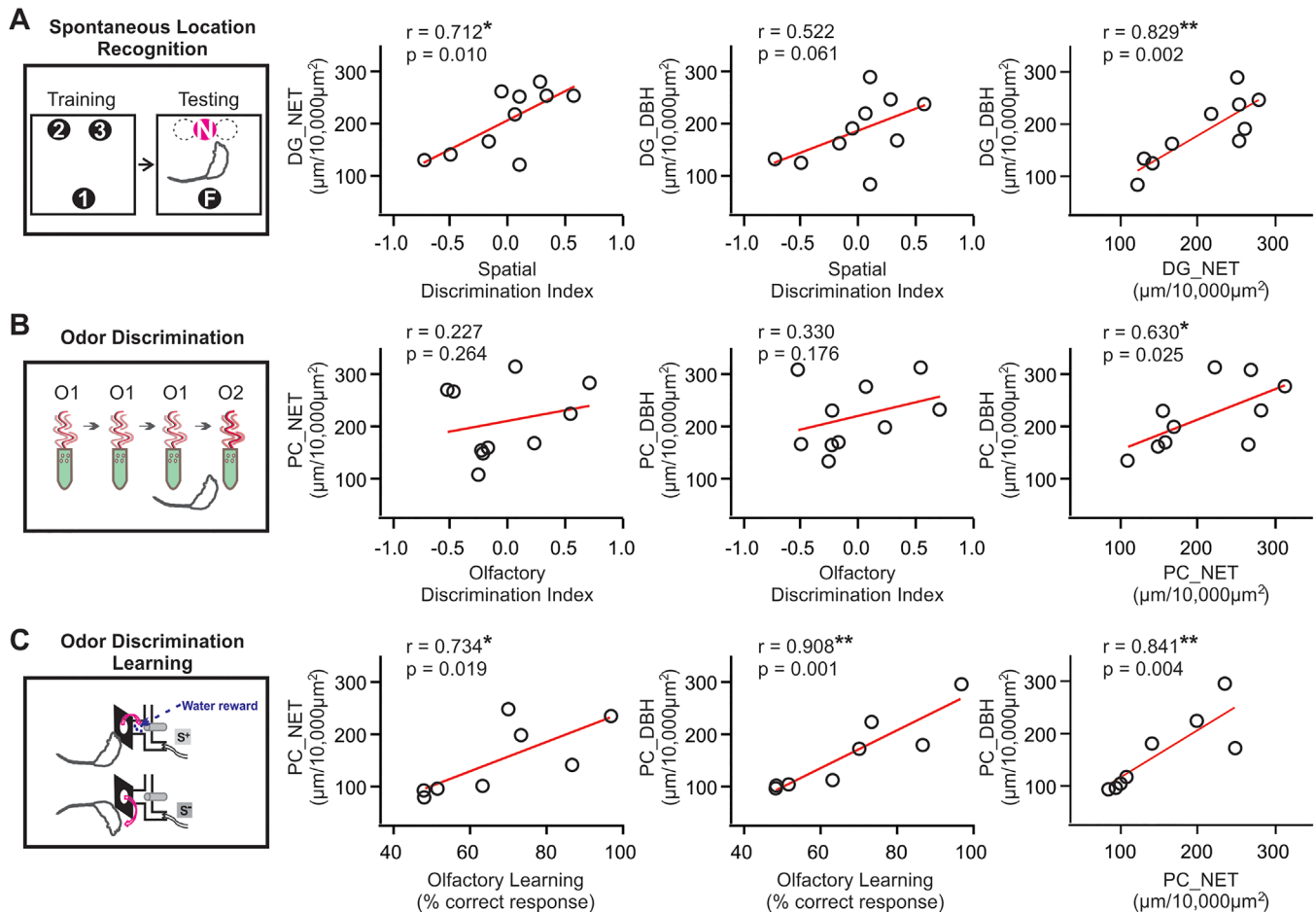


**FIGURE 2** Phasic LC activation prevents LC fiber degeneration induced by pretangle tau. A1-A5, Co-expression of htauE14 (GFP) and Chr2 (mCherry) in the LC. A2-A5, Zoom-in images from the yellow square in A1 (N = 12). B1-B5, LC cell numbers. Non-E14: n = 8; E14: n = 6; E14-P: n = 7; E14-T: n = 7. C1-C5, Active caspase 3 (AC3) cell counts. Non-E14: n = 6; E14: n = 7; E14-P: n = 7; E14-T: n = 8. D1-D5, Dopamine beta-hydroxylase (DBH) fiber density in the piriform cortex (PC). E1-E5, Norepinephrine transporter (NET) fiber density in the PC. F1-F5, DBH fiber density in the dentate gyrus (DG). G1-G5, NET fiber density in the DG. D-G, Non-E14: n = 5; E14: n = 8; E14-P: n = 5; E14-T: n = 5. Scale bars: 50  $\mu\text{m}$  except 500  $\mu\text{m}$  in A1. \* $P < .05$ ; \*\* $P < .01$

### 3.3.2 | Tonic LC stimulation impairs LC neuronal health while phasic stimulation rescues LC fiber degeneration

Chr2 (mCherry) and E14 (GFP) largely co-express in the LC (Figure 2A). On average,  $76.4 \pm 3.45\%$  E14-expressing (GFP<sup>+</sup>) cells also expressed

Chr2 (mCherry<sup>+</sup>), whereas  $82.4 \pm 5.49\%$  mCherry<sup>+</sup> cells were GFP<sup>+</sup> (n = 12). The co-expression levels were not different among the E14 control, E14 phasic, and E14 tonic groups ( $F_{3,9} = 1.461$ ,  $P = .282$ ). LC cell counts by Nissl staining were not different among groups ( $F_{3,24} = 2.098$ ,  $P = .127$ ; Figure 2B1-B5). However, both the E14 control and E14 tonic groups exhibited elevated LC AC-3 ( $F_{3,24} = 27.022$ ,



**FIGURE 3** LC fiber degeneration is correlated with spatial and olfactory learning performance. A, Correlation of LC fiber density (DBH and NET) with spatial discrimination scores in the spontaneous location recognition test ( $n = 10$ ). Schematic of the behavioral test is indicated by the left panel. Middle two panels show the correlation between spatial discrimination scores (x-axis) and DG LC fiber density (y-axis). DBH and NET fiber densities are also highly correlated as shown in the right panel. B, No strong correlation of LC fiber density with acute odor discrimination ( $n = 10$ ). C, Strong correlation of LC fiber density with similar odor discrimination learning performance ( $n = 8$ )

$P < .001$ ; Figure 2C1-5), a marker of apoptosis, with higher expression in E14 tonic rats than the E14 control, suggesting that tonic stimulation exacerbates the negative effect of E14 on LC health.

LC fiber density in the PC and DG was measured using DBH and NET staining. We found that LC E14 induced axonal degeneration in the PC (DBH:  $F_{3,19} = 8.280$ ,  $P < .001$ , Figure 2D1-D5; NET:  $F_{3,19} = 11.711$ ,  $P < .001$ , Figure 2E1-E5) and DG (DBH:  $F_{3,19} = 5.783$ ,  $P = .006$ , Figure 2F1-F5; NET:  $F_{3,19} = 9.443$ ,  $P < .001$ , Figure 2G1-G5), 12 months post-AAV infusion. E14-induced axonal degeneration was rescued by chronic LC phasic stimulation. The E14 phasic group had DBH and NET fibers comparable to that of the non-E14 control group.

### 3.3.3 | Correlations suggest that LC fiber density in target structures positively supports both spatial and olfactory cognitive performance

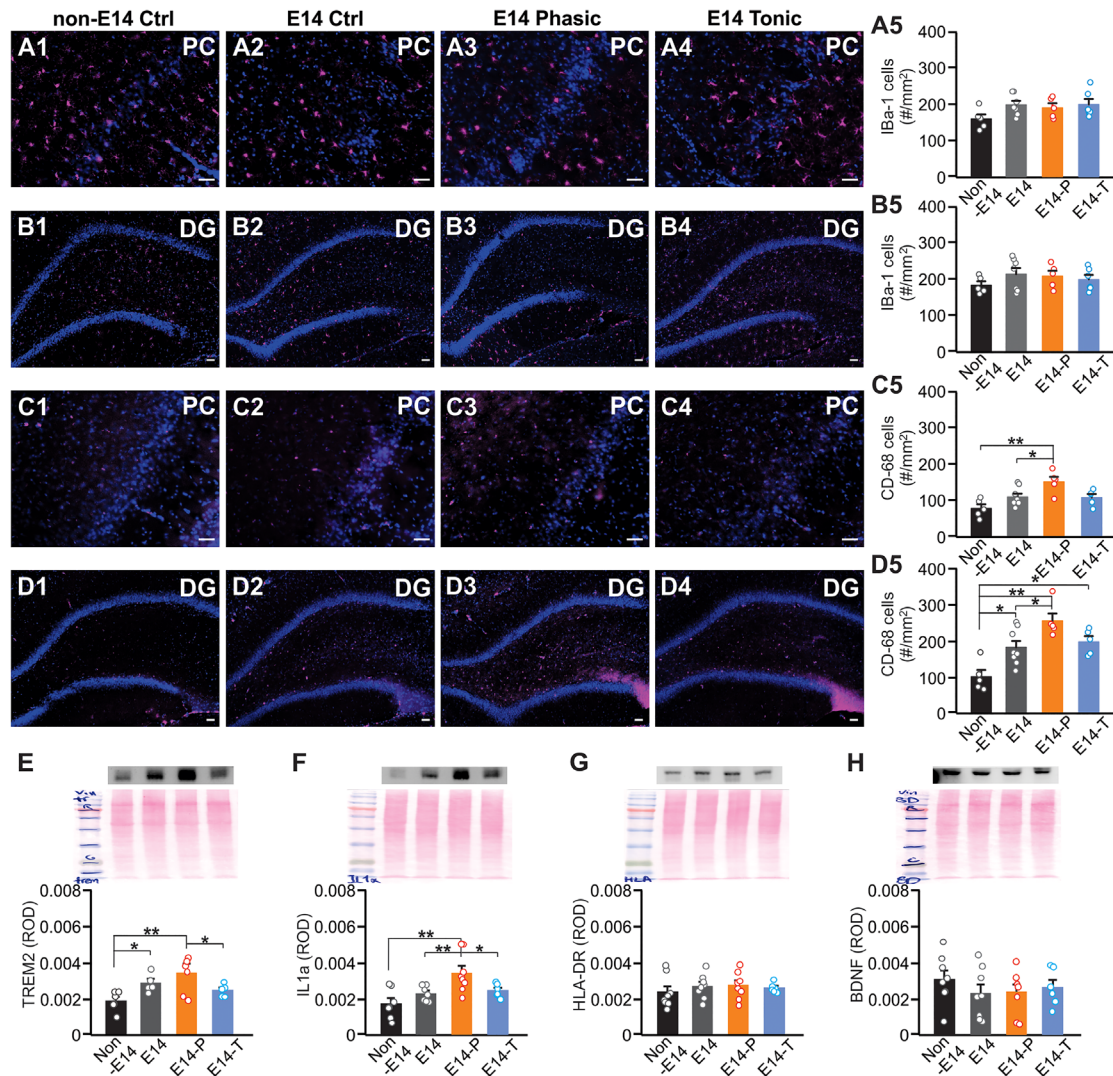
We assessed the correlation between LC fiber density and behavioral performance across all groups (Figure 3). DG NET density correlated

positively with spatial discrimination scores in the spontaneous location recognition task ( $r = 0.712$ ,  $P = .010$ ; Figure 3A). LC fiber density in the PC was not significantly correlated with novel odor discrimination (NET:  $r = 0.227$ ,  $P = .264$ ; DBH:  $r = 0.330$ ,  $P = .176$ ; Figure 3B); however, it did correlate with odor discrimination learning performance (NET:  $r = 0.734$ ,  $P = .019$ ; DBH:  $r = 0.908$ ,  $P = .001$ ; Figure 3C). These results reveal that LC fiber density is positively correlated with spatial discrimination and similar odor discrimination learning.<sup>60</sup> However, discrimination of similar odors itself is not significantly correlated with LC fibers. Nevertheless, the prevention of LC fiber degeneration in DG and PC by LC phasic stimulation was beneficial for spatial and olfactory cognitive functions in our tests.

### 3.3.4 | Chronic phasic LC stimulation activates macrophages

Using IHC, we tested levels of two microglia markers in the DG and PC: IBA-1 (a marker for resting and active microglia) and CD-68





**FIGURE 4** Phasic LC stimulation activates CD68<sup>+</sup> microglia/macrophages. A1-A5, IBA-1 cell counts in the PC. Non-E14:  $n = 5$ ; E14:  $n = 7$ ; E14-P:  $n = 5$ ; E14-T:  $n = 6$ . B1-B5, IBA-1 cell counts in the DG. Non-E14:  $n = 5$ ; E14:  $n = 7$ ; E14-P:  $n = 5$ ; E14-T:  $n = 6$ . C1-C5, CD-68 cell counts in the PC. D1-D5, CD-68 cell counts in the DG. C-D: Non-E14:  $n = 5$ ; E14:  $n = 8$ ; E14-P:  $n = 5$ ; E14-T:  $n = 5$ . Scale bars: 50  $\mu\text{m}$ . \* $P < .05$ ; \*\* $P < .01$ . E-H, Western blotting measuring levels of TREM2 (Non-E14:  $n = 6$ ; E14:  $n = 5$ ; E14-P:  $n = 6$ ; E14-T:  $n = 5$ ), IL1a (Non-E14:  $n = 7$ ; E14:  $n = 7$ ; E14-P:  $n = 8$ ; E14-T:  $n = 6$ ; F), MCHII (Non-E14:  $n = 8$ ; E14:  $n = 8$ ; E14-P:  $n = 8$ ; E14-T:  $n = 6$ ; G), and BDNF (Non-E14:  $n = 8$ ; E14:  $n = 8$ ; E14-P:  $n = 8$ ; E14-T:  $n = 6$ ; H) in the hippocampus. Data were normalized to Ponceau total protein staining

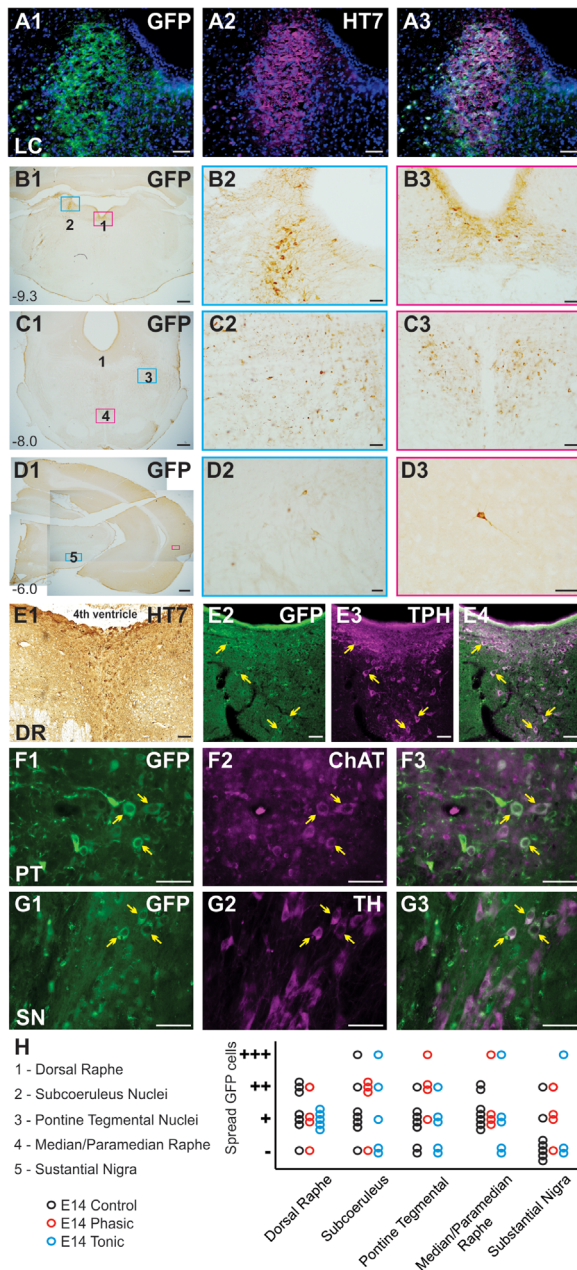
(a marker for active microglia/macrophages). IBA-1 levels did not differ among the groups in PC ( $F_{3,19} = 1.943$ ,  $P = .157$ ; Figure 4A) or DG ( $F_{3,19} = 0.806$ ,  $P = .506$ ; Figure 4B). However, CD-68 levels were significantly higher in the E14 phasic group in both structures (PC:  $F_{3,19} = 6.694$ ,  $P = .003$ , Figure 4C; DG:  $F_{3,19} = 10.316$ ,  $P < .001$ , Figure 4D). The non-E14 group has lower expression than E14 groups in the DG and phasic LC stimulation induced a further increase in CD-68 level. Increased macrophage activation associated with phasic LC stimulation may be beneficial as an active “cleaning” of abnormal proteins such as pretangle tau released from the LC, consistent with the role of macrophages in tau pathology.<sup>61</sup>

WB revealed that TREM2 ( $F_{3,18} = 4.437$ ,  $P = .017$ ; Figure 4E) and IL1a ( $F_{3,24} = 6.496$ ,  $P = .002$ ; Figure 4F) expressions were different among groups. TREM2 was significantly higher in the E14 control and E14 phasic rats compared to the non-E14 rats. Like CD-68, TREM2,

which occurs in microglia/macrophages, is suggested to have therapeutic effects in AD and AD models.<sup>62</sup> IL1a showed the highest expression in E14 phasic rats as well (Figure 4F). IL1a is less studied in the brain than TREM2, but appears neuroprotective following ischemic stroke,<sup>63</sup> and has been proposed to be beneficial in early phases of AD.<sup>53</sup> There were no differences in the HLA-DR ( $F_{3,26} = 0.435$ ,  $P = .730$ ; Figure 4G) and BDNF ( $F_{3,26} = 0.645$ ,  $P = .593$ ; Figure 4H) levels.

### 3.3.5 | Spread of pretangle tau to other neuromodulatory groups is not different with either prior phasic or tonic LC activation

Neuronal activation is associated with acute tau release.<sup>43,44</sup> However, it is not known if neuronal activity affects tau spread in intact



**FIGURE 5** Spread of pretangle tau in the brainstem and mid-brain. A1-A3, GFP and HT7 (human tau) co-labeling in the LC. B1-B3) Spread GFP cells in the brainstem at the level of  $-9.3$ . B2-B3 are enlargements from the squares in B1. C1-C3, Spread GFP cells in the brainstem at the level of  $-8.0$ . C2-C3 are enlargements from the squares in C1. D1-D5, Spread GFP cells in the mid-brain at the level of  $-6.0$ . D2-D3 are enlargements from the squares in D1. E1-E4, Dorsal raphe (DR) neurons staining positive for HT7 (E1) and tryptophan hydroxylase (TPH; E2-E4). F1-F3, GFP neurons in the pontine tegmental (PT) nuclei staining positive for ChaT. G1-G3, GFP neurons in the substantia nigra (SN) area staining positive for tyrosine hydroxylase (TH). Scale bars:  $50 \mu\text{m}$  except  $500 \mu\text{m}$  in B1, C1 and D1. H, GFP spread cells comparison in five areas in the brain stem and SN. +, < 10 cells; ++, 10-20 cells; +++, > 20 cells. E14:  $n = 7$ ; E14-P:  $n = 4$ ; E14-T:  $n = 5$

animals. Here we asked if LC stimulation may influence pre-tangle tau spread (Figure 5). HT7, a human tau antibody, co-localizes with GFP (Figure 5A1-A3). We used GFP as a marker to index pretangle tau spread. We observed spread of pretangle tau in several regions of the brainstem (Figure 5B and 5C), and to mid-brain substantia nigra (Figure 5D1-D2), and occasional sparse spread to the entorhinal region (Figure 5D1 and D3). TPH co-labeling with GFP confirmed mid-line serotonergic raphe neurons (Figure 5E1-E4) as reported earlier.<sup>29</sup> ChAT<sup>+</sup> cholinergic cells in the pontine tegmental nuclei (Figure 5F1-F3) and TH<sup>+</sup> dopaminergic neurons in the substantia nigra (Figure 5G1-G3) were also observed. We compiled and compared GFP cells in five frequent spread areas that we observed, and we found no obvious differences in spread patterns from the different groups, with spread being variable among members of each group (Figure 5H).

AT8 IHC, used for Braak staging of AD,<sup>1,64</sup> revealed reactivity in the brainstem regions. However, AT8<sup>+</sup> cells co-labeled with only a portion of GFP cells, suggesting the AT8 antibody indexed phosphorylated rat tau instead of human E14 pretangle tau. AT8 and PHF staining was not observed in either the PC or hippocampus (Figure S5). Thioflavin S staining confirmed no tangle formation in our rat brains (Figure S6), consistent with our previous observations in this model.<sup>29</sup> These results suggest that the behavioral phenotypes are mainly associated with LC axonal health and NE release, but are not related to differences in pretangle tau spread.

#### ACKNOWLEDGMENT

This work was supported by Canadian Institute of Health Research project grant (#PJT-16124) to Qi Yuan. The authors thank the Dr Karl Deisseroth laboratory at Stanford University for providing Chr2 and control AAVs.

#### CONFLICT OF INTERESTS

Qi Yuan has been appointed as a member of the Natural Sciences and Engineering Research Council of Canada (NSERC) Genes, Cells and Molecules Evaluation Group (Neuroscience) for a 3-year term ending on the June 30, 2021. NSERC funds non-health-related science and engineering research grants. This work was supported by Canadian Institute of Health Research project grant (#PJT-16124) to Qi Yuan, received in April 2019 (5 years) and paid to Memorial University. All other authors have nothing to disclose.

#### HUMAN CONSENT STATEMENT

No human subjects were used in this study; therefore consent was not necessary.

#### ORCID

Qi Yuan <https://orcid.org/0000-0002-9837-8495>

#### REFERENCES

1. Braak H, Thal DR, Ghebremedhin E, Del Tredici K. Stages of the pathological process in Alzheimer disease: age categories from 1 to 100 years. *J Neuropathol Exp Neurol*. 2011;70:960-969.



2. Bancher C, Brunner C, Lassmann H, et al. Accumulation of abnormally phosphorylated tau precedes the formation of neurofibrillary tangles in Alzheimer's disease. *Brain Res.* 1989;477:90-99.
3. Cowan CM, Chee F, Shepherd D, Mudher A. Disruption of neuronal function by soluble hyperphosphorylated tau in a Drosophila model of tauopathy. *Biochem Soc Trans.* 2010;38:564-570.
4. Yoshiyama Y, Higuchi M, Zhang B, et al. Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. *Neuron.* 2007;53:337-351.
5. 2020 Alzheimer's disease facts and figures. *Alzheimers Dement.* 2020.
6. Robertson IH. A noradrenergic theory of cognitive reserve: implications for Alzheimer's disease. *Neurobiol Aging.* 2013;34:298-308.
7. Stern Y, Albert S, Tang MX, Tsai WY. Rate of memory decline in AD is related to education and occupation: cognitive reserve?. *Neurology.* 1999;53:1942-1947.
8. Xu H, Yang R, Dintica C, et al. Association of lifespan cognitive reserve indicator with the risk of mild cognitive impairment and its progression to dementia. *Alzheimers Dement.* 2020;16:873-882.
9. Wilson RS, Begney CT, Boyle PA, Schneider JA, Bennett DA. Vulnerability to stress, anxiety, and development of dementia in old age. *Am J Geriatr Psychiatry.* 2011;19:327-334.
10. Borenstein AR, Copenhaver CI, Mortimer JA. Early-life risk factors for Alzheimer disease. *Alzheimer Dis Assoc Disord.* 2006;20:63-72.
11. Swift KM, Gross BA, Frazer MA, et al. Abnormal locus coeruleus sleep activity alters sleep signatures of memory consolidation and impairs place cell stability and spatial memory. *Curr Biol.* 2018;28:3599-3609 e4.
12. Poe GR, Foote S, Eschenko O, et al. Locus coeruleus: a new look at the blue spot. *Nat Rev Neurosci.* 2020;21:644-659.
13. Sara SJ. The locus coeruleus and noradrenergic modulation of cognition. *Nat Rev Neurosci.* 2009;10:211-223.
14. Abercrombie ED, Jacobs BL. Single-unit response of noradrenergic neurons in the locus coeruleus of freely moving cats. I. Acutely presented stressful and nonstressful stimuli. *J Neurosci.* 1987;7:2837-2843.
15. Takeuchi T, Duszkiwicz AJ, Sonneborn A, et al. Locus coeruleus and dopaminergic consolidation of everyday memory. *Nature.* 2016;537:357-362.
16. Aston-Jones G, Bloom FE. Norepinephrine-containing locus coeruleus neurons in behaving rats exhibit pronounced responses to non-noxious environmental stimuli. *J Neurosci.* 1981;1:887-900.
17. Aston-Jones G, Cohen JD. An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annu Rev Neurosci.* 2005;28:403-450.
18. Aston-Jones G, Chiang C, Alexinsky T. Discharge of noradrenergic locus coeruleus neurons in behaving rats and monkeys suggests a role in vigilance. *Prog Brain Res.* 1991;88:501-520.
19. Kempadoo KA, Mosharov EV, Choi SJ, Sulzer D, Kandel ER. Dopamine release from the locus coeruleus to the dorsal hippocampus promotes spatial learning and memory. *Proc Natl Acad Sci USA.* 2016;113:14835-14840.
20. Ghosh A, Massaeli F, Power KD, et al. Locus coeruleus activation patterns differentially modulate odor discrimination learning and odor valence in rats. *Cerebral Cortex Commun.* 2021;2:tgab026.
21. McCall JG, Al-Hasani R, Siuda ER, et al. CRH engagement of the locus coeruleus noradrenergic system mediates stress-induced anxiety. *Neuron.* 2015;87:605-620.
22. Wilson RS, Nag S, Boyle PA, et al. Neural reserve, neuronal density in the locus ceruleus, and cognitive decline. *Neurology.* 2013;80:1202-1208.
23. Kelly SC, He B, Perez SE, Ginsberg SD, Mufson EJ, Counts SE. Locus coeruleus cellular and molecular pathology during the progression of Alzheimer's disease. *Acta Neuropathol Commun.* 2017;5:8.
24. Theofilas P, Ehrenberg AJ, Dunlop S, et al. Locus coeruleus volume and cell population changes during Alzheimer's disease progression: a stereological study in human postmortem brains with potential implication for early-stage biomarker discovery. *Alzheimers Dement.* 2017;13:236-246.
25. Dutt S, Li Y, Mather M, Nation DA. Alzheimer's disease neuroimaging I. Brainstem volumetric integrity in preclinical and prodromal Alzheimer's disease. *J Alzheimers Dis.* 2020;77:1579-1594.
26. Lee TH, Kim SH, Katz B, Mather M. The decline in intrinsic connectivity between the salience network and locus coeruleus in older adults: implications for distractibility. *Front. Aging Neurosci.* 2020;12:2.
27. Watanabe T, Tan Z, Wang X, Martinez-Hernandez A, Frahm J. Magnetic resonance imaging of noradrenergic neurons. *Brain Struct Funct.* 2019;224:1609-1625.
28. Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol.* 1991;82:239-259.
29. Ghosh A, Torraville SE, Mukherjee B, et al. An experimental model of Braak's pretangle proposal for the origin of Alzheimer's disease: the role of locus coeruleus in early symptom development. *Alzheimers Res Ther.* 2019;11:59.
30. Hoover BR, Reed MN, Su J, et al. Tau mislocalization to dendritic spines mediates synaptic dysfunction independently of neurodegeneration. *Neuron.* 2010;68:1067-1081.
31. Harley CW, Walling SG, Yuan Q, Martin GM. The 'a, b, c's of pretangle tau and their relation to aging and the risk of Alzheimer's Disease. *Semin Cell Dev Biol.* 2021;116:125-134.
32. Stratmann K, Heinsen H, Korf HW, et al. Precortical phase of Alzheimer's disease (AD)-related tau cytoskeletal pathology. *Brain Pathol.* 2016;26:371-386.
33. Doty RL, Shaman P, Applebaum SL, Giberson R, Siksorski L, Rosenberg L. Smell identification ability: changes with age. *Science.* 1984;226:1441-1443.
34. Ding YS, Singhal T, Planeta-Wilson B, et al. PET imaging of the effects of age and cocaine on the norepinephrine transporter in the human brain using (S,S)-[11C]O-methylreboxetine and HRRT. *Synapse.* 2010;64:30-38.
35. Gulyas B, Brockschneider D, Nag S, et al. The norepinephrine transporter (NET) radioligand (S,S)-[18F]FMeNER-D2 shows significant decreases in NET density in the human brain in Alzheimer's disease: a post-mortem autoradiographic study. *Neurochem Int.* 2010;56:789-798.
36. Arve S, Tilvis RS, Lehtonen A, Valvanne J, Sairanen S. Coexistence of lowered mood and cognitive impairment of elderly people in five birth cohorts. *Aging (Milano).* 1999;11:90-95.
37. Diniz BS, Butters MA, Albert SM, Dew MA. Late-life depression and risk of vascular dementia and Alzheimer's disease: systematic review and meta-analysis of community-based cohort studies. *Br J Psychiatry.* 2013;202:329-335.
38. Gatchel JR, Donovan NJ, Locascio JJ, et al. Depressive symptoms and tau accumulation in the inferior temporal lobe and entorhinal cortex in cognitively normal older adults: a pilot study. *J Alzheimers Dis.* 2017;59:975-985.
39. Babulal GM, Roe CM, Stout SH, et al. Depression is associated with tau and not amyloid positron emission tomography in cognitively normal adults. *J Alzheimers Dis.* 2020;74:1045-1055.
40. Ordway GA, Schenk J, Stockmeier CA, May W, Klimek V. Elevated agonist binding to alpha2-adrenoceptors in the locus coeruleus in major depression. *Biol Psychiatry.* 2003;53:315-323.
41. Zhu MY, Klimek V, Dilley GE, et al. Elevated levels of tyrosine hydroxylase in the locus coeruleus in major depression. *Biol Psychiatry.* 1999;46:1275-1286.
42. Delgado PL, Moreno FA. Role of norepinephrine in depression. *J Clin Psychiatry.* 2000;61(1):5-12.
43. Pooler AM, Phillips EC, Lau DH, Noble W, Hanger DP. Physiological release of endogenous tau is stimulated by neuronal activity. *EMBO Rep.* 2013;14:389-394.

44. Yamada K, Holth JK, Liao F, et al. Neuronal activity regulates extracellular tau in vivo. *J Exp Med*. 2014;211:387-393.
45. Feng J, Zhang C, Lischinsky JE, et al. A genetically encoded fluorescent sensor for rapid and specific in vivo detection of norepinephrine. *Neuron*. 2019;102:745-761 e8.
46. Minzenberg MJ, Watrous AJ, Yoon JH, Ursu S, Carter CS. Modafinil shifts human locus coeruleus to low-tonic, high-phasic activity during functional MRI. *Science*. 2008;322:1700-1702.
47. Minzenberg MJ, Carter CS. Modafinil: a review of neurochemical actions and effects on cognition. *Neuropsychopharmacology*. 2008;33:1477-1502.
48. Popescu T, Pernet C, Beisteiner R. Transcranial ultrasound pulse stimulation reduces cortical atrophy in Alzheimer's patients: a follow-up study. *Alzheimers Dement (NY)*. 2021;7:e12121.
49. Sahay A, Wilson DA, Hen R. Pattern separation: a common function for new neurons in hippocampus and olfactory bulb. *Neuron*. 2011;70:582-588.
50. Chapuis J, Wilson DA. Bidirectional plasticity of cortical pattern recognition and behavioral sensory acuity. *Nat Neurosci*. 2011;15:155-161.
51. Shakhawat AM, Harley CW, Yuan Q. Arc visualization of odor objects reveals experience-dependent ensemble sharpening, separation, and merging in anterior piriform cortex in adult rat. *J Neurosci*. 2014;34:10206-10210.
52. Theofilas P, Ehrenberg AJ, Dunlop S, et al. Locus coeruleus volume and cell population changes during Alzheimer's disease progression: a stereological study in human postmortem brains with potential implication for early-stage biomarker discovery. *Alzheimers Dement*. 2017;13:236-246.
53. Shaftel SS, Griffin WS, O'Banion MK. The role of interleukin-1 in neuroinflammation and Alzheimer disease: an evolving perspective. *J Neuroinflammation*. 2008;5:7.
54. Gratzke M, Leyns CEG, Holtzman DM. New insights into the role of TREM2 in Alzheimer's disease. *Mol Neurodegener*. 2018;13:66.
55. Sarlus H, Heneka MT. Microglia in Alzheimer's disease. *J Clin Invest*. 2017;127:3240-3249.
56. Guo JL, Narasimhan S, Changolkar L, et al. Unique pathological tau conformers from Alzheimer's brains transmit tau pathology in nontransgenic mice. *J Exp Med*. 2016;213:2635-2654.
57. Bekinschtein P, Kent BA, Oomen CA, et al. BDNF in the dentate gyrus is required for consolidation of "pattern-separated" memories. *Cell Rep*. 2013;5:759-768.
58. Garcia-Cabezas MA, John YJ, Barbas H, Zikopoulos B. Distinction of neurons, glia and endothelial cells in the cerebral cortex: an algorithm based on cytological features. *Front Neuroanat*. 2016;10:107.
59. Morrison GL, Fontaine CJ, Harley CW, Yuan Q. A role for the anterior piriform cortex in early odor preference learning: evidence for multiple olfactory learning structures in the rat pup. *J Neurophysiol*. 2013;110:141-152.
60. Shakhawat AM, Gheidi A, MacIntyre IT, Walsh ML, Harley CW, Yuan Q. Arc-expressing neuronal ensembles supporting pattern separation require adrenergic activity in anterior piriform cortex: an exploration of neural constraints on learning. *J Neurosci*. 2015;35:14070-14075.
61. Hamano T, Hayashi K, Shirafuji N, Nakamoto Y. The implications of autophagy in Alzheimer's disease. *Curr Alzheimer Res*. 2018;15:1283-1296.
62. Qin Q, Teng Z, Liu C, Li Q, Yin Y, Tang Y. TREM2, microglia, and Alzheimer's disease. *Mech Ageing Dev*. 2021;195:111438.
63. Salmeron KE, Maniskas ME, Edwards DN, et al. Interleukin 1 alpha administration is neuroprotective and neuro-restorative following experimental ischemic stroke. *J Neuroinflammation*. 2019;16:222.
64. Braak H, Del Trecidi K. Neuroanatomy and pathology of sporadic Alzheimer's disease. *Adv Anat Embryol Cell Biol*. 2015;215:1-162.

#### SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

**How to cite this article:** Omoluabi T, Torraville SE, Maziar A, et al. Novelty-like activation of locus coeruleus protects against deleterious human pretangle tau effects while stress-inducing activation worsens its effects. *Alzheimer's Dement*. 2021;7:e12231. <https://doi.org/10.1002/trc2.12231>