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FEATURED ARTICLE

Epigenetic conditioning induces intergenerational resilience to dementia in a mouse model of vascular cognitive impairment

Krystal Courtney D. Belmonte[1,2] | **Eleanor B. Holmgren**[3] | **Tiffany A. Wills**[3,4] | **Jeff M. Gidday**[1,2,4,5]

[1] Department of Ophthalmology, Louisiana State University School of Medicine, LSUHSC, New Orleans, Louisiana, USA

[2] Department of Physiology, Louisiana State University School of Medicine, LSUHSC, New Orleans, Louisiana, USA

[3] Department of Cell Biology and Anatomy, Louisiana State University School of Medicine, LSUHSC, New Orleans, Louisiana, USA

[4] Neuroscience Center of Excellence, Louisiana State University School of Medicine, LSUHSC, New Orleans, Louisiana, USA

[5] Department of Biochemistry and Molecular Biology, Louisiana State University School of Medicine, LSUHSC, New Orleans, Louisiana, USA

Correspondence
Jeff M. Gidday, Department of Ophthalmology, Louisiana State University School of Medicine, LSUHSC, 533 Bolivar St., CSRB Room 454, New Orleans, LA 70112, USA.
Email: jgidda@lsuhsc.edu

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Abstract

Introduction: Epigenetic stimuli induce beneficial or detrimental changes in gene expression, and consequently, phenotype. Some of these phenotypes can manifest across the lifespan—and even in subsequent generations. Here, we used a mouse model of vascular cognitive impairment and dementia (VCID) to determine whether epigenetically induced resilience to specific dementia-related phenotypes is heritable by first-generation progeny.

Methods: Our systemic epigenetic therapy consisted of 2 months of repetitive hypoxic "conditioning" (RHC) prior to chronic cerebral hypoperfusion in adult C57BL/6J mice. Resultant changes in object recognition memory and hippocampal long-term potentiation (LTP) were assessed 3 and 4 months later, respectively.

Results: Hypoperfusion-induced memory/plasticity deficits were abrogated by RHC. Moreover, similarly robust dementia resilience was documented in untreated cerebral hypoperfused animals derived from RHC-treated parents.

Conclusions: Our results in experimental VCID underscore the efficacy of epigenetics-based treatments to prevent memory loss, and demonstrate for the first time the heritability of an induced resilience to dementia.

KEYWORDS
chronic cerebral hypoperfusion, epigenetics, hypoxic conditioning, intergenerational, resilience, vascular cognitive impairment and dementia

1 | INTRODUCTION

Vascular contributions to cognitive impairment and dementia (VCID), secondary to chronic cerebral hypoperfusion (CCH), acute stroke, and other causes, is the second most common form of dementia after Alzheimer's disease (AD).[1–4] Moreover, the prevalence of cerebrovascular disease is in fact higher than the prevalence of AD,[5] suggesting that many cases of VCID remain undocumented. To date, there is no efficacious therapy for VCID.

An epigenetics-based VCID therapeutic strategy may be able to meet this clinical challenge. Specifically, preconditioning the brain to activate changes in gene expression is a well-established approach to promote endogenous pathways of cell survival and tissue resistance to injury.[6,7] We and others have previously shown the efficacy

of hypoxic preconditioning in inducing protection against acute stroke.[8–11] Importantly, our lab has also documented, in a mouse model of focal stroke, that the duration of this inducible neurovascular-protective phenotype can be extended dramatically—from days to months—after the last of a series of repetitive hypoxic conditioning (RHC) stimuli.[9] Recently, we showed that 4 months of RHC in male and female mice, prior to breeding, results in a heritable resilience to retinal ischemic injury in their untreated, first-generation progeny.[12]

Although a number of studies have provided evidence for intergenerational epigenetic inheritance in both animals and humans,[13,14] the vast majority have focused on the heritability of disease risk and disease itself. In the present investigation, we hypothesized that RHC will induce resilience to cognitive deficits in a mouse model of VCID, and that this dementia-resilient phenotype will be inherited. The data we collected support our hypothesis, documenting the therapeutic potential of activating an endogenous resiliency against dementia capable of providing protection that may span multiple generations.

RESEARCH IN CONTEXT

1. **Systematic Review**: Considerable pre-clinical and clinical evidence indicates that repetitive adverse stress can epigenetically promote phenotypes that enhance disease risk. In turn, transient protection against injury can be induced epigenetically by a nonharmful stress. We explored in mice whether repetitive nonharmful stress will induce long-lasting resilience to vascular cognitive impairment and dementia (VCID), and whether this induced, cognition-protective phenotype is heritable.
2. **Interpretation**: Repetitive systemic hypoxia prevented loss of recognition memory in vivo, and impairments in hippocampal long-term potentiation (LTP) ex vivo. Similar resilience to VCID was documented in untreated mice derived from parents repetitively conditioned before mating, indicating that epigenetically induced resilience is heritable.
3. **Future Directions**: Myriad studies are needed to elucidate the molecular mechanisms of epigenetic protection against VCID, and how this new phenotype is passed to the next generation. However, the translational efficacy of an epigenetic approach for establishing within-generation resilience could be tested in clinical trials immediately.

2 | METHODS

2.1 | Animals

Male and female C57BL/6J mice (Jackson Laboratory), age 8 to 9 weeks, were maintained on a 12 hour/12 hour light/dark cycle with food and water provided ad libitum, and randomly assigned into experimental groups after ≈2 weeks of habituation.

2.2 | Experimental design

Based on our experience leveraging RHC as an epigenetic stimulus that promotes long-lasting disease-resilient phenotypes in treated mice[9,15,16] and more recently, in untreated adult offspring from RHC-treated parents,[12] mice were treated with 8 weeks of RHC in the present study, in parallel with age-matched normoxic controls. CCH was induced in separate cohorts of F0-generation mice, with and without prior RHC treatment, to examine the direct and inherited effects of RHC on CCH outcomes. Specifically, males treated with 8 weeks of RHC were paired with 8-week RHC-treated females to produce the [F1: RHC]* generation (asterisk denoting that F1-generation mice were never exposed to RHC themselves), and normoxic males and females were paired to produce the F1: CTL (control) generation (Figure 1). In all cases, both F0 parents remained in the cage until their F1 offspring were weaned at PND21, and housed thereafter in standard cages. All F1 mice breathed ambient air throughout their lives, and were age-matched with their respective F0 generation at the time of carotid microcoil placement, neurocognitive testing, and the collection of hippocampal slices for long-term potentiation (LTP) measurements. All experiments were approved by our institutional animal care and use committee and conformed to Animal Research: Reporting of In Vivo Experiments guidelines.

2.3 | RHC

RHC was performed by exposing F0 mice to mild-to-moderate systemic hypoxia (11% O_2) using an oxygen tension-controlled chamber (BioSpherix). Cages were placed directly in the chamber, with mice allowed free access to food and water during treatment. Mice were treated for 1 hour, every other day (M/W/F), for 8 continuous weeks. Age- and sex-matched mice exposed to normal atmospheric oxygen served as normoxic controls.

2.4 | CCH

To model the effects of VCID, CCH was achieved by bilateral carotid artery stenosis (BCAS) at ≈20 weeks of age, as previously described.[17–19] Control and experimental animals were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) intraperitoneally, and sustained-release buprenorphine (0.1 mg/kg, i.p.) prior to surgery. After a midline incision, gold-plated microcoils (0.18-mm internal diameter; 0.50-mm pitch; 2.5-mm total length; Motion Dynamics Corporation) were wrapped around both common carotids. Incisions were closed using Vetbond tissue adhesive (3M). Body temperature was maintained during the operative procedure and post-operative recovery with a warming pad.

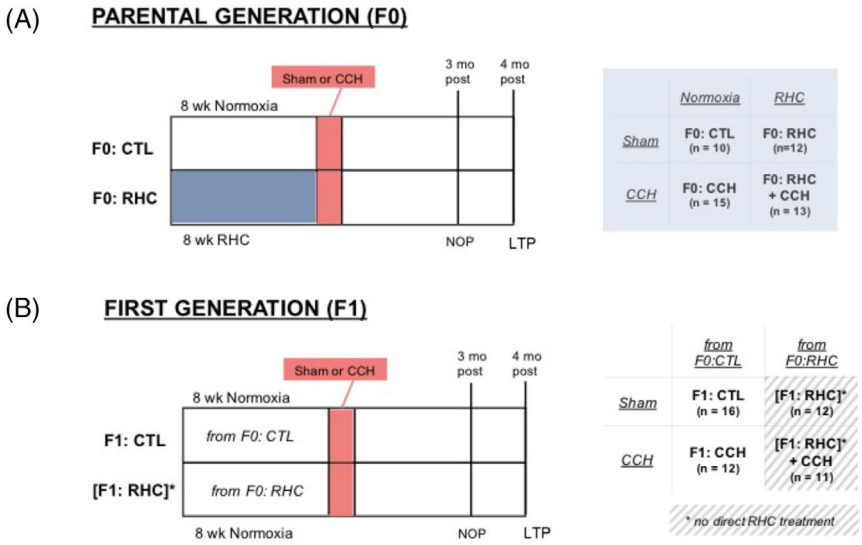

FIGURE 1 Experimental design for repetitive hypoxic conditioning (RHC) studies in mice with chronic cerebral hypoperfusion (CCH). A, Parental generation male and female adult mice were exposed to repetitive hypoxic conditioning (11% O_2 for 1 hour every other day), or normoxia, for 8 weeks. After the last treatment, mice were either subjected to bilateral carotid artery stenosis (BCAS) surgery to induce CCH, or sham surgery. B, First generation offspring were derived from a separate cohort of F0 animals, both males and females, that were only subjected to either 8 weeks of RHC, or normoxia, prior to mating. Male and female F1 offspring originating from these pairings never received RHC directly. At ≈20 weeks of age, all F1-generation animals were subjected to either CCH or sham surgery. In both generations, novel object preference (NOP), a neurobehavioral measurement of recognition memory, was performed after 3 months of CCH, and hippocampal long-term potentiation (LTP) was assessed as an electrophysiological proxy of memory-associated synaptic plasticity,1 month later

2.5 | Demyelination

Brains were removed after 4 months of CCH (or sham surgery) and post-fixed in Z-Fix (ANATECH LTD) for 24 hours at 4°C before cryoprotection in 30% sucrose. Brains were flash-frozen with isopentane at −20°C, and serial, 20 μm-thick coronal sections were obtained (approximately 0.86 mm posterior to Bregma) by cryostat and stored at 4°C in phosphate-buffered saline (PBS). Free-floating sections were rinsed in 0.2% Triton X-100 PBS (PBSt) for at least 20 minutes at room temperature before applying a fluorescent myelin stain (FluoroMyelinTM Green; 1:300 in PBS; Molecular Probes) for 20 minutes at room temperature. Seven to eight sections per animal were mounted onto SuperFrost glass slides (Thermo Fisher Scientific) cover-slipped, and 4x magnification images were captured on an Olympus BX51 fluorescent microscope (Olympus Life Science) using cellSens software (Olympus Life Science). The entire corpus callosum was selected

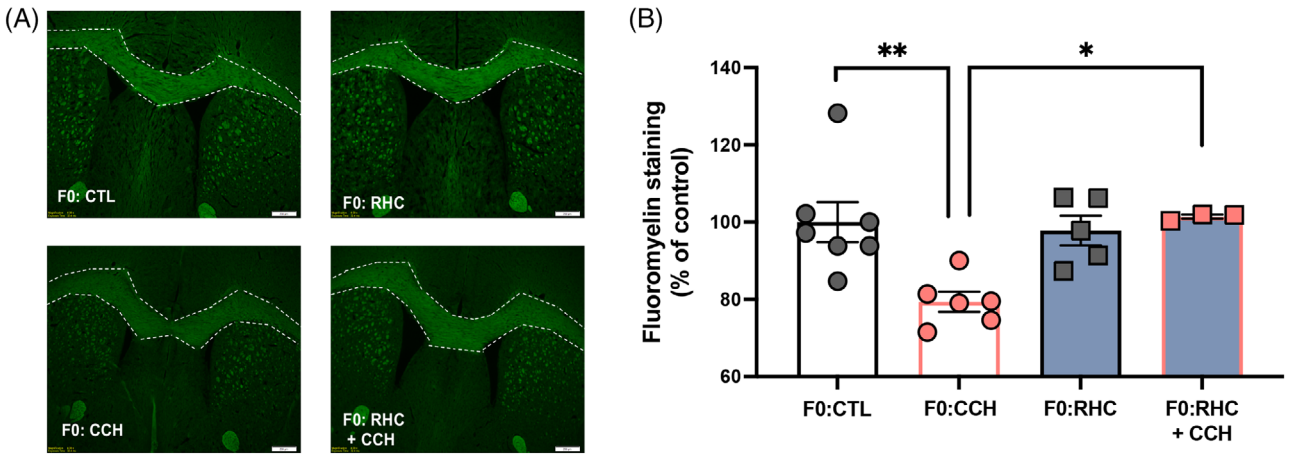

FIGURE 2 Repetitive hypoxic conditioning (RHC) reduces white matter injury in the corpus callosum after 3 months of chronic cerebral hypoperfusion (CCH). A, Representative fluoromyelin images of coronal brain sections from F0 mice after 3 months of CCH, or sham surgery (CTL). Region of corpus callosum used for quantification of fluoromyelin staining intensity is delineated by white lines. B, Fluoromyelin staining intensity, used to quantify corpus callosum myelination, was normalized for each experimental group to untreated controls (F0:CTL). *$P < .05$, ** $P < .01$, by Mann-Whitney ($n = 3$-7/group). Mean ± standard error of the mean. Scale bar = 200 μm

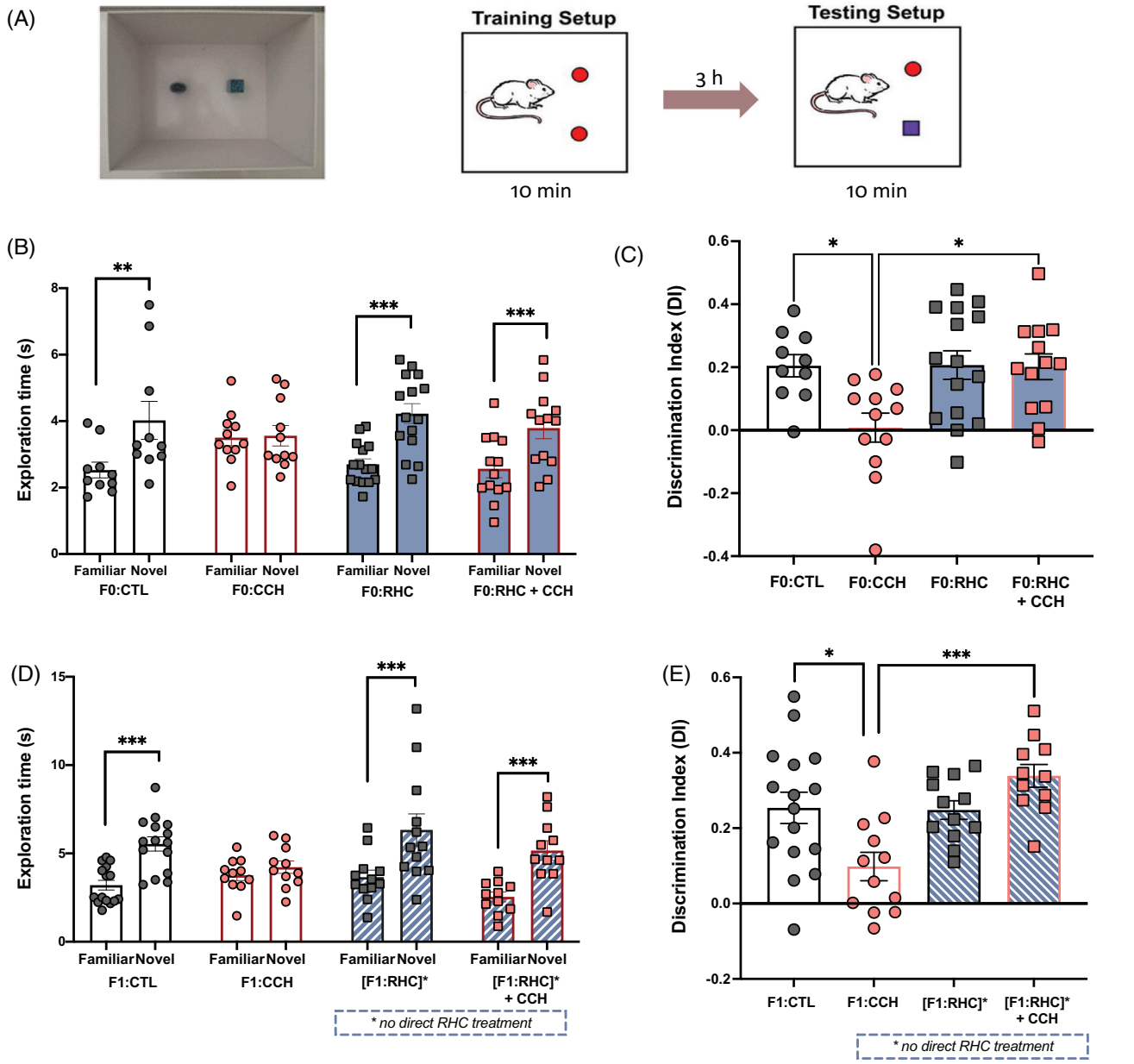

FIGURE 3 Repetitive hypoxic conditioning (RHC) prevents loss of object recognition memory in vivo in F0 mice and in their untreated F1 progeny. Schematic diagram of the spontaneous object recognition testing paradigm (A). Familiar and novel object exploration times (B), and discrimination indices (C), of the four F0 experimental groups. Note, impairments in recognition memory after 3 months of CCH were prevented in F0 mice directly treated with RHC. Familiar and novel object exploration times (D), and discrimination indices (E), of the four F1 experimental groups. Note, in parallel with findings in F0 mice (B,C), loss of recognition memory after 3 months of CCH is prevented in untreated F1 mice derived from F0 parents treated with RHC prior to mating (D,E). $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, by Wilcoxon matched-pairs rank test (B, D) and Mann-Whitney (C, E; $n = 10$-16/group). Mean ± standard error of the mean

(Figure 2A) using the "freehand selection" option and pixel intensity for the seven to eight sections was determined bilaterally and averaged using ImageJ 2 software (National Institutes of Health).

2.6 | Novel object preference

After 3 months of CCH (or sham surgery), mice were individually habituated to a dimly lit black Plexiglass box with a white floor (20 × 20 × 20 cm) for 15 minutes on the day before the test (Figure 3A). The next day, a testing session consisting of two trials was administered: the familiar object exposure and the novel object exposure. Mice were individually placed in the box containing two identical objects for 10 minutes and then returned to their home cage. Three hours later, for the novel object preference (NOP) assessment, mice were returned to the box, which now contained a novel object along with one of the familiar objects (Figure 3A) and allowed to freely explore the objects for 10 minutes. Time spent exploring each object

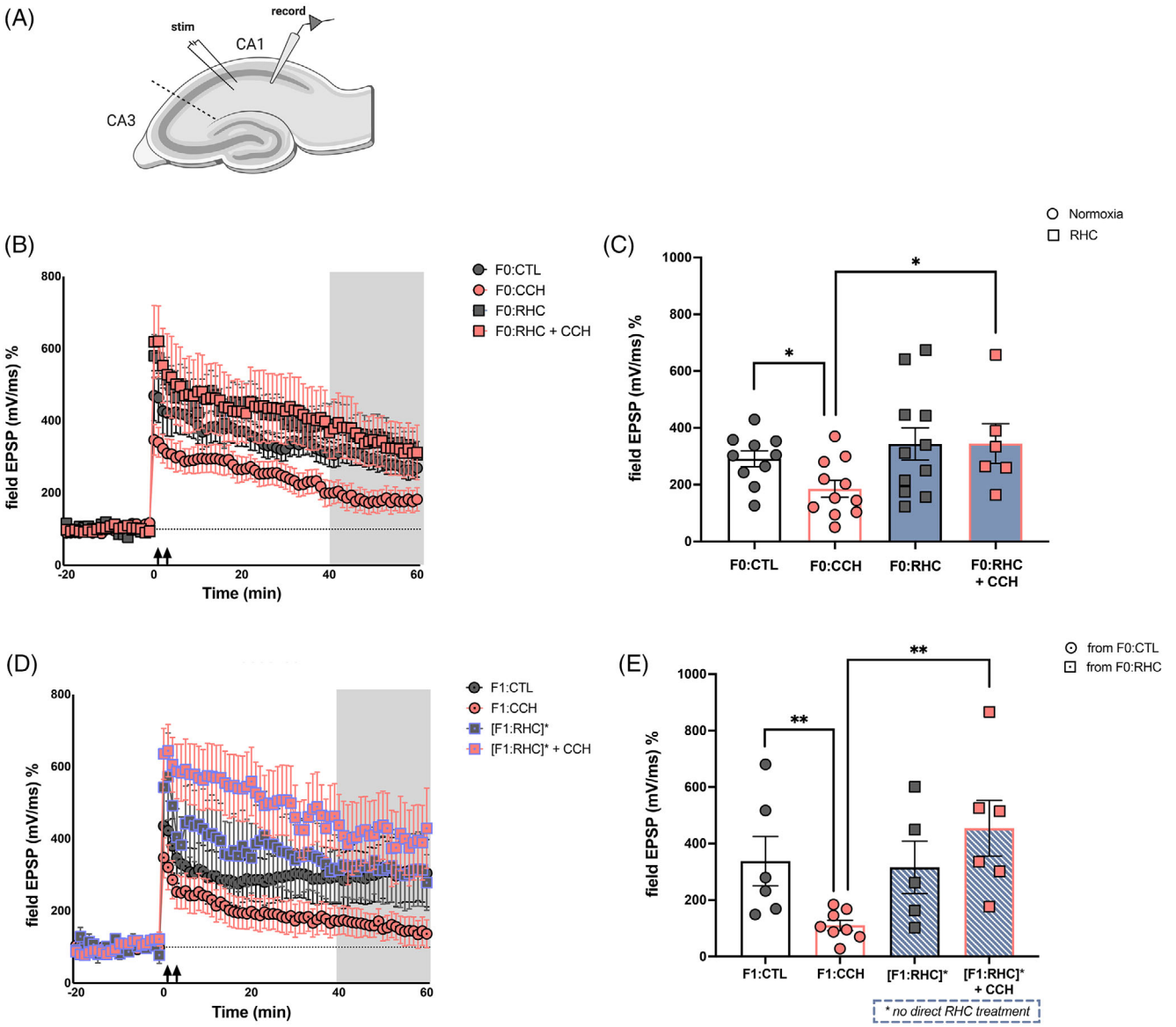

FIGURE 4 Repetitive hypoxic conditioning (RHC) rescues ex vivo long-term potentiation (LTP), a metric of synaptic plasticity, in CA1 hippocampus of F0 mice and their untreated F1 progeny. A, Schematic of a coronal hippocampal slice showing positions of the stimulating (stim) and recording (record) electrodes. B, Field excitatory postsynaptic potential recordings (fEPSPs), normalized to their respective baselines, for the four experimental groups of F0 mice; the increase in fEPSP after theta-burst stimulation (TBS; double arrows) indicates a hippocampal LTP response. The average fEPSP over 40 to 60 minutes post-TBS (shaded in gray) of each F0 group is represented in (C). Similarly, fEPSP responses (D), and their average amplitude over 40 to 60 minutes (E), are shown for the four groups of F1 mice. Note, the loss of LTP as a result of 4 months of CCH was reversed in F0 mice directly treated with RHC, and in their untreated F1 progeny. $^{*}P < .05$, $^{**}P < .01$ by Mann-Whitney ($n = 10$-16/group). Mean ± standard error of the mean

was recorded by video camera; exploration was defined as sniffing or touching either object at a distance of < 2 cm from the object. Analysis of NOP behavior was conducted by video playback by an observer blinded to experimental treatment conditions. Recognition memory was quantified by a discrimination index (DI), as follows: (time exploring novel object – time exploring familiar object)/(time exploring novel + familiar). A larger DI is indicative of more time exploring the novel object, thus reflecting the animal's recognition memory of the familiar object.

2.7 | LTP

Hippocampal slices were prepared from mice after 4 months of CCH (or sham surgery), and 1 month after the completion of NOP testing for that animal. As previously described,[20] mice were anesthetized with isoflurane and decapitated. Hippocampal slices (300 μm) were prepared using a Tissue Slicer (Leica Biosystems), collected in artificial cerebrospinal fluid (ACSF), and transferred to a submerged recording chamber where they were perfused with heated (28°C) and

oxygenated (95% O2/5% CO2) ACSF for 1 hour before recordings were initiated. A stainless steel stimulating electrode and a borosilicate glass recording electrode filled with ACSF were both positioned in the stratum radiatum of the hippocampal CA1 region to elicit and record extracellular field responses (Figure 4A). The field excitatory postsynaptic potential (fEPSP) slope was monitored using Axon Patch-Clamp instrumentation (Molecular Devices), and recordings were analyzed with Clampfit software (Molecular Devices). The test stimulation strength was determined for each input to elicit a field EPSP of 40% maximal slope. Baseline fEPSPs were recorded for 20 minutes. LTP was then induced with a tetanus of theta-burst stimulation (TBS) protocol consisting of two stimulus trains (100 biphasic constant-current pulses per train at 100 Hz, inter-train interval 20 seconds); fEPSPs were recorded for 60 minutes post-TBS and LTP was displayed as the average fEPSP recorded over the last 20 minutes post-TBS.

2.8 | Statistical analyses

GraphPad Prism 9 software was used to analyze data. Differences in exploration times of familiar and novel objects were compared using the Wilcoxon signed rank test. Differences in white matter myelin densities, discrimination indices for NOP, and average fEPSP amplitudes 40 to 60 minutes post-TBS were analyzed using Mann-Whitney tests. The association between fEPSP responses and DIs was analyzed by a Spearman correlation. All values are presented as mean ± standard error of the mean, with $P < .05$ considered significant.

3 | RESULTS

3.1 | RHC prevents CCH-induced white matter injury

Given previous reports documenting a loss of myelin integrity in mice after 1 month of CCH,[17,18,21–24] we assessed myelin density in the corpus callosum after 3 months of CCH in F0 cohorts of mice with and without RHC treatment as a metric for white matter injury and protection (Figures 2A, 2B). Compared to control animals, fluoromyelin intensities were significantly reduced after 4 months of CCH (F0:CTL vs. F0:CCH, $P = .002$), reflecting CCH-induced demyelination. However, animals treated with RHC prior to CCH exhibited significantly greater fluoromyelin intensities compared to CCH animals (F0:RHC+CCH vs. F0:CCH, $P = .024$), and in fact were indistinguishable from control animals (F0:CTL vs. F0:RHC+CCH, $P = .358$), consistent with the hypothesis that RHC completely prevented CCH-induced demyelination.

3.2 | Intergenerational prevention of CCH-induced impairment in object recognition memory by RHC

NOP testing is a well-established neurobehavioral tool to assess recognition memory.[25–28] Control mice from both generations spent significantly more time exploring the novel object compared to the familiar object (F0:CTL familiar vs. novel, $P = .004$; F1:CTL familiar vs. novel, $P = .0001$; Figures 3B, 3D). After 3 months of CCH, both F0 and F1 mice exhibited no significant differences in exploration time between the novel and familiar object (F0:CCH familiar vs. novel, $P = .563$; F1:CCH familiar vs. novel, $P = .067$; Figures 3B, 3D), indicative of a CCH-induced impairment in recognition memory. In contrast, F0 animals directly treated with RHC, as well as their untreated F1 offspring, spent significantly more time exploring the novel object compared to the familiar object (F0:RHC+CCH familiar vs. novel, $P = .0009$; [F1:RHC]*+CCH familiar vs. novel, $P = .0005$), indicating that RHC prevented CCH-induced losses in recognition memory both in animals receiving the treatment, as well as in their untreated offspring (Figures 3B, 3D).

To account for the differences in total exploration time and standardize such total time among all groups for comparison, the DI was used to quantify the animal's preference between the novel and familiar object (Figure 3C, 3E). In both generations, CCH animals demonstrated lower DI compared to that of their generational controls, indicating an impairment in recognition memory in the former (F0:CTL vs. F0:CCH, $P = .001$; F1:CTL vs. F1:CCH, $P = .015$; Figures 3C, 3E). However, in F0 animals directly treated with RHC prior to CCH induction, such recognition memory impairment was prevented, with RHC-treated CCH mice exhibiting a significant increase in DI compared to untreated CCH animals (F0:RHC+CCH vs. F0:CCH, $P = .004$; Figure 3C). Of note, the F1 offspring of RHC-treated parents also exhibited similar resilience to CCH-induced memory loss with DI values significantly increased relative to control offspring derived from untreated parents ([F1:RHC]*+CCH vs. F1:CCH, $P = .0002$; Figure 3E). Thus, RHC prevented CCH-induced losses in recognition memory in animals receiving RHC treatment directly (Figure 3C), as well as in their untreated offspring (Figure 3E).

3.3 | Intergenerational preservation of hippocampal LTP via RHC

LTP is a well-established form of synaptic plasticity that subserves memory function in the mammalian hippocampus, amygdala, and other cortical brain structures.[29,30] Tetanic stimulation led to an initial increase in the slope of fEPSP in all groups (Figures 4B, 4D), with the average of the last 20 minutes post-stimulation (40–60 minutes post-TBS) represented in Figures 4C and 4E. Four months of CCH caused a significant reduction in LTP (F0:CTL vs. F0:CCH, $P = .024$; F1:CTL vs. F1:CCH, $P = .005$). Furthermore, F0 animals directly treated with RHC prior to CCH, and the F1 offspring of RHC-treated parents, exhibited significant increases in LTP compared to untreated CCH animals and the F1 offspring of untreated parents, respectively (F0:RHC+CCH vs. F0:CCH, $P = .037$; [F1:RHC]*+CCH vs. F1:CCH, $P = .001$), indicative of a significant RHC-mediated preservation of LTP in the face of ongoing CCH.

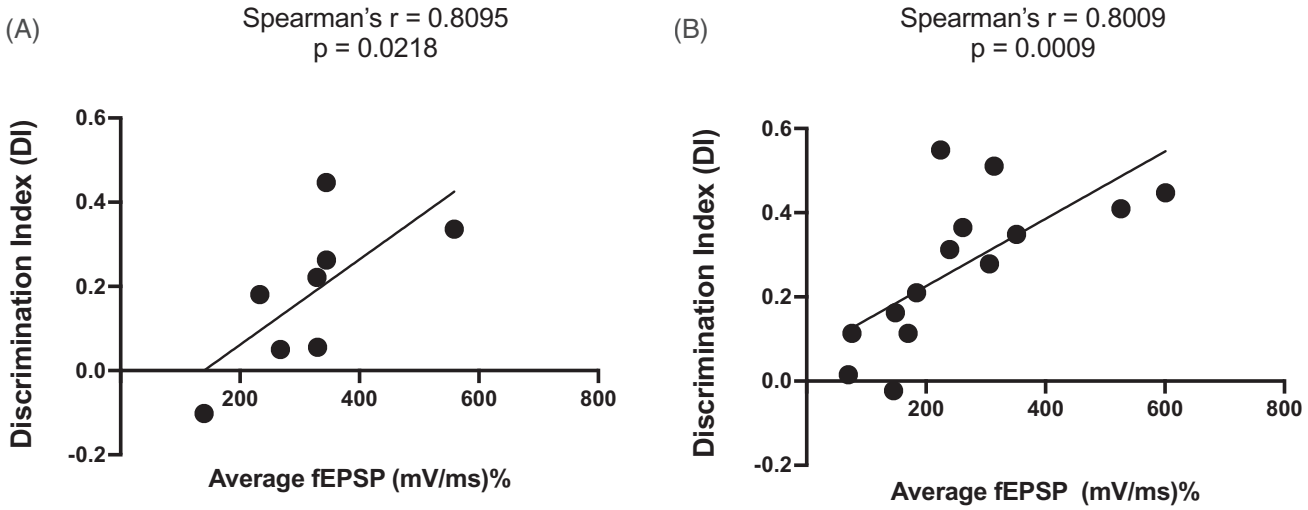

FIGURE 5 Within-animal correlations between ex vivo measurements of synaptic plasticity and in vivo measures of object recognition memory, in each generation. Statistically significant correlations were found between long-term potentiation (LTP; measured ex vivo by average field excitatory postsynaptic potential recordings [fEPSP] magnitude) and discrimination indices (measured in vivo by novel object preference) in both F0 (A) and F1 (B) mice. Spearman's correlation coefficients and *P* -values are shown for each generation

3.4 | In vivo measures of object recognition memory correlate with ex vivo measures of synaptic plasticity

In both F0 and F1 animal groups, there was a significant correlation (F0 Spearman r = 0.810, *P* = .022; F1 Spearman r = 0.801, *P* = .0009) between averaged LTP fEPSP responses and NOP-associated DIs, such that RHC-treated mice with CCH that exhibited higher DI also demonstrated heightened synaptic plasticity, similar to these same values in healthy controls, whereas untreated mice with CCH exhibited decreased synaptic plasticity and lower NOP-associated DIs (Figures 5A, 5B).

4 | DISCUSSION

Herein we provide the first evidence for the heritability of an epigenetically induced resilience to dementia. Specifically, we show in a mouse model of VCID that the protection afforded by an epigenetics-based treatment against two functional metrics of memory impairment after 3 to 4 months of disease is manifested not only in treated animals, but also in their first-generation offspring. These findings support the concept of adaptive epigenetic conditioning as a therapeutic strategy for reducing the burden of dementia both within and across generations.

While extended periods of hypoxia can be pathologic, appropriately titrated, intermittent exposures to hypoxia show therapeutic efficacy in a number of disease states, without evidence of injury by the hypoxia itself.[31–33] We previously demonstrated that 4 months of RHC did not affect the viability of hippocampal CA1 pyramidal cells, known to be highly sensitive to hypoxia.[34] Herein, neither mice treated directly with 2 months of RHC, nor their adult offspring, showed changes in white matter myelin density, neurocognitive function, or synaptic plasticity.

Given impairments in NOP observed in mice after 1 month of CCH,[24,35–37] it is not surprising that, in our study, 3 months of CCH significantly decreased DIs and novelty preferences based on exploration time. Both RHC-treated mice and the adult F1 offspring of RHC-treated parents were resilient to such CCH-induced deficits, with both cohorts exhibiting no loss of object recognition memory. Similarly, although hippocampal LTP was negatively affected after 4 months of CCH, RHC-treated animals and the adult F1 offspring of RHC-treated parents demonstrated no CCH-induced deficits in synaptic plasticity. Finally, we found strong within-animal correlations between ex vivo measures of LTP and in vivo assessments of recognition memory (NOP-associated DI) and in untreated and treated mice, in both generations, consistent with an RHC-induced maintenance of synaptic plasticity in the face of CCH, which in turn manifested functionally as neurocognitive protection.

Our results support growing evidence that epigenetics-based treatments, if repetitively administered, can provide long-lasting changes in phenotype that can counterbalance the pathological changes induced by chronic neurodegenerative diseases like dementia. Indeed, repetitive patterns of hypoxic conditioning lessen disease severity in several preclinical models of AD,[38–40] and two independent labs have documented the efficacy of "remote limb ischemic conditioning" (RLIC) in the same mouse model of VCID as we used herein.[41–43] In fact, given the successful preclinical history of RLIC in protecting against ischemic injury of brain, heart, and other tissues that are physically "remote" from the intermittently ischemic skeletal muscle,[44] small clinical trials of RLIC have reported success in patients with cerebral small-vessel disease,[45] subcortical ischemic vascular dementia,[46] chronic cerebral circulation insufficiency,[47] and poststroke cognitive impairment.[48] Moreover, one clinical study of patients with amnestic mild cognitive impairment showed efficacy of a systemic hypoxia therapy very similar to the one we used in mice (hypoxic challenges 3x/week over

8 weeks) in improving short-term memory and attention.[49] Despite their small sample sizes, studies like these reflect a growing awareness that distinct temporal patterns of systemic stimuli can trigger epigenetic responses in the brain, and perhaps in the immune system as well, that promote resilience to progressive dementia. However, no study prior to ours has documented that such an induced resiliency to disease may manifest across generations.

Although previous investigations have demonstrated intergenerational inheritance of epigenetically modified phenotypes,[50–52] the vast majority of research in this field has focused almost exclusively on the inheritance of unhealthy phenotypes, successfully linking repetitively presented stressors to the inheritance of increased disease susceptibility and disease itself.[53–56] To our knowledge, only three mammalian studies[57–59] have documented the inheritance of induced, beneficial phenotypes in offspring, but these related only to effects on resting, baseline health metrics. Specifically, environmental enrichment in parental mouse housing improved baseline memory in their F1 offspring,[57,58] and exposure of newborn male mice to unpredictable maternal separation and unpredictable maternal stress favored goal-directed behaviors and behavioral flexibility in their adult offspring.[59] However, our findings are the first to document the inheritance of an epigenetically induced phenotype that is protective against disease. We did not investigate whether the epigenetically induced, intergenerational resilience to VCID we documented in F1-generation mice extends to the F2 generation, or beyond, but there is evidence in mammals that, at least for detrimental phenotypes, such effects are possible.[60]

Activating a broad, endogenously driven resiliency to memory loss—rather than attempting to inhibit specific pathological mechanisms with an exogenous pharmaceutical—is a concept worthy of further investigation, particularly in light of our finding that such an epigenetic therapy promotes dementia resilience and perhaps a broader brain health across generations. Elucidating the many complex mechanisms underlying this induced, intergenerational protection against VCID was beyond the scope of our present proof-of-concept study. One obvious contributor would be an enhancement in cerebral blood flow (CBF), secondary to angiogenesis-driven collateralization and/or autoregulatory adjustments, that counters the reduction in perfusion through stenosed carotids; indeed, improvements in CBF (by laser speckle contrast imaging) and increases in capillary density were measured after 1 month of RLIC in this same VCID model.[41] Resilience may also be afforded by direct pro-survival adaptations that RHC induces in cells of the neurovascular unit, rendering them more resistant to the pathological consequences of CCH. The relative contributions of these yet-to-be-identified mechanisms may show generation-dependence as well, given that a previous genome-wide methylome analysis of DNA promoters in the prefrontal cortices of F0 mice treated with RHC, and F1 mice derived from F0 parents treated with RHC, revealed largely distinct methylomes relative to their respective generation-matched controls.[34] It is likely that all three primary epigenetic regulatory mechanisms—DNA methylation, histone post-translational modifications, and long-noncoding RNAs—are involved in establishing and maintaining disease-resilient phenotypes in the somatic cells of the F0 mice, as well as laying down the differentiation blueprint in F0 germ cells such that their F1 progeny recapitulate these disease-resilient phenotypes throughout adulthood.[61,62] Given the robustness of the induced resilience, we hypothesize that germ cell modifications in both parents are necessary to establish the broad and deep modifications in phenotype capable of protecting adult progeny against memory loss caused by CCH; we have yet to test this hypothesis, but such resilience may be transferred solely by the father or mother.[63]

Addressing several limitations of our study in future work will help solidify our novel findings. In addition to a variety of mechanism-focused investigations, expanding the battery of neurocognitive assessments used to assess dementia resilience—including the Barnes maze and Morris water maze tests, the radial arm maze test, the passive avoidance test, and others, would allow for the differentiation of short- and long-term memory, provide measures of working memory, and metrics of spatial learning as well, that our NOP test did not. In addition, the mice in our study and many others using this VCID model experienced CCH at roughly the human equivalent of middle-age; the clinical relevance of our findings would be significantly underscored by showing RHC-induced resilience in much older animals that more closely align with the human age demographic that generally experiences advancing dementia.

In conclusion, our study is the first to document that brief, repetitive exposures to systemic hypoxia can epigenetically reprogram gene expression to establish a dementia-resilient phenotype, resulting in adult offspring that are also protected against memory loss despite sustained cerebral hypoperfusion. The preservation of in vivo and ex vivo functional metrics of memory/synaptic plasticity we demonstrate herein represents the first mammalian evidence for the inheritance of an induced, disease-resilient, cerebroprotective phenotype. Related studies, based upon our findings, can fundamentally impact how current therapeutic approaches for reducing neurocognitive impairment in VCID are conceptualized, provide mechanistic insights into epigenetically mediated resilience to dementia, and set the stage for future investigations to further explore the public health implications of the inheritance of such beneficial phenotypes.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ORCID

Krystal Courtney D. Belmonte https://orcid.org/0000-0003-2997-0572
Jeff M. Gidday https://orcid.org/0000-0002-4641-0465

REFERENCES

1. O'Brien JT, Thomas A. Vascular dementia. *Lancet*. 2015;386:1698-1706.
2. Gorelick PB, Counts SE, Nyenhuis D. Vascular cognitive impairment and dementia. *Biochimica Et Biophysica Acta Bba - Mol Basis Dis*. 2016;1862:860-868.
3. Kalaria RN, Akinyemi R, Ihara M. Stroke injury, cognitive impairment and vascular dementia. *Biochimica Et Biophysica Acta Bba - Mol Basis Dis*. 2016;1862:915-925.
4. Iadecola C, Duering M, Hachinski V, et al. Vascular cognitive impairment and dementia - JACC Scientific Expert Panel. *J Am Coll Cardiol*. 2019;73:3326-3344.
5. Heron M. Deaths: leading Causes for 2017. *Natl Vital Stat Rep*. 2019;68(6):1-77.
6. Sprick JD, Mallet RT, Przyklenk K, Rickards CA. Ischaemic and hypoxic conditioning: potential for protection of vital organs. *Exp Physiol*. 2019;104:278-294.
7. Hao Y, Xin M, Feng L, et al. Review cerebral ischemic tolerance and preconditioning: methods, mechanisms, clinical applications, and challenges. *Front Neurol*. 2020;11:812.
8. Miller BA, Perez RS, Shah AR, Gonzales ER, Park TS, Gidday JM. Cerebral protection by hypoxic preconditioning in a murine model of focal ischemia-reperfusion. *NeuroReport*. 2001;12:1663-1669.
9. Stowe AM, Altay T, Freie AB, Gidday JM. Repetitive hypoxia extends endogenous neurovascular protection for stroke. *Ann Neurol*. 2011;69:975-985.
10. Wacker BK, Perfater JL, Gidday JM. Hypoxic preconditioning induces stroke tolerance in mice via a cascading HIF, sphingosine kinase, and CCL2 signaling pathway. *J Neurochem*. 2012;123:954-962.
11. Gidday JM. Cerebrovascular ischemic protection by pre- and post-conditioning. *Brain Circulation*. 2015;1:97-103.
12. Harman JC, Guidry JJ, Gidday JM. Intermittent hypoxia promotes functional neuroprotection from retinal ischemia in untreated first-generation offspring: proteomic mechanistic insights. *Invest Ophth Vis Sci*. 2020;61:1-22.
13. Pembrey M, Saffery R, Bygren LO, Network in Epigenetic Epidemiology. Human transgenerational responses to early-life experience: potential impact on development, health and biomedical research. *J Med Genet*. 2014;51:563-572.
14. Bowers ME, Yehuda R. Intergenerational transmission of stress in humans. *Neuropsychopharmacol*. 2016;41:232-244.
15. Zhu Y, Zhang Y, Ojwang BA, Brantley MA Jr, Gidday JM. Long-term tolerance to retinal ischemia by repetitive hypoxic preconditioning: role of HIF-1alpha and heme oxygenase-1. *Invest Ophth Vis Sci*. 2007;48:1735-1743.
16. Zhu Y, Zhang L, Schmidt JF, Gidday JM. Glaucoma-induced degeneration of retinal ganglion cells prevented by hypoxic preconditioning: a model of glaucoma tolerance. *Mol Med*. 2012;18:697-706.
17. Shibata M, Yamasaki N, Miyakawa T, et al. Selective impairment of working memory in a mouse model of chronic cerebral hypoperfusion. *Stroke*. 2007;38:2826-2832.
18. Shibata M, Ohtani R, Ihara M, Tomimoto H. White matter lesions and glial activation in a novel mouse model of chronic cerebral hypoperfusion. *Stroke*. 2004;35:2598-2603.
19. Nishio K, Ihara M, Yamasaki N, et al. A mouse model characterizing features of vascular dementia with hippocampal atrophy. *Stroke*. 2010;41:1278-1284.
20. Carzoli KL, Sharfman NM, Lerner MR, Miller MC, Holmgren EB, Wills TA. Regulation of NMDA receptor plasticity in the BNST following adolescent alcohol exposure. *Front Cell Neurosci*. 2019;13:440.
21. Miki K, Ishibashi S, Sun L, et al. Intensity of chronic cerebral hypoperfusion determines white/gray matter injury and cognitive/motor dysfunction in mice. *J Neurosci Res*. 2009;87:1270-1281.
22. Coltman R, Spain A, Tsenkina Y, et al. Selective white matter pathology induces a specific impairment in spatial working memory. *Neurobiol Aging*. 2011;32:2324.e7-2324.e12.
23. Ahn SM, Kim YR, Kim HN, Shin Y-I, Shin HK, Choi BT. Electroacupuncture ameliorates memory impairments by enhancing oligodendrocyte regeneration in a mouse model of prolonged cerebral hypoperfusion. *Sci Rep*. 2016;6:28646.
24. Ben-Ari H, Lifschytz T, Wolf G, et al. White matter lesions, cerebral inflammation and cognitive function in a mouse model of cerebral hypoperfusion. *Brain Res*. 2019;1711:193-201.
25. Antunes M, Biala G. The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cogn Process*. 2012;13:93-110.
26. Vogel-Ciernia A, Wood MA. Examining object location and object recognition memory in mice. *Curr Protoc Neurosci*. 2014;69:8.31.1-8.31.17.
27. Lueptow LM. Novel object recognition test for the investigation of learning and memory in mice. *J Vis Exp*. 2017;(126):e55718. https://doi.org/10.3791/55718
28. Moreno-Castilla P, Guzman-Ramos K, Bermudez-Rattoni F. Object recognition and object location recognition memory—The role of dopamine and noradrenaline. *Handb Behav Neurosci*. 2018;27:403-413.
29. Nicoll RA. A brief history of long-term potentiation. *Neuron*. 2017;93:281-290.
30. Sumner RL, Spriggs MJ, Muthukumaraswamy SD, Kirk IJ. The role of Hebbian learning in human perception: a methodological and theoretical review of the human visual long-term potentiation paradigm. *Neurosci Biobehav Rev*. 2020;115:220-237.
31. Navarrete-Opazo A, Mitchell GS. Therapeutic potential of intermittent hypoxia: a matter of dose. *Am J Physiology-Regulatory Integr Comp Physiology*. 2014;307:R1181-R1197.
32. Burtscher J, Mallet RT, Burtscher M, Millet GP. Hypoxia and brain aging: neurodegeneration or neuroprotection? *Ageing Res Rev*. 2021;68:101343.
33. Mallet RT, Burtscher J, Manukhina EB, et al. Hypoxic-hyperoxic conditioning and dementia. Diagnosis and Management in Dementia (Chapter 47) 2020:745-760.
34. Belmonte KCD, Harman JC, Lanson NA, Gidday JM. Intra- and intergenerational changes in the cortical DNA methylome in response to therapeutic intermittent hypoxia in mice. *Physiol Genomics*. 2020;52:20-34.
35. Patel A, Moalem A, Cheng H, et al. Chronic cerebral hypoperfusion induced by bilateral carotid artery stenosis causes selective recognition impairment in adult mice. *Neurol Res*. 2017;39:910-917.
36. Dominguez R, Zitting M, Liu Q, et al. Estradiol protects white matter of male C57BL6J mice against experimental chronic cerebral hypoperfusion. *J Stroke Cerebrovasc Dis*. 2018;27:1743-1751.
37. Miyanohara J, Kakae M, Nagayasu K, et al. TRPM2 channel aggravates CNS inflammation and cognitive impairment via activation of microglia in chronic cerebral hypoperfusion. *J Neurosci*. 2018;38:3520-3533.
38. Manukhina EB, Downey HF, Shi X, Mallet RT. Intermittent hypoxia training protects cerebrovascular function in Alzheimer's disease. *Exp Biol Med*. 2016;241:1351-1363.
39. Meng S, Wang B, Li W. Intermittent hypoxia improves cognition and reduces anxiety-related behavior in APP/PS1 mice. *Brain Behav*. 2020;10:e01513.
40. Correia SC, Machado NJ, Alves MG, Oliveira PF, Moreira PI. Intermittent hypoxic conditioning rescues cognition and mitochondrial bioenergetic profile in the triple transgenic mouse model of Alzheimer's disease. *Int J Mol Sci*. 2021;22:461.
41. Khan MB, Hafez S, Hoda MdN, et al. Chronic remote ischemic conditioning is cerebroprotective and induces vascular remodeling in a VCID model. *Transl Stroke Res*. 2018;9:51-63.

42. Khan MB, Hoda MN, Vaibhav K, et al. Remote ischemic postconditioning: harnessing endogenous protection in a murine model of vascular cognitive impairment. *Transl Stroke Res*. 2015;6:69-77.
43. Li X, Ren C, Li S, et al. Limb remote ischemic conditioning promotes myelination by upregulating PTEN/Akt/mTOR signaling activities after chronic cerebral hypoperfusion. *Aging Dis*. 2017;8:392-401.
44. Hess DC, Blauenfeldt RA, Andersen G, et al. Remote ischaemic conditioning—a new paradigm of self-protection in the brain. *Nat Rev Neurol*. 2015;11:698-710.
45. Wang Y, Meng R, Song H, et al. Remote ischemic conditioning may improve outcomes of patients with cerebral small-vessel disease. *Stroke*. 2017;48:3064-3072.
46. Liao Z, Bu Y, Li M, et al. Remote ischemic conditioning improves cognition in patients with subcortical ischemic vascular dementia. *BMC Neurol*. 2019;19:206.
47. You J, Feng L, Bao L, Xin M, Ma D, Feng J. Potential applications of remote limb ischemic conditioning for chronic cerebral circulation insufficiency. *Front Neurol*. 2019;10:467.
48. Feng X, Huang L, Wang Z, et al. Efficacy of remote limb ischemic conditioning on poststroke cognitive impairment. *J Integr Neurosci*. 2019;18:377.
49. Wang H, Shi X, Schenck H, et al. Intermittent hypoxia training for treating mild cognitive impairment: a pilot study. *Am J Alzheimer's Dis Other Dementias*. 2020;35:1533317519896725.
50. Skvortsova K, Iovino N, Bogdanović O. Functions and mechanisms of epigenetic inheritance in animals. *Nat Rev Mol Cell Bio*. 2018;19:774-790.
51. Cavalli G, Heard E. Advances in epigenetics link genetics to the environment and disease. *Nature*. 2019;571:489-499.
52. Perez MF, Lehner B. Intergenerational and transgenerational epigenetic inheritance in animals. *Nat Cell Biol*. 2019;21:143-151.
53. Nelson VR, Nadeau JH. Transgenerational genetic effects. *Epigenomics-UK*. 2010;2:797-806.
54. Bohacek J, Mansuy IM. Epigenetic inheritance of disease and disease risk. *Neuropsychopharmacol*. 2013;38:220-236.
55. Guerrero-Bosagna C, Skinner MK. Environmentally induced epigenetic transgenerational inheritance of phenotype and disease. *Mol Cell Endocrinol*. 2012;354:3-8.
56. Saab BJ, Mansuy IM, Davies SA, Dow JAT, Lukowiak K. Neurobiological disease etiology and inheritance: an epigenetic perspective. *J Exp Biol*. 2013;217:94-101.
57. Arai JA, Li S, Hartley DM, Feig LA. Transgenerational rescue of a genetic defect in long-term potentiation and memory formation by juvenile enrichment. *J Neurosci*. 2009;29:1496-1502.
58. Benito E, Kerimoglu C, Ramachandran B, et al. RNA-dependent intergenerational inheritance of enhanced synaptic plasticity after environmental enrichment. *Cell Reports*. 2018;23:546-554.
59. Gapp K, Soldado-Magraner S, Alvarez-Sánchez M, et al. Early life stress in fathers improves behavioural flexibility in their offspring. *Nat Commun*. 2014;5:5466.
60. van Steenwyk G, Roszkowski M, Manuella F, Franklin TB, Mansuy IM. Transgenerational inheritance of behavioral and metabolic effects of paternal exposure to traumatic stress in early postnatal life: evidence in the 4th generation. *Environ Epigenetics*. 2018;4:dvy023.
61. Harrison JS, Cornett EM, Goldfarb D, et al. Hemi-methylated DNA regulates DNA methylation inheritance through allosteric activation of H3 ubiquitylation by UHRF1. *eLife*. 2016;5:e17101.
62. Zhang W, Song M, Qu J, Liu G-H. Epigenetic modifications in cardiovascular aging and diseases. *Circ Res*. 2018;123:773-786.
63. Chan JC, Nugent BM, Bale TL. Parental advisory: maternal and paternal stress can impact offspring neurodevelopment. *Biol Psychiat*. 2018;83:886-894.

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