Aerobic Exercise Preserves Capillary Oxygen Homeostasis and Neurovascular Function in a Mouse Model of Alzheimer's Disease

Alfredo Cardenas-Rivera *[+]; Eda Erdogmus *; Austin Birmingham *[&]; Evan Yee *; Jaime Anton *; Zhengyi Lu *; Yuntao Li *; Matthew Morais *; Nazila Loghmani *; Chang Liu *; Mohammad A. Yaseen *

*Department of Bioengineering, Northeastern University, Boston, MA, USA
[&]Weill Cornell Medical College, New York, NY, USA
[+]Centro de Investigación en Bioingeniería, Universidad de Ingeniería y Tecnología (UTEC), Lima, Peru

Abstract

Age-related cerebrovascular dysfunction is increasingly recognized as a critical contributor to cognitive decline and Alzheimer's disease (AD) progression [1,2], yet the extent to which lifestyle interventions preserve microvascular oxygen delivery remains poorly defined. Physical activity (PA) improves vascular health and cognition in humans [3–7], but its impact on capillary-level oxygenation and functional hyperemia during aging and amyloid pathology is unknown. Here, we longitudinally quantified microvascular oxygen tension and stimulus-evoked oxygen dynamics in awake APP/PS1 mice and wild-type littermates using two-photon phosphorescence lifetime microscopy. Chronic aerobic PA initiated in early adulthood preserved basal arteriolar, capillary, and venular oxygenation, prevented age-dependent increases in microvascular heterogeneity, and mitigated excessive oxygen extraction in preclinical AD mice. While amyloid pathology impaired stimulus-evoked oxygen responses across vascular compartments, PA selectively enhanced capillary dilation and accelerated hyperemic kinetics without altering vascular density or architecture. Notably, sedentary AD mice developed widespread capillary hypoxia and bimodal oxygen distributions, hallmarks of malignant microvascular dysfunction, which were largely absent in physically active animals. These findings demonstrate that routine aerobic PA preserves microvascular oxygen homeostasis and functional responsiveness during aging and amyloid accumulation, supporting a capillary-centric mechanism through which exercise confers neurovascular resilience in preclinical AD.

1. INTRODUCTION

Alzheimer's disease (AD) is traditionally defined by the accumulation of amyloid-β (Aβ) plaques and tau pathology [8–12]; however, mounting evidence indicates that cerebrovascular dysfunction precedes and

accelerates neurodegeneration [1,13–16]. Alterations in cerebral blood flow, oxygen extraction, and neurovascular coupling are detectable in cognitively normal individuals [2,17–21] at risk for AD and are strongly associated with early cognitive impairment [16,22–24]. Functional neuroimaging studies consistently report aberrant blood-oxygen-level-dependent (BOLD) responses during aging and preclinical AD [25–27], yet the microvascular mechanisms underlying these changes remain incompletely understood.

The cerebral microvasculature, particularly the capillary network, plays a central role in oxygen delivery, metabolic exchange, and clearance of toxic metabolites, including Aβ [28,29]. Emerging work suggests that capillary dysfunction—manifested as impaired perfusion, increased flow heterogeneity, and stalled red blood cells—can generate localized hypoxia and metabolic stress even in the absence of overt vascular rarefaction [30,31]. Such microvascular disturbances are not uniformly distributed but instead create microscopic territories of impaired oxygen delivery, a phenomenon increasingly implicated in both normal aging and AD pathogenesis [32]. Despite this, most studies of cerebrovascular aging rely on bulk measures of blood flow or oxygenation, obscuring capillary-scale dysfunction that may critically shape neuronal vulnerability.

Aerobic physical activity (PA) is one of the most robust non-pharmacological interventions known to preserve cognitive function across the lifespan. In humans, PA improves executive function, memory, and processing speed, while reducing the risk of dementia [33–36]. At the vascular level, PA enhances endothelial function, arterial compliance, and systemic oxygen delivery [35,37–42]. In the brain, exercise has been shown to improve cerebral blood flow, reduce neuroinflammation, and attenuate amyloid burden in animal models [43–49]. However, whether PA directly preserves microvascular oxygen delivery and homogenizes capillary oxygenation during aging and AD progression remains unresolved.

Critically, neurovascular coupling—the process by which neuronal activity drives local increases in blood flow and oxygen delivery—relies on coordinated signaling among endothelial cells, vascular smooth muscle cells, and pericytes [18,50–52]. These cellular elements are particularly vulnerable to Aβ, which disrupts vasomotor control, capillary dilation, and oxygen extraction efficiency [16,30,53–59]. At the same time, PA is known to modulate the phenotype and function of these vascular cells, suggesting that exercise may exert its neuroprotective effects, at least in part, through restoration of capillary-level vascular dynamics rather than gross structural remodeling.

Here, we test the hypothesis that chronic aerobic PA preserves microvascular oxygen homeostasis and functional hyperemia during aging and amyloid pathology. Using longitudinal two-photon

65 phosphorescence lifetime microscopy in awake mice, we directly measured intravascular oxygen tension,
66 oxygen extraction fraction, and stimulus-evoked oxygen responses across arterioles, capillaries, and
67 venules in APP/PS1 mice and wild-type controls. By combining high-resolution oxygen measurements
68 with functional stimulation and vascular morphology analysis, we delineate how PA modifies capillary
69 oxygen delivery, heterogeneity, and responsiveness independently of vascular density. Our findings
70 identify capillary oxygen dysfunction as a key target of PA and provide mechanistic insight into how
71 exercise may counteract early neurovascular impairments in preclinical AD.
72
73 **3. RESULTS**
74
75 Physical exercise reportedly enhances systemic vascular function and memory in healthy adult
76 humans [60]. To determine whether physical activity mitigates cerebrovascular alterations during aging
77 and preclinical AD, we longitudinally measured microvascular oxygenation in the somatosensory cortices
78 of awake, head-restrained mice. Male APP/PS1dE9 transgenic (AD) mice and wild-type littermates (WT)
79 were categorized into 4 cohorts. From age 6-12 months, 2 cohorts (AD-PA and WT-PA) underwent
80 structured treadmill running exercise (30 minutes/day, 5 days/week; Fig. 1A), while the remaining cohorts
81 remained sedentary (AD-noPA and WT-noPA). Using two-photon phosphorescence lifetime microscopy
82 and the phosphorescent oxygen sensitizer Oxyphor2P, intravascular oxygen tension (pO_2) was quantified
83 up to 375 μm below the cortical surface in response to somatosensory whisker stimulation after 1 month
84 (phase 1) and 6 months (phase 2). The influences of Aβ pathology, PA, and age were evaluated using
85 linear mixed-effect modelling.
86
87 **3.a Basal oxygenation**

88 Figure 2 displays baseline intravascular pO2 from cortical arterioles, capillaries, and venules of each
89 experimental cohort. After one month (Phase 1), both AD and WT mice undergoing PA demonstrated
90 significantly higher levels of arteriolar oxygen compared to their sedentary counterparts (Fig. 1A).
91 Significant differences in arteriolar pO2 were sustained between PA and sedentary cohorts after 6 months
92 (phase 2); however, arterial pO2 also declined with age in both AD mouse cohorts. In WT animals,
93 arteriolar pO2 at phase 2 was significantly higher for the WT-PA animals relative to WT-noPA.

At the capillary level, where the majority of metabolite exchange and Aβ clearance reportedly transpires [61], PA yielded pronounced differences in AD mice at Phase 2. Both WT-PA and AD-PA animals had significantly higher baseline capillary oxygenation at phase 2 relative to phase 1. Conversely, AD-noPA mice demonstrated a pronounced decline in capillary oxygenation over 6 months, and capillary pO2 did not change significantly in WT-noPA mice. Similar trends were observed in the venous compartment. In AD-noPA mice, baseline venular oxygenation decreased significantly. In all other cohorts, venous pO2 increased significantly between phases 1 and 2.

Microvascular heterogeneity has recently emerged as an important indicator of pathological disturbances, indicating microscopic cortical territories with disrupted metabolic supply [62,63]. The distribution profiles and coefficients of variations (C.V.) of baseline intravascular pO2 in Figs 1.d-g highlight how Aβ pathology, age, and PA alter microvascular heterogeneity through their impacts on dispersity of capillary and venous oxygenation. In all groups during Phase 1, the distributions of capillary oxygen tension exhibited a unimodal Gaussian profile centered around ~50 mmHg with similar C.V. at 7 months. By 12 months, capillary pO2 within sedentary AD mice differed substantially from the other cohorts, displaying a shifted, bimodal distribution. The lower, broader distribution is consistent with widespread, heterogeneous capillary hypoxia. Venular oxygen distributions paralleled these trends, remaining unimodal (~45 mmHg) at 7 months and becoming bimodal in sedentary AD mice at 12 months, with distinct modes at ~23 mmHg and ~45 mmHg. In AD-PA mice, PA mitigated increases in malignant capillary and venous heterogeneity, maintaining values comparable to those observed in age-matched WT controls (Fig. 2E,G).

We calculated oxygen extraction fraction ($OEF = \frac{SO_{2_artery} - SO_{2_vein}}{SO_{2_artery}} \times 100\%$) to quantify the amount of oxygen delivered at each cortical depth. Microvascular pO2 measurements were converted to oxygen saturation using the Hill equation [64]. Figs 2.H-I depict average and depth-resolved OEF in each cohort at each phase. At phase 1, all groups demonstrated OEFs ranging around 40% that decreased slightly with cortical depth, in agreement with prior studies of 3-6 month old awake mice [65–67]. Between phases 1 and 2, AD-noPA animals experienced significant increases in OEF across all layers, while all other cohorts showed slight reductions. At phase 2, OEF in animals with PA appeared lower than their sedentary counterparts.

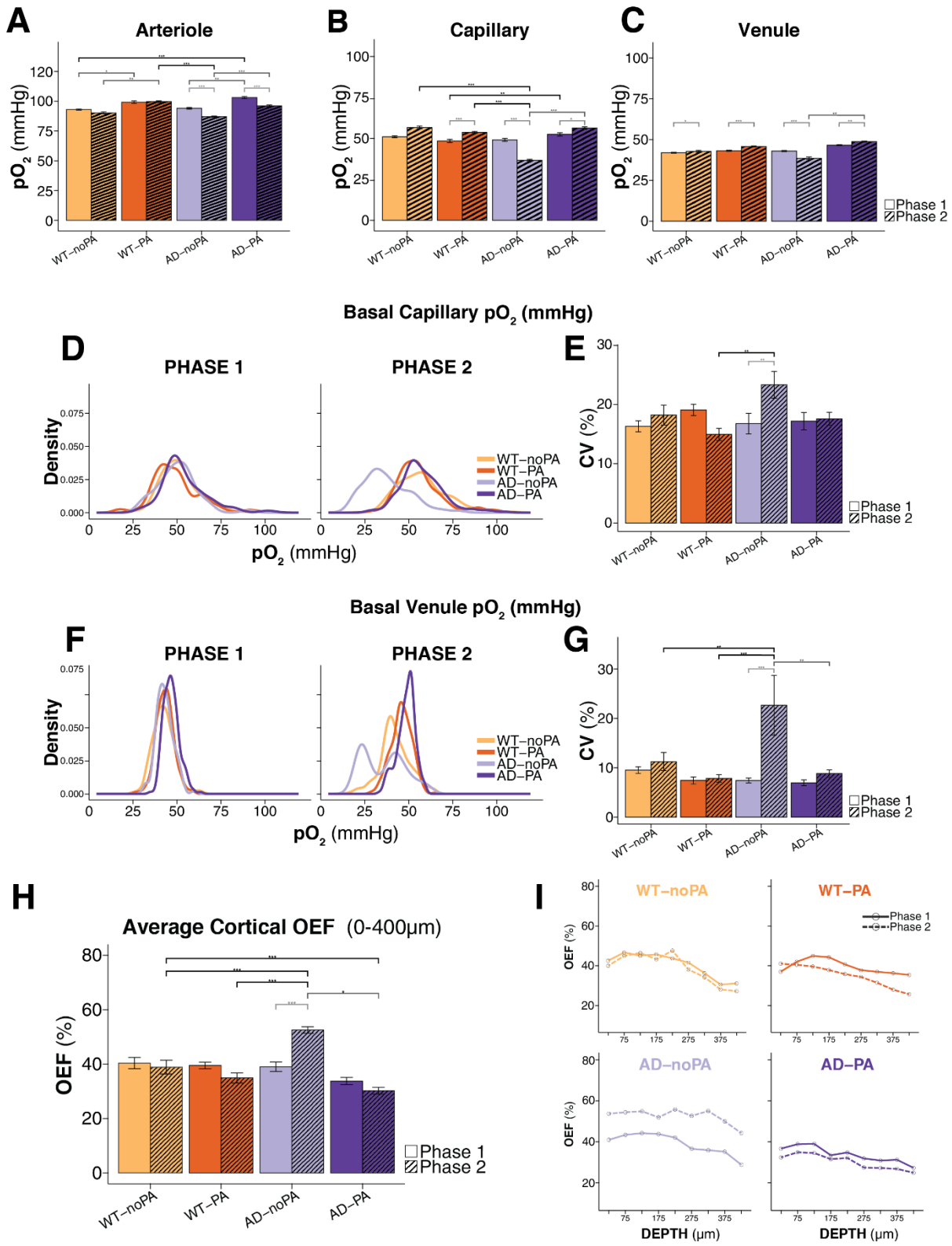
A
Arteriole
B
Capillary
C
Venule
pO2 (mmHg)
WT–noPA
WT–PA
AD–noPA
AD–PA
Phase 1
Phase 2
Basal Capillary pO2 (mmHg)
D
PHASE 1
PHASE 2
Density
pO2 (mmHg)
E
CV (%)
Basal Venule pO2 (mmHg)
F
G
H
Average Cortical OEF (0-400μm)
OEF (%)
I
WT–noPA
WT–PA
AD–noPA
AD–PA
DEPTH (μm)

Figure 1 Basal microvascular pO_2 in AD and wildtype mice ***(A)*** Arteriolar cortical oxygenation remains stable between 7 months (phase 1) and 12 months (phase 2) in wild-type (WT) mice, but is significantly reduced in 12-month-old AD mice. Physical activity (PA) induces an early and sustained increase in arteriolar oxygenation. ***(B)*** Capillary oxygenation increases with aging in WT mice and is markedly reduced in AD-noPA mice at 12 months; this reduction is prevented by PA. ***(C)*** Venular oxygenation remains stable across ages and groups, except for a significant decline in AD-noPA mice at 12 months. ***(D-G)*** Basal pO_2 distributions at the capillary and venular levels are similar for all groups at 7 months. AD-noPA mice at 12 months display a left-shifted, bimodal distribution, while other groups maintain a narrow, unimodal profile. ***(H-I)*** The cortical oxygen extraction fraction (OEF) increases for AD-noPA mice between phases 1 and 2. Routine PA yielded lower cortical OEFs in WT and AD mice at phase 2.

3.b Functional Hyperemia

We applied intravital OISI and 2P-PLIM to assess stimulus-evoked hyperemic responses in microvascular pO_2 and the impacts of physical activity PA, Aβ, and age. We measured transient changes in cortical blood volume and intravascular pO2 in response to 3-second pneumatic whisker deflection. OISI using 532 nm reflectance was first applied to identify the activated cortical region. Stimulation trials were then conducted to measure pO2 in individual microvessels from the cortical surface down to 350 µm in 50 µm increments. Linear mixed effect modeling revealed no significant depth-related differences. As a result, data from cortical layers were combined.

Figs. 3a-c displays OISI-based measurements of cerebral blood volume changes after 0 months (baseline), 1 month (phase 1), and 6-months of aerobic PA. Reductions in 532 nm OISI reflectance intensity correspond to increases in HbT, allowing simple quantification of cerebral blood volume [68]. Linear mixed effects analysis revealed no significant differences between experimental groups or phases for hyperemic blood volume changes. Figs 3d-e highlight group-averaged capillary pO_2 transients at phases 1 and 2, obtained by 2P-PLIM. In all measurements, capillary pO2 reached maximal levels shortly after stimulus cessation, slower than peak changes in blood volume. In WT animals, peak amplitude of capillary pO2 remained unaffected by age or PA. Conversely, both AD-PA and AD-noPA mice demonstrated markedly lower peak responses at 12 months of age compared to 7 months. Figs 3 g-h display maximum stimulus-induced pO2 in arterioles, capillaries, and venules, resolved into baseline pO2 and stimulus-induced hyperemic increases (colored bars and white bars, respectively). Statistical bars in figs 3. g-h indicate significant differences in maximum pO2 during stimulation. In sedentary AD mice, maximal pO2 were significantly lower in older animals (phase 2) within all vascular compartments. While in both sedentary and active WT animals, peak pO2 either increased (capillaries and venules) or remained unchanged.

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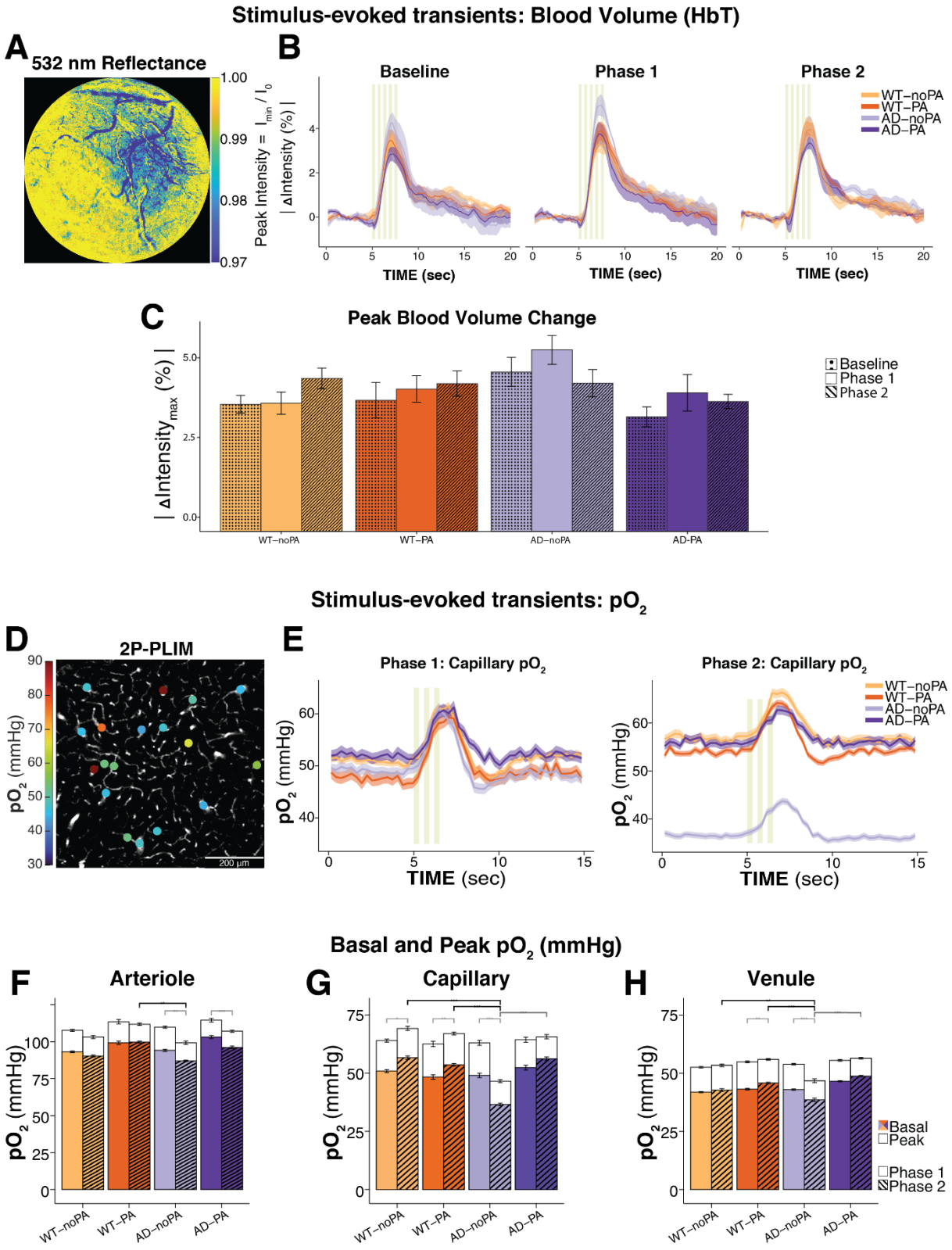
Stimulus-evoked transients: Blood Volume (HbT)
A
532 nm Reflectance
1.00
0.99
0.98
0.97
Peak Intensity = I_min / I_0
B
Baseline
Phase 1
Phase 2
| ΔIntensity (%) |
TIME (sec)
WT–noPA
WT–PA
AD–noPA
AD–PA
C
Peak Blood Volume Change
| ΔIntensity_max (%) |
Baseline
Phase 1
Phase 2
Stimulus-evoked transients: pO2
D
2P-PLIM
pO2 (mmHg)
200 µm
E
Phase 1: Capillary pO2
Phase 2: Capillary pO2
TIME (sec)
Basal and Peak pO2 (mmHg)
F
Arteriole
G
Capillary
H
Venule
Basal
Peak
Phase 1
Phase 2

159

Figure 2 Hyperemia dynamics during functional stimulation (**A-C**) Representative OISI image and group-averaged transients of 532 nm reflectance intensity. Pneumatic whisker stimulation provoked localized increases in blood volume within the somatosensory cortex. Hyperemic blood volume changes did not vary significantly between experimental groups or phases ***(D-E)*** Pointwise 2P-PLIM measurements in cortical microvessels. 6 months of routine aerobic PA protects AD mice from reductions in capillary pO2 ***(F-H)*** Alterations in basal and peak pO2 in arterioles, capillaries, and venules. Statistical bars in figs f-h indicate significant differences in maximum pO2 during stimulus trials. Peak differences in arteriolar oxygenation are higher in sedentary groups of both genotypes. In capillaries, AD-noPA mice display lower baseline pO2 but larger ΔpO2 than AD-PA at both 12 months. Venular oxygenation peaks increased slightly between phases 1 and 2 in WT-PA mice. PA prevented reductions in venous oxygenation in AD-mice.

To comprehensively assess variations in total blood oxygen, vascular pO2 was converted to oxygen saturation (SO2) using the Hill equation [64,66]. Fig 3 displays group-averaged SO2 transients for each vascular compartment and the calculated metrics for stimulus-evoked hyperemia ($\Delta SO2 = SO2_{peak} - SO2_{baseline}$, AUC = area under curve). In arterioles, baseline SO2 levels at phase 2 were slightly higher for WT-PA and AD-PA animals relative to the noPA groups. In all groups except AD-PA, functional stimulation evoked smaller increases in vascular oxygenation at phase 2, as indicated by ΔSO2 and AUC plots (figs 3.a and b).

Our observations indicate more pronounced alterations related to PA, Aβ, and age within capillary vessels. In WT animals, stimulus-evoked capillary SO2 changes were lower at phase 2 relative to phase 1, and WT-PA animals showed slightly higher SO2 changes compared to WT-noPA animals. While AD-PA animals maintained higher baseline SO2 levels, stimulus-evoked changes in SO2 were smaller in AD-PA animals and reduced further between phases 1 and 2. Interestingly, hyperemic responses in AD-PA animals displayed faster kinetics compared to the other groups, evidenced by lower rise times and fall times. Both WT-PA and AD-PA animals showed slightly faster responses than their sedentary counterparts. These differences were more pronounced at phase 2 compared to phase 1. While further investigation is required, the results could indicate PA- and age-related variations in pericyte-mediated capillary constriction and dilation.

In venules, observations of baseline and stimulus-evoked SO2 responses appeared to follow those of the capillary vessels (figs 3.e-f). AD-noPA animals showed lower baseline SO2 compared to AD-PA animals, and these differences were more pronounced at phase 2. Stimulus-evoked hyperemic SO2 changes were lower at phase 2 relative to phase 1. Interestingly in both PA groups, we observed age-related variations in fall time of the SO2 response. Both PA animals showed faster fall times at phase 2 compared to phase 1.

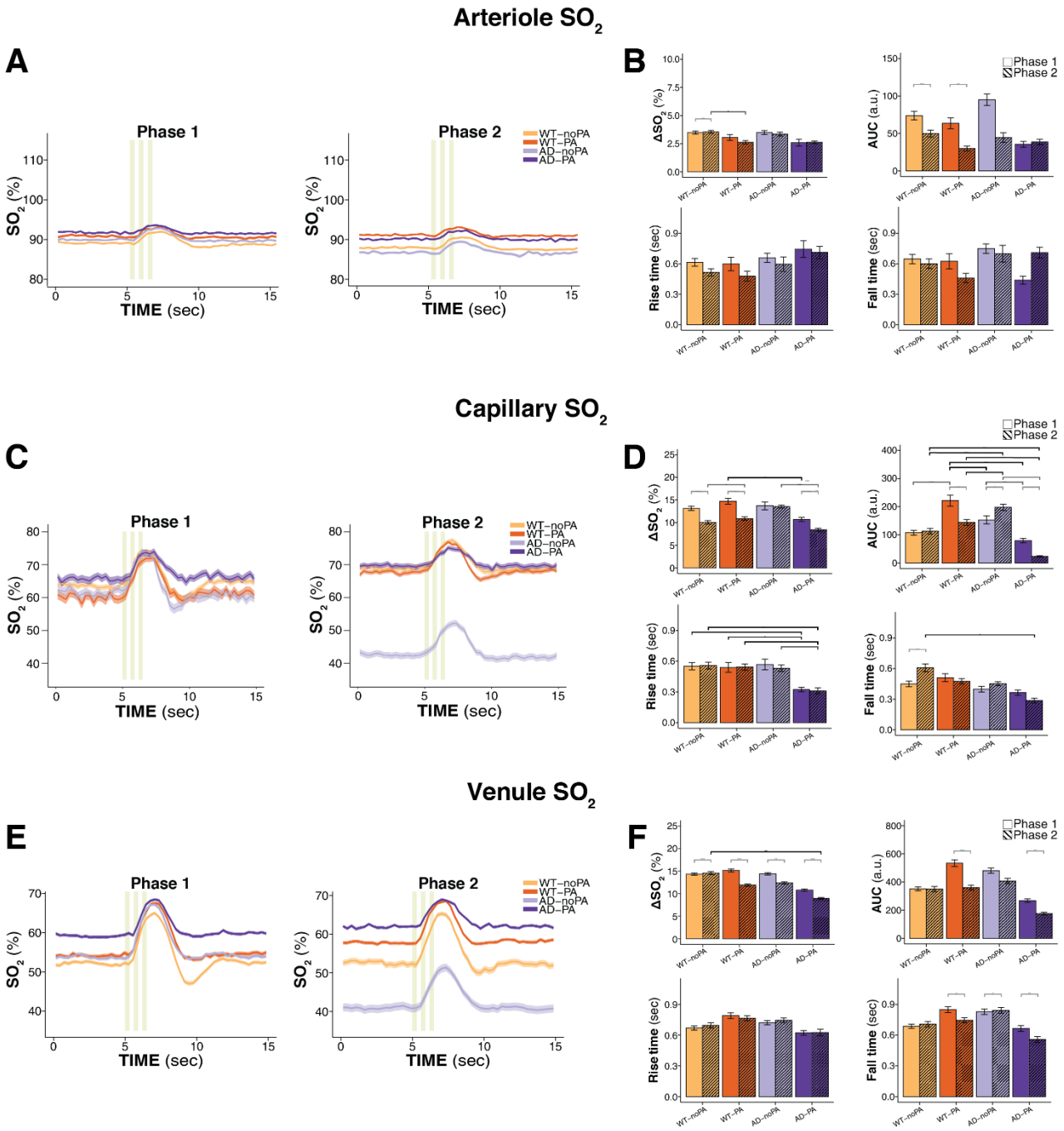

Figure 4 Microvascular SO_2 transients during whisker stimulation in wild-type and AD mice under aging and physical activity: ***(A-B)*** Arteriolar oxygen transients show higher basal SO_2, but smaller stimulus-evoked increases, in both AD-PA and WT-PA groups. For all groups, functional stimulation evoked smaller arteriolar SO_2 changes at phase 2 than phase 1. ***(C-D)*** In capillary vessels, Aβ and aging yielded reductions in basal SO_2 in AD-noPA mice. Routine PA protected AD-PA mice from reductions in basal SO_2. In WT animals, hyperemic increases in SO2 were higher in PA animals compared to sedentary noPA animals, while in AD animals, PA animals responded to stimulation with smaller, yet faster changes in SO2. (**E-F**) In venules, differences in basal SO2 were higher in WT-PA and AD-PA animals at phase 2. Functional stimulation evoked smaller venous SO_2 changes at phase 2 than phase 1.

205 At Phase 2, after 6-months of PA, we applied rapid, high-speed line-scanning to monitor capillary diameter
206 during whisker stimulation trials (Fig. 4A). Within the activated cortical region, we observed two distinct
207 populations of capillaries. One set demonstrated the conventional stimulus-evoked vasodilation in
208 response to pneumatic air puffs ("responsive" capillaries; Fig. 4C), while the other set of capillaries
209 exhibited no dilatory response ("non-responsive" capillaries; Fig. 4B). Fig 4.d-e display resting, pre-
210 stimulus diameters for both capillary populations, along with their relative proportions for each cohort at
211 phase 2. For all four experimental cohorts, no appreciable differences were detected between responsive
212 and non-responsive capillary populations for resting state diameters and their relative proportions. In all
213 cases, average capillaries diameters remained tightly distributed around 4.9 μm. Fig 4.f displays peak
214 stimulus-induced dilation of responsive capillaries in both WT and AD mice. In both cohorts, mice that
215 underwent six months of PA demonstrated significantly larger peak dilation relative to their sedentary
216 counterparts. Intriguingly, responsive capillaries in AD mice demonstrated larger dilations compared to
217 WT mice, despite having similar baseline capillary diameters. The maximal dilation occurred in response
218 to the first air-puff across all experimental groups (Fig. 4C), while the maximal oxygenation peak occurred
219 following the final air-puff (Fig. 4A). The area under the curve (AUC) of capillary diameter change
220 indicates a greater overall vascular response in PA mice, compared to sedentary animals (Fig. 4E). AD
221 mice exhibited larger AUC values than WT counterparts, with the most pronounced response observed in
222 the AD-PA group. Fig 4.H quantifies the heterogeneity in peak dilation among responsive capillaries. AD
223 mice showed higher, yet not significantly different, heterogeneity in stimulus-evoked diameter changes.

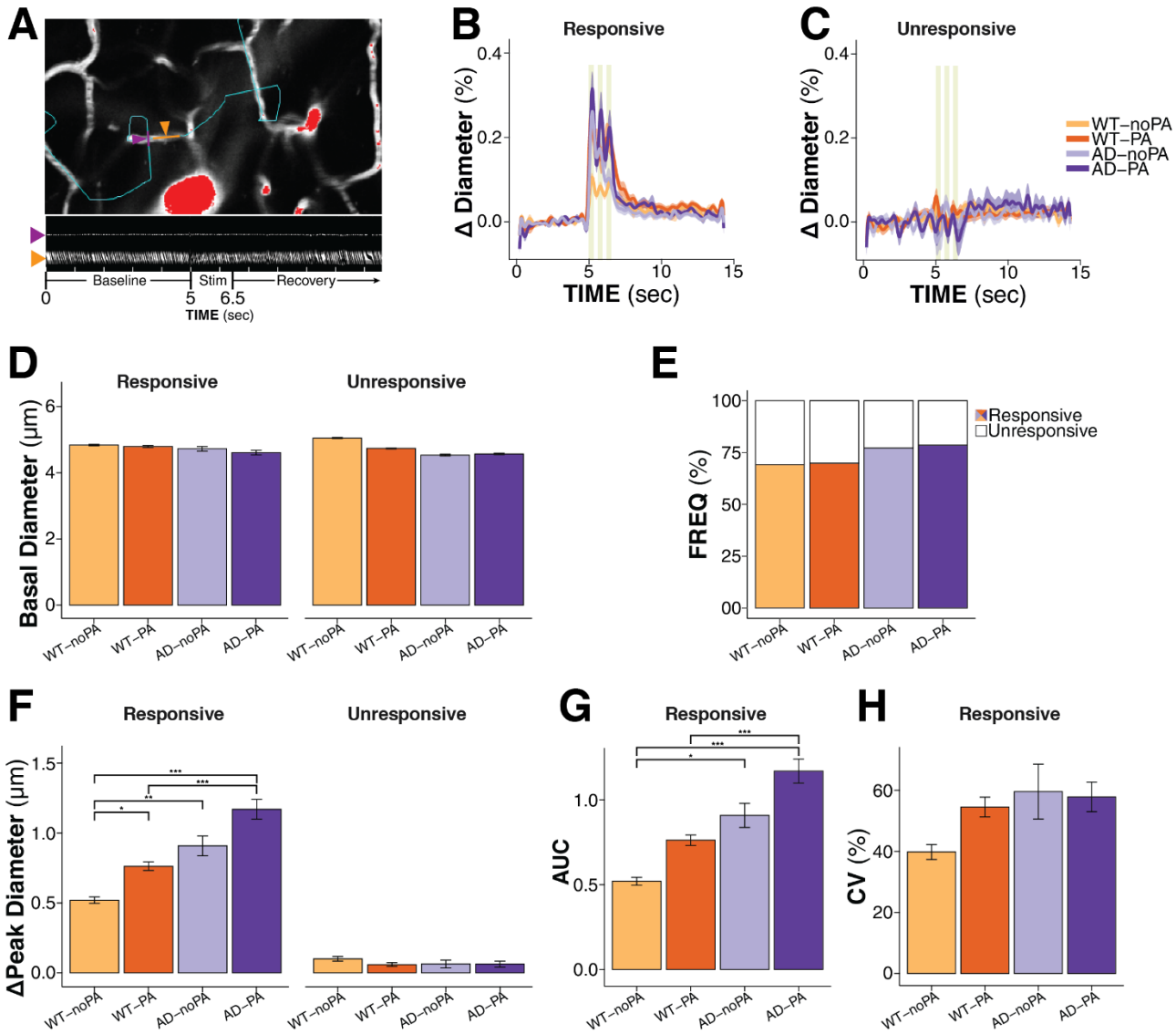

Figure 5 Capillary dilation responses during functional activation in wild-type and AD mice at Phase 2 (age 12 months). ***(A)*** Line-scan imaging protocol used to measure rapid vascular dynamics during whisker stimulation. ***(B–C)*** Two distinct subtypes of capillary responses were identified: responsive vessels exhibiting rapid, stimulus-locked dilations to each pneumatic air-puff and (**C**) non-responsive vessels displaying no discernible change in diameter. ***(D, E)*** Baseline diameters for responsive and non-responsive capillaries at phase 2. ***(F-G)*** Quantitative analysis of stimulus-evoked capillary dilation, area under the curve (AUC), and heterogeneity revealed larger vasodilatory responses in trained AD-PA and WT-PA mice after 6 months of regular physical activity ***(H)*** computed coefficients of variation from responsive capillaries indicate no differences in heterogeneity of capillary dilation.

238 **3.c Microvascular morphology**

239 At phase 2, we quantified features of microvascular angiography to assess the influences of Aβ pathology
240 and 6 months routine PA. High-resolution z-stacks of cortical microvessels (~450 μm thick at 1 μm
241 intervals) were acquired using Dextran-labeled fluorescein. We performed segmentation and analysis of
242 the vascular image stacks using Matlab-based VIDA software package [69]. Supplementary figure #
243 displays example stack images for each cohort and computed morphological metrics for the vascular
244 stacks. Although linear mixed effect modeling identified differences at specific cortical depths between
245 some experimental groups, we observed no discernible trends in vascular density, vessel length, cross
246 sectional area of microvessels, or total vascular sufrace between sedentary and trained groups, irrespective
247 of genotype. These results indicate that neither Aβ accumulation nor chronic exercise appreciably alter
248 microvascular architecture in the barrel cortex at age 12 months.

249 **DISCUSSION**

250 Numerous studies have demonstrated that routine engagement in aerobic physical activity (PA) helps
251 maintain and improve cognition and memory in aging humans and animals. In particular PA, offers
252 benefits for processing speed, attention, and executive control [70,71]. However, a complete
253 understanding of PA's effects on cellular and vascular physiology in the brain remains unclear. Our
254 findings provide compelling evidence that routine aerobic PA alleviates Aβ's detrimental effects on
255 cortical oxygen supply, oxygen extraction fraction, vascular heterogeneity, and vascular responses to
256 functional stimulation. These results offer novel insights into PA's neurovascular implications highlighted
257 in previous studies in healthy humans and AD patients [60,72–74]. Specifically, our findings indicate that
258 both AD pathology and PA do not affect the vascular network uniformly. The results offer greater insight
259 regarding variations between healthy aging and AD progression observed via fMRI BOLD neuroimaging.

260 We observed age-associated reductions in basal oxygen tension across the cortical vascular network in
261 sedentary APP/PS1 mice (AD-noPA), extending from arteriolar inflow to venular outflow. Though the
262 mechanism remains unclear, the persistent reduction in arteriolar pO_2 is consistent with earlier reports that
263 attribute vascular abnormalities to cerebral amyloid angiopathy (CAA) along cerebral arteries. In the
264 APP/PS1 mouse model, CAA begins to manifest around six months of age and has been shown to promote
265 multiple impairments to arterial structure and function, including degeneration of vascular smooth muscle

266 cells (vSMCs), compromised integrity of the periarterial basement membrane, impaired vessel wall
267 compliance, and vasomotor function [75]. Our basal pO2 observations in AD-noPA mice demonstrated
268 more substantial alterations in the capillary and venous compartments at age 12 months (phase 2). In
269 addition to significant age-related reductions in basal pO_2, the observed increases in heterogeneity of
270 capillary and venous pO_2 reflect variable, uneven perfusion and functional shunting within the capillary
271 beds. Such alterations purportedly result in microscopic territories of hypoxic tissue as blood flow and
272 oxygen availability drops [62,63,76]. We observed no depth-dependent differences; however, our OEF
273 observations agree well with prior 2P-PLIM observations in awake female C57BL/6, 3–5 months
274 mice [65,66]. In our AD-noPA mice, OEF increased substantially between ages 7 months (phase 1) and
275 12 months (phase 2). Conversely, based on bolus tracking measurements and Jespersen & Østergaard's 3-
276 compartment model, Gutiérrez-Jimenez et al computed lower OEF values in anesthetized APP/PS1 mice
277 and wildtype littermates at age 18-months [62,77]. The OEF disparities are likely attributable to different
278 measurement methods, anesthesia-induced alterations to cerebral energy metabolism and blood flow, and
279 age-related variations in cerebral blood flow. When taken together, however, the findings from our study
280 and Gutiérrez-Jimenez highlight how capillary function and perfusion each vary independently with age
281 and during AD progression. The resultant age-dependent alterations in cerebral blood flow, metabolism,
282 and oxygen extraction likely account for differences in fMRI BOLD response for pre-symptomatic,
283 subclinical, and demented AD patients [76].

284 Routine aerobic PA yielded significant increases in basal arteriolar pO2 in both WT and AD mice at
285 phases 1 and 2. In capillaries and venules, PA prevented or mitigated reductions in basal pO2 in AD mice
286 at phase 2. Our analyses indicate no PA-induced changes to microvascular density, morphology, or surface
287 area. For stimulus-induced hyperemic responses, the impacts of both Aβ and PA were most notably
288 pronounced within the capillary bed, which accounts for the majority of vascular resistance and metabolite
289 exchange [61]. In WT mice, PA yielded larger changes in stimulus- evoked capillary dilation and
290 oxygenation changes; while in AD mice, PA yielded increased capillary dilation and faster, but smaller
291 changes in capillary oxygenation. Our observed differences in arterioles and capillaries support prior
292 reports highlighting vulnerability of vascular smooth muscle cells, endothelial cells, and pericytes to Aβ,
293 as well as the potential influences of PA on each of these cell types [59,60,76,76,78,79]. Importantly,
294 while Aβ and PA alter their cellular phenotypes, our data reflects the resultant impact on vascular

295 physiology. In a pilot study, the impact of PA on capillaries on capillary stalls and other stuff appeared
296 less evident through.

297 The differential response of capillary oxygenation in WT and AD mice to PA constitutes a notable finding.
298 In WT mice, capillary SO_2 exhibited a transient decline following one month of PA, which fully recovered
299 by six months. This initial dip likely reflects a progressive physiological adaptation, where early increases
300 in oxygen demand could outpace microvascular supply before to completely compensation with prolonged
301 moderate exercise, a process associated with improvements in cerebral blood flow [80]. However, abrupt
302 or excessive exercise may exceed the adaptive capacity of the cortical vasculature, potentially impairing
303 the beneficial effects of moderate exercise [81]. This suggests that the neurovascular benefits of PA are
304 contingent on both duration and intensity, emphasizing the importance of controlled exercise regimens in
305 mitigating cerebrovascular deficits. Further studies are warranted to delineate the threshold at which
306 exercise transitions from being beneficial to detrimental, particularly in the context of AD-related vascular
307 dysfunction

308 Our findings strongly support emerging hypotheses that emphasize the role of capillary dysfunction for
309 AD progression [76,82–84]. Although it provoked no changes to capillary density or morphology in either
310 WT or AD mice, routine engagement in aerobic PA shows great promise for strongly mitigating
311 disturbances to basal capillary oxygenation and homogenize perfusion. Taken together, our basal pO2
312 measurements and functional hyperemia measurements offer insight to the "hyperactivation" phenomenon
313 observed in fMRI BOLD studies of AD patients [85]. Although PA did not substantially affect absolute
314 changes in SO2, the relative change is augmented by the reduced baseline in AD-noPA. When compared
315 to other cohorts, our data indicates that reduced basal oxygen in the capillary and venous compartment
316 account for a larger stimulus-evoked relative change.

317 While Aerobic PA has proven useful for mitigating aging related alterations to vascular function,
318 neuroinflammation, and numerous pathological hallmarks of preclinical AD [73,74,86–89], its protective
319 mechanisms and capacity to prevent functional decline associated healthy aging and AD progression are
320 still highly nuanced, multifaceted, and highly variable. Confounding parameters such as age, sex, intensity
321 levels, and duration drastically affect PA's effectiveness for neuroprotection. Our study highlights the
322 benefits of maintaining routine aerobic PA throughout early adulthood and middle age to mitigate
323 disruptions to cortical arteriolar and capillary oxygenation. Our findings motivate future investigations

exploring PA's effects on endothelial cells, vascular smooth muscle cells, and pericytes within cortical and subcortical brain regions.

Materials and Methods

Mice and Experimental Design

All male mice used in this study were on a C57BL/6 background. The APP/PS1 transgenic mouse model ( )85Dbo/Mmjax, MMRRC Strain #034829-JAX, Jackson Laboratory) was utilized. Genotypes were confirmed via standard PCR reactions performed by TransnetXY Company, employing proprietary platforms and pipelines to ensure high-quality read depth and reproducibility. Mice were housed in a temperature- and humidity-controlled animal facility with a 12-hour light/dark cycle and provided ad libitum access to food and water. Experimental groups consisted of 4–6 mice each, analyzed at 6 months of age. Wild-type (WT) and Alzheimer's disease (AD) mice were randomly assigned to sedentary (noPA) or physically active conditions (PA). All mice were bred and housed at the Northeastern University (NEU) vivarium, and all procedures were conducted in compliance with protocols approved by the NEU Institutional Animal Care and Use Committee (NE-IACUC).

Treadmill Training

The experimental design was performed as previously described [90]. At the age of 6 months, male APP/PS1 transgenic mice and wild-type littermate controls were randomly assigned to one of four groups (n = 4-6 each group), previously assigned. A motor-driven treadmill (Ugo Basile, cat#47303) was used to train the animals at fixed speeds. To ensure recovery after cranial window surgery, treadmill training commenced 1-month post-surgery. Exercise mice underwent a 2-day acclimatization period, running for 10 minutes each day (Day 1 at 5 m/min; Day 2 at 8 m/min). Following acclimatization, exercise groups engaged in treadmill running at progressively increasing speeds (5–11 m/min) for 30 minutes per session, 5 days per week, for 5 months (from 6 to 12 months of age). Each session began at 5 m/min, with the speed incrementally increased to 11 m/min within the first minute. The maximum speed (11 m/min) was maintained for the remaining 29 minutes of the session. Mice in the sedentary groups were placed on the stationary treadmill for the same duration as the exercise groups but were not required to run. Animals that repeatedly failed to complete exercise sessions were excluded from the experiment. This long-duration training protocol has been reported to maintain exercise intensity at 45%–55% of VO_2 max [91].

Mapping the center of cortical activation

353 Cortical activation mapping was performed using optical intrinsic signal imaging (OISI) integrated into a
354 multimodal two-photon microscope (Ultima 2Pplus, Bruker) equipped with a high-resolution
355 monochromatic camera (acA1300-200µm, Part No. 106752, Basler aceU). A continuous broadband LED
356 light source (KL1600 LED, Part No. 1500-600, Schott), filtered at the isosbestic wavelength of oxy- and
357 deoxyhemoglobin (bandpass filter, 568/4 nm, cat# 65160, Edmund Optics), provided illumination.
358 Hemodynamic responses were recorded at 5 Hz [92] to capture stimulus-evoked cortical activity.
359 Activation ratio maps were generated by normalizing the post-stimulus signal (0–1.5 s after stimulus
360 onset) to the baseline period (1–4 s prior to stimulus onset). The spatial localization of cortical activation
361 (300 × 300 µm) was determined using a custom algorithm that mapped the entire cranial window. After
362 applying the 3D median and 3D Gaussian filters to the image series, the activation center was determined
363 by averaging the vectorized output of the region of interest (ROI) across successive imaging frames.

364 ***Chronical Cranial Window Implantation***

365 All animal handling and surgical procedures were approved by the Northeastern University Institutional
366 Animal Care and Use Committee (NE-IACUC). Male C57BL/6 mice (16 weeks old, 19–24 g) were used
367 in this study. The surgical procedure was performed as previously described [93]. Briefly, mice were pre-
368 treated with dexamethasone (4.8 mg/kg) and cefazolin (0.5 g/kg) 4–6 hours before surgery to prevent brain
369 edema and infection, respectively. Mice were anesthetized with isoflurane (2% for induction and 1%–2%
370 during surgery). A circular incision was made on the scalp over the frontal and sagittal bones, and the
371 exposed periosteum was carefully removed with a No. 15 blade to ensure proper fixation. A custom
372 holding bar was attached to the skull using cyanoacrylate adhesive (Loctite-401, Henkel, Cat#135429) to
373 immobilize the animal during imaging. A craniotomy (~3 mm in diameter) was performed on the left
374 hemisphere over the primary somatosensory cortex (coordinates: A–P: 2.0 mm; M-L: 3.0 mm) while
375 leaving the dura intact. The craniotomy was sealed with a glass plug and secured with dental cement. Post-
376 surgical care included administering Sulfamethoxazole/Trimethoprim (0.5 mg/0.1 mg/mL) and
377 Carboprofen (1 mg/mL) in the drinking water for 5 days. Additionally, cefazolin (0.5 g/kg) was given via
378 intraperitoneal injections twice daily for 5 days, and Buprenorphine (0.05 mg/kg) was administered
379 subcutaneously once daily for the first 3 days to manage pain. Mice were provided with softened food
380 pellets immersed in water in a petri dish placed on the cage floor to ensure adequate nutrition and hydration
381 during recovery.

382

383 ***Training for awake imaging***

384 Two weeks after surgery, to ensure full recovery, mice were trained to tolerate head fixation in a custom-
385 designed cradle for imaging sessions lasting up to 2 hours. Training sessions were conducted three times
386 per week over a 2-week period, with the duration of each session gradually increased. During training and
387 imaging sessions, mice were rewarded with sweetened milk approximately every 15 minutes. To promote
388 comfort and natural behavior, mice were placed on a suspended fabric bed, allowing them to engage in
389 natural grooming behaviors

390 ***Functional activation protocol***

391 Functional hyperemia in the somatosensory primary cortex (Barrel cortex) was induced by gentle
392 stimulation of the whisker pad, following a previously established protocol [94]. Briefly, a Picospritzer
393 microinjection device (PDES-01DXH-E-LA-4, npi electronic GmbH). Air puffs (10–20 psi) were
394 delivered to the contralateral whisker pad relative to the cranial window implantation. To ensure effective
395 activation of the whisker Barrel cortex while minimizing the eye-blink reflex, air puffs were applied in an
396 inferoforward direction. A 3D-printed custom nozzle directed the air puffs toward the E1 whisker. As
397 other whiskers were not trimmed, the stimulus affected multiple whiskers simultaneously. Each trial
398 consisted of a 5-second baseline period followed by a 1.5-second stimulation period. During stimulation,
399 air puffs were delivered at a frequency of 3.3 Hz, with each puff lasting 300 ms and separated by a 300-
400 ms interval. The inter-stimulation interval (ISI, onset-to-onset) was set to 35 seconds.

401 ***Oxygen Measurements***

402 Systemic oxygen delivery and exchange were quantified using OxyPhor-2P, a phosphorescent oxygen-
403 sensitive dye injected intravascularly (10 μL of 20 μM solution). Two-photon excitation fluorescence
404 microscopy was conducted with a scanning microscope (Ultima 2Pplus, Bruker), integrated with a tunable
405 Ti:sapphire laser (80 MHz, <120 ps pulse width, Insight X3+, Spectra-Physic) tuned to 950 nm for
406 OxyPhor-2 excitation. The imaging system employed a 25× water-immersion objective (380–1050 nm,
407 1.1 NA, 2.0 mm WD, N25X-APO-LWD, Nikon), ensuring precise optical focus. System control,
408 including motorized alignment with the cortical surface, image acquisition, and adjustments to laser power
409 and PMT voltage, was managed using embedded software (Prairie View, Bruker), fully integrated with
410 the scan head, laser power and PMT voltage control.

411 Measurements of partial pressure of oxygen (pO2) were derived from the phosphorescent lifetime of
412 OxyPhor-2, utilizing the Stern-Volmer equation as previously described [95]. Each phosphorescence
413 excitation/collection cycle comprised a 10 μs pulse train (800 femtosecond pulses) followed by a 290 μs
414 interval, culminating in a total cycle duration of 300 μs. Data acquisition was conducted across 10

415 locations per iteration, with 100 cycles per location. This process was repeated 72 times to accumulate
416 ~20 seconds of measurement per iteration, for a total of 18 iterations (~6 minutes of data collection per
417 trial). Emission light was routed through an infrared blocker (short-pass filter, 900 nm) and a dichroic
418 mirror (bins splitter, 565 nm) into a four-channel detection system. The phosphorescence emission was
419 isolated with a 795±75 nm bandpass filter and detected via a highly sensitive photomultiplier tube (PMT;
420 H10770PA-50/001, Hamamatsu). The PMT signal was digitized using an analog-to-digital module (SPC-
421 150N TCSPC, Becker & Hilks). The PMT signal was digitized using an analog-to-digital converter.
422 Phosphorescence lifetimes were determined by fitting decay curves to single-exponential models using
423 non-linear least squares regression. Standard errors for lifetime measurements were estimated through a
424 bootstrap resampling methodology implemented in a custom executable file [95]. The calculated lifetimes
425 and associated standard errors were converted to pO2 values using the Stern-Volmer relationship. Oxygen
426 saturation (SO2) was calculated from pO2 measurements using the Hill equation, with a Hill coefficient
427 (h) of 2.59 and a P50 value of 40.2 mmHg, as previously described [94].

428 ***Capillary blood flow imaging and vascular dynamic with two-photon laser-scanning fluorescence***
429 ***microscopy***

430 Capillary flow dynamics were quantified using a custom-built two-photon laser-scanning microscope as
431 previously detailed (refer to Oxygen Measurement section). Vascular labeling was achieved through
432 retroorbital injection of 10 μL FITC–Dextran 70 (50 mg/mL in saline; Sigma-Aldrich, cat# 46945). This
433 fluorescent marker delineated the plasma as bright regions in the images, with red blood cells (RBCs)
434 appearing as dark shadows. Imaging was performed within a selected region of interest (600 μm × 600
435 μm) defining by OISI protocol, targeting approximately 10 capillaries (range: 7–13 capillaries; diameter
436 <10 μm) across a depth range of 0–400 μm, with measurements obtained at 50 μm intervals. Velocity
437 calculations involved tracing a straight line parallel to the longitudinal axis of each capillary, while internal
438 diameter measurements were obtained by drawing perpendicular straight line across the lumen at
439 approximately 50 points of length each one. Data acquisition was conducted continuously during a
440 functional activation protocol. Velocity computation was performed using a MATLAB-based dedicated
441 function: HydroVel [96], and lumen diameter analysis was facilitated by a custom image processing
442 routine (Supplementary Figure 1). Acquired data were aggregated into 1/100-second time bins for
443 downstream analyses.

444

445 ***Microvascular Morphology Measurements***

446 Microvascular angiograms were recorded within the same regions of interest (ROIs) used for functional
447 measurements (e.g., intravascular pO2, blood flow, and arteriolar diameter transients). Imaging was
448 performed on a separate day in awake mice at rest. Three-dimensional fluorescence intensity stacks were
449 acquired down to a depth of 400 μm from the cortical surface.
450
451 ***Data Analysis***
452 Oxygen saturation levels were quantified in arterioles, capillaries, and venules, alongside measurements
453 of blood flow velocity, vascular dynamics, and vessel density, across defined cortical layers. To assess
454 vascular heterogeneity under both resting and stimulus-evoked conditions, the coefficient of variation
455 (CV) of microvascular oxygen tension was calculated within 50-μm cortical depth bins for each individual
456 animal. Statistical comparisons across experimental groups were performed using linear mixed-effects
457 models implemented with the lme4 package (version 1.1.37) in R (version 4.4.2, Pile of Leaves). The
458 models included group and vascular segment as fixed effects and animal identity as a random intercept,
459 accounting for repeated measures within subjects. Post hoc pairwise comparisons were conducted using
460 estimated marginal means with Tukey adjustment for multiple comparisons, implemented via the
461 emmeans package (version 1.11.0). All results are reported as mean ± standard error of the mean (SEM),
462 and statistical significance was defined as p: *< 0.05, **<0.01 and ***<0.001.

463

464 **ACKNOWLEDGMENTS**

465 This work was performed with generous support from the Northeastern College of Engineering and the
466 National Institutes of Health: NIH R01AA27097, NIH R56AG058849, and R21AG085655. We thank Dr.
467 Sava Sakadzic and Allen Alfadhel from the MGH Martinos Center for Biomedical Imaging for
468 consultations with data processing and interpretation. We thank the Institute for Chemical Imaging of
469 Living Systems (RRID:SCR_022681) at Northeastern University for consultation and imaging support,
470 and for the assistance of confocal imaging and wide-field fluorescence imaging.

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