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ORIGINAL ARTICLE

Pharmacologically induced impairment of neurovascular coupling responses alters gait coordination in mice

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Abstract There is correlative evidence that impaired cerebral blood flow (CBF) regulation, in addition to promoting cognitive impairment, is also associated with alterations in gait and development of falls in elderly people. CBF is adjusted to neuronal activity via neurovascular coupling (NVC) and this mechanism becomes progressively impaired with age. To establish a direct cause-and-effect relationship between impaired NVC and gait abnormalities, we induced neurovascular uncoupling pharmacologically in young C57BL/6 mice by inhibiting the synthesis of vasodilator mediators involved in NVC. Treatment of mice with the epoxygenase inhibitor MSPPOH, the NO synthase inhibitor L-NAME, and the COX inhibitor indomethacin significantly decreased NVC mimicking the aging phenotype. Pharmacologically induced neurovascular uncoupling significantly decreased the dynamic gait parameter duty cycle, altered footfall patterns, and significantly increased phase dispersion, indicating impaired interlimb coordination. Impaired NVC also tended to increase gait variability. Thus, selective experimental disruption of NVC causes subclinical gait abnormalities, supporting the importance of CBF in both cognitive function and gait regulation.

Keywords Neurovascular coupling · Gait · Catwalk · Neurovascular uncoupling

Introduction

Vascular contributions to cognitive impairment and dementia (VCID) in aging have garnered much interest in

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the past decade (Corriveau et al. 2016; Csiszar et al. 2017; Gorelick et al. 2011; Snyder et al. 2015; Toth et al. 2017; Tucsek et al. 2017). There are numerous age-related vascular pathologies underlying VCID. It has become evident that in addition to pathologies affecting the larger cerebral arteries (e.g., atherosclerosis promoting cerebral ischemia) and cerebral microvessels (including arteriosclerosis, blood-brain barrier disruption, microvascular rarefaction, microvascular amyloid deposition, microhemorrhages) (Tucsek et al. 2014a, b, 2017; Ungvari et al. 2017b), functional impairment of cerebral microvessels resulting in dysregulation of cerebral blood flow (CBF) also has a critical role in the age-related decline of brain function (Csiszar et al. 2017; Tarantini et al. 2016; Toth et al. 2017; Zlokovic 2011).

There is growing evidence that functional impairment of the neurovascular unit develops early during aging before manifestation of other pathologies (Balbi et al. 2015), and that it importantly contributes to age-related impairment of brain function (Tarantini et al. 2016; Toth et al. 2017). The energetic demand of active neurons is high and their proper function depends on constant, tightly controlled delivery of oxygen and nutrients via the microcirculatory network. With increased neuronal activity there is a requirement for rapid compensatory increases in oxygen and glucose delivery to the active brain regions. This "functional hyperemia" is elicited through the process of neurovascular coupling, a feed-forward control mechanism orchestrated by cells of the neurovascular unit, which adjusts CBF, via regulating microvascular resistance, to the energy requirements of activated neurons (Attwell et al. 2010; Tarantini et al. 2016; Toth et al. 2017). Functional hyperemia is not only responsible for increased delivery of oxygen and nutrients, it also enables effective washout of noxious substances, ensuring an optimal humoral microenvironment in the cerebral tissue. The cellular mechanisms underlying neurovascular coupling include synthesis of nitric oxide (NO) by activated neurons and/or endothelial of nitric oxide (NO) and astrocytic production of vasodilator eicosanoid metabolites, including epoxygenase-derived epoxyeicosatrienoic acids (EETs) and cyclooxygenase-derived prostaglandins(Chen et al. 2014; Ma et al. 1996; Peng et al. 2002; Stobart et al. 2013; Takano et al. 2006; Tarantini et al. 2015, 2016, 2017; Toth et al. 2014, 2015a, b, 2017; Tucsek et al. 2014b; Ungvari et al. 2017a; Zonta et al. 2003). There is strong evidence that aging is associated with impairment of functional hyperemia (termed "neurovascular uncoupling") due to dysregulated release and/or increased degradation of NO, EETs, and prostaglandins (Stefanova et al. 2013; Tarantini et al. 2016; Topcuoglu et al. 2009; Toth et al. 2014). Neurovascular uncoupling is also manifested in pathophysiological conditions associated with accelerated cerebromicrovascular aging and cognitive impairment, including hypertension (Kazama et al. 2004), post-irradiation cognitive decline (Ungvari et al. 2017a), and obesity (Tucsek et al. 2014) both in human and laboratory models. Neurovascular dysfunction is also an early alteration in animal models of Alzheimer's disease (Girouard and Iadecola 2006; Lourenco et al. 2017; Tarantini et al. 2017).

Impaired delivery of nutrients and oxygen due to neurovascular dysfunction is expected to adversely affect neuronal function. Indeed, impaired neurovascular coupling responses in the elderly were shown to associate with impaired higher cognitive function (Sorond et al. 2013). Experimental studies in mouse models with pharmacological inhibition of the synthesis of NO, EETs, and prostaglandins confirm that a causal link exists between neurovascular dysfunction and cognitive impairment. In particular, pharmacologically induced neurovascular coupling was shown to result in impairment of spatial and recognition memory, mimicking the aging phenotype (Tarantini et al. 2015).

Human studies suggested that age-related neurovascular uncoupling also associates with gait abnormalities (Sorond et al. 2011). The significance of this observation is threefold. First, gait is no longer considered merely an automated motor activity (Atkinson et al. 2007). There is strong evidence that gait coordination requires normal executive function. Importantly, cognitive impairment and gait abnormalities frequently coexist in the elderly and in patients with neurodegenerative disease. In fact, gait disorders could manifest long before cognitive impairment is clinically evident (Mielke et al. 2013; Verghese et al. 2002). It is predicted that shared mechanisms, including microvascular pathologies that similarly affect brain regions involved in cognition and motor coordination, contribute to both cognitive impairment and gait dysfunction. Based on the complex interaction between brain regions involved in cognition and gait coordination in recent years, the concept has emerged that gait abnormalities may predict cognitive decline (Belghali et al. 2017; Callisaya et al. 2017; Fitzpatrick et al. 2007; Holtzer et al. 2006; Mielke et al. 2013; Verghese et al. 2007). Although there is growing evidence that neurovascular uncoupling contributes to cognitive decline, the role of neurovascular health in gait abnormalities

remains elusive. Second, gait dysfunction in older adults is a major cause of functional impairment, contributes to falls, and predicts increased risk of institutionalization and mortality (Mignardot et al. 2014; Nakamura et al. 1996; Sorond et al. 2010; Verghese et al. 2009). Third, gait abnormalities were shown to be associated with survival in older adults (Studenski et al. 2011). Identification of potentially preventable causes of gait dysfunction has great relevance for maintaining functional independence in late life, preventing falls and possibly preserving cognition before impairment ensues. However, because senile gait disorders are likely multifactorial, in previous studies, it has been challenging to establish the mechanistic link between neurovascular dysfunction and gait abnormalities.

The present study was designed to test the hypothesis that neurovascular dysfunction per se results in alterations of gait coordination. To achieve this goal, neurovascular dysfunction was induced experimentally in healthy young control mice by treatment with specific pharmacological inhibitors of synthesis of nitric oxide, epoxyeicosatrienoic acids, and prostaglandins followed by assessment of gait coordination. To verify treatment efficiency, whisker stimulation-induced neurovascular coupling responses were measured by assessing CBF in the somatosensory whisker barrel cortex using a laser Doppler flow probe. To assess the effects of experimentally induced neurovascular uncoupling in laboratory mice, we analyzed gait parameters that have direct translational relevance (e.g., gait speed, swing speed, cadence, stride length, stride time, base of support) and also developed the methods to analyze stride time and stride length variability, which are considered sensitive indices of human gait abnormalities.

Methods

All the performed procedures were approved by the Institutional Animal Care and Use Committee of the University of Oklahoma Health Sciences Center.

Animals and pharmacological treatments

To study the effects of neurovascular uncoupling, young male C57BL/6J mice (5 months old, $n = 10$ per group) were obtained from the Jackson Laboratories (Bar Harbor, ME). Mice were kept under specific pathogen-free barrier conditions in the Rodent Barrier Facility at University of Oklahoma Health Sciences Center under a controlled photoperiod (12 h light; 12 h dark). The animals were divided into two groups: (1) mice with experimentally induced neurovascular dysfunction were administered pharmacological inhibitors to disrupt production of mediators involved in functional hyperemia (nitric oxide, epoxyeicosatrienoic acids, and prostaglandins) (Tarantini et al. 2015); and (2) sham controls receiving vehicle treatment. To inhibit the production of EETs, mice were treated with *N*-(methylsulfonyl)-2-(2-propynyloxy)-benzenehexanamide (MS-PPOH), a specific inhibitor of EET-producing epoxidases (Brand-Schieber et al. 2000), as described (Tarantini et al. 2015). Alzet osmotic minipumps (7 days, 1 μl/h, ~ 200 μl total volume, Cat No.: 2001; Durect Corp., Cupertino, CA) were filled with MS-PPOH (20 mg/kg/day, dissolved in DMSO and diluted to final concentration with 45% cyclodextrin (Brand-Schieber et al. 2000)) and implanted subcutaneously. Sham operated control animals received vehicle. To inhibit synthesis of vasodilator NO, mice were treated with the NO synthase inhibitor N(G)-Nitro-L-arginine methyl ester (L-NAME, 100 mg/kg/day; in drinking water) (Wakisaka et al. 2010). Indomethacin (INDO; 7.5 mg/kg/day, p.o.), a non-selective inhibitor of cyclooxygenases, was used to disrupt NVC responses by cyxlooxygenase-derived vasodilator arachidonic acid metabolites. Indomethacin was dissolved in ethanol and diluted in 5% (*w*/*v*) sodium bicarbonate solution. The maximum administered daily volume of ethanol was 3 μl per animal. The treatments were continued throughout the entire experimental period (7 days). Blood pressure of the animals was recorded before the treatment, and on day 3 of the treatment period, by the tail cuff method, as previously described (Toth et al. 2013).

Analysis of gait function

To determine the impact of impaired NVC responses on gait coordination, we tested the experimental groups of mice using an automated computer-assisted method (CatWalk; Noldus Information Technology Inc.) before and 3 days after the initiation of the pharmacological treatment, when the animals completely recovered from surgery. Using the CatWalk system, the detection of paw print size and paw placement patterns during volunteer running on an illuminated glass walkway by a camera placed under the glass surface provides an automated analysis of gait function and the spatial and temporal aspects of interlimb coordination (Tarantini et al. 2015;

Ungvari et al. 2017a). Briefly, animals were trained to cross the walkway and then, in a dark and silent room (< 20 lx of illumination), animals were tested in 20 consecutive runs (to obtain > 200 steps per animal). Data were averaged across ~ 20 runs in which the animal maintained a constant speed across the walkway. After manual identification and labeling of each footprint, the variability of the data has been assessed using quartile dispersion. We adopted a common outlier definition, labeling points more than 1.5 interquartile ranges away from the sample median as extreme values. After variability analyses, spatial and temporal gait parameters were calculated.

Base of support is the average width between either the front paws or the hind paws. Swing speed is the speed (cm/s) of the paw during swing. Stride length is the distance (in cm) between successive placements of the same paw. The regularity index (%) is a fractional measure of interpaw coordination, which expresses the number of normal step sequence patterns relative to the total number of paw placements. The formula of regularity index is as follows: (normal step sequence patterns) × 4/(total number of paw placements) × 100 (%). In healthy, fully coordinated animals, its value is close to 100%. Phase dispersion provides a quantitative metric of interpaw coordination. Phase dispersion characterizes the placement of two paws ("target" and "anchor") during the cycle of consecutive initial contacts with an anchor paw. In a step cycle, base of support gives the distance between the mass-midpoints of the fore prints at maximal contact. The results are averaged and expressed in centimeter. Duty cycle (%) is expressed as stand time as a percentage of step cycle (Duty Cycle = Stand time / (Stand time + Swing time × 100%)).

Terminal dual stance (in seconds) is a measure of simultaneous contralateral support, calculated as the duration of ground contact for both hind paws simultaneously; it is the second step in a step cycle of a hind paw that the contralateral hind paw also makes contact with the glass plate. Cadence is the rate at which a mouse walks, expressed in steps per second.

Investigating gait variability (Beauchet et al. 2017; Decker et al. 2016), the stride-to-stride fluctuations in gait parameters offers a sensitive, novel method of quantifying subtle changes in locomotion in mice. Step time and step length variability were analyzed by computing the median absolute deviation (MAD) for datasets that contained > 200 steps for each animal, obtained in consecutive runs at similar speeds. MAD is a robust measure of statistical dispersion, which is more resilient to outliers in a data set than the standard deviation.

Measurement of neurovascular coupling responses and somatosensory-evoked field potentials

After behavioral testing, mice in each group were anesthetized (α-chloralose (50 mg/kg, i.p.)/urethane (750 mg/kg, i.p.), endotracheally intubated, and ventilated (MousVent G500; Kent Scientific Co, Torrington, CT). A thermostatic heating pad (Kent Scientific Co, Torrington, CT) was used to maintain rectal temperature at 37 °C (Toth et al. 2014). End-tidal CO_2 (including dead space) was controlled between 3.2 and 3.7% to keep blood gas values within the physiological range as described (Tarantini et al. 2015; Toth et al. 2014; Ungvari et al. 2017a). The right femoral artery was cannulated for arterial blood pressure measurement (Living Systems Instrumentations, Burlington, VT) (Toth et al. 2014). The blood pressure was within the physiological range throughout the experiments (90–110 mmHg). Mice were immobilized and placed on a stereotaxic frame (Leica Microsystems Inc., Buffalo Grove, IL) as the scalp and periosteum were pulled aside. The animals were equipped with an open cranial window as described (Tarantini et al. 2015; Toth et al. 2014) and a glass-insulated tungsten microelectrode (impedance, 2–3 MΩ, Kation Scientific, LLC, Minneapolis, MN) was inserted stereotaxically into the left barrel cortex (3 mm lateral and 1.5 mm caudal to bregma; depth of 0.6 mm) through the ACSF-perfused open cranial window for recording local field potentials. An Ag/AgCl electrode inserted into the neck muscles served as reference electrode. Changes in cerebral blood flow (CBF) were assessed above the left barrel cortex using a laser Doppler probe (Transonic Systems Inc., Ithaca, NY) as described (Tarantini et al. 2015; Toth et al. 2014; Ungvari et al. 2017a).

After basal activity was recorded, the right whisker pad was stimulated by a bipolar stimulating electrode placed to the ramus infraorbitalis of the trigeminal nerve and into the masticatory muscles. The stimulation protocol used to investigate neurovascular coupling and somatosensory-evoked field potentials consisted of 10 stimulation presentation trials with an intertrial interval of 70 s, each delivering a 30-s train of electrical pulses (2 Hz, 0.2 mA, intensity, and 0.3 ms pulse width) to the mystacial pad after a 10-s prestimulation baseline period.

Changes in CBF were averaged and expressed as percent (%) increase from the baseline value (Kazama et al. 2004). The electrical signal was amplified with an AC/DC differential amplifier (high pass at 1 Hz, low pass at 1 kHz) (Model 3000, A-M Systems, Inc. Carlsborg, WA) and digitalized by the PowerLab/Labchart data acquisition system (ADInstruments, Colorado Springs, CO) with the sampling rate of 40 kHz. Analyses were performed on the average of 10 stimulation trials. The negative amplitude in the somatosensory-evoked field potential response was considered as the excitatory postsynaptic potential (fEPSP) (Lind et al. 2013). Experiments lasted ~20–30 min per mouse, which permitted stable physiological parameters to be obtained. The experimenter was blinded to the treatment of the animals.

Statistical analysis

Statistical analysis was carried out by unpaired or paired *t* test, as appropriate, using Prism 5.0 for Windows (Graphpad Software, La Jolla, CA). A *p* value less than 0.05 was considered statistically significant. Data are expressed as mean ± S.E.M.

Results

Pharmacologically induced neurovascular uncoupling

Changes in CBF in the whisker barrel cortex in response to contralateral whisker stimulation were significantly decreased by in vivo treatment with MSPPOH+NAME+INDO (Fig. 1a) (Park et al. 2007). Pharmacological treatments could potentially attenuate CBF responses by impairing neural activity evoked by whisker pad stimulation. To examine this possibility, we assessed the effects of treatment with MSPPOH+NAME+INDO by recording spontaneous and evoked neural activity. We found that the somatosensory field potentials produced by activation of the whisker pad do not differ between control and MSPPOH+NAME+INDO-treated mice (Fig. 1b). Therefore, treatment with MSPPOH+NAME+INDO is unlikely to contribute to impaired functional hyperemia by modulating the neural activity evoked by whisker stimulation (Tarantini et al. 2015). The blood pressure of the two groups of animals did not differ significantly (data not shown).

Effects of neurovascular uncoupling on gait coordination

Gait coordination is a higher integrative process of the sensorimotor system. Clinical studies suggest that neurovascular coupling may be involved in preservation of gait function in elderly people (Sorond et al. 2011). With advanced age, balance and gait speed are reduced (Abellan van Kan et al. 2009; Atkinson et al. 2007; Callisaya et al. 2015; Fitzpatrick et al. 2007; Liu et al. 2017; Nadkarni et al. 2014; Sorond et al. 2010, 2011; Verlinden et al. 2013; Watson et al. 2010), while many other mobility parameters remain unchanged in humans.

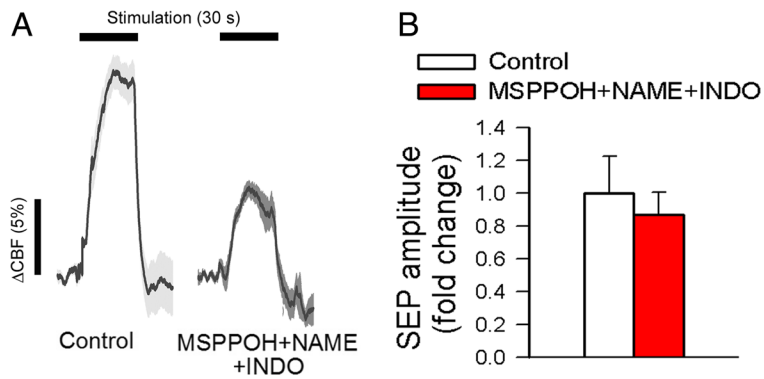

Fig. 1 Pharmacologically induced neurovascular uncoupling in mice. **a** Time course of cerebral blood flow (CBF) responses measured with a laser Doppler probe above the whisker barrel cortex during electrical stimulation of the contralateral whisker pad (current 0.2 mA, pulse duration 0.3 ms, at 2 Hz for a 30-s period) obtained in control mice and mice treated chronically with MSPPOH, L-NAME, plus indomethacin (INDO). Shaded areas denote 95% confidence intervals. **b** Bar graphs showing the effect of treatment with MSPPOH+NAME+INDO on somatosensory-evoked potential (SEP) responses in the primary somatosensory cortex in response to electrical stimulation of the contralateral whisker pad in control and treated groups. The amplitudes of the negative waves were unaffected by chronic treatment of the mice with MSPPOH+NAME+INDO. Data are mean ± SEM

In the present study, we did not observe differences between control mice and mice treated with MSPPOH+NAME+INDO in the following parameters indicative of gait: speed (Fig. 2a), swing speed (Fig. 2b), cadence (Fig. 2c), stride length (Fig. 2d), stride time (Fig. 2e), base of support (front paws; Fig. 2g), base of support (hind paws; Fig. 2h), and terminal dual stance (Fig. 2i). Pharmacologically induced neurovascular uncoupling significantly decreased the dynamic gait parameter duty cycle, which represents stance duration as a percentage of step cycle duration (Fig. 2f).

The regularity index tended to decrease in mice treated with MSPPOH+NAME+INDO (Fig. 3a). Mice in both groups predominantly used the four most common footfall patterns (Fig. 3b). The primary differences in patterns used by control mice and mice with pharmacologically induced neurovascular uncoupling were in the frequency of the radial "giraffe walk" AA pattern. Mice with neurovascular uncoupling used more frequently the AA pattern than control mice, and compensated with a decreased use of the alternating AB pattern and CB pattern.

Interlimb coordination was also analyzed by phase dispersion analysis. Mean homologous, ipsilateral, and diagonal phase dispersion values, obtained in animals studied at comparable walking speeds, were calculated for the respective limb pairs and their deviations from the expected phase dispersion values were computed. As shown in Fig. 3c, significantly higher phase dispersion was evident in mice treated with MSPPOH+NAME+INDO as compared to controls.

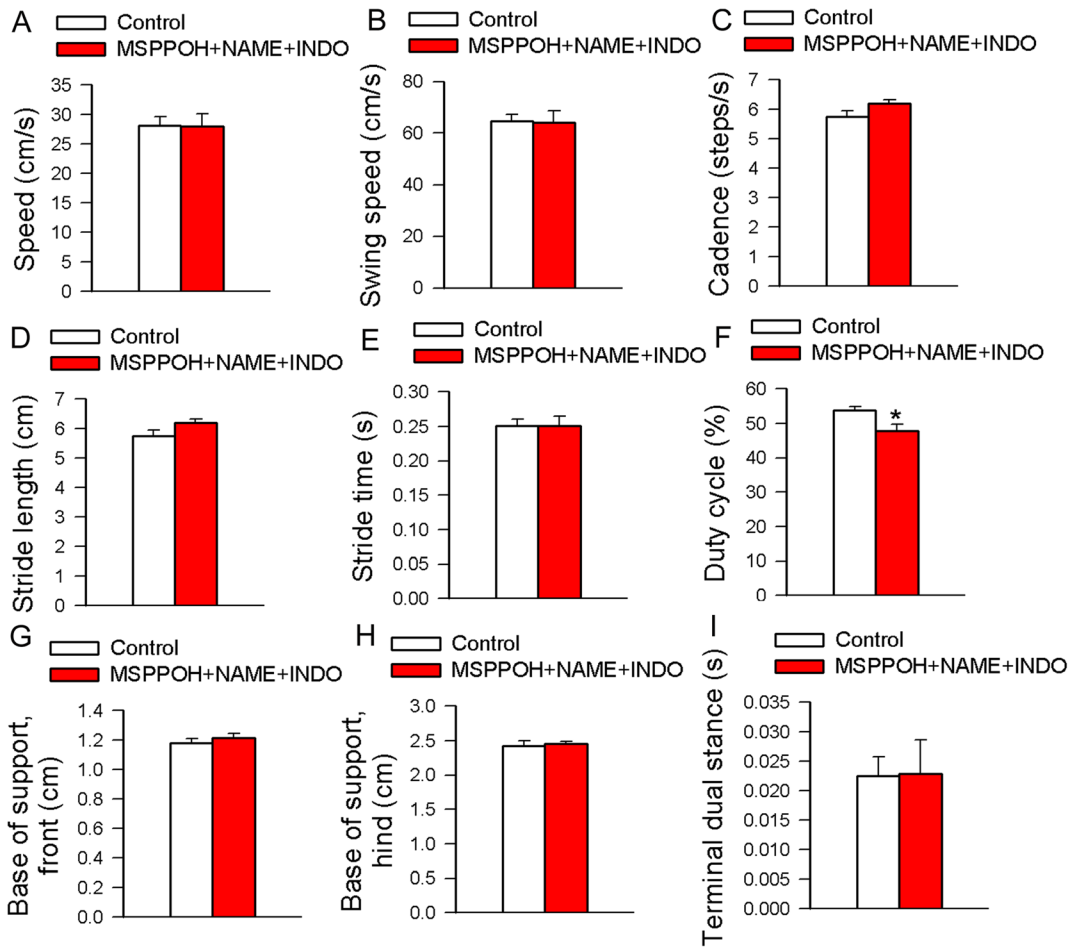

Fig. 2 Effects of neurovascular uncoupling on gait parameters**. a** Body speed, **b** swing speed, **c** cadence, **d** stride length, **e** stride time, **f** duty cycle, **g** and **h** base of support (**g** front paws; **h** hind paws), and **i** terminal dual stance in control mice and mice treated chronically with MSPPOH, L-NAME, plus indomethacin (INDO). Data are mean ± SEM ($n = 10$ in each group). *$P < 0.05$ vs. control

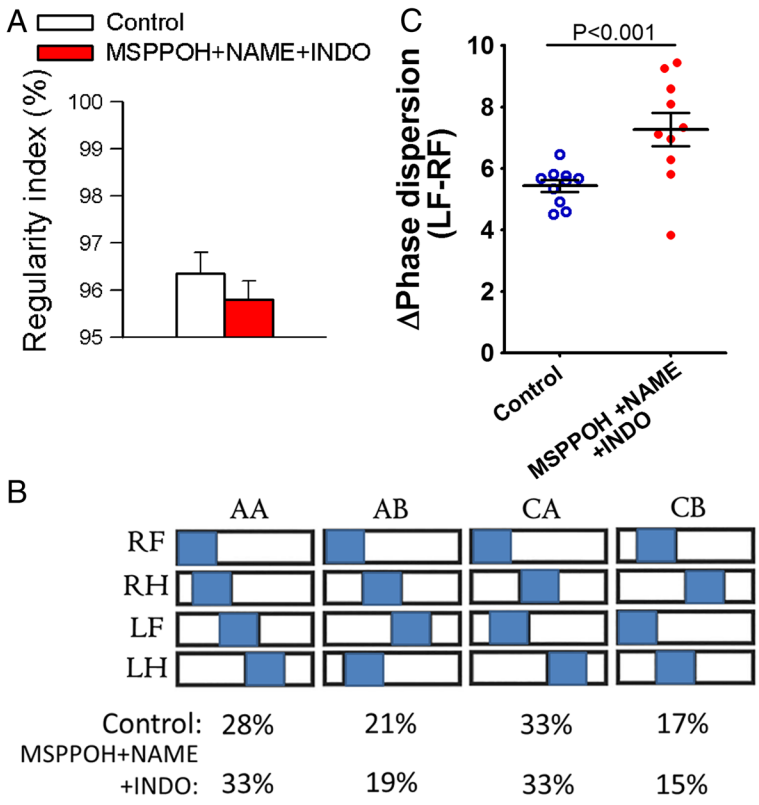

Fig. 3 Neurovascular uncoupling impairs gait coordination. **a** A similar sequence regularity index reflects no change in step patterns in mice treated with MSPPOH+NAME+INDO as compared to control mice. **b** Hildebrand plot of the common gait patterns: AA (RF-RH-LF-LH), AB (LF-RH-RF-LH), CA (RF-LF-RH-LH), and CB (LF-RF-LH-RH). Percentages indicate relative use of each step pattern in each mouse group. The most common step pattern in both was the CA pattern. Mice with neurovascular uncoupling used more frequently the radial AA pattern than control mice and compensated with a decreased use of the alternating AB pattern and CB pattern. **c** The average deviation of phase dispersion (calculated between the right front paw [RF] and left front paw [LF]) from the expected value (50%) under baseline conditions and after treatment with MSPPOH+NAME+INDO. Data are mean ± SEM.*$P < 0.05$ vs. control ($n = 10$ in each group)

The study of gait variability, the stride-to-stride fluctuations in walking first observed by von Vierordt (1881), offers a sensitive method of quantifying locomotion. Previous studies demonstrated that measures of gait variability may be more closely related to cognitive decline or falls than other measures based on the mean values of other gait parameters. Stride length variability is in fact a strong predictor of falls and cognitive impairment in elderly patients (Montero-Odasso et al. 2014; Nakamura et al. 1996; Rosso et al. 2014; Studenski et al. 2011; Verghese et al. 2007, 2008, 2009; Verlinden et al. 2013; Visser, 1983; Wittwer et al. 2013). We found that in mice treated with MSPPOH+NAME+INDO there was a discernable trend for increased stride length variability (Fig. 4a) and stride time variability (Fig. 4b).

Discussion

The present study provides evidence that neurovascular uncoupling is associated with changes in mouse gait coordination. This is complementary to previous data demonstrating that pharmacologically induced neurovascular uncoupling in mice also promotes detectable cognitive impairment (Tarantini et al. 2015).

Gait and balance disorders are ubiquitous in aging. In addition to being a major cause of falls, they are also associated with increased morbidity and mortality, as well as reduced level of function and increased risk of institutionalization. Clinically diagnosed gait abnormalities in older adults involve multiple contributing factors, including neurological diseases (e.g., strokes, Parkinson's disease) (Hajjar et al. 2009). Clinical

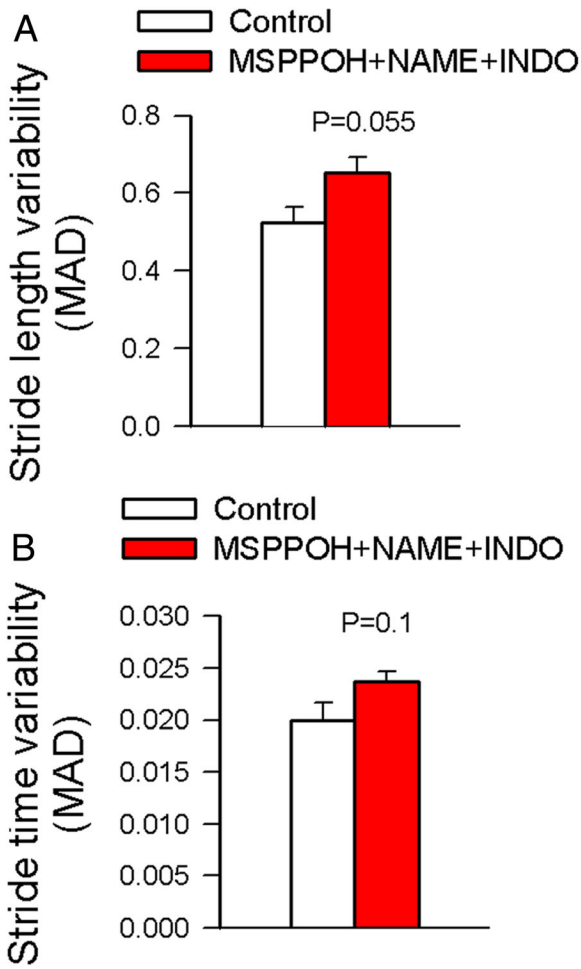

Fig. 4 Neurovascular uncoupling tends to increase gait variability. **a** Stride length variability and **b** stride time variability in spontaneously walking control mice and mice treated chronically with MSPPOH, L-NAME, plus indomethacin (INDO). Data are mean ± SEM ($n = 10$ in each group). MAD median absolute deviation

studies, including the MOBILIZE Boston study (Sorond et al. 2011), link neurovascular uncoupling to subtle gait dysfunction (e.g., slowing of gait) in humans. Recent studies extend these findings, demonstrating that neurovascular coupling, assessed using transcranial Doppler, is related to activation of brain regions within the executive network, which predicts gait alterations in older adults (Jor'dan et al. 2017). While these findings regarding the association between neurovascular uncoupling and gait abnormalities were intriguing, a cause-and-effect relationship cannot be established given the cross-sectional nature of these studies. In the present study, short-term experimentally induced isolated neurovascular uncoupling resulted in significant change in duty cycle, phase dispersion, and gait pattern, whereas no significant changes in gait speed, cadence, base of support, stride length, and stride time. These findings provide direct evidence that a cause-and-effect relationship likely exists between impairment of neurovascular coupling responses and alteration in gait coordination, supporting the conclusions of earlier clinical studies (Sorond et al. 2011). Our results also warrant further studies on different modalities of gait (e.g., indices reflecting gait coordination) in the context of neurovascular uncoupling in humans. There are several lines of evidence in support of the concept that induction of neurovascular uncoupling is the main mechanism by which inhibition of synthesis of EETs, prostaglandins, and NO affects brain function (Leithner et al. 2010; Tarantini et al. 2015). Although we cannot exclude the possibility that the inhibition of synthesis of nitric oxide, prostaglandins, and EETs may also affect other aspects of neural, glial, or vascular mechanisms, which were not investigated in this study, it did not affect somatosensory-evoked potentials in the barrel cortex (Tarantini et al. 2015). Previous investigations also did not observe alterations in basic synaptic transmission parameters and observed normal long-term potentiation response of fEPSPs in the hippocampus using inhibitors of synthesis of nitric oxide, EETs, and prostaglandins (Leithner et al. 2010; Tarantini et al. 2015).

Healthy neural control systems can fine tune the stride-to-stride fluctuations of gait. Gait variability is a sensitive parameter, which in older humans was reported to predict cognitive decline (Brach et al. 2008; Callisaya et al. 2010; Cedervall et al. 2014; Decker et al. 2016; Gillain et al. 2016; Herman et al. 2005; Verghese et al. 2002, 2007, 2008; Wittwer et al. 2013) and survival(Verlinden et al. 2013) and associated with risk of falls (Verghese et al. 2010). There is strong evidence that vascular pathologies promote gait variability. For example, subclinical brain vascular abnormalities, measured on brain MRIs as infarcts and white matter hyperintensities, were reported to associate with greater variability of spatial gait parameters (step length) (Rosano et al. 2007). In older adults, gait variability is thought to be associated with areas important for sensorimotor integration, coordination (Tian et al. 2017), and memory and executive function (Rosso et al. 2014). In the present study, there was a discernible trend for increased gait variability after induction of neurovascular uncoupling. It will be interesting to determine whether a similar relationship exists

between gait variability and neurovascular uncoupling in older humans. If this is the case, then it could be proposed that even subclinical impairment of neurovascular function in aging may exacerbate the complex gait abnormalities in older patients. In that regard, it is important to point out that in aging, and age-related pathophysiological conditions (e.g., hypertension), neurovascular uncoupling often co-occurs with other microvascular pathophysiological alterations (e.g., microhemorrhages (Ungvari et al. 2017b), white matter hyperintensities (Sorond et al. 2013)), which are also known to cause gait abnormalities (Toth et al. 2015c). It should be thus emphasized that specific patterns of gait variability may imply different underlying causes (Brach et al. 2008).

Our findings have important clinical relevance. Experimentally induced neurovascular uncoupling mimics impairment of functional hyperemia observed in aging (Toth et al. 2014) and pathophysiological conditions associated with accelerated cerebromicrovascular aging. Advanced age promotes neurovascular uncoupling, at least in part, by decreasing NO bioavailability (Park et al. 2007; Tarantini et al. 2016; Toth et al. 2014). A wide range of pathophysiological conditions in the elderly was shown to adversely affect the synthesis and/or release of NO and astrocyte-derived vasoactive eicosanoid mediators, promoting neurovascular uncoupling (Girouard and Iadecola 2006; Kazama et al. 2004; Tucsek et al. 2014). Cardiovascular risk factors, including hypertension (Kazama et al. 2003, 2004), dyslipidemia, smoking, low circulating IGF-1 levels (Toth et al. 2015a), and obesity (Tucsek et al. 2014b), which are all important risk factors for cognitive decline in elderly patients (Gorelick et al. 2011; Iadecola et al. 2009; Miralbell et al. 2013), also inhibit NO mediation of neurovascular coupling and microvascular dilations by promoting oxidative stress, uncoupling endothelial NO synthase and/or by upregulating asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO synthase. In that regard, it is important that a significant association between serum ADMA level and slower gait speed was demonstrated among elderly individuals (Obayashi et al. 2016). Type 2 diabetes mellitus (Duarte et al. 2015) also leads to neurovascular dysfunction, which is associated with increased risk for brain function loss and long-term cognitive impairment (Biessels and Reijmer 2014; Brundel et al. 2012, 2014; de Bresser et al. 2010; Palta et al. 2014; Reijmer et al. 2011; Ruis et al. 2009; Ryan et al. 2014, 2016; van den Berg et al. 2008, 2009, 2010). Increased oxidative stress and heightened inflammation associated with cardiovascular risk factors may also affect arachidonic acid metabolism, decreasing production of vasodilator prostaglandins and EETs and/or increasing synthesis of vasoconstrictor eicosanoids, such as 20-hydroxyeicosatetraenoic acid (20-HETE), contributing to neurovascular dysfunction.

In recent years in gait research, there has been a substantial increase in the use of methodologies that dynamically assess cerebral blood flow during walking. Methods such as functional near-infrared spectroscopy (fNIRS) can be used to assess functional hyperemia through monitoring of blood oxygenation and blood volume in the cortex during neuronal activation, which are profoundly affected by age-related alterations in cerebrovascular hemodynamics. Thus, using these methodologies, the link between impaired functional hyperemia and gait alterations can be elucidated.

On the basis of our present and previous (Tarantini et al. 2015) findings, we posit that pharmacological and non-pharmacological interventions that promote microvascular health and improve neurovascular coupling responses may exert beneficial effects on higher cortical function (Sorond et al. 2013). Recent findings provide initial support for this concept showing that pharmacological or dietary interventions (e.g., treatment with resveratrol in mouse models (Toth et al. 2014; Witte et al. 2014), consumption of cocoa in elderly patients (Sorond et al. 2013)) that rescue endothelial function and neurovascular coupling can improve cognitive function in aging. Further, our recent studies also demonstrate that rescue of neurovascular coupling responses by treatment with the mitochondria-targeted antioxidative compound SS-31 improves gait coordination in aged mice (Tarantini and Ungvari, under review). Second, pharmacological treatments and dietary and lifestyle factors that impair neurovascular coupling (e.g., by inhibiting the synthesis of nitric oxide, epoxyeicosatrienoic acids, and/or prostaglandins) may adversely affect gait coordination and/or cognitive function. For example, pharmacological inhibitors of the synthesis of vasodilator eicosanoids, including indomethacin, were shown to impair neurovascular coupling in humans (Bruhn et al. 2001; Szabo et al. 2014), decreasing BOLD cerebral MRI contrast by over 50%. The available data from the Baltimore Longitudinal Study on Aging also suggest that use of the cyclooxygenase inhibitor aspirin is associated with greater prospective cognitive decline (Waldstein et al. 2010); however, its effect on gait function was not investigated.

In that regard, the results of the Aspirin in Reducing Events in the Elderly (ASPREE) trial (a placebo-controlled trial of aspirin treatment that will determine the effects of 5 years of daily 100 mg aspirin on gait function in the elderly (McNeil et al. 2017)) will be informative.

Collectively, combined inhibition of production of nitric oxide, epoxyeicosatrienoic acids, and prostanoids significantly reduces CBF responses to neuronal activation in mice, which mimics neurovascular uncoupling observed in aging and pathophysiological conditions associated with accelerated cerebromicrovascular aging. The results of this study provide experimental evidence in support of the concept that neurovascular uncoupling per se promotes subclinical gait abnormalities. Our findings, taken together with the results of earlier studies (Hamel et al. 2016; Ongali et al. 2014; Papadopoulos et al. 2017; Tong et al. 2012), point to potential benefits of pharmacological and non-pharmacological (e.g., dietary (Sorond et al. 2013)) interventions targeting neurovascular coupling pathways and promoting microvascular health to preserve gait function in aging.

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