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Interaction between Nitric Oxide Synthase Inhibitor Induced Oscillations and the Activation Flow Coupling Response

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Abstract

The role of nitric oxide (NO) in the activation-flow coupling (AFC) response to periodic electrical forepaw stimulation was investigated using signal averaged laser Doppler (LD) flowmetry. LD measures of calculated cerebral blood flow (CBF) were obtained both prior and after intra-peritoneal administration of the non-selective nitric oxide synthase (NOS) inhibitor, N^G-nitro-L-arginine (L-NNA) (40 mg/kg). Characteristic baseline low frequency vasomotion oscillations (0.17 Hz) were observed after L-NNA administration. These LD_{CBF} oscillations were synchronous within but not between hemispheres. L-NNA reduced the magnitude of the AFC response ($p < 0.05$) for longer stimuli (1 minute) with longer inter-stimulus intervals (2 minutes). In contrast, the magnitude of the AFC response for short duration stimuli (4 seconds) with short inter-stimulus intervals (20 seconds) was augmented ($p < 0.05$) after L-NNA. An interaction occurred between L-NNA induced vasomotion oscillations and the AFC response with the greatest increase occurring at the stimulus harmonic closest to the oscillatory frequency. Nitric oxide may therefore modulate the effects of other vasodilators involved in vasomotion oscillations and the AFC response.

Keywords

nitric oxide; oscillations; laser-Doppler flowmetry; cerebral blood flow

1. Introduction

The free radical nitric oxide (NO) is an important modulator of the activation-flow coupling (AFC) response, the coupling of neuronal activity and cerebral blood flow (CBF) for a functional task (Faraci and Breese, 1993; Iadecola et al., 1994; Villringer and Dirnagl, 1995). NO is a potent vasodilator that is readily abundant; can easily diffuse; and has a relatively short half-life (Magistretti and Pellerin, 1999). It has shown to be involved in hypercapnia associated CBF increases (Iadecola and Zhang, 1996).

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NO is synthesized by a family of isoenzymes termed NO synthases (NOS). Three main isoforms of NOS exist including neuronal (nNOS), inflammatory (iNOS), and endothelial (eNOS). Both nNOS and eNOS are constitutively expressed under normal physiological conditions; whereas iNOS is produced during immunological stress (Moore and Handy, 1997; Szabo, 1996; Valko et al., 2007; Wiesinger, 2001).

The role of NO in the AFC response has been assessed in genetically engineered mice lacking either nNOS or eNOS. The AFC response for vibrissae stimulation was affected in nNOS knockout (Ma et al., 1996) but not eNOS knockout mice (Ayata et al., 1996). nNOS rather than eNOS may modulate the AFC. However, the absence of complete elimination of the AFC response in these knockout mice suggests that involvement of additional vasodilators in this coupling response (Peng et al., 2004).

The role of NO in the AFC response can also be studied using nitric oxide synthase inhibitors such as: N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME) (a non-selective NOS inhibitor), Nⁿ-nitro-L-arginine (L-NNA) (a non-selective NOS inhibitor), and 7-nitroindazole (7NI) (a selective nNOS inhibitor). The magnitude of the AFC response due to sciatic nerve stimulation in rats was significantly reduced after topical administration of L-NAME but restored with infusion of the NO precursor, L-arginine (Northington et al., 1992). Both topical and systemic application of L-NNA reduced the magnitude of the AFC response with systemic dispensation primarily affecting the early portion of the AFC response while topical administration dampening the entire AFC response (Dirnagl et al., 1993a; Dirnagl et al., 1993b; Dirnagl et al., 1994; Lindauer et al., 1999; Ngai et al., 1995; Peng et al., 2004). Systemic administration of 7-NI has also been shown to reduce the amplitude of the AFC response (Liu et al., 2008; Yang et al., 1999; Yang and Chang, 1998). However, these studies have typically used a protracted stimulus (1 minute) separated by relatively long inter-stimulus intervals (> 1 minute) (Dirnagl et al., 1993a; Dirnagl et al., 1993b; Dirnagl et al., 1994; Lindauer et al., 1999; Ngai et al., 1995; Peng et al., 2004) to assess the effects of NOS inhibitors on the AFC response. When a relatively short duration stimulus (< 10 seconds) with small inter-stimulus intervals (< 30 seconds) applied, the magnitude of the AFC response has been shown to be either unaltered (Adachi et al., 1994) or in fact slightly increased (Matsuura and Kanno, 2002). The effects of both stimulus duration and inter-stimulus interval may affect the magnitude of the AFC response. Further characterization of the effects of NOS inhibition on the AFC response with various periodicities is therefore required.

Systemic administration of non-selective NOS inhibitors not only decreases baseline CBF but also leads to the pronounced enhancement of characteristic ~ 0.1 Hz low frequency oscillations (Biswal and Hudetz, 1996; Dirnagl et al., 1993b; Hudetz et al., 1995; Lindauer et al., 1999; Matsuura and Kanno, 2002; Morita-Tsuzuki et al., 1993; Peng et al., 2004). The physiological basis of these vasomotion oscillations remains unknown (Golanov and Reis, 1995; Mayhew et al., 1996). There appears to be no correlation between the frequency, amplitude, and phase of these oscillations with systemic parameters such as heart rate or respiration (Guy et al., 1999). These vasomotion oscillations can be suppressed by cerebral vasodilation induced by mild hypercapnia (inhalation of 5% CO₂) (Hudetz et al., 1992).

Laser Doppler (LD) flowmetry has become a common method for studying the AFC since it can be easily performed; is non-invasive; and can dynamically measure cerebral blood flow (CBF) changes (Lacza et al., 2000). However, these CBF changes are only relative and not absolute measures (Dirnagl et al., 1989; Fabricius and Lauritzen, 1996; Fabricius et al., 1996; Haberl et al., 1989; Skarphedinsson et al., 1988) as LD signal measures red cell velocity and volume from which CBF is then calculated (Dirnagl et al., 1993b) (Stern, 1975). Previous studies have demonstrated that relative changes in LD_{CBF} correlate with blood flow

measurements by radioactive microspheres (Eyre et al., 1988) or the hydrogen clearance technique (Haberl et al., 1989; Skarphedinsson et al., 1988).

In the present study we investigated the effects of systemic administration of the non-selective NOS inhibitor L-NNA on LD_{CBF} in the somatosensory cortex of rats. We used both single and dual LD flowmetry probes over the somatosensory cortex to characterize baseline vasomotion oscillations after L-NNA. We studied NO's role in both modulating vasomotion oscillations and the AFC response by varying the periodicity of forepaw stimulation. Our results demonstrate a possible interaction between L-NNA induced vasomotion oscillations and the AFC response depending on stimulus length and inter-stimulus interval.

2. Results

2.1. Physiological variables were not affected by L-NNA

Physiological parameters from rats ($n=15$) during resting conditions (probes placed unilaterally or bilaterally over the somatosensory cortices) were obtained before and after administration of L-NNA (mean $\pm$ SD) (Table 1). These same parameters were also obtained from rats ($n=20$) during the baseline portions of stimulation paradigms (Table 1). Administration of L-NNA did not significantly affect these parameters, including systemic arterial blood pressure.

2.2. Large amplitude low frequency vasomotion oscillations were observed after administration of L-NNA

Dual probe measures of resting LD_{CBF} were obtained from rats ($n=15$) before and one hour after L-NNA to assess the spatial coherence of the vasomotion oscillations. LD probes were placed either unilaterally (< 1 mm apart from each other over the forepaw area of the somatosensory cortex) or bilaterally (over each of the somatosensory cortices). Prior to administration of L-NNA oscillations were not seen within either a single (Fig. 1A) or both hemispheres (Fig. 1B). The fast Fourier transform (FFT) was evenly distributed overall frequencies both within and between hemispheres (insets for Fig. 1A and B). LD_{CBF} was not correlated for the probes either unilaterally ($r=0.14$, $p=0.76$) or bilaterally ($r=0.21$, $p=0.58$). One hour after systemic administration of L-NNA, large amplitude baseline low frequency vasomotion oscillations were seen throughout the brain (Figs. 1C and D). After L-NNA, the FFT showed a major peak at 0.17 Hz for probes placed either unilaterally or bilaterally (inset Fig. 1C and D). LD_{CBF} was significantly correlated when the two probes were placed over a single somatosensory cortex ($r=0.86$, $p<0.05$) but asynchronous between somatosensory cortices ($r=0.08$, $p=0.71$). The maximum cross-correlation between bilateral somatosensory cortices after L-NNA was 0.20 ± 0.11 suggesting a random phase relationship between hemispheres.

2.3. The magnitude of the AFC response for a long stimulus (1 minute) with a long inter-stimulus interval (2 minutes) was attenuated after L-NNA

The signal averaged AFC response for a 1 minute stimulus with a 2 minute inter-stimulus interval ($n=5$) was obtained before and one hour following L-NNA administration. Stimuli were applied 30 seconds into the 3 minute iteration. Prior to L-NNA the AFC response consisted of an initial peak (first 10 seconds after stimulation) followed by a lower amplitude plateau (last 30 seconds of stimulation) portion that persisted for the length of the stimulus. Administration of L-NNA induced vasomotion oscillations that remained even after signal averaging. The overall magnitude of the AFC response, as measured by the area under the curve, was significantly attenuated (Fig. 2) by L-NNA. The peak, and not the plateau, portion of the AFC response was primarily affected.

2.4. The magnitude of the AFC response for a short stimulus (4 seconds) with a fixed shorter inter-stimulus interval was augmented after L-NNA

The AFC response for a 4 second stimulus was obtained prior to and one hour after L-NNA in a separate group of rats ($n=5$). Stimuli were always applied 8 seconds into the 24 second length iteration. A characteristic peak AFC response was observed (Fig 3). L-NNA induced vasomotion oscillations that remained even after signal averaging. Both the magnitude and area under the curve of the AFC response were significantly augmented after L-NNA.

2.5 The AFC responses cancelled for a short stimulus (4 seconds) with a random inter-stimulus interval both before and after L-NNA

To exclude the possibility that observed increases in the AFC responses were due to an occasional, extremely large response within a single iteration, we applied a short 4 second stimulus at various time points during the 24 second length iteration in a separate set of rats ($n=5$). Stimuli were always applied at either 16 second (Fig. 4A) or randomly (Fig. 4B) along the iteration in separate trials. Prior to L-NNA a characteristic peak AFC response was observed soon after the stimulus was applied (Fig. 4A). One hour after L-NNA low frequency LD_{CBF} oscillations were observed that did not cancel even after signal averaging. Similar to the above studies, both the magnitude and the area under the curve of the AFC response were significantly augmented after L-NNA.

Prior to L-NNA when a 4 second stimulus was randomly applied throughout the 24 second length iteration the AFC responses canceled each other with no single response dominating (Fig 4B). A similar reaction was seen after L-NNA with no single AFC response dominating after L-NNA.

2.6. The magnitude of the AFC response for a short stimulus (4 second) with various periodic shorter inter-stimulus intervals was augmented by L-NNA

To further characterize the interaction between stimulus periodicity of the AFC response and L-NNA induced vasomotion oscillations, a short stimulus (4 seconds) was applied with various iteration lengths (16 seconds, 24 seconds, or 36 seconds) for separate group of rats ($n=5$). Stimuli were always applied 8 seconds into these various length iterations. Prior to L-NNA the peak height (PH) or magnitude of the AFC response was similar for the various iteration lengths (Fig 5). After L-NNA, the PH of the AFC response was significantly increased for the 4 second stimuli applied during 16 second and 24 second length iterations. A similar increasing trend in the PH of the AFC response was seen for the 36 second length iteration after L-NNA ($p=0.11$).

2.7. An interaction occurred between stimulus frequency of the AFC response and L-NNA induced vasomotion oscillations

A FFT was performed on raw unaveraged data from rats used to investigate the effects of functional stimulus periodicity on the AFC response (Section 2.6). When a 4 second stimulus was periodically applied during the 16 second length iteration prior to L-NNA, a peak was observed at the stimulus frequency (fundamental frequency) of 0.063 Hz with smaller peaks seen at harmonics of this frequency (0.125 Hz and 0.188 Hz) (Fig. 6A). After administration of L-NNA, both the fundamental frequency and stimulus harmonics of this frequency were augmented. A characteristic oscillation peak was also observed at 0.17 Hz along with an increase at the second harmonic (0.188 Hz) after L-NNA. Similar results were seen for a 4 second stimulus applied during the 24 second length iteration (Fig. 6B) with L-NNA leading to an increase in both the fundamental frequency and stimulus harmonics of this frequency. A characteristic oscillation peak was also observed at 0.17 Hz with a large increase seen at the third harmonic (0.167 Hz). For 4 second stimuli applied during the 36 second length interval

(Fig. 6C), before L-NNA the largest peak was at the fundamental frequency (0.028 Hz) with smaller peak seen at harmonics of this frequency (0.056 Hz, 0.083 Hz, 0.111 Hz, 0.139 Hz, 0.167 Hz, and 0.194 Hz). After L-NNA there was no significant augmentation at the fundamental frequency, the first stimulus harmonic, or second stimulus harmonic. A characteristic oscillation peak though was also observed at 0.17 Hz with a larger increase in the spectral power at the fifth stimulus harmonic (0.167 Hz). These results suggest a possible interaction between the stimulus frequency or its harmonic and the L-NNA induced oscillations. At shorter iteration lengths an increase was seen at the fundamental frequencies and at the stimulus harmonic closest to the vasomotion oscillation frequency. As the iteration length increased there was a reduction in the amplitude of the AFC response with less interaction occurring between L-NNA induced oscillations and the AFC response.

3. Discussion

The major findings of this paper are: 1) the administration of the non-selective NO synthase inhibitor, L-NNA, led to vasomotion oscillations that were in phase when the two LD probes were close to each other over the same hemisphere, but were asynchronous when the LD probes were placed over bilateral somatosensory cortices, 2.) after administration of L-NNA the AFC response was significantly reduced for stimuli repeatedly applied at relatively longer inter-stimulus intervals. The greatest decrease occurred at the early peak segment of the AFC response 3.) for stimuli applied at shorter inter-stimulus intervals the magnitude of the AFC response was augmented after L-NNA, 4.) spectral analysis demonstrated an interaction between the AFC response and L-NNA induced vasomotion oscillations with the greatest increase occurring at the stimulus harmonic closest to the vasomotion oscillation frequency.

Our study confirms previous findings that have demonstrated the presence of characteristic oscillations (between 4–11 cycles per minute or between 0.07 Hz and 0.18 Hz) (Biswal and Hudetz, 1996; Hudetz et al., 1992; Hudetz et al., 1995; Lacza et al., 2000; Lindauer et al., 1999; Mayhew et al., 1996; Morita-Tsuzuki et al., 1993; Vern et al., 1988). We have further characterized these 0.17 Hz oscillations using dual LD probes. After L-NNA, oscillations were present bilaterally and were correlated locally (within a hemisphere) but not with increasing distance (between hemispheres) (Dirnagl et al., 1989; Morita-Tsuzuki et al., 1993). Systemic administration of L-NNA may inhibit NOS production leading to oscillations within a common feeding vessel that are propagated to downstream branches (Behzadi and Liu, 2005; Griffith, 1996; Hudetz et al., 1992; Hudetz et al., 1995; Vern et al., 1988).

The exact etiology of these low frequency vasomotion oscillations remains unknown (Vetri et al., 2007). These oscillations may increase the diffusion of oxygen from the capillaries (Tsai and Intaglietta, 1993); improve the mean driving pressure across arterioles (Hudetz et al., 1992); and improve cerebral blood flow within activated cortical regions (Funk et al., 1983). Two mechanisms have been proposed to explain their etiology: 1) a neurogenic basis with bursts originating from either the cerebellar fastigial nucleus or the rostral ventrolateral medulla (Golanov and Reis, 1995). 2) a myogenic source (Mayhew et al., 1996) due to tonic fluctuations in intracellular concentrations in K^+ and Ca^{+2} from sympathetic nerves surrounding the arterioles (Morita-Tsuzuki et al., 1993). Mathematical models using a myogenic mechanism have demonstrated that vasomotion oscillations can be propagated locally and self-sustained within a group feeding of vessels (Behzadi and Liu, 2005; Ursino et al., 1996). Our results are in agreement with a myogenic model.

In our studies we also examined the effect of variations in stimulus periodicity on the interaction between L-NNA induced oscillations and the AFC response. The magnitude of the LD CBF response to 1 minute of stimulation with a 2 minute inter-stimulus interval was reduced after L-NNA, with the greatest decrease occurring in the early peak segment of the AFC response

(Fig 3). Distinct neurovascular coupling mediators may be responsible for the peak and plateau portions of the AFC response (Berwick et al., 2008). NO may modulate the early portion of the AFC response with an initial diffuse response becoming more spatially restricted with protracted stimulation (Dirnagl et al., 1993a).

In contrast, we observed an augmentation of the AFC response for short stimuli (4 seconds) with shorter inter-stimulus after administration of L-NNA (Fig 3, 4, and 5). Our results are similar to a previous study that showed a trend toward an increase in the AFC response for shorter periodicities after L-NNA (Matsuura and Kanno, 2002). This observed increase after L-NNA occurred through an interaction between the stimulus frequency and vasomotion oscillations. In particular, a frequency dependent transmission occurred at the stimulus harmonic closest to the 0.17 Hz L-NNA induced vasomotion oscillation frequency (Figures 5 and 6). The degree of modulation depended on the number of the stimulus harmonic with a lower stimulus harmonic number leading to a greater increase in the AFC response (Obrig et al., 2000). These results are consistent with the observation that the degree of augmentation of the AFC response is modulated by the periodicity of stimulation (Jones et al., 2007). Typically increases in neuronal activity with AFC response will dominate over the vast background oscillatory activity. However, a significant interaction can emerge if the stimulus frequency is triggered at a certain periodicity (Baselli et al., 2006).

While we did not measure neuronal activity using evoked field potentials, a study using a similar systemic concentration of L-NNA did not observe significant changes in the spike firing using this drug (Matsuura and Kanno, 2002). Our results suggest that non selective NOS inhibition by L-NNA may affect the vascular resistance but not the coupling between CBF and neuronal activity. NO is most likely a modulator of both the AFC response (Iadecola et al., 1994; Lindauer et al., 1999) and low frequency baseline oscillations (Fujii et al., 1990). Under normal conditions basal release of NO may modulate vascular relaxation permitting vasodilation mediated by other agents. These NO independent metabolic or neurogenic influences of vascular tone may therefore gain importance after administration of L-NNA. These same factors may also be also involved in AFC and could augment coupling as seen with short periodic stimuli. (Lindauer et al., 1999).

Our current study does have limitations. First, we used relatively low frequency sinusoidal stimulation. Even larger changes in the amplitude of the AFC response would be seen if either short triangular pulses or high frequency sinusoidal wave were applied (Goloshesky et al., 2008) as both will increase the power in the high frequency harmonics. At these higher frequencies electrical stimulation of the forepaw may bypass peripheral nerve receptors and lead to direct stimulation of both fast and slow adapting afferent axons of the sensory nerves. Future studies using various frequencies are required. Second, the type of anesthetic may interfere with NOS inhibition and influence the AFC response (Gerrits et al., 2001). The effect of alpha chloralose on vasomotion oscillations remains unresolved. A previous study has shown no impact of this anesthetic on the occurrence of vasomotion oscillations (Jones et al., 1995). However, others have demonstrated that alpha chloralose can suppress vasomotion oscillations and interfere with the translocation of calcium (Adachi et al., 1992). We cannot exclude the possibility that vasomotion oscillations could be even more pronounced in the awake rats (Fujii et al., 1990).

4. Experimental procedures

4.1 General Preparation

Thirty-five adult male Sprague-Dawley rats (320–400g) obtained from Charles River (Wilmington, MA) were initially anesthetized with 2–4% halothane in 70% N₂O, 30% O₂ by face mask. Subcutaneous 2% lidocaine was used to elevate the tail dermis away from the tail

artery before incision and prevent vasospasm during catheter insertion. A polyethylene catheter (PE-50) was used to cannulate the tail artery to measure the arterial blood pressure and monitor arterial blood gasses. Rats were tracheostomized; mechanically ventilated (Harvard Rodent Ventilator, Harvard, South Natick, MA); and maintained on 1% halothane in 70% N₂O, 30% O₂. The head of the rat was placed in a stereotactic frame, a midline incision performed, and the scalp over the frontoparietal cortex retracted. An area 4 mm × 5 mm overlying the forepaw area of the somatosensory cortex was thinned on both sides of the skull using a saline-cooled dental drill until a thin translucent cranial plate remained (Ances et al., 1998). Halothane and nitrous oxide were discontinued following surgery and for at least 45 minutes prior to data acquisition. Anesthesia was maintained by intraperitoneal injections of α -chloralose (60 mg/kg) with supplemental doses (30 mg/kg) administered hourly. Body temperature was monitored using a rectal probe and maintained at 37.0 ± 0.5 °C by a feedback controlled heating pad. P_aO₂ and P_aCO₂ were monitored by hourly blood gasses. Ventilation parameters were adjusted to maintain P_aCO₂ between 30–35 mm Hg. After stabilizing the blood pressure and ensuring that the tail pinch response was absent, LD_{CBF} was recorded.

4.2 Forepaw stimulation

Electrical forepaw stimulation in rats (n= 20) was performed using two needle electrodes inserted subdermally into the forepaw contralateral to the single LD flowmetry probe. A function generator (Global Specialties, New Haven, CT) was used to control the stimulus frequency with frequency fixed at 5 Hz (Ances et al., 1998) and sinusoidal pulse width maintained at 1 millisecond. The stimulus amplitude was set at 1.0 mA using a stimulus isolation device (World Precision Instruments A-36V, San Diego, CA).

4.3 Laser Doppler Flowmetry

LD_{CBF} measures were obtained from either a single 0.8 mm diameter LD flowmetry (Vasamedics, St. Paul, MN) or a dual 1.0 mm diameter LD flowmetry system (Perimed, Sweden). Micromanipulators were used to place the single or dual LD probes normal to the thinned skull. Probes were positioned approximately 4–5 mm lateral to Bregma. All LD measurements using the dual probe studies were recorded with a time constant of 0.2 seconds while a time constant of 0.5 seconds was used for single probe studies. For all trials using either the single or dual probes, arterial blood pressure was monitored to insure that observed LD_{CBF} changes were not the result of fluctuations in systemic blood pressure (Ances et al., 1998). LD_{CBF} measurements were obtained both prior to and one hour after intra-peritoneal injection of L-NNA (40 mg/kg).

4.4 Data Acquisition and Analysis

On-line signal averaging of all LD_{CBF} data was accomplished using software written in Labview running on a PC equipped with an analog to digital converter (National Instruments, Austin, TX). Baseline LD_{CBF} studies consisted of 600 data points collected at 5 Hz. All stimulation trials consisted on ten signal averaged iterations for either a longer (1 minute) or shorter (4 seconds) periodic stimulus. Longer stimulation periodicity trials consisted of a 1 minute stimulus with a 2 minute inter-stimulus interval. These trials were acquired at 5 Hz with a single iteration consisting of 900 data points. Shorter stimulation periodicity trials consisted of a 4 second stimulus periodically applied during 16, 24, or 36 second length iteration. These trials were acquired at 10 Hz with a single iteration consisted of 160, 240, or 360 data points respectively. At least two baseline or stimulation trials were performed for each rat.

Both raw and signal averaged LD data were analyzed using software written in Interactive Data Language (IDL, Research Systems, Boulder, CO). For stimulation studies LD voltages were corrected to relative percent changes in LD_{CBF} from baseline by dividing by the average baseline value obtained prior to stimulation (either 30 seconds for the 1 minute stimuli or 8

seconds for the 4 seconds stimuli). The magnitude or PH of the AFC response due to stimulation was calculated by averaging 1 second of data surrounding the peak response for each trial. This procedure allowed for averaging across identical time points for all rats and avoided errors in peak picking due to residual noise within individual trials. The area under the curve for all stimulation studies was obtained by summing all points above the baseline for the signal averaged responses. Data from each rat for the various stimulation conditions was collated and an overall average for all rats was determined. Correlation coefficients from baseline studies were determined for each rat prior to and after L-NNA. Spectral analysis using the FFT was performed on raw unaveraged LD data from both baseline and stimulation studies to determine possible phase and frequency changes due to L-NNA.

4.5 Statistical Analysis

All data are expressed as mean $\pm$ intrasubject standard deviation (SD). A one-way analysis of variance (ANOVA) with repeated measures was performed for both the PH, area under the curve, and the peak values of the vasomotion oscillation frequency obtained for the various stimulation paradigms (Sigma Stat, SPSS Inc., Chicago, IL). A Tukey's test was performed when a significant difference ($p < 0.05$) was observed.

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Figure 1.

Laser Doppler (LD) measurements in arbitrary units over the ipsilateral and contralateral somatosensory cortices prior to and one hour after administration of the non selective nitric oxide synthase (NOS) inhibitor- N^G -nitro-L-arginine (L-NNA). Before L-NNA no oscillations were observed when both probes were placed either adjacent to each other over the same hemisphere (A) or between hemispheres (B) for a single rat. L-NNA induced oscillations were seen within each hemisphere (C) and between hemispheres (D) for the same rat. Insets shows the FFT of unaveraged data from rats ($n=15$) before and after L-NNA. Prior to L-NNA the power spectrum is evenly distributed with no frequency dominating when the probes were placed either adjacent to each other (inset to Figure 1A) or bilaterally (inset to Figure 1B). After L-NNA a major peak at 0.17 Hz ($P < 0.05$) was present both unilaterally (inset to Figure 1C) and bilaterally (inset to Figure 1D). For all figures black lines are measurements from Probe #1 and gray lines are from Probe #2.

Figure 2.

The activation flow coupling (AFC) response for periodic forepaw stimulation (1 minute) at longer inter-stimulus intervals (2 minutes) both prior to and after L-NNA. The early peak portion of the AFC response, as measured by % LD_{CBF} change from baseline, was diminished after L-NNA ($n=5$). Solid gray line shows the AFC response before L-NNA while solid black line shows the AFC response after L-NNA. The hashed lines represent the stimulus duration with all errors bars indicating standard deviation (SD).

Figure 3.

The AFC response for periodic forepaw stimulation (4 seconds) at shorter inter-stimulus intervals (20 seconds) both prior to and after L-NNA. The magnitude of the AFC response was significantly augmented after L-NNA (n=5). The solid gray line shows the AFC response before L-NNA while the solid black line shows the AFC response after L-NNA. The hashed lines represent the stimulus and error bars indicate SD.

Figure 4.

Interaction between AFC response for various inter-stimulus intervals and L-NNA induced vasomotion oscillations. Changes in LD_{CBF} expressed as a percentage of baseline for 4 second stimuli applied 16–20 seconds into a 24 second iteration (A) or randomly (B) within 24 second length iteration both before and after L-NNA (n=5). The AFC response was augmented after L-NNA for periodic stimuli (A) but cancelled for random stimuli (B). The solid gray line represents the AFC responses to 4 second stimulus before L-NNA for both conditions while the solid black line shows the AFC response for 4 second stimulus presented after L-NNA for both conditions. For Figure 4A the hashed lines represent the stimulus duration with all errors bars indicating SD. For Figure 4B the stimulus was randomly applied throughout the iteration.

Figure 5. Peak heights (PH) of the AFC response for 4 second stimuli obtained prior to and after L-NNA for various inter-stimulus intervals (n=5). After L-NNA administration, the PH was significantly increased for 4 second stimuli during the 16 second (*= $p < 0.01$) and 24 second (**= $p < 0.05$) length iterations. The PH was also increased, but not significantly ($p = 0.11$), for 4 second stimuli during the 36 second length iteration after L-NNA. Error bars indicate SD.

Figure 6.

Spectral analysis using the FFT for various inter-stimulus intervals both before and after L-NNA. Power spectrum for 4 second stimuli for iterations lengths of 16 seconds (A), 24 seconds (B), and 36 seconds (C) (n=5). The FFT for 4 second stimuli applied every 16 seconds prior to L-NNA has a peak at 0.063 Hz, the fundamental frequency, with additional peak seen at stimulus harmonics of this frequency (0.125 Hz and 0.188 Hz). After L-NNA, the fundamental frequency and its harmonics were augmented with the second stimulus harmonic (0.188 Hz) having the greatest augmentation. Also after L-NNA a vasomotion oscillation peak was observed at 0.17 Hz (Fig. 6A). Similar results were seen with 4 second stimuli for both 24 seconds (Fig. 6B). As seen in Figure 6B the fundamental frequency was 0.042 Hz with peaks observed at stimulus harmonics of this frequency (0.083 Hz, 0.125 Hz, and 0.167 Hz). After L-NNA the fundamental frequency and its harmonics were augmented with the third stimulus harmonic (0.167 Hz) having the greatest augmentation. A vasomotion oscillation peak was also observed at 0.17 Hz. In Figure 6C for 4 second stimuli applied every 36 seconds, the fundamental frequency was 0.028 Hz with peaks observed at stimulus harmonics of this frequency (0.056 Hz, 0.083 Hz, 0.111 Hz, 0.139 Hz, 0.167 Hz and 0.194 Hz). After L-NNA there was no significant change in the fundamental frequency and its harmonics. The greatest change was at the fifth stimulus harmonic (0.167 Hz) but this was not significant. In all figures solid gray lines show the FFT prior to L-NNA while solid black lines show the FFT after L-NNA.

Table 1

Physiological variables both before and after L-NNA.

Study	SAP (mm Hg)	PaCO ₂ (mmHg)	PaO ₂ (mmHg)	pH
<i>Baseline (n=15)</i>				
Before L-NNA	112 ± 5	33 ± 2	118 ± 3	7.41 ± 0.02
After L-NNA	117 ± 6	34 ± 3	116 ± 2	7.40 ± 0.01
<i>Stimulation (n=20)</i>				
Before L-NNA	113 ± 6	35 ± 3	121 ± 6	7.39 ± 0.03
After L-NNA	117 ± 4	34 ± 3	118 ± 7	7.40 ± 0.02

Values are the mean ± standard deviation (SD) with n= number of animals for each study. SAP = systemic arterial pressure; PaCO₂= arterial PCO₂; PaO₂=arterial PO₂.