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# BRAIN HEMODYNAMIC RESPONSE TO SOMATOSENSORY STIMULATION IN NEUROLIGIN-1 KNOCKOUT MICE

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**Abstract—Neuroligin 1 (NLGN1) is a postsynaptic adhesion molecule that determines N-methyl-D-aspartate receptor (NMDAR) function and cellular localization. Our recent work showed that *Nlgn1* knockout (KO) mice cannot sustain neuronal activity occurring during wakefulness for a prolonged period of time. Since NMDAR-dependent neuronal activity drives an important vascular response, we used multispectral optical imaging to determine if the hemodynamic response to neuronal stimulation is modified in *Nlgn1* KO mice. We observed that *Nlgn1* KO mice show a 10% lower response rate to forepaw electrical stimulation compared to wild-type (WT) and heterozygote (HET) littermates on both the contra- and ipsilateral sides of the somatosensory cortex. Moreover, *Nlgn1* mutant mice showed an earlier oxyhemoglobin peak response that tended to return to baseline faster than in WT mice. Analysis of the time course of the hemodynamic response also showed that HET mice express a faster dynamics of cerebrovascular response in comparison to WT. Taken together, these data are indicative of an altered immediate response of the brain to peripheral stimulation in *Nlgn1* KO mice, and suggest a role for NLGN1 in the regulation of cerebrovascular responses.**
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**Key words: multispectral intrinsic optical imaging, cerebrovascular response, N-methyl-D-aspartate receptors, synaptic adhesion molecules, rodents.**

## INTRODUCTION

The brain is one of the most oxygen-consuming organs because the oxidation of glucose provides the energy required for neuronal activity (Siesjo, 1978). Neuronal activity, oxygen consumption and metabolism are thus strongly linked together. Cerebral blood flow (CBF) regulates the oxygen supply to brain tissue and increases during neuronal activity (Uludag et al., 2004; Leithner et al., 2010). CBF typically increases within 1 s following electrical stimulation, along with other hemodynamic changes such as oxyhemoglobin (HbO) increases and deoxyhemoglobin (HbR) decreases (Dunn et al., 2005; Dubeau et al., 2011). Dysfunctions in the vascular response of the brain will impact neuronal activity and were shown to associate with cognitive impairment (Iadecola, 2013).

Glutamate is the main excitatory neurotransmitter of the brain (Nicholls, 1992; Attwell and Laughlin, 2001). Glutamatergic neurotransmission thus represents an important energy-consuming process (Attwell and Iadecola, 2002). The hemodynamic response to somatosensory stimulation has been shown to depend on the activity of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and N-methyl-D-aspartate receptors (AMPAR and NMDAR) (Gsell et al., 2006). More precisely, blocking NMDAR and AMPAR not only modulates synaptic responses but also alters CBF in the rat somatosensory cortex (Norup Nielsen and Lauritzen, 2001). Also, a selective NMDAR antagonist was shown to block the cerebral dilator response induced by NMDA in rodents (Faraci and Breese, 1994; Pelligrino et al., 1995, 1996).

Specific adhesion proteins could mediate the relationship between cerebrovascular response and glutamatergic neurotransmission. Indeed, members of both the Ephrin/Eph and Neurexin/Neuroligin (NRXN/NLGN) families have been linked to neurovascular physiology. For instance, the absence of EphA4 led to smaller diameter brain blood vessels (Goldshmit et al., 2006), whereas NRXN and NLGN were shown to be expressed in blood vessels and to modulate angiogenesis (Bottos et al., 2009; Graziano et al., 2013). NLGN1 is a post-synaptic protein that regulates synaptic function through its association with pre-synaptic NRXN (Ichtchenko et al., 1995). NLGN1 has been shown to regulate NMDAR functioning and sub-cellular localization (Kim et al., 2008; Barrow et al., 2009; Wittenmayer et al., 2009; Jung et al., 2010). Recent work shows that *Nlgn1* knockout (KO) mice exhibit deficits in social novelty and

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*Abbreviations:* ANOVA, analysis of variance; CBF, cerebral blood flow; EEG, electroencephalographic; HbO, oxyhemoglobin; HbR, deoxyhemoglobin; HET, heterozygote; KO, knockout; LED, light-emitting diode; LTP, long-term potentiation; NMDAR, N-methyl-D-aspartate receptor; NRXN, Neurexin; NLGN, Neuroligin; NLGN1, Neuroligin 1; NLGN3, Neuroligin 3; ROI, region of interest; SEM, standard error of the mean; WT, wild-type.

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fear-conditioning tasks, reduced wakefulness duration, and alterations in electroencephalographic (EEG) activity during wakefulness and sleep (Kim et al., 2008; El Helou et al., 2013; Massart et al., 2014).

Here, we hypothesized that altered NMDAR function, behavior, cognition and EEG activity observed in *Nlgn1* KO mice would associate with modifications in the cerebrovascular response to somatosensory stimulation since neurovascular coupling depends on NMDAR activity (Gsell et al., 2006), NLGN1 regulates NMDAR-mediated transmission (Kim et al., 2008; Jung et al., 2010) and our preliminary analyses suggested a modified brain hemodynamic response in *Nlgn1* KO mice (El Helou et al., 2013). We characterized in detail the cerebrovascular response of the contra- and ipsilateral somatosensory cortex to two different intensities of electrical stimulation of the forepaw in *Nlgn1* KO mice using intrinsic optical imaging. Results support altered dynamics of the cerebrovascular response in *Nlgn1* KO mice and suggest an implication of NLGN1 in the regulation of the hemodynamic response to neuronal activity.

## EXPERIMENTAL PROCEDURES

### Animals

Male mice used in this study were B6;129 *Nlgn1$^{mutant}$*/J mice (absent NLGN1 protein due to deletion of coding exons 1 and 2) bred on site and studied between 8 and 16 weeks of age ($n = 11$ wild-type [WT, $+/+$], 16 heterozygous [HET, $+/-$], 19 homozygous mutant [KO, $-/-$]). Mice were maintained under standard housing conditions (free access to food and water, 12 h-light/12 h-dark cycle, 22–25 °C ambient temperature, housed 2–3 per cage) from birth to testing. Experiments were approved by the Ethics Committee for Animal Experimentation of the Research Center of the Hôpital du Sacré-Coeur de Montréal in accordance with Guidelines from the Canadian Council on Animal Care, which are recognized as an international reference by the International Council for Laboratory Animal Science. A sample of the animals studied here ($n = 27$) has previously been submitted to preliminary analyses, and therefore part of the methodological design has been described in our previous publication (El Helou et al., 2013).

### Surgeries and protocol

The brain hemodynamic response to somatosensory stimulation was assessed using multispectral optical imaging similar to previously described (Dubeau et al., 2011; El Helou et al., 2013). Animals were anesthetized using an intraperitoneal injection of Ketamine/Xylazine, (120/10 mg/kg), and half the initial dose was supplemented when the animal responded to a hindpaw toe-pinch at any instance throughout the protocol. Mice were anesthetized for the entire duration of the experiments (45–75 min) and sacrificed at the end. Mice were positioned on a stereotaxic frame, and temperature was monitored and maintained at 37 °C with a heating blanket (Harvard Apparatus Canada, St-Laurent, QC, Canada). A section of the skull of about 1 cm$^2$ was exposed, and the skin was held by surgical clamps. Mineral oil was added to the exposed skull to prevent drying and to ensure stable camera focus. Two electrodes were placed on each side of the left forepaw for stimulation using an electrical stimulator (A-M Systems; #2200, Sequim, WA, USA).

The threshold for muscular excitation was determined under anesthesia using a 0.3-ms pulse and increasing current intensity. For imaging, the first session included the use of a twofold-threshold intensity (between 1 and 2.6 mA) for electrical stimulation (3 Hz, 300 µs). Stimulations (minimum of 15) were repeated every 20 s with a 1- to 3-s random jitter to avoid systemic synchronization. For some animals ($n = 3$ WT, 4 HET, 9 KO), a second session using a fourfold-threshold intensity (between 2 and 5.2 mA) was also performed (minimum of 15 stimulations) in order to investigate the impact of the *Nlgn1* mutation on the cerebrovascular response to very high/saturated stimulation.

### Data acquisition

Functional images (typical truncated size 450 × 400 pixels) were recorded under three wavelength flashing illuminations (525, 590 and 637 nm) produced by light-emitting diodes (LED) Optek Technologies, TX, USA) at a sampling rate of 5 Hz per wavelength using a 12-bit CCD camera (CS3960DCL; Toshiba Teli). Reflectance signals from the LED collected with the camera were converted into changes in absorption for the three wavelengths, followed by extraction of relative changes in HbO and HbR using a modified Beer–Lambert law (Dunn et al., 2003; Dubeau et al., 2011).

### Data analysis

Spatial *t*-statistic HbO and HbR response maps were constructed from the mean and standard error of the mean (SEM) of the early and filtered response to all twofold-threshold stimulations (Fig. 1A). This was calculated for each pixel by taking the mean between 1 and 3 s after stimulation, expressed relative to the median of 2 s before stimulation on temporally high-pass-filtered (0.03-Hz cutoff) and spatially low-pass-filtered data (Gaussian, 0.5 pixel SD). Animals that did not respond on these maps were removed from further analysis because a contralateral region of interest (ROI) could not be defined ($n = 3$; 1 HET, 2 KO). For each mouse, a ROI (fixed size of 75 × 75 pixels) was selected to cover the maximum response area located above the right somatosensory cortex (contralateral; Fig. 1A). The ipsilateral ROI was defined as the corresponding position over the left somatosensory cortex (Fig. 1A). Then, after standard bandpass filtering of the raw data between 0.01 and 0.67 Hz, time courses of the relative changes were built for HbO and HbR for the selected ROI as a function of the median of 2 s before stimulation. Response failures (absence of HbO peak combined with HbR trough between 1 and 5 s poststimulation)

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