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; Leitner 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...
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 Nlgn
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 (Dubéau 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 cm² 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; Dubéau 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 (contralateral; 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)
were excluded to construct the mean time course of one animal in order for the time course to solely reflect distinguishable vascular responses. This was done separately for contra- and ipsilateral ROI as well as for twofold- and fourfold-threshold stimulation. A response rate (percentage of response) was calculated as [total number of stimulations minus response failures] divided by the total number of stimulations multiplied by 100. To ensure the contribution of a minimum number of responses to individual hemodynamic parameters, mice that did not show either a 50% or greater response rate or 10 or more distinct contralateral responses were excluded (1 HET, 1 KO). The final number of mice for the twofold-threshold intensity was thus 11 WT, 14 HET, and 16 KO; and for the fourfold-threshold intensity, 3 WT, 4 HET, and 9 KO.

Four properties were computed from the time course of hemodynamic responses: peak time, peak amplitude, rise to half-peak and fall to half-peak. All parameters were calculated per mouse by taking the mean of all good responses. Peak time was measured as the time from stimulation onset to the maximum response level (i.e., peak amplitude; Fig. 2A, denoted B). Peak amplitude was defined as the highest level reached within 5 s after stimulation and represented as a percent change from the mean of 2 s before stimulation (Fig. 2A, denoted D). The rise to half-peak was calculated as the first time to reach half the peak amplitude after stimulation, and the fall to half-peak as the time to reach half the peak amplitude value after peak time (Fig. 2A, denoted A and C respectively).

Statistical analyses

Muscular threshold, percent response and response properties were compared between genotypes using one- or two-way analyses of variance (ANOVA) followed by Tukey’s multiple comparison tests when appropriate. Time courses of HbO and HbR were compared for the first 10 s using Genotype-by-Time repeated-measure ANOVAs with degrees of freedom adjusted for nonsphericity using Huynh–Feldt corrections. Significant effects were decomposed, when appropriate, using planned comparisons. Statistical analyses were performed using Statistica (StatSoft Inc., Oklahoma, USA) or SPSS (SPSS Inc., Chicago, USA). The threshold for statistical significance was set to 0.05, and data are reported as mean ± SEM.

RESULTS

Decreased percent response to peripheral stimulation in Nlgn1 KO mice

To characterize the impact of NLGN1 absence on cerebrovascular response evoked by peripheral muscle stimulation, the number of hemodynamic responses observed for the ROI covering the somatosensory cortex was initially compared between Nlgn1 KO mice and WT and HET littermates (representative ROI shown for the

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**Fig. 1.** (A) Representative HbO response maps to twofold-threshold forepaw stimulations for each genotype (WT: +/+; HET: +/−; KO: −/−). Yellow rectangles represent regions of interest (ROI) selected to cover the left (ipsilateral; lower ROI) and right (contralateral; upper ROI) somatosensory cortex. (B) Percentage of brain hemodynamic responses in both HbO and HbR identified over contra- and ipsilateral ROI for the twofold-threshold forepaw stimulation in WT (n = 11), HET (n = 14) and Nlgn1 KO (n = 16) mice. A significant ROI effect (contralateral vs. ipsilateral; F1,76 = 27.4, P < 0.001) and a significant genotype effect were found (F2,76 = 9.5, P < 0.001). *P < 0.05 compared to WT and HET. (C) Threshold intensity for observable muscular response, measured in the same WT, HET and KO as above, did not differ with genotype (F2,38 = 1.4, P = 0.3).
As expected, percent response to stimulations was higher in the contralateral than in the ipsilateral cortex (Fig. 1B). Importantly, Nlgn1 KO mice showed a 10% decrease in percent response to stimulation in the targeted ROI when compared to HET and WT littermates on both contra- and ipsilateral sides of the cerebral cortex (Fig. 1B). This may indicate that the brain ability to respond to peripheral stimulation is compromised in mice lacking NLGN1. This difference should not originate from differences in stimulation intensity because threshold intensity did not significantly differ between genotypes (Fig. 1C).

### Altered time course of hemodynamic response in Nlgn1 KO mice

The detailed time course of the hemodynamic response of the somatosensory cortex was compared between genotypes, separately for HbO and HbR and for the contralateral and ipsilateral cortex. The time course of HbO response significantly differed between genotypes in both the contralateral and ipsilateral regions (Fig. 2A). In general, HET mice displayed an elevated HbO percent change after stimulation onset and until maximum change was reached in comparison to WT mice (between 0 and 1.8 s in contra, and between 0 and

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**Fig. 2.** Mean hemodynamic response of the regions of the somatosensory cortex following twofold-threshold forepaw stimulation in WT (+/+), n = 11), HET (+/−, n = 14) and Nlgn1 KO (−/−, n = 16) mice. (A) Time course of HbO and HbR responses of the contra- and ipsilateral cerebral cortex regions. Analysis of the first 10 s after stimulation revealed a significant Genotype-by-Time interaction for HbO in both contra- and ipsilateral ROI (F_{98,1862} = 2.8, P < 0.01) and for HbR in the ipsilateral ROI (F_{98,1862} = 2.7, P < 0.05). Dark blue symbols: P < 0.05 and light blue symbols: P < 0.1 between HET and WT; light red symbols: P < 0.1 between KO and WT. Response properties computed in panel (B) are indicated by letters in the left contralateral panel: A for rise to half peak, B for peak time, C for return to half peak and D for peak amplitude. (B) Response properties of HbO and HbR time course for the contra- and ipsilateral ROI. A significant Genotype effect was found for contralateral HbO peak time (F_{2,38} = 3.3, P < 0.05) and ipsilateral HbR return to half peak (F_{2,37} = 3.2, P = 0.05). Tendencies for a significant Genotype effect were observed for contralateral HbO and HbR rise to half peak and for ipsilateral HbR peak amplitude (F_{2,38} ≥ 2.8, P ≤ 0.07), *P < 0.05 compared to WT, †P < 0.1 compared to WT. One KO mouse was an outlier for certain HbR response properties and was removed from subsequent analyses (contra and ipsilateral peak amplitude and ipsilateral return to half peak). Absolute HbR values are shown to facilitate representation.

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Three genotypes in Fig. 1A). As expected, percent response to stimulations was higher in the contralateral than in the ipsilateral cortex (Fig. 1B). Importantly, Nlgn1 KO mice showed a 10% decrease in percent response to stimulation in the targeted ROI when compared to HET and WT littermates on both contra- and ipsilateral sides of the cerebral cortex (Fig. 1B). This may indicate that the brain ability to respond to peripheral stimulation is compromised in mice lacking NLGN1. This difference should not originate from differences in stimulation intensity because threshold intensity did not significantly differ between genotypes (Fig. 1C).
2.4 s in ipsilateral). *Nlgn1* KO mice tended to have lower contralateral HbO response than WT mice but only after maximum response was reached (6.0–7.4 s after stimulation onset), which may indicate a faster return to baseline. For HbR, the time course significantly differed with genotype only for the ipsilateral region (Fig. 2A).

Again, HET mice tended to have a greater response between 1.6 and 2.2 s after stimulation compared to WT.

To further characterize alterations in the hemodynamic response in mice lacking NLGN1, specific response properties drawn from the response time course were compared between genotypes. In the contralateral

![Image](https://example.com/fig3.png)

**Fig. 3.** Mean hemodynamic response of the regions of the somatosensory cortex following fourfold-threshold forepaw stimulation in WT (+/+), *Nlgn1* KO (−/−), n = 9) mice. (A) Percentage of brain hemodynamic responses in both HbO and HbR identified over contra- and ipsilateral ROI for the fourfold-threshold forepaw stimulation in the three genotypes. No significant Genotype effect was observed (*F*2,13 = 1.8, *P* > 0.2). (B) Time course of HbO and HbR responses of the contra- and ipsilateral cerebral cortex regions. Analysis of the first 10 s after stimulation revealed no significant Genotype-by-Time interaction (*F*98,637 = 1.0, *P* > 0.4). (C) Response properties of HbO and HbR time course for the contra- and ipsilateral ROI. A significant Genotype effect was found for rise to half peak in contralateral HbR (*F*2,13 = 4.3, *P* < 0.05) and ipsilateral HbO (*F*2,13 = 4.5, *P* < 0.05). A trend for a Genotype effect was observed for ipsilateral HbR time to reach half peak (*F*2,13 = 3.6, *P* < 0.06). *P* < 0.05 compared to HET; *P* < 0.08 compared to HET. Absolute HbR values are shown to facilitate representation.
region. Nlgn1 KO showed a 0.5 s faster time to reach response peak in HbO compared to WT mice (Fig. 2B). This faster time-to-peak may reside from a shorter response duration or a faster response onset. Both these parameters were calculated, and no difference in response duration was observed (data not shown). However, a trend for a genotype effect in the time to reach half peak was observed for both HbO and HbR contralaterally (Fig. 2B), suggesting a faster response onset in Nlgn1 KO. No other hemodynamic response parameters differed between genotypes in the contralateral region. In the ipsilateral region, a significant genotype effect was found for HbR half peak amplitude, with HET mice returning to half peak HbR level faster and showing a trend for higher HbO peak amplitude than WT littermates (Fig. 2B). Together, these findings support that NLGN1 modulates the cerebrovascular response to peripheral stimulation.

Hemodynamic response to high stimulation intensity in Nlgn1 KO mice

To evaluate if elevated somatosensory stimulation could compensate for alterations in cerebrovascular response observed in Nlgn1 KO mice, hemodynamic time course was measured following forepaw electrical stimulation to fourfold-threshold intensity. Although the percent response to peripheral stimulation seemed to be decreased in Nlgn1 KO mice as shown for the twofold-threshold stimulation, no significant genotype effect was found for both contralateral and ipsilateral regions (Fig. 3A). In addition, the time course of HbO and HbR responses on either side of the somatosensory cortex was not significantly affected by the absence of NLGN1 for the fourfold-threshold stimulation (Fig 3B). Nevertheless, analyses of the individual properties characterizing the hemodynamic time course revealed some genotype effects (Fig. 3C). More precisely, Nlgn1 KO mice reached both contralateral HbR and ipsilateral HbO half peak faster than HET mice. A similar trend was observed for ipsilateral HbR time to reach half peak. No other hemodynamic response parameters differed between genotypes after fourfold-threshold stimulation.

DISCUSSION

The present study characterized the brain hemodynamic response to forepaw electrical stimulation in Nlgn1 KO mice using multispectral intrinsic optical imaging. The results seem to support that the absence of either one or two copies of the Nlgn1 gene alters cerebrovascular properties. More specifically, mice lacking NLGN1 expressed a lower rate of hemodynamic response following stimulation, a faster HbO peak, and a trend for a faster return to pre-stimulation values. Interestingly, mice lacking only one Nlgn1 allele also showed a faster HbO increase prior to response peak. Taken together, our findings suggest a role for NLGN1 in the regulation of cerebral hemodynamics.

Our previous (El Helou et al., 2013) and current works show that the brain hemodynamic response following sensory stimulation is modified in Nlgn1 KO mice compared to WT littermates. This was anticipated due to the acknowledged implication of NMDAR in the regulation of cerebrovascular responses (Gsell et al., 2006) and the role of NLGN1 in regulating NMDAR activity. Specifically, NMDAR are recruited and co-transported with NLGN1 (Barrow et al., 2009; Wittenmayer et al., 2009; Jung et al., 2010), and the absence of NLGN1 reduces NMDAR-mediated currents as well as the magnitude of long-term potentiation (LTP) (Kim et al., 2008; Blundell et al., 2010; Jung et al., 2010). In addition, the lack of NLGN1 affects NMDAR activity but leaves AMPAR activity mostly preserved, and indeed was shown to reduce the NMDAR/AMPAR current ratio (Chubykin et al., 2007; Jung et al., 2010). Thus, less NMDAR activity could explain the lowered response rate in Nlgn1 KO mice but since AMPAR have a faster kinetics (Blanke and Van Dongen, 2009), this shifted ratio may be responsible for the faster hemodynamic response observed.

One mechanism by which NMDAR can directly regulate CBF resides in their capacity to drive the release of vasoactive metabolites such as nitric oxide. Indeed, NMDAR activation results in calcium influx, which activates neuronal nitric oxide synthase (Garthwaite, 1991). This produces the release of nitric oxide that will expand blood vessel size (Busija et al., 2007). Accordingly, nitric oxide synthase inhibition in the cortex was shown to reduce the neuronal activity-dependent increase in blood flow (Ma et al., 1996). Therefore, it is possible that altered NMDAR functioning in Nlgn1 KO mice changes the production of nitric oxide driven by neuronal activity, which would be responsible for the modification in the cerebrovascular response to sensory stimulation. This mechanism may require astrocytes as well since they have also been proposed to be involved in cerebrovascular changes in response to nitric oxide (Roman, 2002). In addition, astrocytes directly respond to neuronal glutamate release (Porter and McCarthy, 1996; Zonta et al., 2003). As NLGN1 was shown to modulate pre-synaptic glutamate release (Peixoto et al., 2012), its absence may directly modify the response of astrocytes to synaptic activity. Of note, astrocytes express Neuroligin 3 (NLGN3) (Gilbert et al., 2001). Since NLGN1 and NLGN3 are both expressed on excitatory synapses and have similar synaptic function (Song et al., 1999; Budreick and Scheiffele, 2007), NLGN3 may compensate for the absence of NLGN1 in the KO studied here. This could also represent a manner by which either neuronal or astrocytic control of cerebrovascular dynamics was modified in the present model.

Even if the differences observed in both HET and KO mice point to a faster cerebrovascular response to peripheral stimulation, it is important to mention that the overall phenotype of HET mice did not systematically stand intermediate to that of WT and KO mice. This may be explained by the fact that specific functions of NLGN1 seem to depend on NLGN1 dosage. Indeed, a recent study showed that defects in synaptogenesis and thus in synapse number observed in NLGN1-deficient neurons depend on the level of NLGN1 expression in neighboring neurons (Kwon et al., 2012). Using this model, cells with a higher NLGN1 level were favored in synapse formation.
and thus in establishing connections with presynaptic neurons in comparison to neighboring neurons not expressing NLGN1 (Kwon et al., 2012). This may have produced some degree of between-cell competition for neuronal targets in HET mice, where different levels of NLGN1 were expressed, which could have involved units regulating cerebrovascular parameters. Interestingly, synaptic plasticity as measured using LTP was shown to be impaired in conditions where NLGN1 is either absent (Blundell et al., 2010) or overexpressed (Dahlhaus et al., 2010). Hence, intermediate NLGN1 level such as those observed in HET mice (El Helou et al., 2013) might be beneficial to synaptic potentiation, which could be linked to enhanced vascular need and faster hemodynamic response.

One limitation of the present study concerns measurements of the hemodynamic response under Ketamine/Xylazine anesthesia, because it was shown to reduce the cerebrovascular response in comparison to other anesthetics (Franceschini et al., 2010). However, discrepancies exist regarding the use of anesthetics when measuring hemodynamic responses (Berwick et al., 2002). For instance, isoflurane, which is often used because of its simplicity, reduces neuronal excitation and acts as a vasodilator at doses higher than 1.6% (Eger, 1984). Moreover, it was also shown to attenuate hemodynamic responses (Franceschini et al., 2010; Takuwa et al., 2012). Alpha-chloralose anesthesia allows the largest hemodynamic response following sensory stimulation (Franceschini et al., 2010), and urethane anesthesia (with tracheotomy) also seems to importantly preserve hemodynamic function (Moldestad et al., 2009; Guevara et al., 2013). Alpha-chloralose was not approved for mice by our local Ethics Committee due to insufficient analgesic properties (Holzgrefe et al., 1987; Zuurbier et al., 2002). In addition, we observed that urethane anesthesia (with or without tracheotomy) prematurely killed Nlgn1 KO mice making it impossible to perform data acquisition. Thus, we cannot exclude the possibility that the anesthetic used in the present study could have masked additional between-genotype differences because of NMDAR inhibition by Ketamine. Nevertheless, we believe that the observed genotype differences are valid because high-amplitude cerebrovascular responses were observed under Ketamine/Xylazine anesthesia (Wey et al., 2010; Kim and Jeong, 2013), and the overall magnitude of hemodynamic responses reported here (3–4% change) was similar to previous studies performed with alpha-chloralose (Dubeau et al., 2011). More importantly, the application of NMDAR and AMPAR antagonists blocks the hemodynamic response to somatosensory stimulation under Ketamine/Xylazine anesthesia (Scott and Murphy, 2012), which supports that glutamatergic transmission is preserved under such anesthetic conditions and that our findings of altered cerebrovascular parameters in Nlgn1 KO mice could be linked to modifications in glutamate receptor functions. Future experiments should optimally perform measurements in awake animals to validate the observations of changes in cerebrovascular dynamics in Nlgn1 KO mice.

In this study, the use of fourfold-threshold stimulation was performed to compare different stimulation intensities but also to maximize the between-genotype differences in hemodynamic response by counteracting the issues related to the selected anesthetic mentioned above. Using this second intensity, the hemodynamic time course seemed again advanced in Nlgn1 KO mice but this time in comparison to HET mice. Increased stimulation intensity may have been necessary to reveal the faster rise in HbO and Hbr as mice lacking NLGN1 only tended to show a faster rise to half peak after the twofold-threshold stimulation (Fig. 2B). In general, the time course of brain HbO and Hbr was similar between KO and WT mice following this higher electrical forepaw stimulation, which may indicate that the response to such stimulation reached a plateau that is not controlled by NLGN1. This, however, needs to be interpreted with caution as fewer mice were submitted to this second stimulation intensity, which led to slightly elevated variability. In addition, tested mice in the fourfold-threshold stimulation had already undergone at least 15 trains of twofold-threshold stimulation previously, which could have induced some attenuation of the hemodynamic response.

CONCLUSION

The present findings suggest that cerebral hemodynamics are altered in mice lacking NLGN1. Indeed, Nlgn1 mutant mice exhibited a lower cerebrovascular response rate accompanied by a faster hemodynamic response to somatosensory stimulation in comparison to littermates revealing an additional manner by which neuronal function could be altered. In fact, because disruption in the NRXN-NLGN system was shown to associate with autism spectrum disorder (Sudhof, 2008), and because cognitive impairments correlate with cerebrovascular response (Dickstein et al., 2010), highlighting a role of NLGN1 in cerebrovascular dynamics points to a new mechanism by which cognitive function can be modulated in some psychiatric diseases. The functional analyses presented here importantly complete the imaging observations of alterations in white matter volume in other animal models of autism spectrum disorder, such as in mice with genetically engineered modifications in NRXN1 and NLGN3 (Ellegood et al., 2011; Voineskos et al., 2011).

AUTHOR CONTRIBUTIONS

E.B., F.L., and V.M. designed research; E.B.N. and M.F. performed research; E.B.N., M.F. and V.M. contributed new reagents/analytic tools; E.B.N., M.F., P.P., F.L. and V.M. analyzed data; and E.B.N., M.F., P.P. and V.M. wrote the paper.

Acknowledgments—The authors would like to thank Simon Dubeau, Jean Paquet, Caroline Bouchard, and Gaétan Tremblay for their help in setting up the protocol, surgery and analyses. Funding sources: Canadian Institutes of Health Research (231095-111021), Fonds de la recherche du Québec – Santé (22210), Research Center of the Hôpital du Sacré-Coeur de Montréal.
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(Accepted 24 December 2014)
(Available online 13 January 2015)