Regulation of cortical blood flow responses by the nucleus basalis of Meynert during nociceptive processing.

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Abstract:

Cerebral blood flow (CBF) is essential for neuronal metabolic functions. CBF is partly regulated by cholinergic projections from the nucleus basalis of Meynert (NBM) during cortical processing of sensory information. During pain-related processing, however, this mechanism may be altered by large fluctuations in systemic mean arterial pressure (MAP). The objective of this study was to investigate the contribution of NBM to CBF responses evoked by nociceptive electrical stimuli and how it may be affected by systemic MAP. CBF was recorded in isoflurane-anesthetized rats (n=8) using laser speckle contrast imaging, in two conditions (intact vs left NBM lesion). Electrical stimulation was applied to the sciatic nerve. Sciatic stimulation produced intensity dependent increases in MAP ($p<0.001$) that were almost identical between conditions (intact vs left NBM lesion; $p=0.96$). In both conditions, sciatic stimulation produced intensity dependent CBF increases ($p<0.001$). After NBM lesion, CBF responses were decreased in the left somatosensory cortex ipsilateral to NBM lesion ($p=0.02$) but not in the right somatosensory cortex ($p=0.46$). These results indicate that NBM contributes to CBF responses to nociceptive stimulation in the ipsilateral, but not contralateral somatosensory cortex and that CBF response attenuation by NBM lesion is not compensated passively by systemic MAP changes. This highlights the importance of NBM's integrity for pain-related hemodynamic responses in the somatosensory cortex.

Keywords: Cerebral blood flow; Nucleus basalis of Meynert; Basal forebrain; Nociception; Pain; Laser speckle contrast imaging.

Abbreviation: ACh, acetylcholine; CBF, cerebral blood flow; MAP, mean arterial pressure; NBM, nucleus basalis of Meynert.

1. Introduction:

The intimate relationship between cerebrovascular functions and neuronal activity is fundamental to functional brain imaging methods (Raichle and Mintun, 2006). Techniques such as fMRI assess hemodynamic responses to estimate neuronal activity evoked by different tasks, based on the neurovascular coupling (Logothetis and Pfeuffer, 2004). This coupling is driven by multiple mediators released locally by neurons and astrocytes (Hillman, 2014; Lecrux and Hamel, 2016). Moreover, cerebral blood flow (CBF) is regulated by acetylcholine (ACh) released by projections from the basal forebrain, including the nucleus basalis of Meynert (NBM) (Adachi et al., 1992; Sato and Sato, 1995; Lacombe et al., 1997; Vaucher et al., 1997; Liu et al., 2015; Lecrux and Hamel, 2016; Lecrux et al., 2017). Notwithstanding, regional CBF responses can be altered by changes in systemic blood pressure as demonstrated in previous neurovascular coupling studies (Jeffrey-Gauthier et al., 2013; Uchida et al., 2017) (see (Paquette et al., 2018) for review). For instance, nociceptive stimuli evoke sudden increases in systemic blood pressure that are not compensated by vascular autoregulation, therefore adding to the regional CBF response evoked by neuronal activity. This leads to CBF responses that are a mixture of CBF evoked by local neuronal activity and CBF changes due to systemic blood pressure increases. In contrast, cortical neuronal activity evoked by nociceptive stimuli is either unaffected by MAP or even attenuated (Jeffrey-Gauthier et al., 2013).

In a previous laser speckle contrast imaging study, CBF responses evoked by light brushing stimulation were decreased by pharmacological inactivation of the NBM (Piché et al., 2010). This effect was only observable in the hemisphere ipsilateral to the affected NBM. Consistent with these findings, the increase of ACh release by NBM

potentiates CBF responses to whisker stimulation in the barrel cortex, while a chronic ACh deprivation reduces CBF responses (Lecrux et al., 2017). However, it is unclear how NBM regulates CBF responses during nociceptive processing. NBM receives somatosensory inputs, which may inhibit or activate NBM neurons in the rat (Akaishi et al., 1990). In this study, light somatosensory stimulation (brushing) was generally ineffective to affect NBM neurons' activity, while nociceptive stimulation (hind paw pinching) generally excited NBM neurons. In addition, MAP increases without nociceptive stimulation (phenylephrine i.v. injection) produced mixed effects on NBM neurons: on 12 recorded neurons, 4 (25 %) were inhibited, 1 was excited and 7 were unresponsive. Although most NBM neurons are unaffected by MAP changes, a significant proportion was inhibited. This may serve NBM to regulate CBF responses in the somatosensory cortex when systemic blood pressure is increased by nociceptive processing. However, this remains to be clarified.

The objective of the present study was to investigate the role of NBM in the regulation of cortical blood flow responses in the somatosensory cortex during nociceptive stimulation. CBF responses were measured using laser speckle contrast imaging to visualize responses in the somatosensory cortex bilaterally. Based on the previous studies mentioned above, we hypothesized that unilateral NBM lesion would decrease CBF responses to nociceptive stimuli in the somatosensory cortex ipsilateral, but not contralateral to an electrolytic lesion of NBM. Moreover, we hypothesized that the association between MAP and CBF changes would be altered by NBM lesion, based on the effect of MAP on NBM neurons' activity.

2. Materials and methods

2.1 Ethical approval

Experiments were performed on eight male Wistar rats (body weight 350–475 g; age 18–22 weeks; Charles River Laboratories, Saint-Constant, Québec, Canada). Animals were housed in the animal facilities of Université du Québec à Trois-Rivières. A light–dark cycle of 14–10 h was maintained. Experimental procedures were conducted in accordance with the animal care committee of Université du Québec à Trois-Rivières and in accordance with the guidelines of the Canadian Council on Animal Care.

2.2 General experimental conditions

All animals were in good health on the day of the experiment. Before surgical procedures were initiated, the animal was deeply anesthetized with isoflurane (5% for induction, 2% for maintenance). The depth of anesthesia was routinely assessed with paw pinching to confirm the absence of paw withdrawal. Body temperature was monitored and was maintained at 37.5 ± 0.5 °C with a custom-made temperature control system preventing electrophysiological artefacts. A tracheotomy was performed to ventilate the animal mechanically. The respiratory rate and tidal volume was adjusted to maintain the end-tidal CO_2 around 3% (CAPSTAR-100 Carbon dioxide analyzer, CWE inc., Ardmore, PA, USA). A cannula was inserted into the right jugular vein for solution administration. The left femoral artery was also catheterized to monitor mean arterial pressure and heart rate continuously (Harvard Apparatus, Holliston, MA, USA) (MAP). Fifteen minutes before recording, isoflurane was adjusted to 1.2%. Five minutes before

both conditions (before and after NBM lesion) rats were paralyzed with gallamine triethiodide (20 mg/kg i.v.) to avoid motion artefacts induced by electrical stimulation. When the experiment was terminated, a lethal dose of urethane was administered i.v. and the animal was perfused through the heart to extract the brain.

2.3 Experimental design

This study is based on a repeated-measure design to compare CBF and MAP responses to electrical stimulation of the sciatic nerve at graded intensities, before and after an electrolytic lesion of the NBM.

2.4 Laser speckle contrast imaging

The animal was placed in a prone position with the head fixed in a stereotaxic frame (Model 900, Kopf Instruments, Tujunga, CA, USA). The skull was made transparent with a micro-drill to allow visualization of CBF. CBF changes evoked by sciatic nerve stimulation were then measured with a laser speckle contrast imaging (LSCI) camera positioned over the brain (PeriCam PSI System, Perimed AB, Järfälla, Sweden). The LSCI device consists in an infrared laser diode (785 nm wavelength) and a high-resolution camera. The field of view covered about 570mm^2 (20 mm × 27 mm) with a matrix of 1388×1038 pixels, giving a resolution of 20 µm per pixel. Images were sampled at 1 Hz and signal was filtered temporally using a 2 s moving window.

2.5 Stimulation protocol

The right sciatic nerve was exposed, submerged in warm mineral oil and mounted on a custom-made bipolar stimulation electrode connected to a constant-current stimulator (Model DS7A, Digitimer Ltd, Welwyn Garden city, UK). The stimulation consisted in a 10 s train with 1 ms pulse at 5 Hz. The inter-train interval of 90 s allowed response recovery before the subsequent stimulus. Ten intensities (0.05, 0.075, 0.1, 0.15, 0.3, 0.6, 1.2, 2.4, 4.8, 9.6mA) were delivered in ascending or descending sequence and sequences were counterbalanced between animals to limit intensity order effects. It should be noted that electrical stimulation does not allow a selective activation of specific fibre groups. However, MAP increases associated with sciatic stimulation depend on fibres from groups III and IV in the rat, and most reports on MAP changes induced by somatic afferent stimulation have demonstrated that group I and II afferent fibres are ineffective to produce MAP increases (Sato et al. 1997). Therefore, based on MAP changes observed in the present protocol, stimulus intensities of 0.15 mA and below are considered as non-nociceptive and stimulus intensities above 0.15 mA are considered as nociceptive, for the present experimental conditions.

2.6 Electrolytic lesion of NBM and histological confirmation

A tungsten needle was inserted caudally over the frontal cortex at a 30-degree angle relative to the coronal plane as described previously (Piché et al., 2010). A small hole was made in the skull to allow penetration of the needle into the left NBM (contralateral to the stimulated sciatic nerve), located 1.4mm posterior, -2.5mm lateral and 7.6mm ventral to bregma (Paxinos and Watson, 2007), while the reference electrode

was inserted in the left temporal muscle. This approach was chosen to avoid potential lesions of the thalamocortical afferents localized more posteriorly. A current of 2 mA over 15 seconds was used to perform the NBM electrolytic lesion (Kesner et al., 1986; Vale-Martínez et al., 2002). At the end of the experiment, the brain was then extracted and coronal slices of 100 µm thickness were made using a freezing microtome. Electrolytic lesion of the NBM was confirmed under a microscope (see figure 1).

2.7 Data Analysis

Images were analyzed using Pimsoft software (PimSoft 1.5, Perimed AB, Järfalla, Sweden). The selection of ROIs was based on relative position to bregma to assure reproducibility between subjects. CBF data from regions of interest (ROI) was then exported to spike2 software for quantification. MAP data was analyzed with spike2 software (Cambridge Electronic Design, Cambridge, UK,version 6.15). CBF and MAP changes were measured for each stimulus intensity within a 30s window post-stimulus. Responses amplitude was quantified as the peak value relative to the mean signal value of the 30 s artifact-free baseline prior to stimulus onset.

2.8 Statistical analyses

All results are expressed as means ± SEM. Statistical analyses were performed with Statistica (TIBCO Software Inc. 2017. Statistica version 13) with a significance threshold of $p \leq 0.05$. CBF responses were compared using a Greenhouse-Geisser corrected repeated-measures ANOVA, with intensity (10 levels: 0.05, 0.075, 0.1, 0.15, 0.3, 0.6, 1.2, 2.4, 4.8, 9.6mA), NMB (intact vs NBM lesion) and hemisphere (left vs right) as within-subject

factors. MAP responses were compared using a Greenhouse-Geisser corrected repeated-mesures ANOVA, with intensity (10 levels: 0.05, 0.075, 0.1, 0.15, 0.3, 0.6, 1.2, 2.4, 4.8, 9.6mA) and NMB (intact, NBM lesion) as within-subject factors. Significant effects were decomposed with the Fisher post-hoc test. Effect sizes are reported based on partial eta-squared (η^2_p).

To examine if the association between MAP and CBF changes would be affected by NBM lesion, correlation coefficients were compared between the intact and NBM lesion conditions across animals. In this analysis, correlation coefficients were calculated within subject for the association between MAP and CBF changes across intensities, leading to 2 correlation coefficients per hemisphere for each animal. These coefficients were treated as continuous variables and compared with paired t-tests to test whether the association between MAP and CBF would increase following NBM lesion in the ipsilateral hemisphere, based on a previous study reporting that MAP could inhibit some NBM neurons (Akaishi et al. 1990).

3. Results

3.1 Cerebral blood flow responses

As shown by the individual example in Figure 2, right sciatic nerve stimulation produced cerebral blood flow responses in the somatosensory cortex that increased with intensity, that were of greater amplitude in the left hemisphere and that extended to most of the cortical surface in both hemispheres at high intensity. In this individual example, NBM lesion decreased both the amplitude and the extent of CBF responses. Group

analyses of CBF in the hind paw representation of the somatosensory cortex (ROIs) revealed that right sciatic nerve stimulation evoked significant intensity dependent CBF increases bilaterally (main effect $F_{9,63} = 12.0$, $p<0.001$; $\eta^2_p = 0.63$; see Figure 3). As expected, CBF responses were greater in the left hemisphere, contralateral to the stimulation (main effect: $F_{1,7} = 17.1$, $p<0.01$; $\eta^2_p = 0.71$; see Figure 3). In addition, the left NBM lesion affected CBF responses differently between hemispheres (interaction: $F_{1,7} = 7.0$, $p=0.03$; $\eta^2_p = 0.51$; see Figure 3). Indeed, the Fisher posthoc test revealed that CBF responses were decreased in the left hemisphere, contralateral to stimulation and ispilateral to NBM lesion ($p=0.02$), while no significant effect was produced in the right hemisphere ($p=0.45$).

In order to examine whether these changes may be due, at least in part, to changes in basal CBF, the mean CBF values in the hind paw representation of the somatosensory cortex (ROIs) preceding each stimulation were compared between hemispheres and conditions. Group analyses indicate that basal CBF was affected differently by NBM lesion in the right and left hemispheres (interaction $F_{1,7} = 18.3$, $p<0.01$; $\eta^2_p = 0.72$, see Figure 4). Fisher posthoc test revealed that basal CBF decreased significantly after NBM lesion in the left hemisphere, ispilateral to NBM lesion ($p<0.001$), while no significant change occurred in the right hemisphere ($p=0.2$).

Subsequently, a covariance analysis was performed on CBF responses with basal CBF changes as covariable. This covariance analysis revealed that the decrease in CBF response amplitude in the left hemisphere after NBM lesion did not reach significance after controlling for basal CBF changes (interaction: $F_{1,5} = 4.7$, $p=0.08$; $\eta^2_p = 0.48$). This

indicates that basal CBF changes partly contributed to the effects of NBM lesion on CBF responses evoked by sciatic nerve stimulation.

3.2 Mean arterial pressure changes

Right sciatic nerve stimulation produced the expected MAP response that increased with intensity, as shown in the individual example of Figure 2. Group analyses revealed that right sciatic nerve stimulation evoked significant intensity dependent MAP increases (main effect: $F_{9,63} = 78.8$, $p<0.001$; $\eta^2_p = 0.92$; see Figure 5). These responses were not significantly different between conditions (main effect: $F_{1,7} < 0.1$, $p=0.96$; $\eta^2_p < 0.01$; see Figure 5) and the intensity dependent changes were not significantly different after NBM lesion (interaction: $F_{9,63} = 0.4$, $p=0.94$; $\eta^2_p = 0.05$; see Figure 5). These results confirm that MAP responses were comparable between conditions, ruling out a potential confound for the reported effects of NBM lesion on CBF.

To examine if the association between MAP and CBF changes would be changed by NBM lesion, their correlation coefficients were compared between the intact and NBM lesion conditions across animals. The strength of the association between MAP and CBF changes was significantly increased after left NBM lesion for the left somatosensory cortex ($r = 0.33 \pm 0.18$ vs 0.73 ± 0.04; $T(7) = 2.4$, $p = 0.049$, $\eta^2_p = 0.45$), but not for the right somatosensory cortex ($r = 0.63 \pm 0.17$ vs 0.84 ± 0.04; $T(7) = 1.5$, $p = 0.17$, $\eta^2_p = 0.25$). These results suggest an ipsilateral effect of NBM on the regulation of CBF due to MAP increases.

4. Discussion

The novel findings of the present study are that CBF responses to nociceptive stimulation of the sciatic nerve evoked in the left somatosensory cortex are attenuated by a lesion of the left NBM. Moreover, results indicate that changes in basal CBF following NBM lesion contribute to these effects. In addition, while MAP was comparable before and after NBM lesion, the association between MAP and CBF changes in the left but not right somatosensory cortex was increased following left NBM lesion. These results indicate that the NBM contributes to the regulation of CBF in the ipsilateral somatosensory cortex during nociceptive stimulation.

4.1 Nucleus basalis of Meynert and cortical blood flow

In the present study, an electrolytic lesion of the left NBM decreased basal CBF in the left but not right somatosensory cortex. These results differ from those of a previous laser speckle contrast imaging study in which basal CBF remained comparable after pharmacological inactivation of NBM by muscimol, a gabaergic receptor agonist (Piché et al., 2010). The difference between studies may be due to methodological issues. In the case of an electrolytic lesion, neurons are destroyed. In the case of muscimol injection, some neurons may still be active or only partially inhibited. The present results also differ from those reported in a laser Doppler flowmetry study in which a bilateral electrolytic lesion of NBM was performed in spinal rats (Uchida et al., 2000). In this case, methods are similar to produce the NBM lesion, but spinalization produces a major drop in MAP, which may affect basal CBF and further changes following NBM lesion.

Nonetheless, considering that basal CBF was affected by the NBM lesion similarly to changes in CBF responses evoked by sciatic stimulation, this raises the possibility that the former may contribute to the later. This was examined by comparing CBF responses before and after the NBM lesion while controlling for basal CBF changes. This covariance analysis indicates that basal CBF changes partly contributed to the observed CBF response attenuation. However, based on the effect sizes with and without basal CBF as covariable (η^2_p = 0.48 vs 0.51), the effects of NBM lesion on CBF responses to sciatic nerve stimulation are mostly due to mechanisms other than changes in basal CBF. This is consistent with the previous studies mentioned above in which CBF responses to somatosensory stimulation were attenuated after pharmacological inactivation or electrolytic lesion of NBM without significant changes in basal CBF (Piché et al., 2010; Uchida et al., 2000).

The present findings indicating that NBM lesion decreases basal CBF and CBF responses to sciatic stimulation are consistent with several studies showing that NBM stimulation increases CBF or CBF responses to somatosensory stimuli (Adachi et al., 1990; Dauphin et al., 1991; Adachi et al., 1992; Kocharyan et al., 2008; Lecrux and Hamel, 2016). Although ACh release was not measured in the present study, the present results are also coherent with the body of literature showing that the release of ACh by NBM projections to the cortex is essential for CBF regulation (Sato et al., 2001). In addition, a recent study examined the effect of ACh tone on whisker-evoked CBF changes in the barrel cortex by pharmacological interventions (increasing or decreasing ACh tone) or by subthreshold basal forebrain stimulation (including the NBM), which increases ACh tone (Lecrux et al., 2017). In accordance with our results, they

demonstrated the essential role of ACh tone for somatosensory-evoked responses and for neurovascular coupling. Indeed, a chronic decrease of ACh tone (by an intracerebroventricular injection of Saporin) reduced whisker-evoked CBF responses. In contrast to the present findings, however, basal CBF was unaffected in these conditions. This may be due to some form of adaptation of the cerebrovascular system in basal conditions. Consistent with this idea and with the present findings, a subacute basal forebrain lesion (2 days) produced a decrease in basal CBF in the hemisphere ipsilateral to the lesion (Iadecola et al., 1983). Therefore, a possible explanation for the discrepancy between results is the difference between an acute and a chronic decrease of ACh tone. In conditions of chronic decreased ACh tone, mechanisms other than those related to NBM may overcome the ACh decrease to maintain basal CBF within a physiological range. This is consistent with a previous study showing that four days after an electrolytic lesion of the NBM in non-human primates, the resting state cerebral glucose utilization (indicative of cerebral metabolism) was decreased ipsilateral to the lesion, while 39 days after the lesion, basal glucose metabolism recovered (Kiyosawa et al., 1987).

4.2 Blood pressure and hemodynamic responses

Sciatic nerve stimulation produced robust intensity dependent MAP responses and these changes were comparable before and after the left NBM lesion. Therefore, changes in basal CBF and CBF responses following NBM lesion cannot be explained by MAP changes. Importantly, systemic MAP fluctuations during nociceptive stimulation could prevent the observation of CBF decrease in the somatosensory cortex ipsilateral to NBM lesion, by overriding cerebral vascular autoregulation. Indeed, cerebral vascular

autoregulation is not sufficient to maintain cerebral perfusion in the normal physiological range when MAP increases over 150 mm Hg (Paulson et al., 1990). The present results suggest that systemic MAP increases don't override cerebral vascular autoregulation completely even when the NBM is lesioned. This is consistent with a study by Iadecola et al. (1983) indicating that unilateral NBM lesion in the rat did not alter autoregulation in either hemisphere for systemic arterial pressure between 90-150 mm Hg (Iadecola et al., 1983).

A novel finding of this study is the role of NBM in the association between MAP and CBF. MAP increases without nociceptive stimulation inhibits around 25 % of NBM neurons (Akaishi et al., 1990). Although this has never been investigated, these neurons may play a role in limiting the effect of systemic blood pressure increases on cortical blood flow. The present findings are consistent with this idea, showing that the association between MAP increases and CBF changes during nociceptive processing is improved after NBM lesion, suggesting that although NBM promotes vasodilation and increases cortical blood flow, some of its neurons limit this increase through regulation by systemic MAP. Results also indicate that this regulation is specific to the hemisphere ipsilateral to NBM, consistent with the results described above. Future studies are needed to examine more directly the role of these MAP-inhibited NBM neurons in CBF regulation. A useful approach to test the role of NBM neurons in the regulation of somatosensory-evoked CBF changes during MAP increases would be to use chemogenetic or optogenetic methods to activate or inhibit NBM neurons.

4.3 Significance and limitations

The present findings have implications for brain imaging studies in populations in which NBM integrity is compromised, including patients with Alzheimer's disease (Whitehouse et al., 1981; Rinne et al., 1987; Vogels et al., 1990; Liu et al., 2015). In these patients, vascular responses used to infer neuronal activity should take into account the attenuation CBF responses due to NBM degeneration, since the standard analysis models are not suited to these conditions. It is worth mentioning, however, that CBF measures in the present study are limited to superficial layers of the cortex and may not reflect vascular responses in the entire cortex (Dunn, 2012). It remains to be determined to what extent NBM lesion or degeneration affects cortical CBF responses.

4.4 Conclusion

In summary, the present results show that CBF responses to nociceptive stimulation of the sciatic nerve are attenuated by NBM lesion in the hemisphere ispilateral to NBM lesion, specifically. Moreover, results indicate that changes in basal CBF following NBM lesion contribute to these effects. This highlights the importance of NBM integrity for pain-related hemodynamic responses in the somatosensory cortex. These findings have implications for brain imaging studies of pain in healthy young participants, in which NBM activity may vary and influence cortical hemodynamic responses used to infer neuronal activity. They also have implications for clinical populations such as patients with Alzheimer in which cholinergic functions related to NBM are compromised.

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Conflicts of interest

The authors declare no competing interests and no relationship that may lead to any conflict of interest.

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Author's contributions

Mathieu Piché and Hugues Leblond contributed to all aspects of the research and obtained funding. Thierry Paquette contributed to data collection, analyses and interpretation as well as manuscript writing. Ryota Tokunaga and Sara Touj contributed

to data collection, analyses and interpretation. Each author read and approved the final version of the manuscript.

References

Adachi, T., Baramidze, D.G., Sato, A., 1992. Stimulation of the nucleus basalis of Meynert increases cortical cerebral blood flow without influencing diameter of the pial artery in rats. Neurosci Lett. 143, 173-176.

Adachi, T., Biesold, D., Inanami, O., Sato, A., 1990. Stimulation of the nucleus basalis of Meynert and substantia innominata produces widespread increases in cerebral blood flow in the frontal, parietal and occipital cortices. Brain Res. 514, 163-166.

Akaishi, T., Kimura, A., Sato, A., Suzuki, A., 1990. Responses of neurons in the nucleus basalis of Meynert to various afferent stimuli in rats. Neuroreport. 1, 37-39.

Dauphin, F., Lacombe, P., Sercombe, R., Hamel, E., Seylaz, J., 1991. Hypercapnia and stimulation of the substantia innominata increase rat frontal cortical blood flow by different cholinergic mechanisms. Brain Res. 553, 75-83.

Dunn, A.K., 2012. Laser Speckle Contrast Imaging of Cerebral Blood Flow. Ann Biomed Eng. 40, 367-377.

Hillman, E.M.C., 2014. Coupling Mechanism and Significance of the BOLD Signal: A Status Report. Annu Rev Neurosci. 37, 161-181.

Iadecola, C., Mraovitch, S., Meeley, M.P., Reis, D.J., 1983. Lesions of the basal forebrain in rat selectively impair the cortical vasodilation elicited from cerebellar fastigial nucleus. Brain Res. 279, 41-52.

Jeffrey-Gauthier, R., Guillemot, J.P., Piche, M., 2013. Neurovascular coupling during nociceptive processing in the primary somatosensory cortex of the rat. Pain. 154, 1434-1441.

Kesner, R.P., Crutcher, K.A., Measom, M.O., 1986. Medial septal and nucleus basalis magnocellularis lesions produce order memory deficits in rats which mimic symptomatology of Alzheimer's disease. Neurobiol Aging. 7, 287-295.

Kiyosawa, M., Pappata, S., Duverger, D., Riche, D., Cambon, H., Mazoyer, B., Samson, Y., Crouzel, C., Naquet, R., MacKenzie, E.T., et al., 1987. Cortical hypometabolism and its recovery following nucleus basalis lesions in baboons: a PET study. J Cereb Blood Flow Metab. 7, 812-817.

Kocharyan, A., Fernandes, P., Tong, X.K., Vaucher, E., Hamel, E., 2008. Specific subtypes of cortical GABA interneurons contribute to the neurovascular coupling response to basal forebrain stimulation. J Cereb Blood Flow Metab. 28, 221-231.

Lacombe, P., Sercombe, R., Vaucher, E., Seylaz, J., 1997. Reduced cortical vasodilatory response to stimulation of the nucleus basalis of Meynert in the aged rat and evidence for a control of the cerebral circulation. Ann N Y Acad Sci. 826, 410-415.

Lecrux, C., Hamel, E., 2016. Neuronal networks and mediators of cortical neurovascular coupling responses in normal and altered brain states. Philos Trans R Soc Lond B Biol Sci. 371.

Lecrux, C., Sandoe, C.H., Neupane, S., Kropf, P., Toussay, X., Tong, X.-K., Lacalle-Aurioles, M., Shmuel, A., Hamel, E., 2017. Impact of altered cholinergic tones on the neurovascular coupling response to whisker stimulation. J Neurosci. 37, 1518-1531
Liu, A.K.L., Chang, R.C.-C., Pearce, R.K.B., Gentleman, S.M., 2015. Nucleus basalis of Meynert revisited: anatomy, history and differential involvement in Alzheimer's and Parkinson's disease. Acta Neuropathol. 129, 527-540.
Logothetis, N.K., Pfeuffer, J., 2004. On the nature of the BOLD fMRI contrast mechanism. Magn Reson Imaging. 22, 1517-1531.
Paquette, T., Jeffrey-Gauthier, R., Leblond, H., Piche, M., 2018. Functional neuroimaging of nociceptive and pain-related activity in the spinal cord and brain: Insights from neurovascular coupling studies. Anat Rec. 301, 1585-1595.
Paulson, O.B., Strandgaard, S., Edvinsson, L., 1990. Cerebral autoregulation. Cerebrovasc Brain Metab Rev. 2, 161-192.
Paxinos, G., Watson, W., 2007. The rat brain in stereotaxic coordinates, 6 ed. Elsevier/Academic Press, New York.
Piché, M., Uchida, S., Hara, S., Aikawa, Y., Hotta, H., 2010. Modulation of somatosensory-evoked cortical blood flow changes by GABAergic inhibition of the nucleus basalis of Meynert in urethane-anaesthetized rats. J Physiol. 588, 2163-2171.
Raichle, M.E., Mintun, M.A., 2006. Brain work and brain imaging. Annu Rev Neurosci. 29, 449-476.
Rinne, J.O., Paljärvi, L., Rinne, U.K., 1987. Neuronal size and density in the nucleus basalis of Meynert in Alzheimer's disease. J Neurol Sci. 79, 67-76.
Sato, A., Sato, Y., 1995. Cholinergic neural regulation of regional cerebral blood flow. Alzheimer Dis Assoc Disord. 9, 28-38.
Sato, A., Sato, Y., Schmidt, RF. 1997. The impact of somatosensory input on autonomic functions. Rev Physiol Biochem Pharmacol. 130, 1-328.
Sato, A., Sato, Y., Uchida, S., 2001. Regulation of regional cerebral blood flow by cholinergic fibers originating in the basal forebrain. Int J Dev Neurosci. 19, 327-337.
Uchida, S., Bois, S., Guillemot, J.P., Leblond, H., Piché, M., 2017. Systemic blood pressure alters cortical blood flow and neurovascular coupling during nociceptive processing in the primary somatosensory cortex of the rat. Neuroscience. 343, 250-259.
Uchida, S., Kagitani, F., Suzuki, A., Aikawa, Y., 2000 Effect of acupuncture-like stimulation on cortical cerebral blood flow in anesthetized rats. Jpn J Physiol.50:495-507.
Vale-Martínez, A., Guillazo-Blanch, G., Martí-Nicolovius, M., Nadal, R., Arévalo-García, R., Morgado-Bernal, I., 2002. Electrolytic and ibotenic acid lesions of the nucleus basalis magnocellularis interrupt long-term retention, but not acquisition of two-way active avoidance, in rats. Exp Brain Res. 142, 52-66.
Vaucher, E., Borredon, J., Bonvento, G., Seylaz, J., Lacombe, P., 1997. Autoradiographic evidence for flow-metabolism uncoupling during stimulation of the nucleus basalis of Meynert in the conscious rat. J Cereb Blood Flow Metab. 17, 686-694.
Vogels, O.J.M., Broere, C.A.J., Ter Laak, H.J., Ten Donkelaar, H.J., Nieuwenhuys, R., Schulte, B.P.M., 1990. Cell loss and shrinkage in the nucleus basalis Meynert complex in Alzheimer's disease. Neurobiol Aging. 11, 3-13.
Whitehouse, P.J., Price, D.L., Clark, A.W., Coyle, J.T., DeLong, M.R., 1981. Alzheimer disease: Evidence for selective loss of cholinergic neurons in the nucleus basalis. Ann Neurol. 10, 122-126.

Figure captions

Figure 1: NMB lesion confirmation

Left: coronal section of a representative rat brain showing the location of the NBM electrolytic lesion. Right: schematic representation of the brain section replicated from Paxinos and Watson (2007).

Figure 2: Individual example of laser speckle contrast imaging data

(A) Representative recordings of CBF responses in the region of interest (ROI) corresponding to the somatosensory cortex and MAP responses to electrical stimulation. (B) Upper row: Raw signal of laser speckle contrast imaging, representing the peak response to seven different stimulus intensities in an intact rat. Bottom row: Subtraction analysis showing the signal represented above after subtracting baseline CBF. (C) Upper row: Raw signal of laser speckle contrast imaging, representing the peak response to seven stimulus intensities in an NBM lesioned rat. Bottom row: Subtraction analysis showing the signal represented above after subtracting baseline CBF.

Figure 3: CBF responses

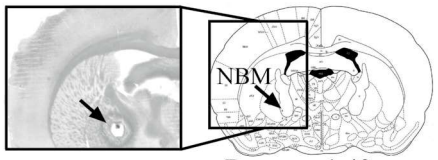
Electrical stimulation produced intensity dependent increases in CBF. NBM lesion significantly reduced CBF responses in the left, but not in the right hemisphere. *$p<0.05$ for the main effect of NBM lesion.

Figure 4: Basal CBF

Basal CBF was altered differently between hemispheres after the NBM lesion. The NBM lesion did not significantly affect basal CBF in the right somatosensory cortex (contralateral to NBM lesion) ($p=0.2$). In contrast, it was significantly reduced in the left somatosensory cortex (ipsilateral to NBM lesion) ($p<0.001$) and this effect was significantly greater compared with the non-significant change in the right hemisphere ($p=0.004$). Basal CBF was normalized to the intact condition for each hemisphere and is expressed as percentage.

Figure 5: MAP responses

Graded stimulus intensities produced intensity dependent MAP increases ($p<0.001$) in both conditions. MAP changes were comparable before and after NBM lesion.



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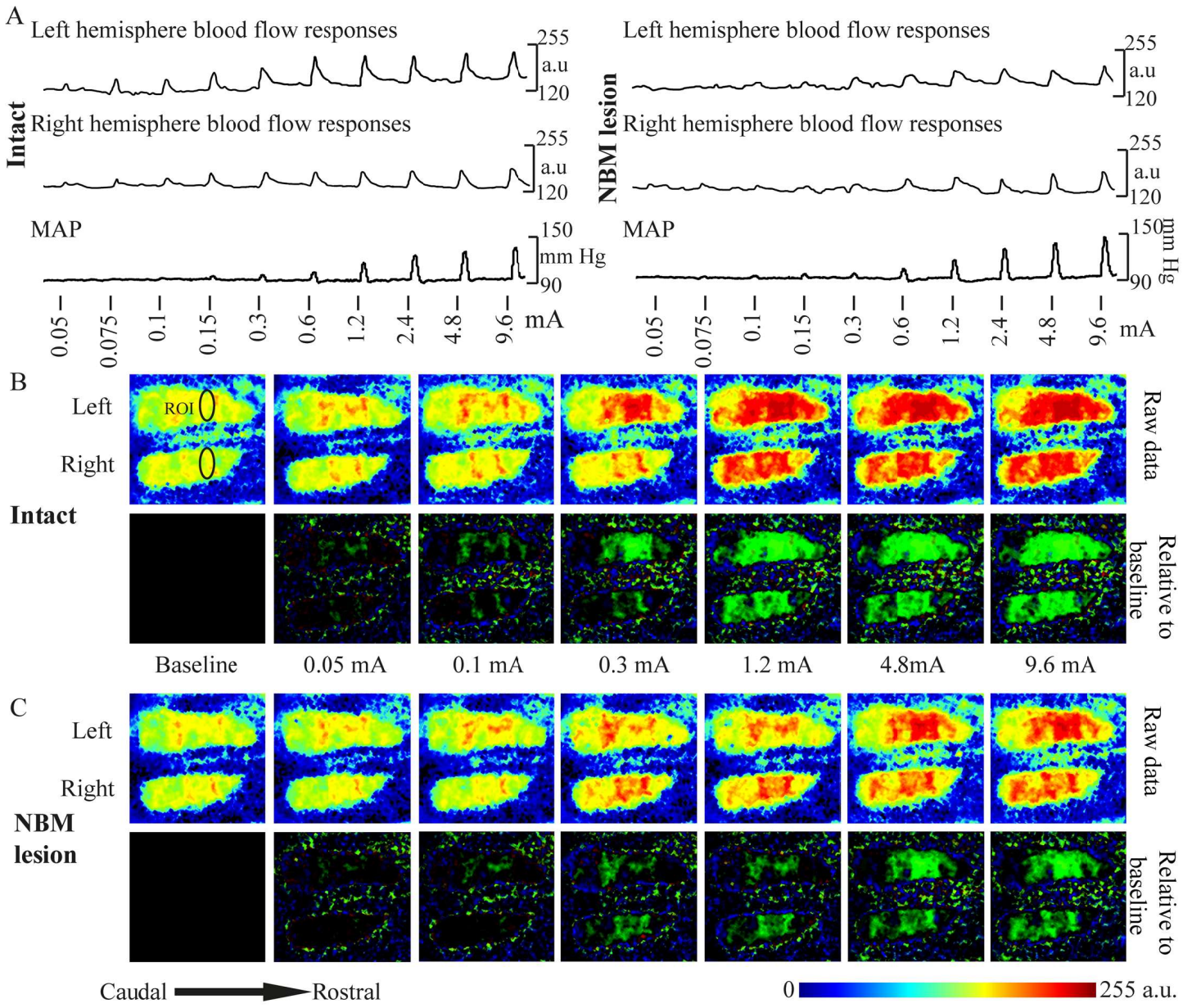
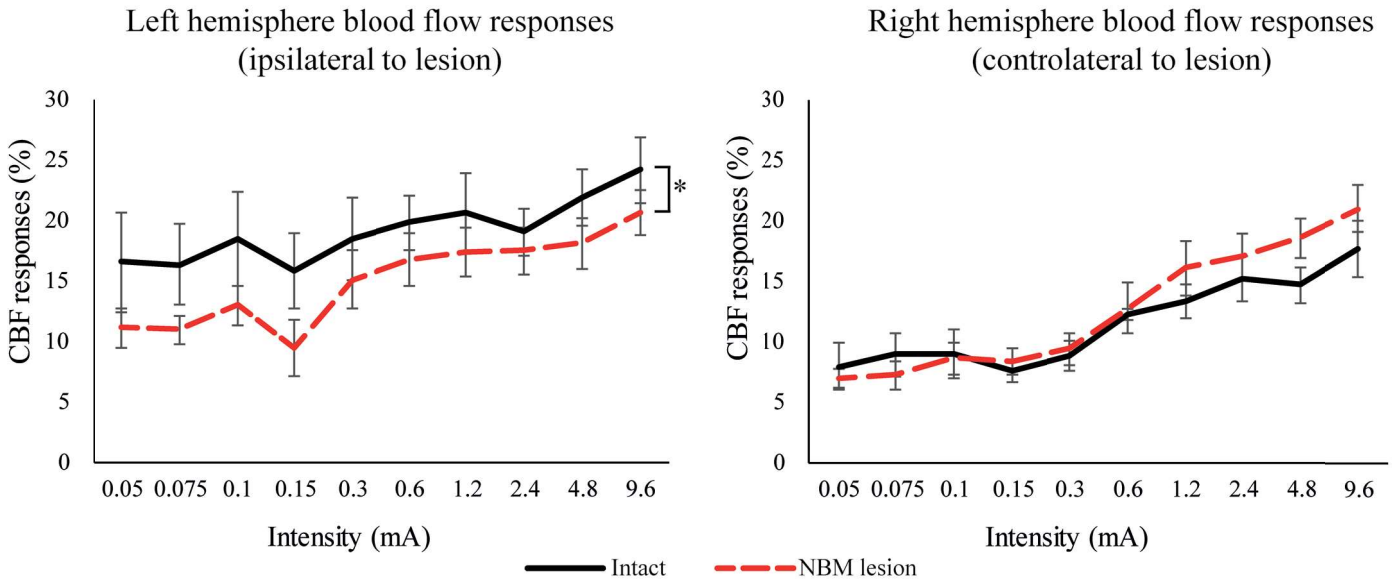
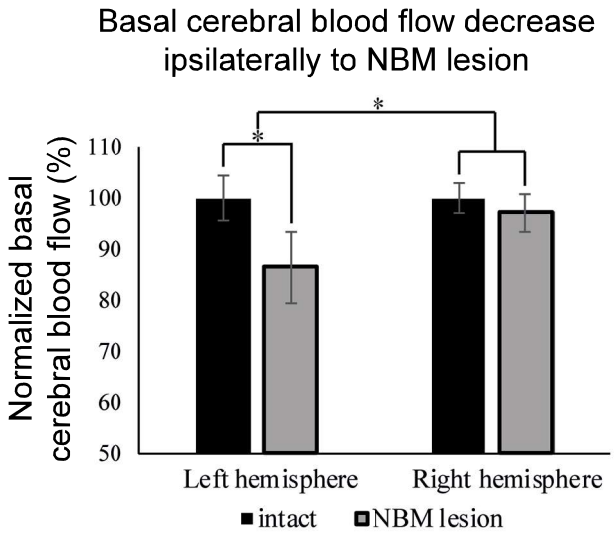

A
Left hemisphere blood flow responses
Right hemisphere blood flow responses
MAP
Intact
NBM lesion
255
a.u
120
150
mm Hg
90
0.05
0.075
0.1
0.15
0.3
0.6
1.2
2.4
4.8
9.6
mA
B
Left
Right
ROI
Intact
Raw data
Relative to baseline
Baseline
0.05 mA
0.1 mA
0.3 mA
1.2 mA
4.8mA
9.6 mA
C
NBM lesion
Caudal
Rostral
0
255 a.u.


Left hemisphere blood flow responses
(ipsilateral to lesion)
Right hemisphere blood flow responses
(controlateral to lesion)
CBF responses (%)
30
25
20
15
10
5
0
0.05 0.075 0.1 0.15 0.3 0.6 1.2 2.4 4.8 9.6
Intensity (mA)
*
Intact
NBM lesion


Basal cerebral blood flow decrease
ipsilaterally to NBM lesion
Normalized basal
cerebral blood flow (%)
110
100
90
80
70
60
50
*
*
Left hemisphere
Right hemisphere
intact
NBM lesion