Fasting prevents medetomidine-induced hyperglycemia and alterations of neurovascular coupling in the somatosensory cortex of the rat during noxious stimulation.

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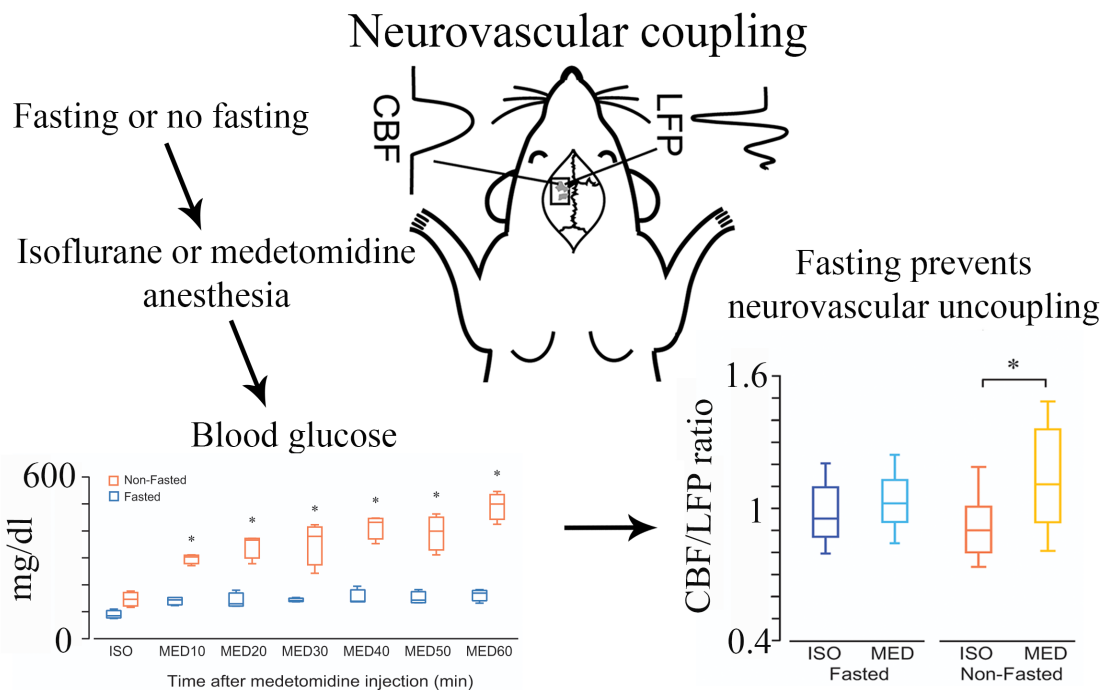
Abstract

Medetomidine and isoflurane are commonly used for general anesthesia in fMRI studies, but they alter cerebral blood flow (CBF) regulation and neurovascular coupling (NVC). In addition, medetomidine induces hypoinsulinemia and hyperglycemia, which also alter CBF regulation and NVC. Furthermore, sudden changes in arterial pressure induced by noxious stimulation may affect NVC differently under medetomidine and isoflurane anesthesia, considering their different effects on vascular functions. The first objective of this study was to compare NVC under medetomidine and isoflurane anesthesia during noxious stimulation. The second objective was to examine whether fasting may improve NVC by reducing medetomidine-induced hyperglycemia. In male Wister rats, noxious electrical stimulation was applied to the sciatic nerve in fasted or non-fasted animals. CBF and local field potentials (LFP) were recorded in the somatosensory cortex to assess NVC (CBF/LFP ratio). The CBF/LFP ratio was increased by medetomidine compared with isoflurane ($p=0.004$), but this effect was abolished by fasting ($p=0.8$). Accordingly, medetomidine produced a threefold increase in blood glucose ($p<0.001$), but this effect was also abolished by fasting ($p=0.3$). This indicates that isoflurane and medetomidine anesthesia alter NVC differently, but the undesirable glucose dependent effects of medetomidine on NVC can be prevented by fasting.



Graphical abstract. Neurovascular coupling (NVC) was measured in the somatosensory cortex of the rat as the ratio of cerebral blood flow and local field potential response amplitude (CBF/LFP). Medetomidine increased NVC through blood glucose increases, but these effect were abolished by fasting.

Introduction

Functional magnetic resonance imaging (fMRI) allows the investigation of a wide variety of brain functions in human and animal models, including pain mechanisms (Lowe *et al.*, 2007; Apkarian *et al.*, 2011; Duerden & Albanese, 2013; Wager *et al.*, 2013; Paquette *et al.*, 2018; Da Silva & Seminowicz, 2019; van der Miesen *et al.*, 2019). The most common fMRI methods rely on the blood oxygen level-dependent (BOLD) signal (Ogawa *et al.*, 1990). Using this method, neuronal activity can be inferred from hemodynamic changes and oxygen metabolism, based on the neurovascular coupling (NVC).

In animals, anesthesia is generally required to collect BOLD fMRI data in order to prevent movement artefacts, especially when noxious stimuli are applied. Isoflurane and medetomidine are two anesthetic agents commonly used in fMRI studies (Masamoto *et al.*, 2007; Tsurugizawa *et al.*, 2016). Isoflurane is a general anesthetic that is easy to administer, that allows control of the depth of anesthesia and fast recovery. However, it has vasodilating proprieties (Crystal *et al.*, 1995; Iida *et al.*, 1998), it alters brain metabolism (Boretius *et al.*, 2013) and suppresses neuronal activity dose-dependently (Tsurugizawa *et al.*, 2016), which may alter NVC (Masamoto *et al.*, 2009). Medetomidine is an α2-adrenergic receptor agonist (Scheinin *et al.*, 1989; Sinclair, 2003). It allows sedation of small animals for several hours, it leads to robust hemodynamic responses (Fukuda *et al.*, 2013) , and it can be used for multiple fMRI scanning (Weber *et al.*, 2006; Pawela *et al.*, 2009; Adamczak *et al.*, 2010). However, it affects sympathetic activity (Shirasaka *et al.*, 2007), the cardiovascular system (Eisenach *et al.*, 1994; Tan *et al.*, 2002; Leino *et al.*, 2009) and vascular smooth muscles (Fukuda *et al.*, 2013), which may alter NVC (Link *et al.*, 1996; Talke *et al.*, 2003). Therefore, isoflurane and medetomidine may affect vascular functions and NVC differently and may produce differences in stimulus-related BOLD signal changes, leading to different conclusions on brain activity (Nasrallah *et al.*, 2014). In addition, noxious stimulation evokes increases in mean arterial pressure (MAP), which may not be compensated by autoregulation (Jeffrey-Gauthier *et al.*, 2013; Uchida *et al.*, 2017; Paquette *et al.*, 2018; Paquette *et al.*, 2019b). This may affect NVC differently under medetomidine and isoflurane anesthesia, considering their different effects on vascular functions.

In addition to these potential alterations of NVC, medetomidine induces hypoinsulinemia and hyperglycemia, caused by the activation of pancreatic α2-adrenergic receptors (Angel & Langer, 1988; Kanda & Hikasa, 2008; Callahan *et al.*, 2014). Since hyperglycemia has opposite effects on cerebral blood flow (CBF) and neuronal excitability, it may also alter NVC. Indeed, hyperglycemia decreases CBF (Duckrow, 1995) while producing neuronal hyperexcitability (Huang *et al.*, 2007) and even seizures (Margineanu *et al.*, 1998). Fasting may be a simple way to limit medetomidine-induced hyperglycemia and the potential alteration of NVC. Blood glucose is lower and insulin secretion is inhibited during fasting, so this may reduce the effect of medetomidine on insulin and glucose levels, but it has never been investigated.

The first objective of the present study was to compare NVC under medetomidine and isoflurane anesthesia during noxious stimulation. The second objective was to examine whether fasting may improve NVC. Noxious electrical stimulation was applied to the

sciatic nerve during isoflurane and medetomidine anesthesia in fasting or non-fasting rats. CBF and local field potentials (LFP) were recorded in the somatosensory cortex to measure NVC (CBF/LFP ratio). Based on the different mechanisms and effects of isoflurane and medetomidine on neuronal and vascular function, we hypothesized that NVC would be different under isoflurane and medetomidine anesthesia. We also anticipated that this difference would be attenuated by fasting, by the reduction of medetomidine-induced hyperglycemia.

Methods

Ethical approval

All experimental procedures were approved by the Université du Québec à Trois-Rivières animal care committee, in accordance with the guidelines of the Canadian Council on Animal Care and adhered to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (IASP). Data is reported in compliance with the ARRIVE guidelines.

Animals

Experiments were performed on 21 male Wistar rats (body weight: 300–450 g; age: 20-24 weeks; Charles River Laboratories, Saint-Constant, as Québec, Canada). Thirteen animals were used for the NVC experiments. In a separate experiment, eight additional animals were used to monitor blood glucose during medetomidine anesthesia, to avoid the effect of tail puncture every 10 minutes on neuronal and CBF responses during the NVC experiment. Animals were kept in the animal facilities of Université du Québec à Trois-Rivières, where a light-dark cycle of 14 h–10 h was maintained. Rats were divided into two groups. The non-fasted group (n=10; 6 for the NVC experiment and 4 for the blood glucose experiment) had access to food and water ad libitum until anesthesia was administered. The fasted group (n=11; 7 for the NVC experiment and 4 for the blood glucose experiment) had access to water but no food during the 15 hours preceding the anesthesia.

General experimental procedures

Surgical procedures were initiated after animals were deeply anesthetized with 2.5% isoflurane. In addition to stable systemic MAP, the depth of anesthesia was routinely confirmed during the surgery by the absence of withdrawal reflexes (paw pinching). A schematic representation of the experimental set-up is shown in Figure 1. Briefly, the right jugular vein was catheterized for intravenous injections and MAP was continuously recorded from the left femoral artery with an intra-arterial cannula connected to a pressure transducer (Harvard Apparatus, Holliston, MA, USA). Animals were artificially ventilated (SAR-830/P Ventilator, CWE Inc., Ardmore, PA, USA) using a tracheal cannula to maintain end-tidal CO2 between 3.0 and 3.5% (CAPSTAR-100 Carbon dioxide analyzer, CWE Inc., Ardmore, PA, USA). Body temperature was monitored with a rectal probe (TCAT-2LV controller, Physitemp Instruments Inc., NJ, USA) and was maintained at 37.5 ±0.5 ℃ with a custom-made temperature control system preventing electrophysiological artifacts. Rats were placed in a stereotaxic frame (Model 900, Kopf Instruments, Tujunga, CA, USA). A craniotomy was performed over the left primary somatosensory cortex (SI) according to stereotaxic coordinates: A–P: 1 to -4 mm; L: -1 to -5 mm (Paxinos and

Watson, 2005). This window included the hind paw representation of the left SI for electrophysiological and CBF recordings. After the dura matter was removed, warm mineral oil was applied on the surface of the brain and was added during the experiment as needed. For electrical stimulation, the right sciatic nerve was exposed and immersed in a warm paraffin oil pool made with skin flaps attached to a metal holder placed over the right limb.

After all surgical procedures were completed, isoflurane concentration was decreased to 1.2% for electrophysiological recordings and the animal was paralyzed with gallamine triethiodide (20 mg/kg, i.v.) after confirming the absence of withdrawal reflexes with paw pinching. After the recording protocol was completed (see Figure 2A for protocol illustration), isoflurane anesthesia was switched to medetomidine. Before reducing isoflurane to 0.5%, a subcutaneous bolus of medetomidine (200 µg/kg) (Sigma-Aldrich, NY, USA) was injected in the back. Five minutes after the bolus injection, a syringe pump was used to provide a continuous subcutaneous infusion of medetomidine at a rate of 120 µg/kg/h (SPLG101, World Precision Instruments, FL, USA) and isoflurane was discontinued. After, the depth of anesthesia was confirmed by the absence of paw withdrawal, 60 minutes was allowed for the physiological parameters to stabilize and to have a complete isoflurane washout. The same recording protocol was then conducted. After completion of the experiment, rats were killed by increasing isoflurane anesthesia to 5% until no heartbeat could be observed.

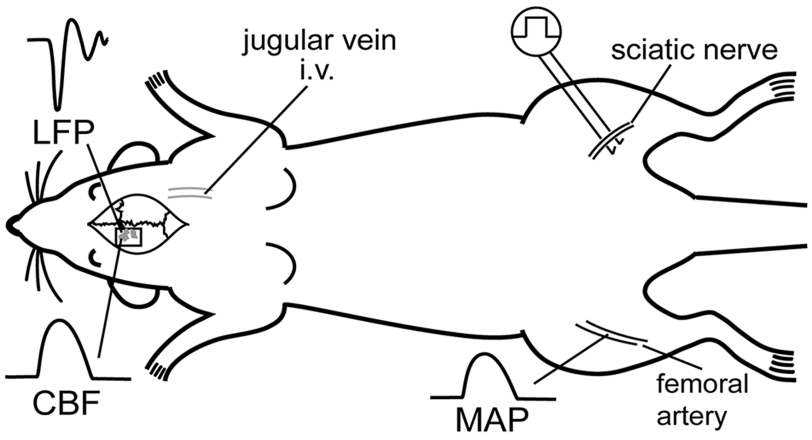


Figure 1. Schematic diagram of the surgical procedures.
Local field potentials (LFP) and regional cerebral blood flow (CBF) were recorded from the left somatosensory cortex. Mean arterial pressure (MAP) was measured from the left femoral artery. The right jugular vein was catheterized for intravenous injections (i.v.) of drugs. The right sciatic nerve was stimulated with a bipolar electrode at various intensities.

Extracellular recordings

Using a micromanipulator (Model 960, David Kopf Instruments, Tujunga, CA, USA), a 16-channel recording electrode (Model A1X16-10 mm-100-177-A16, Neuronexus Technologies Inc., Ann Arbour, Michigan, USA) was inserted below the cortical surface of SI, at the coordinates of the hind paw representation (A–P: -1.1 mm, L: 2.8 mm) (Paxinos & Watson, 2007). The tip of the electrode was inserted to a depth of

1600 µm. The 1500 µm recording span allowed covering all cortical layers. To confirm that some neurons were responsive to hind paw stimulation before the recording, multiunit activity was visualized and monitored with a loudspeaker during tactile stimulation and pinching (bandpass: 300–3 000 Hz, gain: 192 V/V). After this confirmation, the stimulation protocol began and the signal was filtered with a broad band (1-10 000 Hz), sampled at 20 kHz (Smartbox, Neuronexus Technologies Inc., Ann Arbour, MI, USA) and recorded on a personal computer for offline analyses.

Cortical blood flow recordings

A laser-Doppler probe (Micro-needle probe TSD145, Biopac systems, Goleta, CA, USA) was placed on the cortical surface of SI, as close as possible to the microelectrode, without achieving contact. The probe was carefully positioned to avoid large blood vessels. CBF was sampled at 100 Hz with a time constant of 2 s (Power 1401 acquisition system, Cambridge Electronic Design, Cambridge, UK) and recorded on a personal computer for offline analyses. Laser Doppler flowmetry allows the measurement of blood flow at a depth of approximately 1 mm (Shih *et al.*, 2012). In the cortex of the rat, this includes superficial layers and covers the area of interest where neuronal activity is measured simultaneously in layer IV (Hersch & White, 1981; Harris *et al.*, 2010).

Electrical stimulation

Electrical stimulation of the sciatic nerve (30 s trains of 3 ms pulses at 10 Hz with an inter-train interval of 120 s; see Figure 2B) was delivered by a constant-current stimulator (Model DS7A, Digitimer Ltd, Welwyn Garden city, UK), triggered by a computer-controlled sequencer (Power 1401 acquisition system, Cambridge Electronic Design, Cambridge, UK), using a custom-made bipolar hook electrode.

Experimental protocol

LFP and CBF recordings began after a 60 min stabilization period following the surgery. A 60 min stabilization period was also allowed after the switch from isoflurane to medetomidine anesthesia. The stimulation protocol consisted of a series of electrical stimuli at graded intensities in ascending or descending order (0.05, 0.075, 0.1, 0.15, 0.3, 0.6, 1.2, 2.4, 4.8, 9.6 or 9.6, 4.8, 2.4, 1.2, 0.6, 0.3, 0.15, 0.1, 0.075, 0.05 mA, respectively). The order of stimulus intensities was randomized between animals to avoid sequence order effects. This stimulation protocol was repeated once to compare responses between isoflurane and medetomidine anesthesia. The same order was kept for both series.

Blood glucose levels

In a separate experiment, we confirmed that blood glucose level increased with medetomidine anesthesia in four fasted and four non-fasted rats. Blood glucose was measured from tail-tip blood using a blood glucose meter (Contour Next One, Ascensia Diabetes Care Inc., CANADA) just before the initiation of medetomidine anesthesia and at 10, 20, 30, 40, 50 and 60 minutes after switching to medetomidine anesthesia.

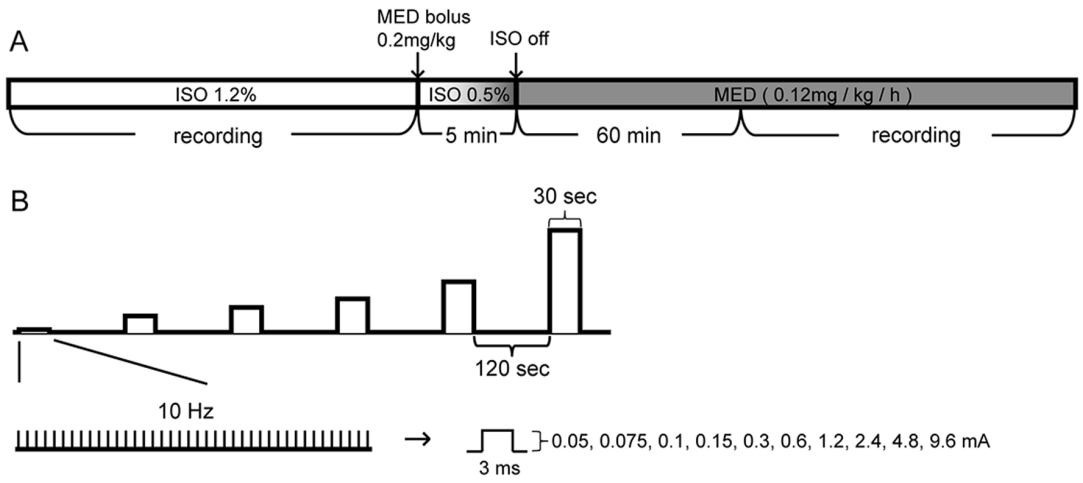


Figure 2. Experimental protocol.
A. After recording under isoflurane anesthesia (1.2 %), a subcutaneous bolus of medetomidine was injected (0.2 mg/kg) and isoflurane concentration was reduced (0.5 %). Five minutes after the bolus injection of medetomidine, isoflurane was discontinued and a continuous subcutaneous infusion of medetomidine was initiated, at a rate of 120 µg/kg/h. Sixty minutes were allowed for the animal to stabilize and for a complete isoflurane washout before recording resumed. B. Stimulation protocol. A train of 3 ms pulses was delivered at 10 Hz during 30 seconds at different intensities, ranging between 0.05 and 9.6 mA. A rest period of 120 s was allowed between each stimulus train.

Data analyses

LFP, CBF, and MAP data were analyzed using Spike2 software (Cambridge Electronic Design, Cambridge, UK, version 8.09b). The raw electrophysiological signal was down sampled at 5 kHz and band pass filtered to obtain LFP (1–300 Hz). For LFP and NVC, only one of the 16 channels was used for analyses. The selected channel was located at approximately 600 µm from the cortical surface (consistent with the location of layer IV), where LFP amplitude was greater, as in previous studies (Verdier & Dykes, 2001; Uchida *et al.*, 2017). For the quantification of LFP amplitude, the peak-to-peak value of averaged potentials (300 responses induced by the stimulus train of 30 s at 10 Hz) was extracted with a custom-made Spike2 script within a 50 m window, from -10 ms to 40 ms following the stimulus onset. Current source density (CSD) analyses were carried out using a step inverse CSD method with the CSD plotter toolbox (Pettersen *et al.*, 2006) running in MATLAB (2016a, MathWorks, Inc, MA). The CSD analysis converts the multilayer field potentials to current sinks and sources in relation to cortical depth (Nicholson & Freeman, 1975). This allowed confirming the pattern of cortical activity evoked by thalamocortical projections to SI. For CBF and MAP changes, the onset-to-peak value was extracted for each stimulus intensity within a 50 s window and response was calculated as the change relative to the mean signal value for the 30 s baseline preceding stimulus onset. MAP responses were calculated as the raw change (mm Hg) while CBF responses were calculated as a percent change, as described previously (Paquette *et al.*, 2019a). The CBF and LFP values were converted into T scores and used to index of NVC calculated as the CBF/LFP ratio.

Statistical analyses

All results are expressed as means ± SD. Statistical analyses were performed with Statistica (TIBCO software Inc. 2017. Statistica version 13) with a significance threshold of $p \leq 0.05$. The Kolmogorov-Smirnof test confirmed that distributions did not significantly deviate from normality and data was analyzed with parametric tests. CBF, MAP, LFP and NVC data were analyzed with mixed ANOVAs and significant effects were decomposed with the Tuckey HSD test. The effect size is reported as partial eta-squared (η^2_p).

Results

Neuronal and vascular measurements

An individual example of recordings is shown in Fig.3. Electrical stimulation of the left sciatic nerve elicited the expected current sinks and sources in the SI. A large current sink was observed between 600 and 1100 µm from the surface (Figure 3A), consistent with projections from the thalamus to layer IV and Va (Hersch & White, 1981; Harris *et al.*, 2010). The stimulation also produced robust increases in CBF (Figure 3B) and MAP (Figure 3C).

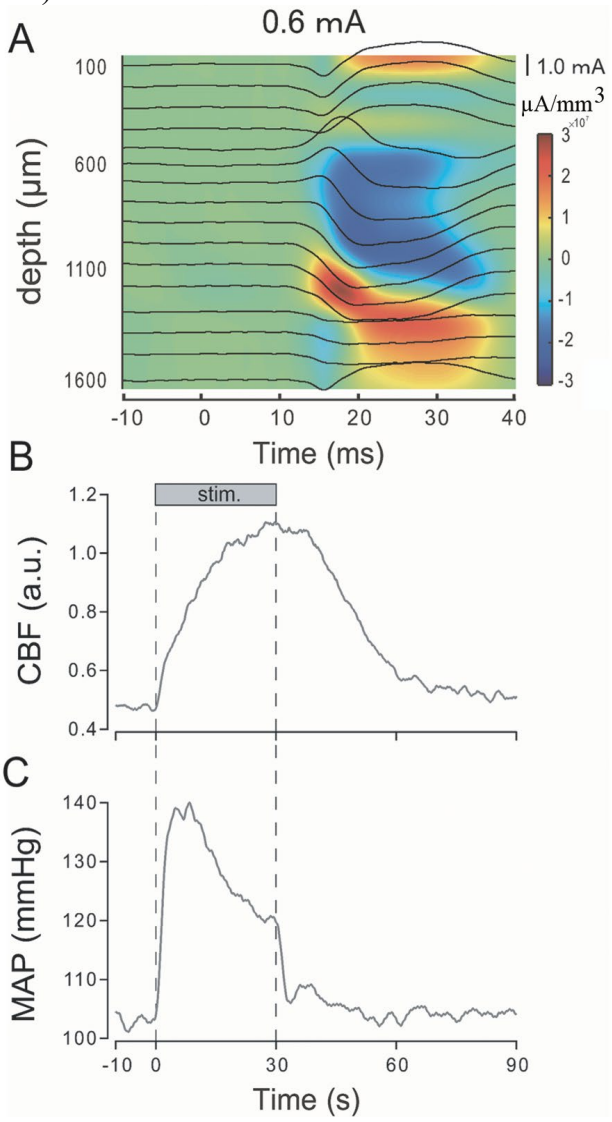


Figure 3. Individual example of neuronal and vascular recordings.

A. Representative example of the current source density (CSD) map and the mean local field potentials (LFP) time-locked to train pulses applied to the sciatic nerve (0 indicates the onset of the 3 ms pulse; 0.6 mA intensity in this example). Cerebral blood flow (CBF) (B) and mean arterial pressure (MAP) (C) responses induced by the 30 s stimulus train. The CBF response extended over the stimulation period while the MAP response shows a sharp peak followed by a progressive decline and sharp recovery after stimulus offset.

Cerebral blood flow

Basal CBF was compared between groups and anesthesia conditions with the average of baseline CBF values preceding each stimulus. Basal CBF was significantly lower under medetomidine compared with isoflurane anesthesia (main effect:$F_{1,11}$=4.8, p=0.05,η_p^2=0.30; mean±SD: 0.34±0.16 vs 0.41±0.15), but was not significantly different between fasted and non-fasted rats (main effect:$F_{1,11}$=0.2,p=0.6,η_p^2=0.02; mean±SD:0.40 ± 0.17 vs 0.35±0.15) or between fasted and non-fasted rats across anesthesia conditions (interaction:$F_{1,11}$=0.1,p=0.7;η_p^2=0.01). CBF responses to sciatic stimulation were compared between groups, anesthesia conditions and stimulation intensities (Figure 4A). CBF responses were not significantly affected by fasting (main effect:$F_{1,11}$=0.2,p=0.7;η_p^2 = 0.02) or the type of anesthesia (main effect:$F_{1,11}$=3.2, p=0.10;η_p^2=0.23), although medetomidine tended to produce larger responses compared with isoflurane. CBF responses significantly increased with intensity (main effect:$F_{9,99}$=11.4,p<0.001;η_p^2=0.51;linear trend:p=0.002). These intensity dependent effects were not significantly affected by the type of anesthesia (interaction:$F_{9,99}$=0.9,p=0.5;η_p^2=0.08) or fasting (interaction: $F_{1,11}$ = 0.7, p=0.4; η_p^2 =0.06).

Mean arterial pressure

Baseline MAP was compared between groups and anesthesia conditions with the average of baseline MAP values preceding each stimulus. Baseline MAP was not significantly different under medetomidine compared with isoflurane anesthesia (main effect: $F_{1,11}$=1.1, p=0.3, η_p^2=0.09; mean ± SD: 84.7±31.6 vs 93.4±16.7 mm Hg), between fasted and non-fasted rats (main effect: $F_{1,11}$=1.1, p=0.3, η_p^2=0.09; mean±SD: 94.8 ± 20.2 vs 83.3 ± 20.2 mm Hg) or between fasted and non-fasted rats across anesthesia conditions (interaction: $F_{1,11}$ = 1.2, p=0.3; η_p^2 = 0.10). MAP responses to sciatic stimulation were compared between groups, anesthesia conditions and stimulation intensities (see Figure 4B) and showed effects similar to those of CBF responses. MAP responses were not significantly affected by fasting (main effect: $F_{1,11}$=0.2, p=0.7; η_p^2=0.02) or the type of anesthesia (main effect: $F_{1,11}$=3.2, p=0.10; η_p^2=0.23). However, MAP responses increased with intensity (main effect:$F_{9,99}$=46.2,p<0.001;η_p^2 =0.81; linear trend:p<0.001). These intensity dependent effects were not significantly affected by the type of anesthesia (interaction:$F_{9,99}$=2.0,p=0.19;η_p^2=0.15) or fasting (interaction:$F_{9,99}$=0.9,p=0.4;η_p^2 =0.07).

Local field potentials

LFP amplitude was compared between groups, anesthesia conditions and stimulation intensities (see Figure 4C and Figure 4D). LFP amplitude was not significantly affected by fasting (main effect: $F_{1,11}$=0.2, p=0.7; η_p^2 =0.02) or the type of anesthesia (main effect: $F_{1,11}$=2.5, p=0.14; η_p^2 =0.19), although LFP amplitude tended to be lower in the medetomidine compared with isoflurane condition. LFP amplitude increased with intensity (main effect: $F_{9,99}$=16.5, p<0.001; η_p^2 =0.60) and these changes were affected by the type of anesthesia (interaction: $F_{9,99}$=4.6, p<0.001; η_p^2 =0.30), but not by fasting (interaction: $F_{9,99}$=0.3, p=0.9; η_p^2=0.03). For the interaction of intensity and anesthesia, the Tuckey HSD test revealed that LFP amplitude was significantly larger under isoflurane compared with medetomidine anesthesia for intensities between 0.6 and 4.8 mA (all p's <0.05; see Figure 4C).

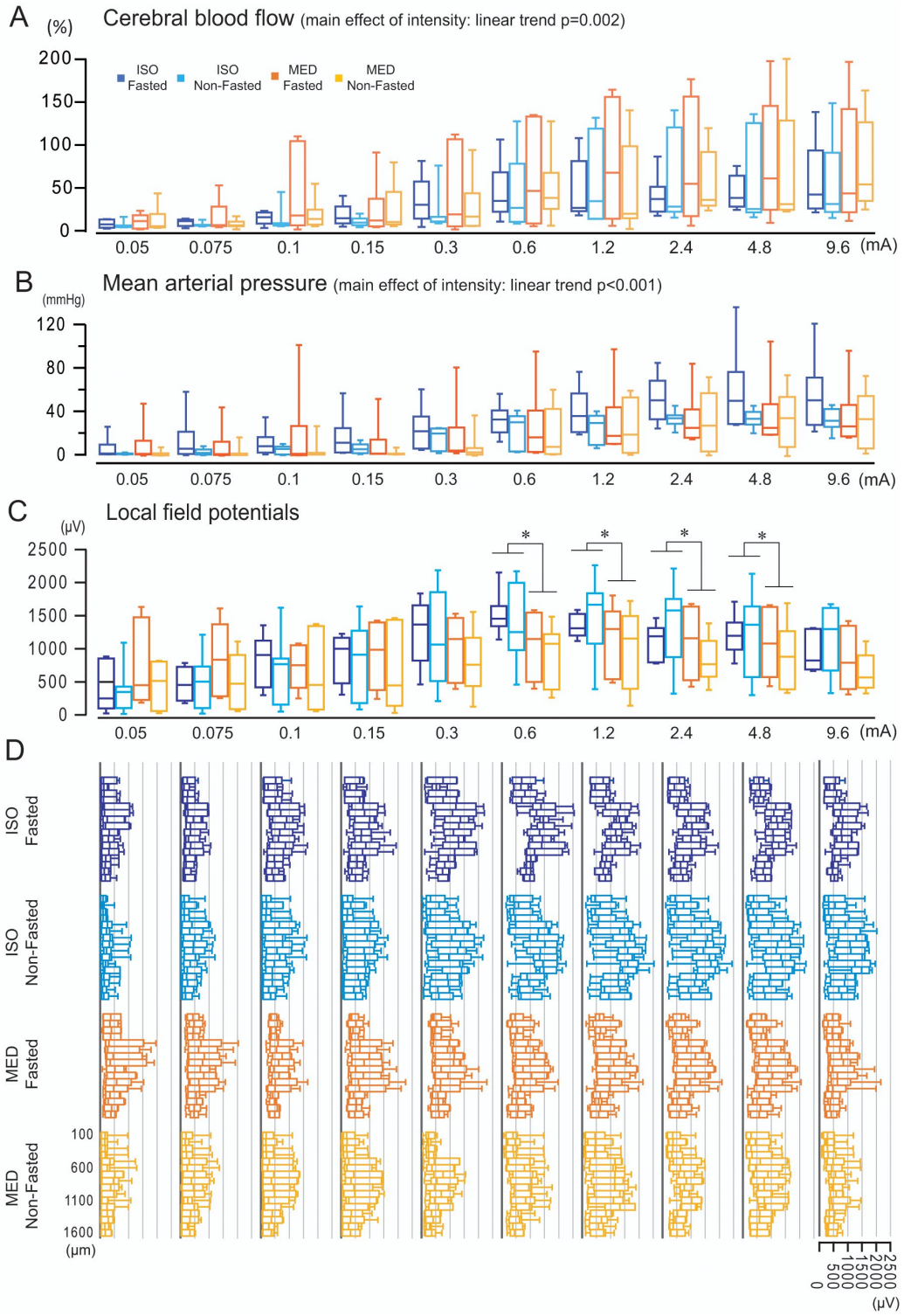


Figure 4. Vascular and neuronal responses to sciatic stimulation.
A. Cerebral blood flow (CBF) responses (% change) induced by electrical stimulation of right sciatic nerve at graded stimulus intensities, under isoflurane and medetomidine anesthesia, in non-fasted and fasted rats. B. Mean arterial blood pressure (MAP) responses (change in mm Hg). C. Amplitude of local field potentials (LFP). D. Amplitude of LFP at each recording site (every 100 μm from cortical surface). Vertical axis shows the cortical depth. Horizonal axis shows LFP amplitude. Box plots indicate the median, 25 % - 75 % range and 10 % - 90% range.

Neurovascular coupling

In order to examine the NVC, the CBF/LFP ratio was compared between groups, anesthesia conditions and intensities (see Figure 5A). The CBF/LFP ratio was significantly different between isoflurane and medetomidine anesthesia (main effect: $F_{1,11} = 14.9$, $p = 0.003$; $\eta_p^2 = 0.57$) and this effect was affected by fasting (interaction: $F_{1,11} = 6.0$, $p=0.03$; $\eta_p^2 = 0.35$), regardless of intensity (interaction: $F_{9,99} = 0.9$, $p=0.5$; $\eta_p^2 = 0.07$), although intensity affected the CBF/LFP ratio (main effect: $F_{9,99} = 5.1$, $p<0.001$; $\eta_p^2 = 0.32$) and this effect was significantly different between isoflurane and medetomidine anesthesia (interaction: $F_{9,99} = 0.9$, $p=0.5$; $\eta_p^2 = 0.07$). For the interaction of anesthesia and fasting, the Tuckey HSD test revealed that the CBF/LFP ratio was larger for medetomidine compared with isoflurane in non-fasted rats (mean ± SD; 0.93 ± 0.05 vs 1.13 ± 0.05, p = 0.004), but this effect was abolished by fasting (mean ± SD; 1.00 ± 0.05 vs 1.04 ± 0.05, p = 0.8) (see Figure 5B), where a ratio of 1 corresponds to linear NVC. Besides, the difference in the CBF/LFP ratio between fasted and non-fasted animals did not reach significance for medetomidine (mean ± SD; 1.00 ± 0.05 vs 0.93 ± 0.05, p=0.069) and was not significantly different for isoflurane (mean ± SD; 1.04 ± 0.05 vs 1.13 ± 0.05, p=0.8). For the interaction of anesthesia and intensity, the Tuckey HSD test revealed that the CBF/LFP ratio was not significantly different for 0.075-9.6 intensities compared with the 0.05 intensity for medetomidine (all p>0.8) and for isoflurane (all p>0.8).

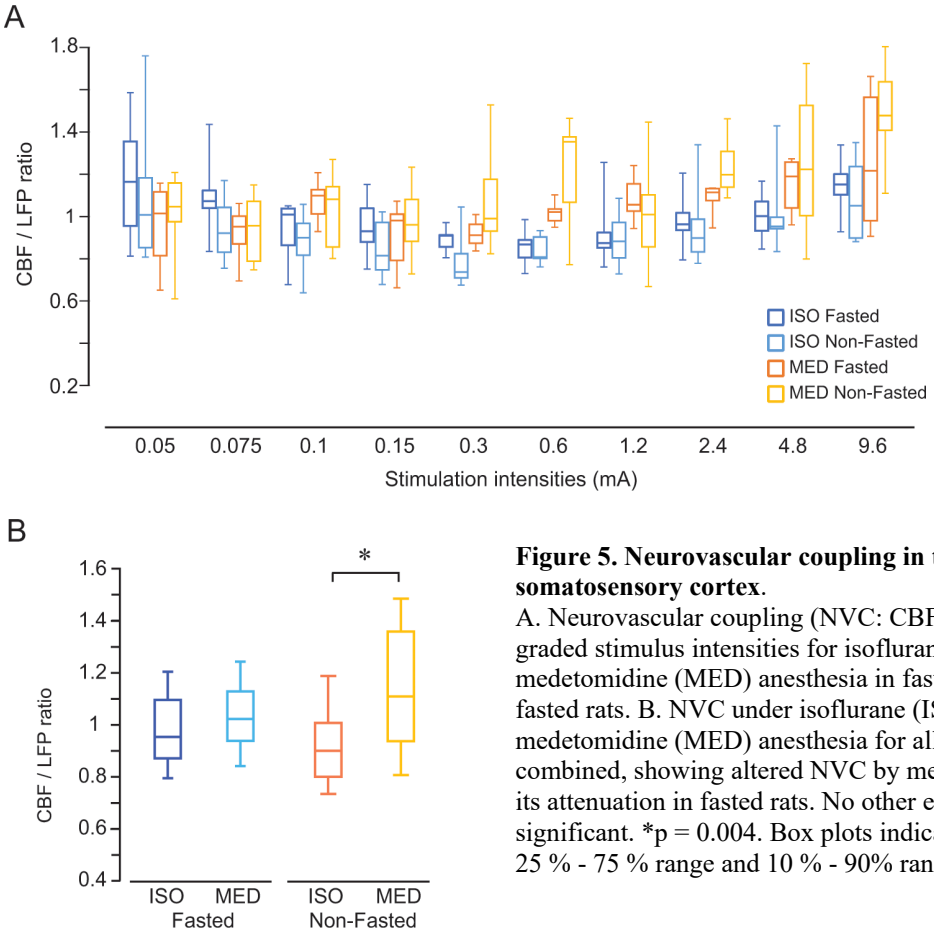


Figure 5. Neurovascular coupling in the somatosensory cortex.
A. Neurovascular coupling (NVC: CBF/LFP ratio) at graded stimulus intensities for isoflurane (ISO) and medetomidine (MED) anesthesia in fasted and non-fasted rats. B. NVC under isoflurane (ISO) and medetomidine (MED) anesthesia for all intensities combined, showing altered NVC by medetomidine and its attenuation in fasted rats. No other effect was significant. *p = 0.004. Box plots indicate the median, 25 % - 75 % range and 10 % - 90% range.

Blood glucose levels

The Mann-Whitney U-test confirmed that blood glucose was lower in fasted (n=4) compared with non-fasted (n=4) rats (mean ± SD: 89.2 ± 14.7 vs 145.9 ± 25.7 mg/dl, respectively; p=0.03). In order to confirm that medetomidine produced blood glucose increases in these rats, blood glucose was monitored for 1 hour during medetomidine anesthesia (see Figure 6). As expected, medetomidine anesthesia significantly increased the blood glucose level over time (main effect: $F_{6,36} = 22.0$, $p<0.001$; $\eta_p^2 = 0.79$) and this effect was abolished by fasting (interaction: $F_{6,36} = 9.4$, $p<0.001$; $\eta_p^2 = 0.61$). The Tuckey HSD test revealed that blood glucose progressively increased over time compared with baseline, in non-fasted rats (all $p<0.001$). In contrast, blood glucose did not change significantly over time compared with baseline in fasted rats (all $p>0.3$).

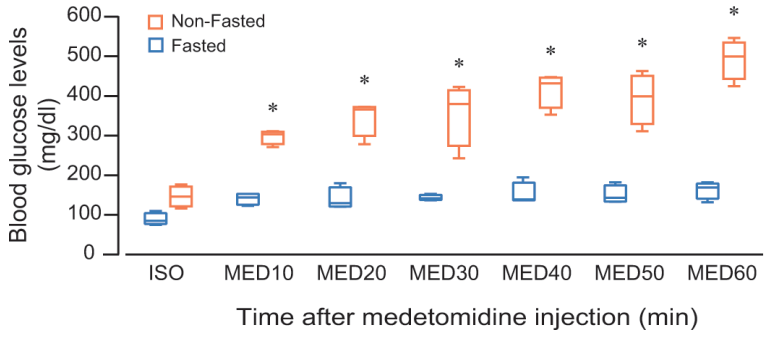


Figure 6. Blood glucose levels
Blood glucose levels showed a threefold increase during medetomidine anesthesia in non-fasted rats. This acute hyperglycemia was largely attenuated by fasting.*p<0.001 compared with isoflurane anesthesia. Box plots indicate the median, 25 % - 75 % range and 10 % - 90% range.

Discussion

In the present study, we investigated the effects of fasting on NVC in the somatosensory cortex during noxious stimulation of the sciatic nerve, under isoflurane and medetomidine anesthesia. The novel finding is that the CBF/LFP ratio was increased under medetomidine compared with isoflurane anesthesia, but this effect was abolished by fasting, which prevented medetomidine-induced hyperglycemia.

Preventing medetomidine-induced hyperglycemia by fasting

Blood glucose decreases during fasting due to the lack of food intake and thereby, the lack of glucose absorbed by the intestines (Secor & Carey, 2016). As expected, in the present study, rats that were fasted overnight showed significantly lower blood glucose levels, with values comparable to those from previous studies (Guezennec *et al.*, 1988; Zardooz *et al.*, 2010; Nowland *et al.*, 2011; Dakic *et al.*, 2017). However, medetomidine anesthesia induced large changes in blood glucose. In non-fasted rats, blood glucose levels already doubled 10 minutes after the medetomidine bolus injection, reaching a threefold increase after 60 minutes. This time course of blood glucose is consistent with previous studies in rats anesthetized with medetomidine or other α2-adrenoceptor agonists (Angel & Langer, 1988; Hoffman *et al.*, 1991; Saha *et al.*, 2005). It is well known that α2-adrenoceptor agonists inhibit the secretion of insulin by their postsynaptic action on pancreatic β-cells (Angel *et al.*, 1990; Niddam *et al.*, 1990). In turn, this leads to hyperglycemia. The present results indicate that this effect can be prevented by overnight fasting, which is known to lower blood glucose and glycogen storage (Fleming & Kenny,

1964; Geisler *et al.*, 2016), while the contribution of glycogenolysis to increase blood glucose levels is limited in fasted rats (Saha *et al.*, 2005).

Impact of hyperglycemia and fasting on neurovascular coupling

In the present study, CBF responses tended to be larger and LFP responses tended to be smaller under medetomidine compared with isoflurane anesthesia, although the effects did not reach significance. Notwithstanding, the combined effects resulted in neurovascular uncoupling with a significantly increased CBF/LFP ratio for medetomidine compared with isoflurane anesthesia. In line with our hypothesis and the blood glucose results, this effect was abolished by fasting. The mechanisms by which fasting prevents medetomidine-induced NVC alterations are not clear but we will consider three possibilities.

Firstly, the α2-adrenoceptor agonist effect of medetomidine produces cerebral vasoconstriction (Nakai *et al.*, 1986) and it was suggested that the high vascular tone may underlie the larger CBF responses to somatosensory stimulation (Fukuda *et al.*, 2013). In the present study, we observed a lower basal blood flow under medetomidine compared with isoflurane anesthesia, consistent with cerebral vasoconstriction. However, there is currently no evidence showing that the adrenergic effect of medetomidine is altered by fasting.

Secondly, hyperglycemia is known to reduce CBF (Duckrow RB et al., 1995). This was shown to rely on cerebral vasoconstriction induced by purinergic signaling (Martin-Aragon Baudel *et al.*, 2020), which increases smooth muscle excitability and vascular reactivity, in addition to the α2-adrenergic effect of medetomidine. The normalization of glucose levels by fasting during medetomidine anesthesia may therefore decrease vascular reactivity, CBF responses to sciatic stimulation and NVC alterations. Consistent with this idea, the alteration of NVC during hyperglycemia is prevented by sodium nitroprusside in the brain of the zebrafish (Chhabria *et al.*, 2020). Sodium nitroprusside is a nitric oxide donor used for hypertension that produces vasodilation (Page *et al.*, 1955), which offsets vasoconstriction and increased vascular reactivity induced by hyperglycemia.

Thirdly, medetomidine may affect astrocyte functions, which are key mediators of NVC (Zonta *et al.*, 2003; Takano *et al.*, 2006). Indeed, cortical astrocytes express α2-adrenergic receptors (Lee *et al.*, 1998) and respond to α2-adrenergic agonists by enhancing energy metabolism (Hertz & Peng, 1992; Chen & Hertz, 1999; Chen *et al.*, 2000). With fasting, glucose metabolism is altered and this may reduce the medetomidine-induced increase in energy metabolism, which in turn would reduce its vascular effects through astrocytes.

Altogether, the present results and the current literature suggests that fasting may prevent medetomidine-induced NVC alterations by normalizing blood glucose levels, which affects vascular reactivity and astrocyte metabolism. This warrants future studies to examine the independent contribution of α2-adrenergic effects, hyperglycemia and astrocyte function regulation on vascular tone and reactivity, in order to clarify the exact mechanism by which medetomidine alters NVC during noxious stimulation.

Regardless of fasting, differences in NVC between isoflurane and medetomidine anesthesia may also be explained by medetomidine effects on the locus coeruleus (LC). During noxious stimulation, the LC is activated by C-fibre-related inputs (Hirata & Aston-Jones, 1994). The LC regulates CBF and its activation produces vasoconstriction. This

effect was likely produced by sciatic nerve stimulation in the present study. However, medetomidine decreases the firing rate of locus coeruleus (LC) neurons (Jorm & Stamford, 1993; Chiu *et al.*, 1995), which in turn may decrease the phasic vasoconstriction induced by noxious inputs and therefore increase CBF responses to sciatic stimulation. To our knowledge, isoflurane has no specific effect on LC neurons and produces vasodilation (Iida *et al.*, 1998), which decreases vascular reactivity and may produce smaller CBF responses compared with medetomidine anesthesia.

Neuronal activity in the somatosensory cortex under medetomidine anesthesia

In addition to the vascular effects, medetomidine regulates neuronal activity. The present results show that the somatosensory evoked LFP were significantly smaller under medetomidine compared with isoflurane anesthesia. This is somewhat unexpected based on previous studies. For example, somatosensory LFP evoked by forepaw stimulation were lager under a combination of dexmedetomidine (the active enantiomer of medetomidine) and isoflurane (0.5%) anesthesia compared with isoflurane alone (1.5 %) (Fukuda *et al.*, 2013). However, one major difference between studies is the stimulation intensity. Fukuda et al. used low-intensity electrical stimulation that did not evoke systemic MAP changes (Fukuda *et al.*, 2013). In the present study, we used graded intensities including noxious stimuli. Indeed, the evoked LFP at intensities ranging from 0.3 to 9.6 mA elicited MAP increases and LFP amplitude at most of these intensities was significantly smaller under medetomidine compared with isoflurane. Thus, medetomidine anesthesia decreased LFP amplitude, but only in the noxious range. This may explain the differences between studies. Accordingly, α2-adrenergic receptor agonists produce analgesia by acting on receptors located in the pain pathways in the spinal cord and brain (Sinclair, 2003). Indeed, a high concentrations of α2-adrenergic receptor binding sites was observed in the dorsal horn of the spinal cord where nociceptive fibres make synapse (Unnerstall *et al.*, 1984) and where nociceptive transmission can be regulated. Therefore, medetomidine anesthesia poses an additional challenge for NVC when noxious stimuli are used to examine cerebral pain-related responses.

It is also known that general anesthesia produced by isoflurane and medetomidine are due to different mechanisms. Isoflurane is thought to produce anesthesia by the activation of $GABA_A$ receptors, while medetomidine is thought to be mediated by the suppression of neuronal activity and the release of noradrenaline in the LC (Jorm & Stamford, 1993; Chiu *et al.*, 1995). Considering that the LC response to noxious stimulation results from C-fibre activation in the rat (Hirata & Aston-Jones, 1994), that stimulation of the LC increases the neuronal response to skin stimulation in the somatosensory cortex (Snow *et al.*, 1999) and that medetomidine decreases the firing rate of LC neurons, it is expected that responses to noxious stimuli in the somatosensory cortex be inhibited by medetomidine, consistent with the present findings.

Methodological considerations

A few methodological issues should be discussed for data interpretation and future studies. Firstly, laser Doppler flowmetry allows the measurement of blood flow at a depth of approximately 1 mm (Shih *et al.*, 2012). In the cortex of the rat, this includes superficial layers and covers the area of interest where neuronal activity is measured simultaneously in layer IV (Hersch & White, 1981; Harris *et al.*, 2010). Thus, the CBF measure covers a

larger area compared with the LFP measure. However, the type of stimulation used in the present study produces intensity dependent changes in all layers of the cortex, so it is unlikely that NVC is biased by the differences in covered areas between the two measures, although it cannot be ruled out. Secondly, Various concentrations of isoflurane, doses of medetomidine (or dexmedetomidine), and mixtures of both anesthetics may be used for different purposes and experiments (Masamoto *et al.*, 2009; Fukuda *et al.*, 2013; Tsurugizawa *et al.*, 2016; Tsurugizawa *et al.*, 2017; Paasonen *et al.*, 2018). In the present study, the anesthesia level was appropriate for major surgery and the delivery of painful electrical stimuli. During data collection, the concentration of isoflurane was 1.2 % while medetomidine was infused subcutaneously at a rate of 120 µg/kg/h. This should be taken into consideration when comparing the results with those of other studies, since different levels of anesthesia may affect NVC differently.

In addition, we chose a within-subject design with repeated measures. This has the advantage of comparing NVC between isoflurane and medetomidine in the same animal. The main disadvantage is that it increases the length of anesthesia, which may affect NVC. However, in a recent study on spinal NVC, after 5 hours of surgical procedures and over 5 hours of recordings, we observed stable NVC (Paquette *et al.*, 2021). Thus, prolonged anesthesia is unlikely to affect the present results. Thirdly, the fasting duration of 15 hours was chosen from a range used in previous studies and was adapted to local animal facilities. Previous studies using fasting duration ranging between 6 and 48 hours obtained similar values of fasting blood (Guezennec *et al.*, 1988; Zardooz *et al.*, 2010; Nowland *et al.*, 2011; Dakic *et al.*, 2017). Therefore, fasting duration may be shortened or extended depending on the objective of the study, although it remains unclear how other metabolic functions may affect the present results with longer duration. In the present study, no significant change in blood pressure was observed between fasted and non-fasted rats. However, the 15-hour fasting may not be sufficient to induce significant MAP changes. Previous studies have shown that longer fasting or intermittent fasting may progressively decrease MAP through peripheral and central noradrenergic changes (El Fazaa *et al.*, 1999; Williams *et al.*, 2000). Whether the metabolic state during longer or intermittent fasting may alter NVC remains to be investigated.

Conclusion

In summary, the present results show that the CBF/LFP ratio in the somatosensory cortex is increased under medetomidine anesthesia during noxious stimulation, but this effect may be attenuated by fasting, which prevents medetomidine-induced hyperglycemia and its consequences on the vascular system. This has implications for fMRI studies in which neuronal activity may be overestimated under medetomidine anesthesia. Overnight fasting is a simple procedure that may be implemented to address this issue in future studies. In pain studies, medetomidine poses an additional challenge because of its inhibitory effects on neuronal activity. Although every anesthetic agent may potentially alter NVC and the outcome of fMRI, the present results should be considered when conducting fMRI studies on pain mechanisms under medetomidine anesthesia.

Data availability statement
The dataset supporting the conclusions of this article is available from the corresponding author on reasonable request.

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Authors' contributions
Each author has contributed significantly to this work and has read and approved the final version of the manuscript. RT contributed to data collection, analysis and interpretation and wrote the preliminary version of the manuscript. TP contributed to data collection and manuscript writing. HL contributed to data collection and interpretation. TT contributed to study design and data interpretation. MP contributed to study design, data collection, analyses, and interpretation, wrote the final version of the manuscript and obtained funding for the study.

Conflict of interest disclosure
The authors declare that they have no competing interests.

Ethics approval statement
All experimental procedures were approved by the Université du Québec à Trois-Rivières animal care committee, in accordance with the guidelines of the Canadian Council on Animal Care and adhered to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (IASP). Data is reported in compliance with the ARRIVE guidelines

References
Adamczak, J.M., Farr, T.D., Seehafer, J.U., Kalthoff, D. & Hoehn, M. (2010) High field BOLD response to forepaw stimulation in the mouse. *Neuroimage*, **51**, 704-712.

Angel, I. & Langer, S.Z. (1988) Adrenergic-induced hyperglycemia in anaesthetized rats: involvement of peripheral alpha 2-adrenoceptors. *Eur J Pharmacol*, **154**, 191-196.

Angel, I., Niddam, R. & Langer, S.Z. (1990) Involvement of alpha-2 adrenergic receptor subtypes in hyperglycemia. *J Pharmacol Exp Ther*, **254**, 877-882.

Apkarian, A.V., Hashmi, J.A. & Baliki, M.N. (2011) Pain and the brain: specificity and plasticity of the brain in clinical chronic pain. *Pain*, **152**, S49-64.

Boretius, S., Tammer, R., Michaelis, T., Brockmoller, J. & Frahm, J. (2013) Halogenated volatile anesthetics alter brain metabolism as revealed by proton magnetic resonance spectroscopy of mice in vivo. *Neuroimage*, **69**, 244-255.

Callahan, L.M., Ross, S.M., Jones, M.L. & Musk, G.C. (2014) Mortality associated with using medetomidine and ketamine for general anesthesia in pregnant and nonpregnant Wistar rats. *Lab Anim (NY)*, **43**, 208-214.

Chen, Y. & Hertz, L. (1999) Noradrenaline effects on pyruvate decarboxylation: correlation with calcium signaling. *J Neurosci Res*, **58**, 599-606.

Chen, Y., Zhao, Z., Code, W.E. & Hertz, L. (2000) A correlation between dexmedetomidine-induced biphasic increases in free cytosolic calcium concentration and energy metabolism in astrocytes. *Anesth Analg*, **91**, 353-357.

Chhabria, K., Plant, K., Bandmann, O., Wilkinson, R.N., Martin, C., Kugler, E., Armitage, P.A., Santoscoy, P.L., Cunliffe, V.T., Huisken, J., McGown, A., Ramesh, T., Chico, T.J. & Howarth, C. (2020) The effect of hyperglycemia on neurovascular coupling and cerebrovascular patterning in zebrafish. *J Cereb Blood Flow Metab*, **40**, 298-313.

Chiu, T.H., Chen, M.J., Yang, Y.R., Yang, J.J. & Tang, F.I. (1995) Action of dexmedetomidine on rat locus coeruleus neurones: intracellular recording in vitro. *Eur J Pharmacol*, **285**, 261-268.

Crystal, G.J., Czinn, E.A., Silver, J.M. & Salem, M.R. (1995) Coronary vasodilation by isoflurane. Abrupt versus gradual administration. *Anesthesiology*, **82**, 542-549.

Da Silva, J.T. & Seminowicz, D.A. (2019) Neuroimaging of pain in animal models: a review of recent literature. *Pain reports*, **4**, e732.

Dakic, T.B., Jevdjovic, T.V., Peric, M.I., Bjelobaba, I.M., Markelic, M.B., Milutinovic, B.S., Lakic, I.V., Jasnic, N.I., Djordjevic, J.D. & Vujovic, P.Z. (2017) Short-term fasting promotes insulin expression in rat hypothalamus. *Eur J Neurosci*, **46**, 1730-1737.

Duckrow, R.B. (1995) Decreased cerebral blood flow during acute hyperglycemia. *Brain Res*, **703**, 145-150.

Duerden, E.G. & Albanese, M.C. (2013) Localization of pain-related brain activation: a meta-analysis of neuroimaging data. *Human brain mapping*, **34**, 109-149.

Eisenach, J.C., Shafer, S.L., Bucklin, B.A., Jackson, C. & Kallio, A. (1994) Pharmacokinetics and pharmacodynamics of intraspinal dexmedetomidine in sheep. *Anesthesiology*, **80**, 1349-1359.

El Fazaa, S., Somody, L., Gharbi, N., Kamoun, A., Gharib, C. & Gauquelin-Koch, G. (1999) Effects of acute and chronic starvation on central and peripheral noradrenaline turnover, blood pressure and heart rate in the rat. *Experimental physiology*, **84**, 357-368.

Fleming, W.W. & Kenny, A.D. (1964) The Effect of Fasting on the Hyperglycaemic Responses to Catechol Amines in Rats. *Br J Pharmacol Chemother*, **22**, 267-274.

Fukuda, M., Vazquez, A.L., Zong, X. & Kim, S.G. (2013) Effects of the alpha(2)-adrenergic receptor agonist dexmedetomidine on neural, vascular and BOLD fMRI responses in the somatosensory cortex. *Eur J Neurosci*, **37**, 80-95.

Geisler, C.E., Hepler, C., Higgins, M.R. & Renquist, B.J. (2016) Hepatic adaptations to maintain metabolic homeostasis in response to fasting and refeeding in mice. *Nutrition & Metabolism*, **13**.

Guezennec, C.Y., Nonglaton, J., Serrurier, B., Merino, D. & Defer, G. (1988) Hormonal and metabolic response to physical exercise, fasting and cold exposure in the rat. Effects on ketogenesis in isolated hepatocytes. *Eur J Appl Physiol Occup Physiol*, **57**, 114-119.

Harris, S., Jones, M., Zheng, Y. & Berwick, J. (2010) Does neural input or processing play a greater role in the magnitude of neuroimaging signals? *Front Neuroenergetics*, **2**.

Hersch, S.M. & White, E.L. (1981) Thalamocortical synapses involving identified neurons in mouse primary somatosensory cortex: a terminal degeneration and golgi/EM study. *J Comp Neurol*, **195**, 253-263.

Hertz, L. & Peng, L. (1992) Effects of monoamine transmitters on neurons and astrocytes: correlation between energy metabolism and intracellular messengers. *Prog Brain Res*, **94**, 283-301.

Hirata, H. & Aston-Jones, G. (1994) A novel long-latency response of locus coeruleus neurons to noxious stimuli: mediation by peripheral C-fibers. *J Neurophysiol*, **71**, 1752-1761.

Hoffman, E., William, Kochs, E., Werner, C., Thomas, C. & Albrecht, F., Ronald (1991) Dexmedetomidine Improves Neurologic Outcome from Incomplete Ischemia in the Rat Reversal by the α2-Adrenergic Antagonist Atipamezole. *Anesthesiology*, **75**, 328-332.

Huang, C.W., Huang, C.C., Cheng, J.T., Tsai, J.J. & Wu, S.N. (2007) Glucose and hippocampal neuronal excitability: role of ATP-sensitive potassium channels. *J Neurosci Res*, **85**, 1468-1477.

Iida, H., Ohata, H., Iida, M., Watanabe, Y. & Dohi, S. (1998) Isoflurane and Sevoflurane Induce Vasodilation of Cerebral Vessels via ATP-sensitive K+Channel Activation. *Anesthesiology*, **89**, 954-960.

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

Jorm, C.M. & Stamford, J.A. (1993) Actions of the hypnotoc anaesthetic, dexmedetomidine, on noradrenaline release and cell firing in rat locos coeruleus slices. *British Journal of Anaesthesia*, **71**, 447-449.

Kanda, T. & Hikasa, Y. (2008) Effects of medetomidine and midazolam alone or in combination on the metabolic and neurohormonal responses in healthy cats. *Can J Vet Res*, **72**, 332-339.

Lee, A., Rosin, D.L. & Van Bockstaele, E.J. (1998) alpha2A-adrenergic receptors in the rat nucleus locus coeruleus: subcellular localization in catecholaminergic dendrites, astrocytes, and presynaptic axon terminals. *Brain Res*, **795**, 157-169.

Leino, T., Viitamaa, T., Haapalinna, A., Lehtimaki, J. & Virtanen, R. (2009) Pharmacological profile of intrathecal fadolmidine, a alpha2-adrenoceptor agonist, in rodent models. *Naunyn Schmiedebergs Arch Pharmacol*, **380**, 539-550.

Link, R.E., Desai, K., Hein, L., Stevens, M.E., Chruscinski, A., Bernstein, D., Barsh, G.S. & Kobilka, B.K. (1996) Cardiovascular regulation in mice lacking alpha2-adrenergic receptor subtypes b and c. *Science*, **273**, 803-805.

Lowe, A.S., Beech, J.S. & Williams, S.C. (2007) Small animal, whole brain fMRI: innocuous and nociceptive forepaw stimulation. *Neuroimage*, **35**, 719-728.

Margineanu, D.G., Niespodziany, I. & Wulfert, E. (1998) Hippocampal slices from long-term streptozotocin-injected rats are prone to epileptiform responses. *Neurosci Lett*, **252**, 183-186.

Martin-Aragon Baudel, M., Espinosa-Tanguma, R., Nieves-Cintron, M. & Navedo, M.F. (2020) Purinergic Signaling During Hyperglycemia in Vascular Smooth Muscle Cells. *Front Endocrinol (Lausanne)*, **11**, 329.

Masamoto, K., Fukuda, M., Vazquez, A. & Kim, S.G. (2009) Dose-dependent effect of isoflurane on neurovascular coupling in rat cerebral cortex. *Eur J Neurosci*, **30**, 242-250.

Masamoto, K., Kim, T., Fukuda, M., Wang, P. & Kim, S.G. (2007) Relationship between neural, vascular, and BOLD signals in isoflurane-anesthetized rat somatosensory cortex. *Cereb Cortex*, **17**, 942-950.

Nakai, K., Itakura, T., Naka, Y., Nakakita, K., Kamei, I., Imai, H., Yokote, H. & Komai, N. (1986) The distribution of adrenergic receptors in cerebral blood vessels: an autoradiographic study. *Brain Res*, **381**, 148-152.

Nasrallah, F.A., Lew, S.K., Low, A.S. & Chuang, K.H. (2014) Neural correlate of resting-state functional connectivity under alpha2 adrenergic receptor agonist, medetomidine. *Neuroimage*, **84**, 27-34.

Nicholson, C. & Freeman, J.A. (1975) Theory of current source-density analysis and determination of conductivity tensor for anuran cerebellum. *J Neurophysiol*, **38**, 356-368.

Niddam, R., Angel, I., Bidet, S. & Langer, S.Z. (1990) Pharmacological characterization of alpha-2 adrenergic receptor subtype involved in the release of insulin from isolated rat pancreatic islets. *J Pharmacol Exp Ther*, **254**, 883-887.

Nowland, M.H., Hugunin, K.M. & Rogers, K.L. (2011) Effects of short-term fasting in male Sprague-Dawley rats. *Comp Med*, **61**, 138-144.

Ogawa, S., Lee, T.M., Kay, A.R. & Tank, D.W. (1990) Brain magnetic resonance imaging with contrast dependent on blood oxygenation. *Proc Natl Acad Sci U S A*, **87**, 9868-9872.

Paasonen, J., Stenroos, P., Salo, R.A., Kiviniemi, V. & Grohn, O. (2018) Functional connectivity under six anesthesia protocols and the awake condition in rat brain. *Neuroimage*, **172**, 9-20.

Page, I.H., Corcoran, A.C., Dustan, H.P. & Koppanyi, T. (1955) Cardiovascular actions of sodium nitroprusside in animals and hypertensive patients. *Circulation*, **11**, 188-198.

Paquette, T., Jeffrey-Gauthier, R., Leblond, H. & Piché, M. (2018) Functional Neuroimaging of Nociceptive and Pain-Related Activity in the Spinal Cord and Brain: Insights From Neurovascular Coupling Studies. *Anat Rec (Hoboken)*, **301**, 1585-1595.

Paquette, T., Leblond, H. & Piché, M. (2019a) Isoflurane anesthesia does not affect spinal cord neurovascular coupling: evidence from decerebrated rats. *J Physiol Sci*, **69**, 13-21.

Paquette, T., Piché, M. & Leblond, H. (2021) Contribution of astrocytes to neurovascular coupling in the spinal cord of the rat. *The Journal of Physiological Sciences*.

Paquette, T., Tokunaga, R., Touj, S., Leblond, H. & Piché, M. (2019b) Regulation of cortical blood flow responses by the nucleus basalis of Meynert during nociceptive processing. *Neuroscience research*, **149**, 22-28.

Pawela, C.P., Biswal, B.B., Hudetz, A.G., Schulte, M.L., Li, R., Jones, S.R., Cho, Y.R., Matloub, H.S. & Hyde, J.S. (2009) A protocol for use of medetomidine anesthesia in rats for extended studies using task-induced BOLD contrast and resting-state functional connectivity. *Neuroimage*, **46**, 1137-1147.

Paxinos, G. & Watson, C.l. (2007) *The rat brain in stereotaxic coordinates*. Elsevier, Amsterdam ; Boston.

Pettersen, K.H., Devor, A., Ulbert, I., Dale, A.M. & Einevoll, G.T. (2006) Current-source density estimation based on inversion of electrostatic forward solution: effects of finite

extent of neuronal activity and conductivity discontinuities. *J Neurosci Methods*, **154**, 116-133.

Saha, J.K., Xia, J., Grondin, J.M., Engle, S.K. & Jakubowski, J.A. (2005) Acute hyperglycemia induced by ketamine/xylazine anesthesia in rats: mechanisms and implications for preclinical models. *Exp Biol Med (Maywood)*, **230**, 777-784.

Scheinin, H., Virtanen, R., MacDonald, E., Lammintausta, R. & Scheinin, M. (1989) Medetomidine--a novel alpha 2-adrenoceptor agonist: a review of its pharmacodynamic effects. *Prog Neuropsychopharmacol Biol Psychiatry*, **13**, 635-651.

Secor, S.M. & Carey, H.V. (2016) Integrative Physiology of Fasting. *Compr Physiol*, **6**, 773-825.

Shih, A.Y., Driscoll, J.D., Drew, P.J., Nishimura, N., Schaffer, C.B. & Kleinfeld, D. (2012) Two-photon microscopy as a tool to study blood flow and neurovascular coupling in the rodent brain. *J Cereb Blood Flow Metab*, **32**, 1277-1309.

Shirasaka, T., Qiu, D.L., Kannan, H. & Takasaki, M. (2007) The effects of centrally administered dexmedetomidine on cardiovascular and sympathetic function in conscious rats. *Anesth Analg*, **105**, 1722-1728, table of contents.

Sinclair, M.D. (2003) A review of the physiological effects of alpha2-agonists related to the clinical use of medetomidine in small animal practice. *Can Vet J*, **44**, 885-897.

Snow, P.J., Andre, P. & Pompeiano, O. (1999) Effects of locus coeruleus stimulation on the responses of SI neurons of the rat to controlled natural and electrical stimulation of the skin. *Arch Ital Biol*, **137**, 1-28.

Takano, T., Tian, G.F., Peng, W., Lou, N., Libionka, W., Han, X. & Nedergaard, M. (2006) Astrocyte-mediated control of cerebral blood flow. *Nat Neurosci*, **9**, 260-267.

Talke, P., Lobo, E. & Brown, R. (2003) Systemically administered alpha2-agonist-induced peripheral vasoconstriction in humans. *Anesthesiology*, **99**, 65-70.

Tan, C.M., Wilson, M.H., MacMillan, L.B., Kobilka, B.K. & Limbird, L.E. (2002) Heterozygous alpha 2A-adrenergic receptor mice unveil unique therapeutic benefits of partial agonists. *Proc Natl Acad Sci U S A*, **99**, 12471-12476.

Tsurugizawa, T., Abe, Y. & Le Bihan, D. (2017) Water apparent diffusion coefficient correlates with gamma oscillation of local field potentials in the rat brain nucleus accumbens following alcohol injection. *J Cereb Blood Flow Metab*, **37**, 3193-3202.

Tsurugizawa, T., Takahashi, Y. & Kato, F. (2016) Distinct effects of isoflurane on basal BOLD signals in tissue/vascular microstructures in rats. *Sci Rep*, **6**, 38977.

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.

Unnerstall, J.R., Kopajtic, T.A. & Kuhar, M.J. (1984) Distribution of alpha 2 agonist binding sites in the rat and human central nervous system: analysis of some functional, anatomic correlates of the pharmacologic effects of clonidine and related adrenergic agents. *Brain Res*, **319**, 69-101.

van der Miesen, M.M., Lindquist, M.A. & Wager, T.D. (2019) Neuroimaging-based biomarkers for pain: state of the field and current directions. *Pain reports*, **4**, e751.

Verdier, D. & Dykes, R.W. (2001) Long-term cholinergic enhancement of evoked potentials in rat hindlimb somatosensory cortex displays characteristics of long-term potentiation. *Exp Brain Res*, **137**, 71-82.

Wager, T.D., Atlas, L.Y., Lindquist, M.A., Roy, M., Woo, C.W. & Kross, E. (2013) An fMRI-based neurologic signature of physical pain. *N Engl J Med*, **368**, 1388-1397.

Weber, R., Ramos-Cabrer, P., Wiedermann, D., van Camp, N. & Hoehn, M. (2006) A fully noninvasive and robust experimental protocol for longitudinal fMRI studies in the rat. *Neuroimage*, **29**, 1303-1310.

Williams, T.D., Chambers, J.B., May, O.L., Henderson, R.P., Rashotte, M.E. & Overton, J.M. (2000) Concurrent reductions in blood pressure and metabolic rate during fasting in the unrestrained SHR. *American journal of physiology. Regulatory, integrative and comparative physiology*, **278**, R255-262.

Zardooz, H., Rostamkhani, F., Zaringhalam, J. & Faraji Shahrivar, F. (2010) Plasma corticosterone, insulin and glucose changes induced by brief exposure to isoflurane, diethyl ether and CO2 in male rats. *Physiological Research*.

Zonta, M., Angulo, M.C., Gobbo, S., Rosengarten, B., Hossmann, K.A., Pozzan, T. & Carmignoto, G. (2003) Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. *Nat Neurosci*, **6**, 43-50.