Hemodynamic-based Mapping of Neural Activity in Medetomidine-sedated Rats using a 1.5T Compact Magnetic Resonance Imaging System: A Preliminary Study

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The use of compact magnetic resonance (MR) systems for the neuroimaging of small animals is spreading. We investigated the potential of such systems in functional MR imaging (fMRI) of somatosensory cortex activity elicited by forepaw stimulation in medetomidine-sedated rats. Using a 1.5-tesla compact imager, we detected maximum activity with an electrophysiologically optimized frequency of 9 Hz in 3 appropriately sedated rats. With this compact system, we successfully mapped neural activity by combining optimum stimulation for a large hemodynamic response with appropriate anesthesia, thus demonstrating the utility of such systems in hemodynamic-based fMRI in preclinical and translational research.

Keywords: compact magnetic resonance imaging system, experimental animal neuroimaging, functional magnetic resonance imaging (fMRI), hemodynamic-based neuroimaging, medetomidine sedation

Introduction

Experimental studies using small laboratory animals play a key role in preclinical and translational research. At the same time, reduction in the number of animals in these studies, which together with refinement and replacement are called the “3Rs”, is an international issue underlying the design of various research projects. Noninvasive magnetic resonance (MR) imaging techniques for use with live animal subjects are promoting as effective means to reduce the number of animals used in experiments and thus may be useful for the proper execution of this type of experimental research.

Neuroimaging analysis, such as functional MR imaging (fMRI) studies of the brain, using rats or other small laboratory animals encourages the use of dedicated MR imagers with static magnetic fields above 4.7 tesla (T) and superconducting magnets (ultrahigh-field MR imaging systems) as noninvasive and powerful research tools for preclinical and translational research.1–4 Blood oxygenation level-dependent (BOLD)-based fMRI is a well known technique in which ultrahigh-field MR imaging systems produce superior susceptibility effects and elevated signal-to-noise ratio (SNR) on MR images. These effects are essential for highly sensitive detection of temporal changes in the balance of oxyhemoglobin and deoxyhemoglobin1 that result from the immediate increase in regional cerebral blood flow (rCBF) following neural activation in the brain.5

However, the high performance demanded of MR imaging systems with ultrahigh fields in BOLD-based fMRI greatly increases the cost and size of these dedicated imagers for studies using small laboratory animals, thereby creating a barrier to their installation and more widespread use. Nevertheless, the recent development of compact systems that are constructed with permanent magnets yielding fields up to 2T,6 are reasonably priced, and present fewer installation constraints may be expected to lead to their more extensive employment in the neuroimaging of small laboratory animals in

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various experimental studies.

We believe there are no reports of fMRI studies of small experimental animals using compact MR imaging systems because of the inferior performance of the smaller systems compared with that of ultrahigh-field systems. In particular, the compact systems offer a small SNR and poor susceptibility effects and have poor capacity to use echo planar imaging (EPI). Moreover, findings of previous fMRI studies performed with 1.5T clinical MR imagers suggest the larger contribution of inflow effects to changes in fMRI signals than that with BOLD contrast, and there is concern that the signal source of fMRI performed with compact systems may differ from that with ultrahigh-field systems because of the similar fields of the compact systems.

We therefore focused on detecting the increase in rCBF based on the neurovascular coupling essential in fMRI and hypothesized that the activated region could be mapped with compact MRI systems by using eliciting stimuli that would evoke large hemodynamic response in the region and employing appropriate animal anesthesia. We examined our hypothesis and the potential role of the compact systems in hemodynamic-based mapping of neural activity using a model of activation of the somatosensory cortex produced by electrical stimulation of the forepaw in medetomidine-sedated rats. We explored the optimal paradigm for the stimulation, examined the correlation between the stimuli and the evoked rCBF response in the rat somatosensory cortex, and verified the detection of the hemodynamic-evoked response induced by the stimuli with a 1.5T compact MR imager.

Materials and Methods

Animal preparation

The Animal Care and Use Committee of Fujita Health University approved all animal experiments. We used 13 female Wistar/ST rats (Slc: Wistar/ST, 200 to 230 g, Japan SLC, Inc., Hamamatsu, Japan) that we kept in a specific pathogen-free environment; all 13 underwent the following pretreatment.

The animals inhaled an anesthetic of isoflurane (ISOFLU, DS Pharma Animal Health, Osaka, Japan) mixed with oxygen (induction 4%, maintenance 2%) by spontaneous respiration using a small-animal gas anesthesia system (SN-487-2, Shinano, Tokyo, Japan). The animals were restrained on a temperature feedback controlled heated pad (BWT-100A, Bio Research Center, Nagoya, Japan), and rectal temperature (RT) was maintained at 36.5 ± 0.5°C.

In a state of sufficient sedation and analgesia from the inhalation anesthetic, the rats were placed in a supine position and an incision was made in the left inguinal region. The left femoral artery and vein and nerves running along them were exposed and carefully teased apart under a microscope. Polyethylene catheters (PE-50, Becton Dickinson, Franklin Lakes, NJ, USA) filled with heparinized saline were placed in the femoral artery to measure blood pressure and blood gas in the artery and in the femoral vein as a route for drug administration, the vessels were ligated, and the catheters were fixed within those vessels. The femoral area was then sutured. After confirmation that blood gas values were within the physiological range using a blood gas analyzer (GASTAT-navi, Techno Medical, Yokohama, Japan), the catheter placed in the femoral artery was connected to a pressure-sensitive transducer (MLT0670, ADInstruments, Nagoya, Japan) and amplifier (FE-117, ADInstruments, Nagoya). That analog output was then input into a multi-channel analog-to-digital (AD) converter (PowerLab ML846, ML870, ADInstruments). In addition, the arterial blood pressure (ABP), mean arterial blood pressure (MABP), heart rate (HR), and RT of the animals were all recorded chronologically using an integrated animal monitoring system constructed with interface software (LabChart v7.3.7, ADInstruments, Nagoya) with a high affinity with this AD converter.

We inserted purpose-made stainless steel paired-needle electrodes (Bio Research Center, Nagoya) under the skin of the right forepaw (between digits 2 and 3 and digits 4 and 5) to apply direct current peripheral nerve stimulation to evoke nerve activity in the forelimb region of the primary somatosensory cortex (S1FL).

Measurement of electrophysiological and hemodynamic responses

We used the following methods to detect rCBF and local field potential (LFP) and simultaneously recorded separate electrophysiological and hemodynamic responses to right forepaw direct current stimulation.

We measured rCBF by laser Doppler flowmetry (LDF) using an LDF system (FLO-C1, Omega-wave, Tokyo, Japan) with a flexible fiberoptic probe with a plastic attachment on the tip that we devised. We measured rCBF in the region of the left S1FL as determined with reference to an atlas of the rat brain. Using a brain stereotaxic frame for rats (SR-5R, Narishige, Tokyo, Japan) and a dental drill (φ2 mm) mounted on a micromanipulator, we
carefully drilled the cranial bone above the S1FL and secured the attachment with adhesive to the surrounding cranial bone to position the tip of the LDF probe on the dura mater. The analog output of the LDF system set with a time constant of 0.1 s was input to the AD converter, and rCBF was chronologically recorded (sampling frequency, 100 Hz; low pass filter frequency, 0.5 Hz) using the monitoring system. The rCBF was also measured as a reflection of the hemodynamic response during fMRI study as described below.

Under the microscope, we carefully inserted a fine stainless steel electrode lead (φ0.3 mm, < 1 MΩ, EKC-2003, Bio Research Center, Nagoya) into the attachment using the stereotaxic frame and micromanipulator, advanced the lead along the LDF probe, and inserted the lead 0.6 mm into the cortex from the cortical surface of the left S1FL. We also placed an Ag-AgCl electrode to serve as a reference electrode on the dura mater near the other electrode and affixed it to the cranial bone with dental composite resin. After amplification of the LFP lead potentials with an amplifier (FE-132, ADInstruments, Nagoya), the analog signals were input into the AD converter, and the LFP was chronologically recorded (sampling frequency, one kHz; band pass filter frequency, 8 to 54 Hz) by the monitoring system.

Sedation protocol using medetomidine
Medetomidine hydrochloride (Domitor, nippon-Zenyaku Kogyo, Fukushima, Japan) was used in the sedative during direct current peripheral nerve stimulation. Taking into consideration its pharmacological characteristics, we introduced medetomidine by dorsolateral subdermal administration and maintained administration into the femoral vein. We devised the following sedation protocol with reference to the results of preliminary experiments on changes over time in various physiological parameters in medetomidine-sedated rats (data not shown) and previous research on rat fMRI using medetomidine sedation.

After pretreatment, subject animals were maintained with 2% isoflurane inhalation anesthesia and a bolus of 0.1 mg/kg body weight (b.w.) medetomidine hydrochloride that was manually administered subcutaneously in the dorsolateral area. The isoflurane inhalation anesthesia was discontinued 10 min after the bolus administration. After another 5 min, maintenance administration was started at a rate of 0.1 mg/kg/h b.w. with a syringe pump (TOP-5500, TOP Corporation, Tokyo, Japan) using the drug administration route established in the femoral vein. After a stabilization time of about 70 min after the discontinuation of isoflurane, the peripheral nerve was stimulated with direct current.

In 4 animals, we applied current stimulation to the right forepaw using 9 Hz, 10 ms, and 4 mA, typical parameters used in this study, for 10 s under both the isoflurane 1% anesthesia used in previous research and the medetomidine sedation in the present protocol and then compared the changes in MABP between the 2 conditions of sedation.

Optimization of direct current peripheral nerve stimulation
We measured the somatosensory-evoked potentials (SEP) and somatosensory-evoked cerebral blood flow (SECBF) response to different direct current stimuli of the forepaw and determined the peripheral nerve stimulation parameters that evoked the maximum response (n = 10).

We used an analog stimulus isolator (Model 2200, A-M Systems, Sequim, WA, USA) with high affinity for the AD converter in the direct current stimulation and connected the 2 needle electrodes puncturing the right forepaw to the output lead of the stimulus isolator. For the stimulus waveform, we designed monophasic rectangular pulses with the 3 parameters of frequency, pulse width, and current value. Based on the results of previous research and preliminary experiments (data not shown), we fixed the pulse width at 10 ms and the current value at 4 mA. Frequency was varied at 3, 5, 7, 9, and 12 Hz. SEP and SECBF were measured simultaneously in each case. Stimulus duration was 10 s in all conditions. Each of the direct current stimulation parameters was used one time only with each animal. The parameters were applied randomly for the measurements.

Hemodynamic-based fMRI
All MR imaging was performed with a 1.5T compact MR imager (MRT-A1508AC, DS Pharma Biomedical, Osaka, Japan) in combination with a solenoid-type radiofrequency (RF) transmitting/receiving coil (inner diameter 38.5 mm; MRT-B200, Neomax Engineering, Gunma, Japan). LDF probes were attached to 3 animals different from those above for simultaneous MR imaging and rCBF measurements. These animals were placed in prone position on an acrylic animal bed for head fixation fabricated by the authors, and RT was maintained at 36.5 ± 1°C with a purpose-made silicone rubber heater (O & M Heater Co., Ltd., Nagoya, Japan) attached to the animal bed. The LDF probes fitted on the rats’ heads were carefully inserted into the RF coil to avoid damage and fixed in the center of the magnetic field. During MR...
imaging, we detected respiratory rate with a self-made respiration sensor that combined a nylon pillow-type probe (Nihon Rufuto, Tokyo, Japan) and a fiberoptic pressure sensor (FOP-MIV-PK, FISO Technologies, Quebec City, Quebec, Canada). RT was detected using a fiberoptic temperature probe (FOP-MIV-PK, FISO Technologies, Quebec, Canada) and measured with a special signal conditioner (Evolution FPI-HR-1, FISO Technologies, Quebec City, Quebec, Canada). These analog outputs were connected to the AD convertor and measured over time by the monitoring system simultaneously with the trigger signal controlling the transmission of the RF pulse, which is used for MR signal acquisition in the compact MR imaging system. At the same time, we also measured rCBF and other physiological indicators that serve important roles in fMRI.

As anatomical reference images, we obtained coronal T₁-weighted images (WI) including the primary somatosensory field with 2-dimensional (2D) spin echo imaging. The imaging parameters were repetition time/echo time (TR/TE), 400/9 ms; flip angle (FA), 90°; field of view (FOV), 27 × 54 mm²; slice thickness (ST), 1.5 mm; number of slices, 5; matrix, 128 × 256; and number of excitations (NEX), 8.

For one coronal section determined with T₁WI, we obtained T₂*WI images with 2D gradient echo (GRE) imaging and performed fMRI. The imaging parameters were TR/TE, 78/20 ms; FA, 20°; FOV, 27 × 54 mm²; ST, 1.5 mm; number of slices, one; matrix, 64 × 128; and NEX, one.

The task paradigm in fMRI was a block design in which direct current peripheral nerve stimulation of 20 s was alternated with rest of 90 s, and this was repeated 5 times. The direct current stimulation parameters were pulse width of 10 ms and current value of 4 mA, the same as those used in the investigation outside the MR imaging system, and fMRI data were obtained with each frequency (3, 5, 9, and 12 Hz). In the above investigation, the results with 7 Hz and 9 Hz were similar, so we omitted 7 Hz for efficiency.

Data analysis and neural activity mapping

All data were analyzed using an original program created in MATLAB (MATLAB 8.1, MathWorks, Natick, MA, USA). CBF and LFP were analyzed with the following methods.

For CBF, we took the intersections of the straight lines connecting 90% and 10% of the maximum increase rate and the base line as the times of onset and termination, and we calculated the integrated value of CBF between the 2 points (integral SECBF). For LFP, we calculated the additional value of the total amplitude (ΣSFP).

The obtained fMRI data were statistically analyzed (t-test) with datasets during the task and during rest. P < 0.05 was taken to indicate an active pixel, and a neural activity map fused with an atlas was created. In addition, a region of interest of 4 × 3 pixels was established in the left S1FL in accordance with the rat brain atlas, and the rate of signal increase was calculated.

Results

Figure 1 shows the results of typical simultaneous recording of rCBF (SECBF) and LFP (SEP) in left S1FL, together with recording of ABP, in response to right forepaw direct current stimulation of a medetomidine-sedated rat based on the protocol. Simultaneous measurements of the various physiological parameters revealed a clear relationship between the electrophysiological response to the stimulation and the corresponding hemodynamic response.

A comparison of isoflurane and medetomidine in relation to changes in blood pressure during stimulation (Fig. 2) showed that the rate of blood pressure increase during stimulation was 14.3 ± 6.3% with isoflurane and 1.4 ± 0.7% with medetomidine. The change in blood pressure was thus significantly smaller with medetomidine than isoflurane (P < 0.01, Student’s t-test).

Among the SEP and SECBF response characteristics shown with each stimulation frequency (Fig. 3), the maximum amplitude of SEP (110.48 ± 28.05 mV) and maximum rate of increase of SECBF (76.73 ± 16.6%) were both obtained with a frequency of 9 Hz, pulse amplitude of 10 ms, and current value of 4 mA.

Both the frequency characteristics of ΣSEP normalized with the same direct current stimulation parameters (frequency 9 Hz, pulse amplitude 10 ms, current value 4 mA) for all animals and integral SECBF (Fig. 4A) showed maximum values with a stimulation frequency of 9 Hz. A significant difference was seen with other frequencies except for 7 Hz (P < 0.05, Student’s t-test). The relationship between integral SECBF and ΣSEP at each stimulation frequency (Fig. 4B), that is, between the electrophysiological response and the hemodynamic response during direct current stimulation of peripheral nerves, was shown to be proportional (integral SECBF = 0.9773*ΣSEP − 0.0196, R² = 0.9894).

In the neural activity map obtained with the 1.5T compact MR imager (Fig. 5A), we observed significant activity in the left S1FL with frequencies of 5,
9, and 12 Hz \( (P < 0.05, \text{Student’s t-test}) \). Maximum activity was detected at 9 Hz.

The frequency dependence of fMRI signals (Fig. 5B) was similar to the above characteristics obtained for SEP and SECBF (Fig. 4A), but no significant difference was seen between the frequencies.

Discussion

We simultaneously performed LFP and rCBF measurement and MR imaging in a model of activation of the somatosensory cortex produced by electrical stimulation of the forepaw in medetomidine-sedated rats, and we clarified the relationship between the electrophysiological response in S1FL in this model and the associated hemodynamic response. Our findings indicate similar characteristics even with hemodynamic-based fMRI performed with a compact MR imager. From this series of results, we also induced the relationship between S1FL neural activity, which is difficult to measure directly inside a compact MR imaging system, and fMRI signals.

Medetomidine is an \( \alpha-2 \) adrenergic receptor agonist that has a superior sedating effect that resembles natural sleep and an analgesic effect.\(^9\) In recent years, it has been used in resting-state BOLD-based fMRI.\(^{11}\) Our results showed adequate inhibition of fluctuations in blood pressure attributable to peripheral nerve stimulation through the activation of the postsynaptic spinal \( \alpha-2A \) adrenergic receptor in medetomidine-sedated rats, thus demonstrating the superior antinociceptive action of \( \alpha-2 \) adrenoceptor agonists, including medetomidine.\(^9,^{12}\) From this, we believe that medetomidine is a suitable sedative and analgesic not only in a resting state but also in a model of activation of the somatosensory cortex produced by electrical stimulation of the forepaw.
Fig. 3. Stimulation frequency dependence of somatosensory-evoked potentials (SEP) and percentage of somatosensory-evoked cerebral blood flow (SECBF) in rat brains under medetomidine sedation. Corresponding recorded SEPs and SECBF time courses induced at different stimulation frequencies with a fixed current of 4 mA and pulse width of 10 ms are plotted. Bars under the time courses indicate the 10-second stimulation period, and the insets in SEP show plots extracted from the evoked responses per second during the stimulation.

Fig. 4. Peak-normalized Σ somatosensory-evoked potentials (SEP) and integral cerebral blood flow (CBF) were plotted as a function of stimulation frequency with fixed current of 4 mA and pulse width of 10 ms (A). *P < 0.05. Relationship between peak-normalized ΣSEP and somatosensory-evoked CBF (SECBF) responses (B). The data shows that ΣSEP is closely correlated with SECBF (regression line; SECBF = 0.9773 × ΣSEP − 0.0196, R^2 = 0.9894). Error bars, ± one standard deviation (SD).
left S1FL of medetomidine-sedated rats were maximum with peripheral nerve stimulation of 9 Hz, which we considered the optimal value for evoking the largest neural activity. Although this optimal frequency differed considerably from that of \( \alpha \)-chloralose (3 Hz), it agreed with that of dexmedetomidine, a medetomidine isomer, and was similar to that of isoflurane (12 Hz) and its isomer enflurane (10 Hz) as described by Masamoto and Kanno and references therein. From this, we infer that the differences in the pharmacological properties of agents used in anesthesia and sedation serve important roles in their respective different optimal frequencies. However, the present finding that dexmedetomidine has pharmacological properties very close to those of medetomidine is thought to have high reliability.

Even when the same anesthetic is used, the optimal frequency for electrical stimulation is reported to differ in brain regions other than the S1FL (secondary somatosensory area, thalamus, neostriatum, brainstem, and others). Our investigation measured the S1FL only, so we cannot describe this regional dependence in detail, but the existence of such regional dependence is an important finding of the present investigation that suggests room for further investigation on optimal derived stimulation frequencies.

For the stimulation parameters other than frequency that we used, pulse amplitude differed largely from that frequently used in previous studies (0.3 ms). However, we obtained a suitable SEP in the present results, and recent reports on \( \alpha \)-chloralose anesthesia support the validity of this parameter. The current value was in agreement with previous studies that used medetomidine. In addition, the setting was thought to be appropriate in that no blood pressure fluctuations were produced during stimulation.

The results of hemodynamic-based fMRI with a 1.5T compact MR imager using the above electrical stimulation parameters showed regions of significant activity in the left cerebral cortex of subjects with stimulation frequencies of 5, 9, and 12 Hz. Because we properly inserted the electrical stimulation needle electrodes into the right forepaws of the subject animals and utilized proper current stimulation parameters to avoid blood pressure fluctuations, we believe our results map the neural activity in the contralateral S1FL caused by electrical stimulation of peripheral nerves of the right forepaw with a compact MR imager. The values of frequency dependence of the obtained fMRI signals were also optimal at 9 Hz for both SEP and SECBF, and we believe this dependence reflects the electrophysiological and hemodynamic responses in sites of activity even with a compact MR imaging system.

This study has several limitations. First, our system is not equipped with EPI, so GRE, with its inferior magnetic susceptibility and time resolution, is the standard fast imaging. We also use imaging parameters that further enlarge the contributions of inflow effects because of the constraints related to time resolution. Therefore, as indicated in previous studies with MR imagers with low magnetic fields, inflow effects for the obtained fMRI signals are conjectured to be more dominant than BOLD contrast. A second limitation is that fMRI was performed in only 3 cases in the preliminary investigation and a statistically significant difference could not be detected in the relationship between

![Fig. 5. Hemodynamic-based neural activity maps in rat brains under medetomidine sedation calculated from functional magnetic resonance imaging (fMRI) datasets obtained on a 1.5-tesla compact magnetic resonance imaging system (A). Markedly high t-value activation was displayed in the left primary somatosensory cortex forelimb region (S1FL) induced by electrical stimulation of the right forepaw at 9 Hz. Stimulation frequency dependence of the peak fMRI signal change (B). Maximum signal change (averaged for 3 rats) was observed with a stimulation frequency of 9 Hz. These data have no statistical significance (\( P > 0.05 \), Student’s t-test). Error bars, ± one standard deviation (SD).](image-url)
stimulation frequency and fMRI. To better clarify the utility of compact MR imaging systems for neuroimaging, more detailed investigation of the promising findings obtained in this study will be necessary that remain within the range of an appropriate number of animals in line with current animal experiment ethics related to reduction in experimental animals.

Conclusion

Our preliminary results on fMRI using a 1.5T compact MR imager verified the hypothesis that the activated region could be mapped even with a compact MR imaging system by using eliciting stimuli that evoke a large hemodynamic response in the area of activity in combination with appropriate animal anesthesia. These results suggest that compact MR imaging systems may be useful for neuroimaging in preclinical and translational research.

References