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SHORT COMMUNICATION

Inter-subject registration and application of the SIGMA rat brain atlas for regional labeling in functional ultrasound imaging

Benjamin Vidal[1,2#*], Marine Droguerre[1#], Ludovic Venet[1], Marco Valdebenito[3], Franck Mouthon[1], Luc Zimmer[2,3,4], Mathieu Charvériat[1]

[1] Theranexus, Lyon, France
[2] Lyon Neuroscience Research Center, Bron, France
[3] CERMEP-Université Lyon 1, Lyon, France
[4] Hospices Civils de Lyon, Lyon, France
[#] Both authors contributed equally

[*] **Correspondence**

Dr Benjamin VIDAL
CERMEP – Imagerie du vivant
59, Boulevard Pinel, 69677 Bron, France
Mail : benjamin.vidal@theranexus.fr

Abstract

Background: Recent advances using functional ultrasound (fUS) imaging have opened new avenues to evaluate brain activity through the regional monitoring of cerebral blood volume (CBV) dynamics. In particular, this technology paves the way for understanding physiological or pathological cerebral processes or exploring the pharmacological profiles of new drugs targeting brain disorders. One of the main difficulties of this technology is the lack of standardized and validated tools, in particular relevant brain atlases, to help improving the accuracy, automation and reproducibility of fUS data analysis. New method: Here, we demonstrate the possibility to use the MRI-validated SIGMA brain atlas in rat to perform fast and precise analysis of CBV changes in numerous functionally relevant regions of interest using fUS imaging. We applied this atlas to a dataset obtained in anesthetized rats evaluating the cerebral effects of atomoxetine, a norepinephrine reuptake inhibitor currently marketed in attention-deficit/hyperactivity-disorder. Results: This approach enabled to show the subregional effects of atomoxetine in the rat with very few inter-individual differences in some areas, such as the dentate gyrus. Conclusions: We show the feasibility of inter-individual registration of 2D pharmaco-fUS data and subsequent detailed analysis using the SIGMA atlas.

1. Introduction

The last years have seen the emergence of functional ultrasound (fUS) imaging, a new functional neuroimaging tool (Macé et al., 2011), which enables the imaging of brain activity through the regional monitoring of cerebral blood volume (CBV) dynamics based on neurovascular coupling. fUS imaging is characterized by a high spatiotemporal resolution and sensitivity, along with a high portability as compared to functional magnetic resonance imaging (fMRI). In rodents, fUS offers the possibility to investigate brain activity at a spatial resolution of about 100 µm from the upper layers of the cortex to the deepest brain structures. A small number of studies have confirmed the interest of this technology to monitor the effect of pharmacological molecules (Rabut et al., 2020; Vidal et al., 2020a, 2020b). There is a need for a standardized atlas that would enable the precise delineation of relevant regions of interest, rather than manually drawing the different regions on each individual scan, in order to improve the accuracy and reproducibility of fUS data analysis.

In a recent study, Barriere and collaborators (Barrière et al., 2019) developed a complete set of MRI references and resources (SIGMA rat brain atlas), compatible with common neuroimaging software and enabling researchers to perform unified analyses of both structural and functional data from MRI.

In this study, we present for the first time the feasibility to register different sessions of fUS data in the same space before using the SIGMA rat brain atlas, to perform fast measurement of CBV changes in numerous functionally relevant regions of interest using fUS imaging. We adapted this atlas to a dataset obtained in a pharmaco-fUS study in anesthetized rats evaluating the cerebral effects of atomoxetine, a norepinephrine reuptake inhibitor currently marketed in attention-deficit/hyperactivity-disorder (ADHD).

2. Materials and methods

2.1 Animals

Wistar-Kyoto (8-week old) male rats were purchased from Janvier labs (France) and maintained under controlled environmental conditions (12/12 h light-dark cycle, 22 ± 1°C ambient temperature) with food and water ad libitum. Animal surgery and experimentations were conducted in strict accordance with the recommendations and guidelines of the European Union (Directive2010/63/EU) and strictly followed the policies of the French ethic committee for preclinical research. Procedures and protocols herein described were authorized by the French Ministry of Research (authorization reference: APAFIS19829). All efforts were made to improve animal welfare and minimize animal sufferings.

2.2 Drugs and administration procedures

Atomoxetine (Sigma-Aldrich) was dissolved in saline and administered intraperitoneally (2 mL/kg) at 1 mg/kg (n=3). For the control group, only saline was administered at the same volume (n = 4).

2.3 Surgical preparation

One day prior to ultrasound imaging, a thin-skulled surgery was performed under isoflurane (induction 4% for the first minutes, lowered to 2% in 1 L/min for constant anesthesia) and with subcutaneous buprenorphine (0.05 mg/kg; (Buprecare, Axience)) pretreatment. After shaving and cleaning with betadine, the scalp was incised to visualize the lateral sides of the skull from the bregma to the lambda anatomical landmarks. The bone was thinned from -3.00 mm to -7.00 mm (AP) and +5.00 to -5.00 mm (L) from bregma using a drill at low speed with a micro drill steel burr (Harvard Apparatus, 75-1887). Saline was frequently added between drilling sessions to avoid drought and overheating. When the skull was thin enough to be flexible, the scalp was sutured, betadine and lidocaine were locally applied and the rats were allowed to recover from anesthesia.

2.4 Functional ultrasound imaging

Thin-skulled rats were anesthetized with isoflurane, placed in a stereotaxic frame and sutures were removed to expose the thin-skulled window. Rats were scanned using a system dedicated to small animal ultrasound neuroimaging (Iconeus, Paris, France). An intraperitoneal catheter was placed for atomoxetine injection during the scan. During the imaging session, body temperature was rectally monitored and maintained using a heating blanket, and respiratory and heart rates were also continuously monitored (TCMT, Minerve, France). Using the Ultrafast Compound Doppler Imaging technique (Bercoff et al., 2011) and real-time filtering with Singular Value Decomposition (Demené et al., 2015), high resolution Doppler vascular images were acquired every second during 15 min at bregma -5.3 mm (Fig. 1A), with an injection (atomoxetine or saline) performed at 5 min of acquisition.

Before data processing, all fUS acquisitions were automatically registered together in Matlab on a custom fUS template. This template was obtained by computing the mean pixel-by-pixel image of all acquisitions from the whole dataset, generating a first mean reference image that reflected the gross anatomical properties of the unregistered images. Linear registration of each single initial acquisition was then performed on this global mean reference image. An additional, second, mean image of all those registered acquisitions was computed to generate a new template, which was improved in quality compared to the first (as images were roughly

registered together). Linear registration of all initial unregistered acquisitions was performed again on this new template, before averaging the newly registered images in order to generate a third template that was further optimized compared to the previous iteration (as images were better registered since the reference template was improved). These steps were repeated for several iterations until there was no residual difference between 2 successive means, meaning that the optimal template was obtained (reflecting the global common anatomical properties between acquisitions). All fUS acquisitions were then registered on this final template using linear registration before non-linear registration using the Demons algorithm (Pennec et al., 1999) with a Gaussian smoothing applied on the vector field to only match the global shapes of the images without focusing on the small vessels. This registration method was estimated on the denoised images, obtained after singular value decomposition (SVD) of each fUS acquisition to keep the first component only (enabling to reduce the influence of small artifacts and noise to focus on the important anatomical properties of each acquisition for the calculation), and applied to the original data. The registered data were then temporally filtered (lowpass filter and highpass filter with respective cutoff frequencies of 0.1 Hz and 0.0008 Hz).

The corresponding 2D slice of interest was then extracted from the SIGMA atlas (-5.3 mm from bregma, with a nearest neighbor interpolation in order to keep whole number values for each label) and coregistered on the fUS template using manual transformations in the ITK-Snap software, until the boundaries of the cortex and bottom of the brain matched those from the template (Fig. 1B). The Power Doppler signal was then automatically extracted from each region for each individual scan using a Matlab script and normalized to the baseline (between 0 and 5 minutes), enabling the measurement of the relative cerebral blood volume (rCBV) changes occurring after injection of saline or atomoxetine in the different ROIs (Fig. 1C). For the functional connectivity analysis, the temporal correlation between the different pairs of ROIs was estimated using the dedicated software available with the Iconeus system. The difference between atomoxetine and saline for rCBV (Fig. 2A-B) and functional connectivity (Fig. 2C) changes was calculated in each region.

3. Results

This mapping showed that atomoxetine effects on rCBV differed across cortical areas, with important CBV increases in the dysgranular retrosplenial cortex and the primary visual cortex (maximal increases of +15% and +18% respectively), but limited changes in the granular retrosplenial cortex (+3.5% in part B - see Fig. 1C and 2A-B). Similarly, in the hippocampus, atomoxetine effects were highest in the dentate gyrus (+8.5%) and CA1 (+3%) and absent in CA2. In the midbrain, the superficial and deep parts of the superior collicus displayed pronounced CBV increases following atomoxetine administration, whereas the periaqueductal gray was not impacted. Alternatively, time-dependent brain activation can be summarized as depicted in Fig. 2A (differences between atomoxetine and saline groups). As compared to saline injection, atomoxetine also had complex and various effects on the functional connectivity (Fig. 2C).

4. Discussion

Here we show that the SIGMA atlas is a valuable tool to label fUS images after registration of the data in the same space as the corresponding 2D slices from the atlas, without possible bias induced by the user's definition of ROIs. This is particularly relevant in pharmaco-fUS experiments, as drug effect can be complex and variable across regions, as illustrated here with atomoxetine. The effects of atomoxetine 1 mg/kg have been previously evaluated using large manually defined ROIs such as the visual cortex, the thalamus and the hippocampus (Vidal et al., 2020b). As an example in this last region, the current study confirms this impact but more importantly, at sub-regional resolution, suggests that the dentate gyrus and CA1 are more largely affected than CA3, and CA2 is not impacted. When a large ROI was used to define the hippocampus, we found rather subtle changes of CBV at this dose (maximal change of +4%) in our previous study (Vidal et al., 2020b), whereas the present results highlight the dentate gyrus as one of the most important area in the effect of atomoxetine. Another example is the case of the retrosplenial cortex, with a strong CBV increase in the dysgranular part and almost no effect in the granular part. In our previous study, we found a global maximal increase of 13% in this area with however no indication of the subregional specific effect of atomoxetine (Vidal et al., 2020b). This illustrates how a molecule can impact differently subregions of the same area and shows the importance of taking such subregions into account when analyzing fUS data. These results are consistent with the known strikingly different functional roles of granular and dysgranular retrosplenial cortices (Pothuizen et al., 2009) and are therefore of relevance to understand the effect of atomoxetine.

The aim of the present work was to provide a simplified method for performing group analysis of fUS data, without the need of additional morphological data. The manual registration of the atlas on the final template, although more robust than performing manual definition of ROIs/manual registration of an atlas on each individual data, constitutes a limit. One would replace such manual step by using additional angiography MRI data and their corresponding anatomic MRI images that could be registered on the MRI Sigma anatomical template, making the link between vascular and anatomic images.

As described in Barriere et al., 2019, the particular interest of the SIGMA anatomical atlas is to merge together two distinct 3D rat brain atlases, the Waxholm (Papp et al., 2014) and the Tohoku (Valdés-Hernández et al., 2011) atlases, enabling full brain coverage with a strong emphasis on both the cortical areas (provided by the Tohoku atlas) and the subcortical areas (provided by the Waxholm atlas). In comparison, other resources do not provide full brain coverage which such details, or are unavailable in a format compatible with the usual neuroimaging softwares. Previous fUS studies used the Paxinos atlas to delineate the regions of interest in 2D (Mairesse et al., 2019; Osmanski et al., 2014; Rahal et al., 2020), which provides a high precision on the different areas without offering the possibility of analyzing 3D data. Conversely, the SIGMA atlas could be used on 3D fUS data, either acquired simultaneously (as in (Rabut et al., 2019), which used separately the Waxholm and Tohoku atlases on the same data) or by imaging successive slices using a motorized probe (as in (Gesnik et al., 2017).

As a perspective, the use of SIGMA atlas can be considered as an elegant tool to compare data from multimodal MRI and fUS, further reinforcing reproducibility and confidence in non-clinical neuroimaging studies, but also providing an answer to the recently underlined

potential of fUS to be hybridized with those other neuro-imaging modalities such as positron emission tomography (Tournier et al., 2020).

Contributions

BV and MV performed the fUS experiments. BV and LV analyzed the data. MD and MC designed the experiments and managed the project. BV, MD and MC wrote the first draft. MD, BV, LZ, FM, MC reviewed the article. All authors approved the final manuscript.

Data availability

The Matlab scripts and data are available upon reasonable request.

Competing interests

This work was supported by Theranexus Company. MD, BV, LV, FM and MC are full-time employees of Theranexus company. The other authors (LZ and MV) declare no financial conflict of interest.

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Figures

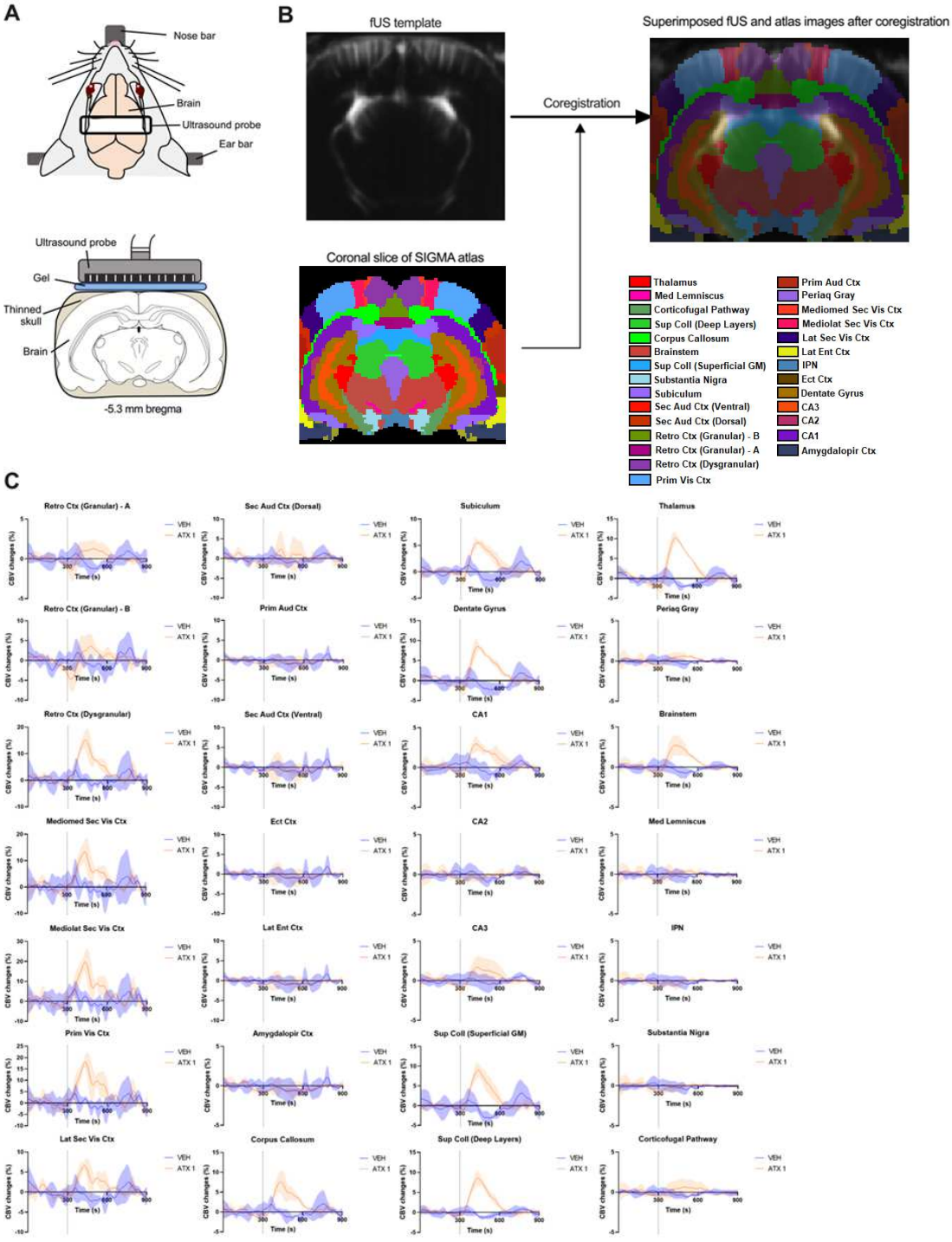

Fig. 1: Precise mapping of atomoxetine impact on CBV using the SIGMA rat brain atlas (A) Schematic representation of the experimental setup during fUS imaging (up) and fUS probe position in a coronal brain plan (down). (B) Steps involved in coregistration between the 2D slice of interest from the SIGMA atlas and the fUS template. (C) rCBV changes after saline or atomoxetine administration in the different brain ROIs. The injection time is shown by a dashed line. ATX: atomoxetine; Aud: Auditory; CA1, CA2, CA3: cornu ammonis areas; CBV: cerebral blood volume; Ctx: Cortex; Ect: Ectorhinal Ent: Entorhinal; IPN:

Interpeduncular nucleus; Lat: Lateral; Med: Medial; Periaq: Periaqueductal; Prim: Primary; Sec: Secondary; Sup Coll: superior colliculus; VEH: vehicle; Vis: Visual

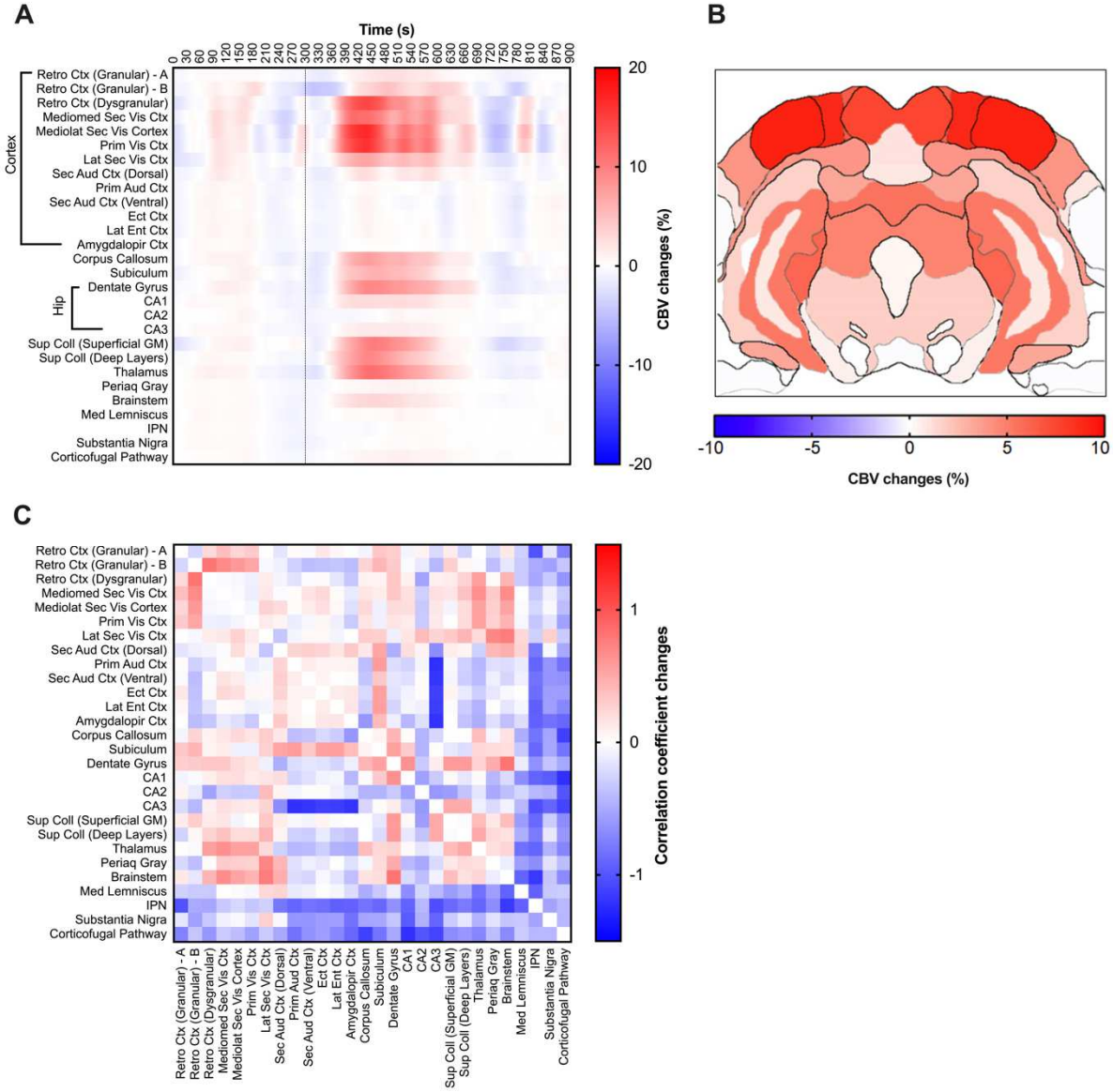

Fig. 2: Time-dependent effects and functional connectivity analysis after atomoxetine challenge

(A) rCBV difference between atomoxetine and saline (B) Schematic representation of changes found in (A). (C) Functional connectivity changes between atomoxetine and saline after the pharmacological challenge in each region. Aud: Auditory; CA1, CA2, CA3: cornu ammonis areas; CBV: cerebral blood volume; Ctx: Cortex; Ect: Ectorhinal Ent: Entorhinal; Hip: Hippocampus; IPN: Interpeduncular nucleus; Lat: Lateral; Med: Medial; Periaq: Periaqueductal; Prim: Primary; Sec: Secondary; Sup Coll: superior colliculus; Vis: Visual