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Clinical short communication

Individual analysis of fMRI data reveals incongruency in a potential CADASIL biomarker

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ABSTRACT

fMRI-based studies on neurodegenerative diseases rarely report single-subject information, which is useful for assessing potential biomarkers. In a previous fMRI study, CADASIL patients showed, at the group level, a significant reduction of the long-lasting visually stimulated hyperaemic response. Here, we used data interpolation and computed a hemodynamic response function from the 20-s visual response to achieve a 40-s response prediction at the individual level. The comparison between the expected and recorded 40-s responses confirmed the occurrence of a late and frequent response reduction among patients. However, this feature was inversely related to age and was also detected in control subjects, which suggests that this potential biomarker cannot be retained for monitoring vascular dysfunction in CADASIL. We showcase an open-source analytical pipeline for single-subject analysis to quickly assess potential biomarkers in fMRI studies.

1. Introduction

A major hurdle to therapeutic development in Cerebral Small Vessel Diseases (SVDs)[1] is the lack of non-invasive methods that can be used in humans to monitor in-vivo the structural or functional changes of cerebral microvessels [2].

Magnetic resonance imaging (MRI) is used daily to assess cerebral tissue lesions resulting from SVDs [1]. However, even using the most advanced imaging scanners, direct examination of microvessels whose diameter can be much lower than 500 μm remains elusive.

An alternative approach might be to probe neurovascular coupling (NVC) changes, as repeatedly performed with different technologies in preclinical models of SVDs, but using functional MRI (fMRI). Recently, promising results were obtained with fMRI in SVD patients, using repeated visual stimulations. A large reduction of the cortical hyperemic response has been detected in patients with Cerebral Amyloid Angiopathy (CAA), the most frequent hemorrhagic SVD [2–4]. Encouraging findings, albeit more subtle, related to changes in the dynamics of the hyperaemic response, have also been detected in the most common form of ischemic cSVD of genetic origin, CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy) [5]. The disease, which shares many clinical and pathological features with common and sporadic forms of ischemic SVDs [4,6], is caused by mutations of the NOTCH3 gene [7], a receptor of microvessels, smooth muscle cells, and pericytes. Stereotyped cysteine mutations in the extracellular EGFr-domains (ECD) of the receptor lead to Notch3-ECD aggregation with sequestration of important proteins involved in vasoreactivity [8], resulting in progressive loss of contractile cells in the vascular wall [9].

To this day, fMRI studies of SVDs mainly reported differences in group-averaged hemodynamic responses. Individual data were not fully explored, although it is crucial to ascertain the applicability of a biomarker at individual level. Indeed, multiple sources of intra-individual variability can impact BOLD fMRI response as the variable anatomical distribution, density of microvessels, cortex thickness and morphology, neuronal sensitivity to stimulation, blood

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deoxyhemoglobin concentration, or even head movements.

Here, we aimed to revisit, at individual level, unreported fMRI data we previously obtained in CADASIL patients. A former group analysis revealed no hemodynamic alteration using 20-s stimulations but a significant drop, hereafter called reduction, in the averaged hyperaemic response of patients using neural stimulations of 40 s, which was proposed as a potential biomarker for CADASIL[9]. For this purpose, we computed the hemodynamic response function from 20-s visually stimulated responses in each individual, which mathematically describes functional hyperemia. The expected curve for 40-s stimulations was then estimated for each subject, assuming that an identical neurovascular-coupling was at work for 20- and 40-s stimuli (i.e. signalling between neurons and vessels) and that other modifications in the microvasculature (as changes in its mechanical properties) could be detected in cerebral SVDs. The theoretical curves were then compared to the actual responses obtained in CADASIL patients and healthy individuals, thus allowing to assess differences in inter-individual features.

2. Methods

Herein, we analyzed unpublished BOLD data already processed in the study of Huneau and colleagues [10]. CADASIL patients were

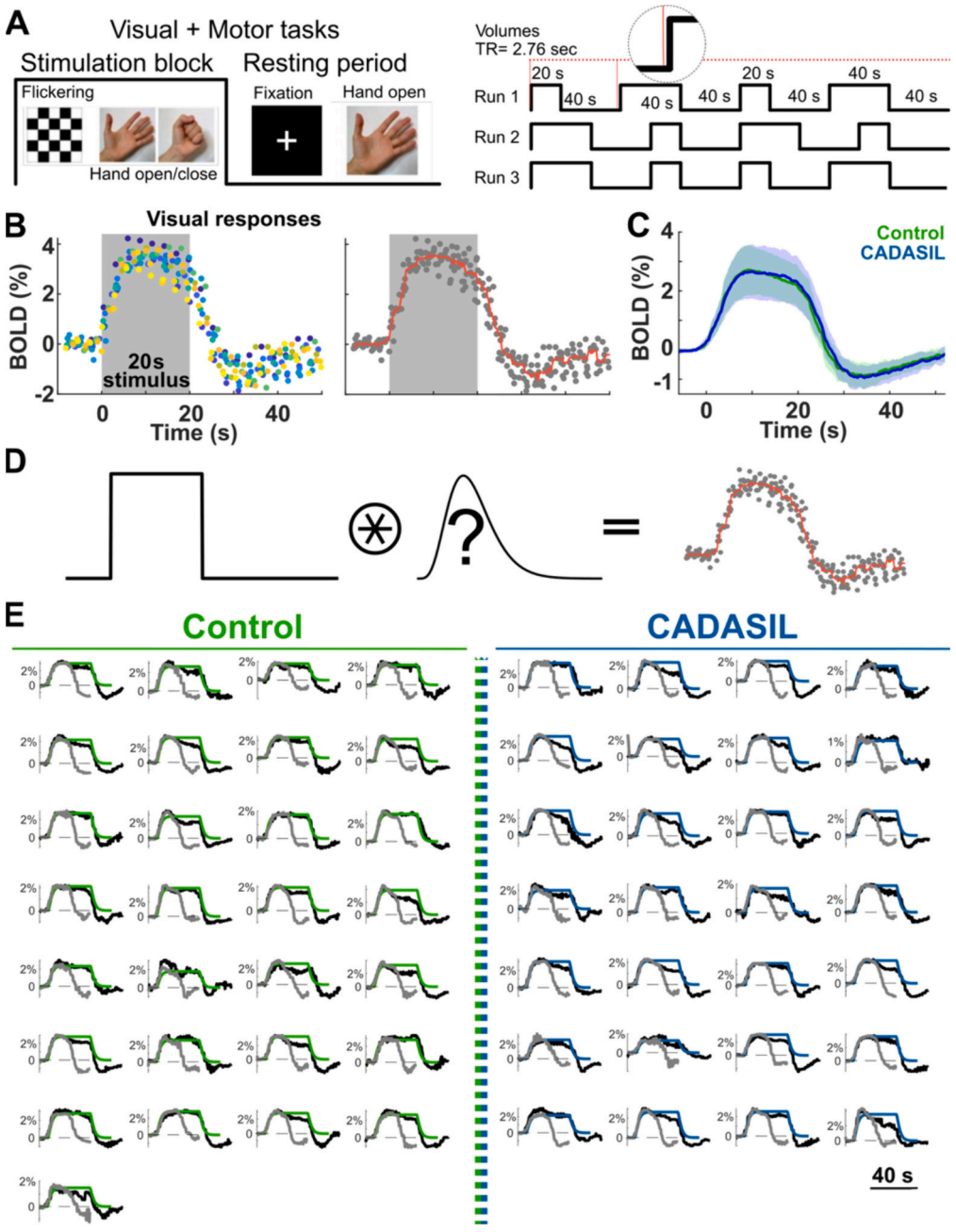

Fig. 1. single-subject analysis (A) Description of the stimulation and requested task protocol for fMRI. Subjects were asked to open and close their non-dominant hand when presented with a flickering chessboard (6 Hz) to ensure awareness. Stimuli lasting 20 or 40 s were randomly presented. (B) Single-subject responses to a 20-s stimulus (12 epochs) are colour-coded and displayed in a single plot. The red line represents the curve obtained in each individual using a 250-ms interpolation. (C) No response difference was detected between CADASIL and control subjects for 20-s visual stimuli. (D) With deconvoluting the reconstructed BOLD responses with a boxcar function representing the 20-s stimulus duration, we were able to compute a TF for each subject to predict the 40-s responses. (E) We assessed the 40-s response rundown with TF predictions. Note the propensity to a stronger rundown in CADASIL subjects versus controls. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

included based on the following criteria [10]: [1] 30 and 60 years, [2] no cognitive or motor complaints, [3] no significant disability with a modified Rankin Scale (mRS) of 0 or 1, and [4] no focal neurological deficit at clinical examination. Healthy control subjects had to be without any history of neurological disorders, age, and sex-matched to the patients' group and not treated for hypertension agents or with drugs with vasoactive properties. An independent Medical Ethics Committee approved the study protocol.

The fMRI protocol consisted of repeated flickering (6 Hz) black/white (100 % contrast) checkerboard visual stimulations, and motor tasks (opening-closing hand movement, Fig. 1a). Each fMRI chunk was composed of four activation periods lasting 20 s (($n = 2$) or 40 s (n = 2), Fig. 1B). Cerebral blood flow and BOLD signal variations were simultaneously acquired using pseudo-continuous Arterial Spin Labelling (pCASL) with T2*weighted echo planar imaging (EPI) as already reported (TR = 2760 msec; TE_{BOLD} = 30 ms [10]. The region of interest (ROI) detection was defined for each subject based on an independent time-domain components criterium previously described [10]. In this study, we only used BOLD fMRI responses obtained with visual stimulations for analysis.

To estimate each individual's hemodynamic response function, we fit the 20-s BOLD fMRI responses with a single gamma 'canonical' function: the cost function being the correlation coefficient between the measured BOLD signal and the BOLD prediction made upon convolution with the boxcar representing the stimulation duration [11]. We used Iliski's [12] simulated annealing algorithm, following the indications of our previous publication [12]. The optimized function can be assumed as a hemodynamic response function except for the onset: the BOLD responses were time-shifted of half a TR (1380 ms) of the pCASL sequence.

We used a chi-square test to compare the percentage of patients and controls with reduced BOLD fMRI response. A multivariate linear model was used in the patient group for analyzing a potential association between the % reduction of the fMRI response and the age, mRS, Mini Mental Status Examination (MMSE) score, number of lacunes, volume of white matter hyperintensities (normalized to the intracranial cavity volume) and number of microbleeds as already assessed [13] after adjustment for age. The Holm-Bonferroni procedure was designed to retain significant p-values due to repeated testing (minimal threshold fixed at $p = 0.05$).

3. Results

The acquired brain volumes were not synchronized with the stimulus presentation (Fig. 1A, right), introducing a variable delay in the repetitions. After merging all time points from the 12 visual responses in the same plot (Fig. 1B, left), we interpolated the resulting scatter distribution and artificially increased the temporal resolution to 250 ms (Fig. 1B, right).

The same pipeline applied to 20 and 40-s datasets showed enough contrast for individual analysis in all subjects. Group-level average of the BOLD responses to 20 s stimuli did not show any difference between CADASIL patients and control subjects (Fig. 1C) and we used these responses as a normalization factor to quantify differences in 40 s responses. A transfer function was obtained from the 20-s response in each subject (Fig. 1D) and used as an estimation of the hemodynamic response function (HRF). The expected curve for 40-s stimulations was then computed for each subject under the assumption of an identical neurovascular coupling for 20- and 40-s stimuli. These results were compared to the measured 40-s response obtained during the examination in CADASIL patients and healthy individuals, thus providing a normalization method to quantify the different neurovascular coupling with respect to stimulus duration to assess inter-individual features (Fig. 1E). The results showed a reduction in the actual response plateau compared to the expected response in different subjects occurring when the visual stimulation was still present and not during the return to baseline phase. A typical reduction during the 20–40 s response timeframe (point-by-point difference between the prediction and 40-s response) was detected in several CADASIL patients but, although less frequently, in healthy individuals too (Fig. 2A).

A reduction larger than 10 % of the predicted response was detected in 86 % of CADASIL patients ($n = 24$) and in 62 % of healthy individuals ($n = 18$) respectively ($p = 0.0395$). Considering all individual results, no significant difference was detected between patients and controls ($p = 0.11$) but a significant age-related decrease of the reduction between 20 and 40 s was observed in the whole population ($\beta = -0.68$; $p = 0.0064$) (Fig. 2C). This age effect was also significant in the patient group ($\beta = -0.79$; p = 0.03) with only a trend detected in the healthy group ($\beta = -0.61$; $p = 0.07$). Analysis restricted to the patient group also showed no significant association between the 20–40 s percentage variation with the mRS score ($p = 0.102$), the MMSE score ($p = 0.91$), the normalized volume of white matter hyperintensities ($p = 0.90$), the number of lacunes ($p = 0.94$) or the number of microbleeds ($p = 0.65$) after adjustment for age.

4. Discussion

In the present study, we showed, using an original analytical approach, that the altered hemodynamic response that we previously detected with a 40-s neural stimulation in CADASIL patients, could no longer be considered a biomarker of vascular dysfunction specific to this genetic disorder. The use of an HRF, determined from a shorter stimulation eliciting similar BOLD responses in CADASIL and healthy subjects,

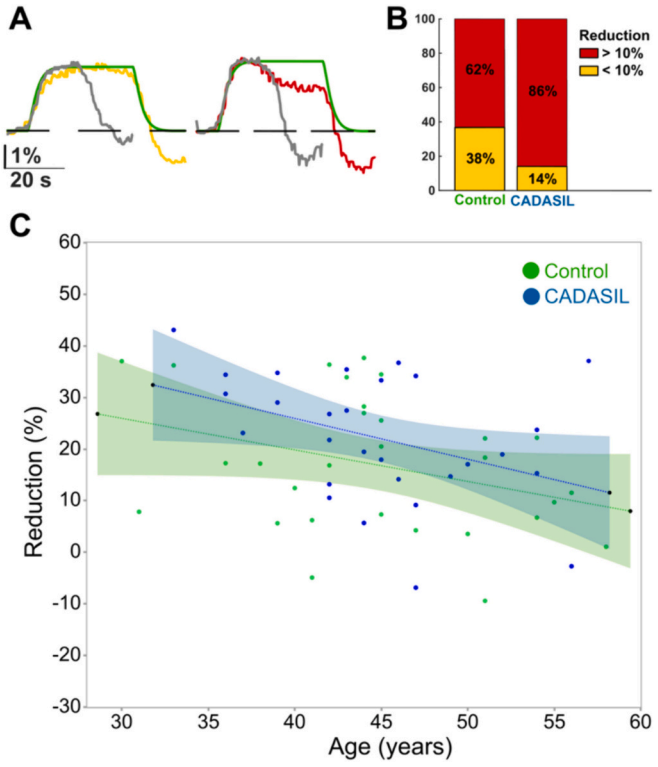

Fig. 2. fMRI response reduction was not exclusively detected in CADASIL patients (A) Two representative fMRI responses. The reduction in amplitude was used to classify the responses (40 s stimulation) as no rundown (< 10 %) and rundown (> 10 %) responses. The 10 % threshold was chosen based on visual inspection of all 40-s responses: the curves displaying no evident reduction, as in the yellow BOLD response of this figure, had a computed reduction lower than 10 %. (B) Frequencies of the two classes of fMRI rundown responses in CADASIL patients and control subjects. (C) The response rundown is correlated with age. The shadows represent the 95 % confidence level of the fits. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

allowed us to predict a reference individual response over 40 s and to observe the late reduction in the BOLD response of several patients, in line with our previous findings [10]. However, individual subject analysis showed that the rundown in the late phase of the 40-s response was not specific to CADASIL and could also be observed, although less frequently, in several healthy individuals. Moreover, the response reduction extent was inversely correlated with age, in contradiction with the age-related increase of vascular wall damage in CADASIL. Finally, the lack of association with the clinical and imaging markers of the disease is in agreement with the non-specificity of the response reduction for the disease.

Our results suggest that the use of long-duration stimulations, lasting 20 or 40 s, does not allow us to measure in-vivo neurovascular dysfunction in CADASIL patients. They also illustrate how the extraction of such key information, which is easily measured at an early stage in the mouse models [14], is particularly challenging when using fMRI in this pathology. Some technical limitations and large variability in the information extracted during our fMRI sequences might have reduced our ability to detect limited changes in the hemodynamic response between patients and controls. Finally, our data do not imply that alterations of neurovascular coupling are not present in CADASIL patients but that further efforts are needed to improve our measures using shorter stimulations whose responses might be more prone to be primarily related to the vascular response and/or innovative approaches for reducing the response variability across individuals.

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CRediT authorship contribution statement

Davide Boido: Writing – review & editing, Writing – original draft, Software, Formal analysis, Conceptualization. **Clément Huneau:** Writing – review & editing, Software, Formal analysis, Data curation. **Jessica Lebenberg:** Writing – review & editing, Data curation. **Ali-Kemal Aydin:** Writing – review & editing, Software, Formal analysis. **Benoit Beranger:** Writing – review & editing, Software, Data curation. **Serge Charpak:** Writing – review & editing, Supervision, Conceptualization. **Hugues Chabriat:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

Clément Huneau and Hugues Chabriat have filed a Patent Application (deposit No. 17305045.1 (Neurovascular coupling studied using fMRI and EEG); the other authors declared no competing interests. All the other Authors declare no conflict of interest.

Data availability

All the data in this study can be shared upon request in compliance with GDPR rules.

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