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Corentin Vallée, Pierre Maurel, Isabelle Corouge, Christian Barillot. Acquisition duration in resting-state arterial spin labeling. How long is enough?. *Frontiers in Neuroscience*, 2020, 14, 10.3389/fnins.2020.00598 . inserm-02129799v2

HAL Id: inserm-02129799

<https://inserm.hal.science/inserm-02129799v2>

Submitted on 18 Sep 2019

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Acquisition duration in resting-state arterial spin labeling. How long is enough?

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2 ABSTRACT

3 Resting-state Arterial Spin Labeling (rs-ASL) is a rather confidential method compared to resting-
4 state BOLD but drives great prospects with respect to potential clinical applications. By enabling
5 the study of CBF maps, rs-ASL can lead to significant clinical subject-scaled applications as
6 CBF is a biomarker in neuropathology. An important parameter to consider in functional imaging
7 is the acquisition duration. Despite directly impacting practicability and functional networks
8 representation, there is no standard for rs-ASL. Our work here focuses on strengthening the
9 confidence in ASL as a rs-fMRI method and on studying the influence of the acquisition duration.
10 To this end, we acquired a long rs-ASL sequence and assessed the quality of typical functional
11 brain networks quality over time compared to gold-standard networks. Our results show that after
12 14 min of duration acquisition, functional networks representation can be considered as stable.

13 **Keywords:** Functional Magnetic Resonance Imaging, Arterial Spin Labeling, Resting-state fMRI, Acquisition duration, Modeling

1 INTRODUCTION

14 Functional MR imaging (fMRI) builds the links between location and function in the brain. The two
15 main sub-domains in fMRI are task-based fMRI and resting-state fMRI. In task-based fMRI, a functional
16 location is considered to be where the acquired signal matches with the task guidelines given to the
17 subject. In resting-state fMRI, as no task is given, the focus is on fluctuations in voxels time-series induced
18 by spontaneous neural activations. Similarities in these time-series in different areas have shown to be
19 not random, but matching function of the brain (1). These similarities define the *functional connectivity*
20 of the brain and show the underlying cerebral architecture organized into functional specialized units
21 communicating with each other. Resting-state functional imaging aims to identify functional areas of the
22 brain and depict how they interact outside any structural connectivity consideration (2). Healthy and
23 diseased subjects differ in functional networks cartography and in the intensity of functional connectivity
24 for major disorders such as Parkinson's disease (3), Alzheimer's disease (4), severe depression (5) or
25 schizophrenia (6).

26 Another subdivision in fMRI concerns the way the signal is obtained. The two major techniques are
27 Blood Oxygen Level Dependent (BOLD) fMRI and functional Arterial Spin Labeling (fASL). Based on
28 neurovascular coupling effects, BOLD techniques rely on the local signal variation induced by the neuron
29 consumption of blood oxygen. Arterial Spin Labeling (ASL) is an MRI perfusion technique which uses
30 magnetically labeled arterial water protons as an endogenous tracer. An inversion pulse labels the inflowing
31 blood and after a delay called post-labeling delay, a labeled image of the volume of interest is acquired. The

32 subtraction of the labeled image from a control image, i.e., non labeled, reflects the quantity of spins that
33 have perfused the imaged volume, producing what is commonly called a perfusion-weighted (PW) image.

34 The PW map can be used to quantify the cerebral blood flow (CBF) under some assumptions (7, 8). The
35 quantification of CBF is the main advantage of ASL over BOLD. Indeed, the latter provides an indirect
36 and non-quantitative measurement of neural activity, as it results from a combination of variations in CBF,
37 cerebral blood volume and cerebral metabolic rate of oxygen. While the pathologies mentioned above were
38 studied with BOLD, ASL allows to study a new set of pathologies with fMRI, such as acute stroke (9) or
39 chronic fatigue syndrome (10) since CBF abnormalities can characterize pathologies.

40 The main drawback of ASL is its lower signal-to-noise ratio compared to BOLD fMRI. The repetition
41 time (TR) is also twice to three times higher in fASL compared to BOLD fMRI, which impacts its temporal
42 resolution. Furthermore, ASL can be implemented through numerous MRI sequences and meta-analyses
43 can be difficult to set up, for ASL shows a high sequence parameter dependency (11, 12). Nevertheless
44 consensus seems to overcome with years (13). Predominant in clinical usage and in academic research,
45 BOLD is still considered as the gold standard in fMRI. However, as it provides quantification of CBF, ASL
46 can be a serious contender to BOLD when it comes to pathologies evaluation, especially for Alzheimer's
47 disease (14, 15, 16). The absence of contrast agent injection makes ASL well suited for longitudinal
48 studies, particularly for pediatric population or for population with poor venous access or contrast agent
49 contraindication.

50 The acquisition duration is an important parameter in an rs-fMRI study with strong practical consequences.
51 Most current studies work with a duration from 8 min to 13 min and a TR from 3 s to 4 s (i.e. 120 to 260
52 images). Intuitively, one would assume the longer the duration, the better the sampling of the signal
53 correlation across the brain and thus the better the acquisition. But this requires to define what "better"
54 actually means and does not consider the practical questions of clinical implementation and subject resting-
55 state upholding. To the best of our knowledge, some papers already studied the influence of duration in
56 rs-BOLD (17, 18, 19, 20, 21), whereas in rs-ASL, it has not been explored yet. In this work, we first focus
57 on the feasibility of detecting functional connected regions of the brain from rs-ASL. We remain as close
58 as what a typical investigator of rs-ASL would experience by implementing usual sequence, processing
59 and functional networks detection methods. We then assess a trend over the duration influence on rs-ASL
60 detected networks quality: we do not directly assess whether an acquisition is good at a given time, but
61 rather how it evolves with longer durations. After describing the scores used and the modeling approach,
62 an in-depth analysis for the Default-Mode Network (DMN) will be presented in order to illustrate scores
63 evolution on the most typical resting-state network. Finally, we will show results for all the functional
64 networks under consideration and discuss an optimal sequence duration in rs-ASL.

2 MATERIAL

65 2.1 Subjects

66 Seven healthy male right-handed subjects aged from 21 to 28 years ($23.5 \text{ yo} \pm 2.5$) were involved in this
67 study. All subjects gave informed written consent before participating in the study. We have maintained the
68 homogeneity of the population in order to limit the influence of factors such as gender or age.

69 2.2 MR Acquisitions

70 The subjects were scanned on a 3.0T whole body Siemens MR scanner (Magnetom Verio, Siemens
71 Healthcare, Erlangen, Germany) with a 32-channel head coil. A 3D anatomical T1-weighted MP2RAGE

72 image was acquired for each subject. The resting-state ASL imaging was performed using a 2D EPI pseudo-
73 continuous (pCASL) sequence. Subjects were asked to keep their eyes closed, to relax (mind-wandering)
74 without falling asleep. We used the most common parameters reported in the literature: TR = 3500 ms,
75 FoV = 224 x 224 mm², TE = 12 ms, LD = 1500 ms and a 1250 ms post-labeling delay (PLD). Volumes were
76 made of 24 slices of 64 × 64 voxels with 5 mm slice thickness with 20% gap for a total resolution of
77 3.5 × 3.5 × 6 mm³. The number of volumes was 420 for a total duration of 24 min 30 s. For the 1250 ms
78 PLD, we chose a balance between a longer duration, about 1800 ms, recommended to optimize the quality
79 of the CBF estimate (13, 22), and a shorter duration, 600 ms, which seems to give a better functional
80 representation (23, 24). We kept the PLD quite long as the main advantage of ASL is ultimately to compute
81 CBF although we will focus on functional areas representation in this paper.

82 2.3 Data preprocessing

83 For each subject, the raw pCASL series is divided into 46 sub-series. The duration of these sub-series
84 ranges from nearly 2 min (34 volumes) to 24 min 30,s (420 volumes) with a time step of 30 s. For the sake
85 of simplicity, we will only mention rounded durations hereafter. All these subdivisions are made *before*
86 any preprocessing: the preprocessing is done independently on each sub-series. For the preprocessing
87 steps and their parameters we chose the most common ones found in bibliography. All steps are shown
88 in Figure 1. For the preprocessing steps we used Matlab CONN toolbox (www.nitrc.org/projects/conn,
89 RRID:SCR_009550) (25).

3 METHODS

90 3.1 Detecting networks with Seed-Based Analysis

91 To obtain the mapping of individual functional networks, we rely on seed-based analysis (SBA) (2). The
92 principle of this method, the first proposed to define functional connectivity (1), is quite straightforward.
93 Considering a similarity measure (usually linear correlation, but many others exist (26)), SBA builds
94 functional areas by gathering voxels which exhibit a matching signal, in the sense of the chosen measure,
95 to that of a ROI, called the seed in this context. Even if SBA is a very polymorphic modeling method, we
96 use its most common form in our work. Hence we consider linear correlation as the similarity measure
97 and use a set of 20 single voxels as seeds. Seeds are spread in the expected location of six usual functional
98 networks: DMN, Sensori-motor, Language, Salience, Visual and Cerebellum. The exact positions of the
99 seeds in the MNI152 space are provided in the appendix section and were suggested by the CONN toolbox.
100 To build a functional map for each seed, we statistically test whether the signal between the seed and a
101 candidate voxel is positively correlated with a risk of 1% FWER-corrected. This is a tough conservative
102 testing compared to most of rs-ASL (even fMRI in general) studies, but we agree with the recommendation
103 of (27) on false positive underestimation in fMRI literature.

104 3.2 Evaluation scores

105 The resting-state BOLD literature suggests different acquisition durations: 6 min (17), 10 min (18),
106 12 min (19), 25 min (20), and even 100 min (21). The main reason of their apparent discrepancy is the
107 modeling. Indeed, there is many ways to properly define a model to assess to role of acquisition duration
108 (a fortiori how much duration is enough), even if they lead to different conclusions. As a pioneer work
109 on rs-ASL, we want our modeling to reflect an investigator experience with the impact of acquisition
110 duration on functional network estimation. Figure 2 illustrates the investigation of acquisition duration
111 we will model. The DMN estimation is validated after 14 min on Figure 2 because it matches with how

112 the functional network is expected to look like. In modeling terms, it is basically assessing the overlap
 113 of the estimated network with a reference network. In order to investigate a trend afterwards and decide
 114 which acquisition duration is enough, the individual functional maps will be compared to a reference, like
 115 process described in Figure 2. For that purpose, we rely on the Multi-Subjects Dictionary Learning atlas
 116 (MSDL) by (28). MSDL is an atlas of 17 resting-state functional networks containing our 6 networks
 117 of interest, and from which our seeds are independent. The key idea is to have functional maps close to
 118 what an expert would expect to observe when looking for the typical functional areas investigated here.
 119 To study the quality of the detected networks as a function of the acquisition duration, we evaluate the
 120 overlap between the SBA estimated functional maps and the MSDL references (simply called "reference"
 121 hereafter) through two measures: the Jaccard's index and the area under curve (AUC).

122 Let \mathbb{E} be a set, let $(v_i)_{i \leq k \in \mathbb{N}}$ be observations in \mathbb{E} and $(M_1; M_2) \in \{0; 1\}^{\mathbb{E}} \times \{0; 1\}^{\mathbb{E}}$ binary categorical
 123 variables. Let A, B, C, D be four sets with respective cardinals a, b, c, d defined by:

$$\begin{cases} A := \{v_i \mid M_1(v_i) = 1, M_2(v_i) = 1\} \\ B := \{v_i \mid M_1(v_i) = 1, M_2(v_i) = 0\} \\ C := \{v_i \mid M_1(v_i) = 0, M_2(v_i) = 1\} \\ D := \{v_i \mid M_1(v_i) = 0, M_2(v_i) = 0\} \end{cases} \quad (1)$$

124 Almost all common similarity measures (Sokal measure family, Sørensen-Dice, correlation etc.) can
 125 be defined with a, b, c, d . If one of the binary categorical variable can be considered as the truth, let's say
 126 M_2 , therefore a becomes the number of *True Positives*, b of *False Positives*, c of *False Negatives* and
 127 d of *True Negatives*. We also trivially have the *Sensitivity*: $a / (a + c)$, *Specificity*: $d / (d + b)$, and the
 128 *Positive Predicted Value* (PPV): $a / (a + b)$. In fMRI, the v_i are the voxels and the variables M_1, M_2 are
 129 the functional maps to be compared (binary here, but the definition can easily be extended to probability
 130 maps).

131 3.2.1 Jaccard's index

132 When comparing two spatially distributed data, the most obvious measure is the Jaccard's index: the ratio
 133 between the size of their intersection and their union. It is defined by $J = a / (a + b + c)$ in our notation
 134 system. It provides intuitive and visual information about the overlap between one tested correlation map
 135 and one reference. It is also test-dependent: changing the risk or the multiple comparisons correction at the
 136 detection step will also change the shape and extent of the functional area, generally modifying Jaccard's
 137 index. This may be considered as a drawback but in fact, a statistical test is usually used at some point
 138 when investigating functional data.

139 3.2.2 Receiver operating characteristic analysis

140 In this section, we assume that the binary categorical variables are parameterized by at least one parameter.
 141 For example, in our case, it could be the risk for the statistical test of correlation α or a threshold on
 142 correlation r . Let r be our parameter, a_r, b_r, c_r , and d_r the previously defined cardinals in (1) now
 143 parametrized by r , and let define a set $\{(x(r), y(r)), r \in [-1, 1]\} \subset [0, 1]^2$ by:

$$\begin{cases} x(r) = 1 - \frac{d_r}{d_r + b_r} \\ y(r) = \frac{a_r}{a_r + c_r} \end{cases} \quad (2)$$

144 The implicitly defined function $f : x \mapsto y$ is called the *Receiver operating characteristic curve* (ROC-
145 curve) and its integral $\int_0^1 f(x)dx$ is simply called the *Area Under Curve* (AUC). In the case where M_2
146 is considered to be the truth, f is just informally $f : 1 - \textit{Specificity} \mapsto \textit{Sensitivity}$. The AUC is not
147 test-dependent as it covers all possible values of the threshold parameter (i.e. risk/correlation). It illustrates
148 how a functional map *can* be close to the reference by considering *all* values of the considered parameter,
149 while the Jaccard's index reflects how it *is* close to the reference by considering *one* value of the given
150 parameter. Hence AUC is a better way to assess the trend of interest from a *theoretical* point of view.
151 However, it is further away from the *practical* proximity of the Jaccard's index modeling offers, so we will
152 eventually consider both scores.

153 3.3 Modeling trend with respect to the duration

154 Both Jaccard's index and AUC are computed for each subject, each seed, each duration and each
155 functional network reference from MSDL. The next step is to model the trend of these two scores evolution
156 according to the acquisition duration for all subjects and for each combination between one seed and
157 one reference. Assuming rs-ASL sequence lasts long enough to cover all usage, extrapolation for a
158 duration longer than 24 min 30 s seems superfluous. There is no theoretical model, even in BOLD, on
159 the dependence between acquisition duration and quality of functional networks detection: we are not
160 interested in an explicit formula. Moreover, even processed independently, neighboring within-subject
161 time-points have a strong dependency as they come from the same acquisition. Under these conditions,
162 a local non-parametric regression is very well-suited. We chose to use the Loess method. Loess can be
163 understood as a local polynomial regression on a subset of the whole dataset, defined by a weighted
164 K-nearest neighbors algorithm. For a more comprehensive description, see (29). We used second degree
165 polynomial functions with a 0.8 span.

4 RESULTS

166 4.1 Effect of the acquisition duration for the Default Mode Network

167 In this section, we present an in-depth analysis of the DMN. In the set of 20 seeds we used, many
168 should not be inspected when used in combination with MSDL DMN. The main reason is that most of the
169 combinations has no objective basis for detecting the DMN. Otherwise, the seed may have failed to detect
170 precisely the networks it was meant to detect, which is expected with very short acquisition duration. A
171 good way to get an idea of the quality of the overlap between the functional maps associated with a seed
172 and a reference for all durations is to check the boxplots of the Jaccard's index as in Figure 3. Boxplots
173 give an overview of the results for rs-ASL: for the DMN reference, Jaccard's indices have higher values
174 for the seeds placed in order to detect it. Prefrontal and posterior seeds seem to work well while lateral
175 DMN seeds provide lower scores but still higher than any other seeds. Figure 4 shows the evolution of the
176 estimated DMN with the prefrontal seed and corresponding scores for one subject. The depiction made by
177 the scores of the overlap between the estimation of the DMN and the MSDL reference match with the four
178 stages identified in Figure 2. Figure 5 shows the Jaccard's index, AUC, Sensitivity and Predicted Positive
179 Value (PPV), for each subject and at each acquisition duration. Loess on Jaccard's index, as well as on
180 AUC, models quite well what can be observed by looking directly at the functional map. Jaccard's index
181 seems to stabilize after 12-13 min and AUC at an earlier acquisition duration around 9-10 min. We could
182 have expected sensitivity and PPV to follow the same trend. Actually, sensitivity just grows over time,
183 but more slowly for longer durations. Interestingly, PPV reaches a peak in the second stage mentioned
184 above. The seven subjects show different level of response but good correlations (except for the subject 2

185 with AUC), i.e. the trend is the same among subjects, rather than an average effect induced by the Loess.
186 Moreover results observed for the DMN, can be generalized for almost every combination of seeds and
187 references as we will see.

188 4.2 Effect of the acquisition duration for all functional networks

189 Visual inspection of the acquisition data and of the estimated functional networks estimation is always
190 a good practice (30) However, with more than 6000 functional maps generated (20 seeds, 7 subjects,
191 46 acquisition durations), a visual checking of all the maps is not practicable. As seen for DMN, many
192 combinations between seeds and reference should not be investigated since they are not functionally
193 meaningful and will yield to very low overlapping scores (e.g. prefrontal seed with visual cortex). For
194 Jaccard's index, we selected combinations for which at least 50% of observations have $J \geq 0.1$. For AUC,
195 the median is also considered, with a threshold of 0.7. These thresholds on the median values may seem
196 rather low, but let us remember that all the acquisition durations are taken into account, even the shortest
197 ones. Figure 6 shows the median values for all the combinations between seeds and references. The two
198 thresholds lead to an almost identical choice for the selection of combinations. All seeds have their best
199 scores with the expected reference, and each of the six functional networks are considered to be sufficiently
200 well detected with SBA for Jaccard's index in accordance with our selection rules. The AUC suggests as
201 good enough one more seed for cerebellum but consider that salience is not detected well enough with our
202 set of seeds.

203 Figure 7 shows the range of durations where scores are not significantly different from their maximum
204 values (5% risk) for each selected reference/seed combination. Colors on heatmap are scaled between
205 minimum and maximum values of the corresponding score and matches with the stages already described
206 for DMN in the previous section. Indeed, for every combination between seeds and references, both
207 scores rapidly increase, and start to stabilize after a certain duration. However, for both measures, the 95%
208 confidence interval around the maximum suggests a later start in the stabilization than suggested directly
209 by the Loess curve values. While some combinations scores look already stabilized at 12 min, almost all of
210 them are close to their maximum value at 16 min. Figure 8 shows a collection of functional areas obtained
211 at a duration of 14 min. While language seed struggles to detect spatial components far from the seeds, all
212 the other ones provide good detection of expected functional networks. The two bottom rows show the
213 same subjects and the same reference with different seeds.

5 DISCUSSION

214 5.1 On methods

215 In order to estimate functional networks, the two most common methods are SBA and Independent
216 Component Analysis (ICA). We did not work with ICA because the association between independent
217 components and functional areas of the brain is intrinsically tedious (31) (32), especially when it comes
218 to comparison between subjects. Moreover, in order to investigate the relationship between quality and
219 duration for all subjects, we must estimate the functional areas in the same way for each subject. Indeed,
220 keeping the same seeds and the same test for SBA is trivial, while keeping the same number of independent
221 components is equivocal, since it should be decided by a goodness of fit criterion.

222 We chose to report the positive predicted value rather than specificity for two main reasons. On the one
223 hand, true negatives can have multiple definitions in fMRI, since it depends on the voxels considered:
224 the whole volume, only the brain or any smaller ROI like grey matter. Although it is logical to consider

225 only brain voxels for functional activity, this implies an extremely high number of true negatives, since
226 the volume of a functional network is ten to a hundred times smaller than the one of the whole brain.
227 Therefore, the specificity reaches values too high to provide relevant information on similarity between
228 functional areas. On the other hand, like specificity, PPV plays a similar role with respect to sensitivity:
229 specificity gives a complementary information to sensitivity in the totality of voxels whereas PPV gives a
230 complementary information in the union of the reference and the estimated functional area.

231 5.2 On results

232 Our two objectives were to confirm the feasibility of resting state ASL and to evaluate the influence of
233 the acquisition duration on the estimation of functional areas. Figure 4 and Figure 8 with corresponding
234 scores in Figure 6 and in Figure 7 confirm that, even with the basic preprocessing and straightforward
235 methodology we used, ASL is fully viable as a resting-state method. Regarding the impact of acquisition
236 duration, the most important result is the stabilization of the functional areas representation after a certain
237 duration for both measures, Jaccard's index and AUC, with a strong inter-subjects correlation (i.e. not
238 a mean-effect induced by the Loess modeling). Since the acquisition should have the shortest duration
239 possible for clinical implementation, the recommended duration eventually corresponds to the start of the
240 stabilization stage. Strict definitions of the stabilization stage lead to longer duration since they would rely
241 heavily on the Loess maximum by considering as stable just a narrow interval around of the maximum.
242 However, since after 12 min to 14 min the score variations are low, a slight change in preprocessing or
243 in the population could also lead to unstable maximum, without changing the trend. Relaxed definitions
244 would keep optimal duration stability, but they may consider a functional area as good enough when an
245 human investigator would not. Actually, early stages of acquisition are associated with poor representation
246 of functional areas disconnected from the seed. Based on our different results, 14 min seems to be an
247 interesting compromise.

248 Note that the DMN, the sensori-motor cortex, and the cerebellum have an almost consensual spatial
249 definition among the authors, unlike language, visual and salience, which show a greater spatial variability
250 (see for example <http://neurosynth.org/>). As we provide an evaluation only with one set of references (from
251 the MSDL), one could have expected this to be a limitation of our work. However the spatial variability
252 of the areas of interest in atlases is low enough to change only the scores but not the trend observed in
253 this paper. Preprocessing influence should also be considered as positive: since we use typical and basic
254 preprocessing, more advanced techniques should provide the same or an earlier stabilization, still keeping
255 our suggestion as a sufficient duration.

256 The same is true for ASL readout approach. Using a 3D readout is probably an improvement in our
257 sequence, as it tends to outperform 2D EPI, but not every investigator has access to 3D sequence (13)¹. Not
258 to mention the resting-state and readout approach, ASL is very sensitive to changes in its parameters. Since
259 we are studying the influence of acquisition duration for a given set of parameters, the optimal duration of
260 14 min could be strongly influenced by the sequence parameters. Although the influence of each of them
261 is to be kept in mind, most of them should not disturb the investigators, as most of them have a specific
262 bibliography that goes well beyond the issue of the optimal acquisition duration. However, two of them
263 may have a deep impact on our results: post-labeling delay and repetition time (TR). As mentioned in
264 section 2.2, we already have some clues on how the PLD can influence functional networks representation.
265 The critical parameter in our opinion is repetition time. It defines the sample frequency of the resting-state

¹ It is anticipated that single-shot 3D readout may be the preferred option in the future, but these methods are not yet sufficiently well tested to recommend for general use at this time. Multislice singleshot 2D echo-planar imaging (EPI) or spiral readout should be considered a viable alternative to segmented 3D sequences, because they are available on all systems and are insensitive to image artifacts from motion.

266 signal and turning our 14 min suggestion into a 240 volumes since we only work numerically on the
267 signal. Its variation may shift the stabilization step toward a higher/lower number of volumes and hence a
268 longest/shortest duration, without changing the stabilization of the functional networks representation after
269 a certain number of volumes (i.e. same signal but different sampling frequency). Moreover in rs-ASL, TR
270 values are typically between 3 s and 5 s, which is too wide to assume the locally linear dependence between
271 TR and optimal duration. As a preliminary work on optimal duration in rs-ASL, we focus more on the
272 modeling rather than investigating the influence of the TR. However, a specific study on the relationship
273 between repetition time, volume number and quality of acquisition would be, in our opinion, highly
274 beneficial to better define the optimal duration and also would be useful when an investigator sets up a
275 sequence.

276 Last, we work on a homogeneous sample of subjects. While it greatly limits the influence of variables
277 related to the population description and not included in our modeling, it also narrows the population
278 represented by our sample of subjects. Hence, a natural perspective is to include new subjects in order
279 to extend the population represented and checks if the influence of variables like gender, age, laterality
280 could be excluded from the population variability. It should be noted that geriatric, pediatric and pathologic
281 populations should be treated separately rather than included. Indeed, these populations can show extreme
282 differences in average (or local) perfusion, which possibly hinders the functional networks estimation, and
283 hence, when the stabilization starts.

6 CONCLUSION

284 We model and process data in order to get results as close as an investigator would do. All the considered
285 functional areas were well detected by rs-ASL. Our results show a quality stabilization after a certain
286 volume number/duration for both scores in all but one combination between seeds and references: very
287 long sequences should not hence be considered. The main objective was to answer "How long is enough?".
288 While we can suggest, for our set of sequence parameters, the optimal volume number of 240 / optimal
289 duration of 14 min, any methods that improves the detection of functional networks are likely to provide an
290 earlier stabilization start, i.e. optimal volume number/duration. Since we use a basic and typical sequence,
291 preprocessing and estimation, 240 volumes / 14 min even if not optimal should be enough for most rs-ASL
292 usage. Last, the exploration of the impact of the TR and PLD on the optimal acquisition duration was
293 beyond the scope of this article but would be highly beneficial for sequence implementation, since we
294 forecast they are the two parameters that may shift the stabilization start toward higher number of volumes.

CONFLICT OF INTEREST STATEMENT

295 The authors declare that the research was conducted in the absence of any commercial or financial
296 relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

297 C.V. acquired the data with the help of the Neurinfo MRI research facility. C.V. designed research,
298 performed research, analyzed data and wrote the paper. P.M, I.C, C.B. supervised all steps and corrected
299 the paper.

ACKNOWLEDGMENTS AND FUNDINGS

300 MRI data acquisition was supported by the Neurinfo MRI research facility from the University of Rennes I.
301 Neurinfo is granted by the the European Union (FEDER), the French State, the Brittany Council, Rennes
302 Metropole, Inria, Inserm and the University Hospital of Rennes. This manuscript has been released as a
303 pre-print at: <https://www.hal.inserm.fr/inserm-02129799v1>

DATA AVAILABILITY STATEMENT

304 In accordance with the consent form signed by the subjects, authors are not allowed to share MRI
305 acquisitions. However, requests to see the raw data can be sent to the corresponding author. For the
306 preprocessing steps we used Matlab CONN toolbox (www.nitrc.org/projects/conn, RRID:SCR_009550)
307 (25). The rest of the code used for evaluation scores and Loess is available upon request from the
308 corresponding author.

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FIGURES

APPENDIX - SEED LOCATION IN MNI152

Expected networks	Seed	Location in MNI152
DMN	Prefrontal	(1,55,-3)
DMN	Left	(-39,-77,33)
DMN	Right	(47,-67,29)
DMN	Posterior	(1,-61,38)
Motor	Left	(-55,-12,29)
Motor	Right	(56,-10,29)
Motor	Superior	(0,-31,67)
Visual	Primary	(2,-79,12)
Visual	Ventral	(0,-93,-4)
400 Visual	Dorsal Left	(-37,-79,10)
Visual	Dorsal Right	(38,-72,13)
Saliency	Cingulate Anterior	(0,22,35)
Saliency	Prefrontal Left	(-32,45,27)
Saliency	Prefrontal Right	(32,46,27)
Language	Frontal Gyrus Left	(-51,26,2)
Language	Frontal Gyrus Right	(54,28,1)
Language	Temporal Gyrus Left	(-57,-47,15)
Language	Temporal Gyrus Right	(59,-42,13)
Cerebellum	Anterior	(0,-63,-30)
Cerebellum	Posterior	(0,-79,-32)

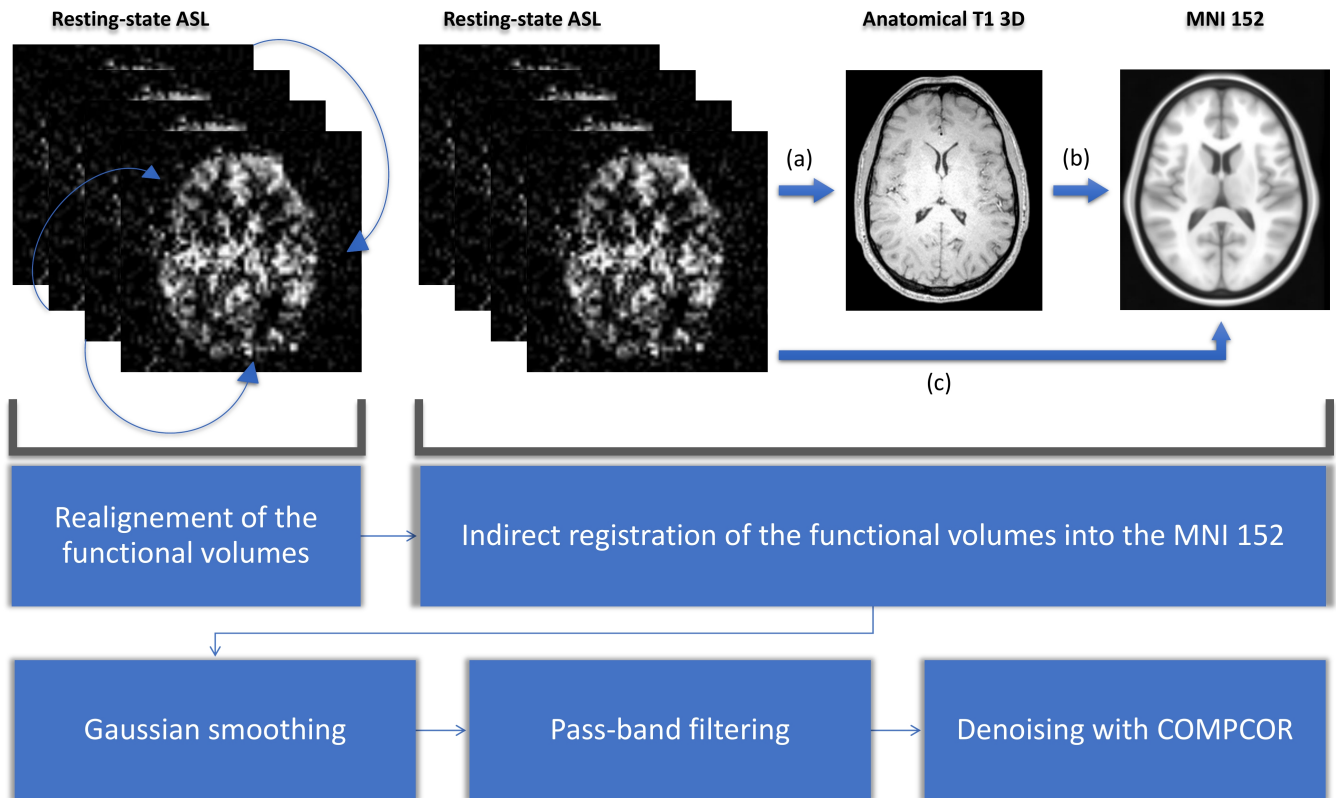


Figure 1. Preprocessing steps of the rs-ASL images. After a surround subtraction of labels and controls, preprocessing starts with the realignment of the perfusion weighted-map. All the functional volumes are registered with the first one. Second step is the indirect normalization of the functional volumes. It starts with registration of the functional data on the anatomical 3D T1 (a). Then comes the registration of the anatomical image on the MNI 152 template (b). Finally, transformations of (a) and (b) are composed to register functional data on the MNI template (c). We then used Gaussian smoothing with typical 6 mm kernel and pass-band filtering with a range from 0.005 Hz to 0.1 Hz. Final denoising was made with COMPCOR (33).

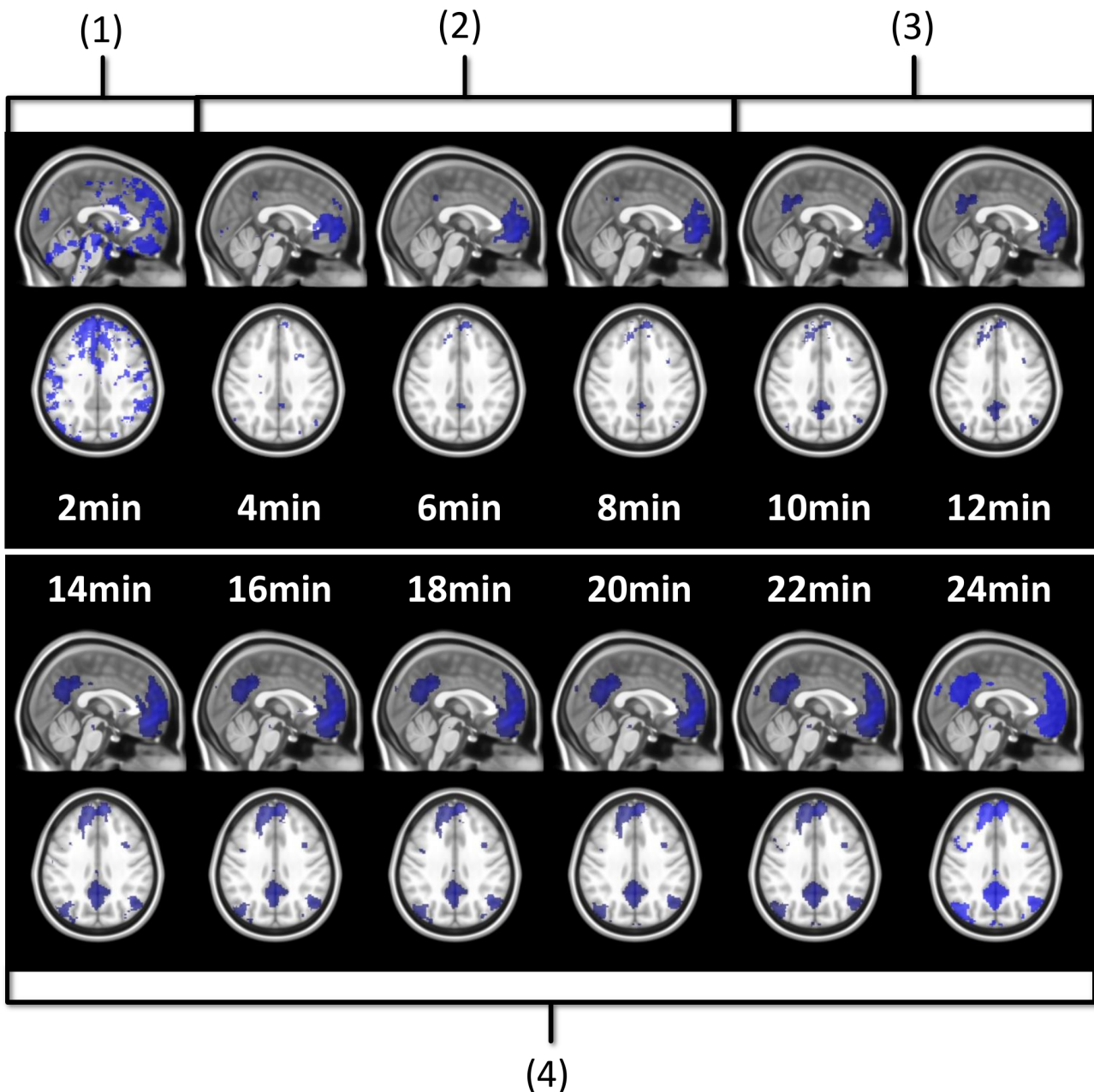


Figure 2. Seed-based estimation of the DMN with prefrontal seed at different acquisition durations. Four stages can be identified. At 2 min (1), the map shows only false positive noise detection. Between 4 min and 8 min (2), the false positive noise has disappeared while the frontal component of the DMN starts growing and the posterior starts being detected. Between 10 min and 12 min (3), the frontal component is well detected, the posterior grows and the lateral components are barely being detected. At 14 min and after (4), DMN detection is good and interestingly, stable.

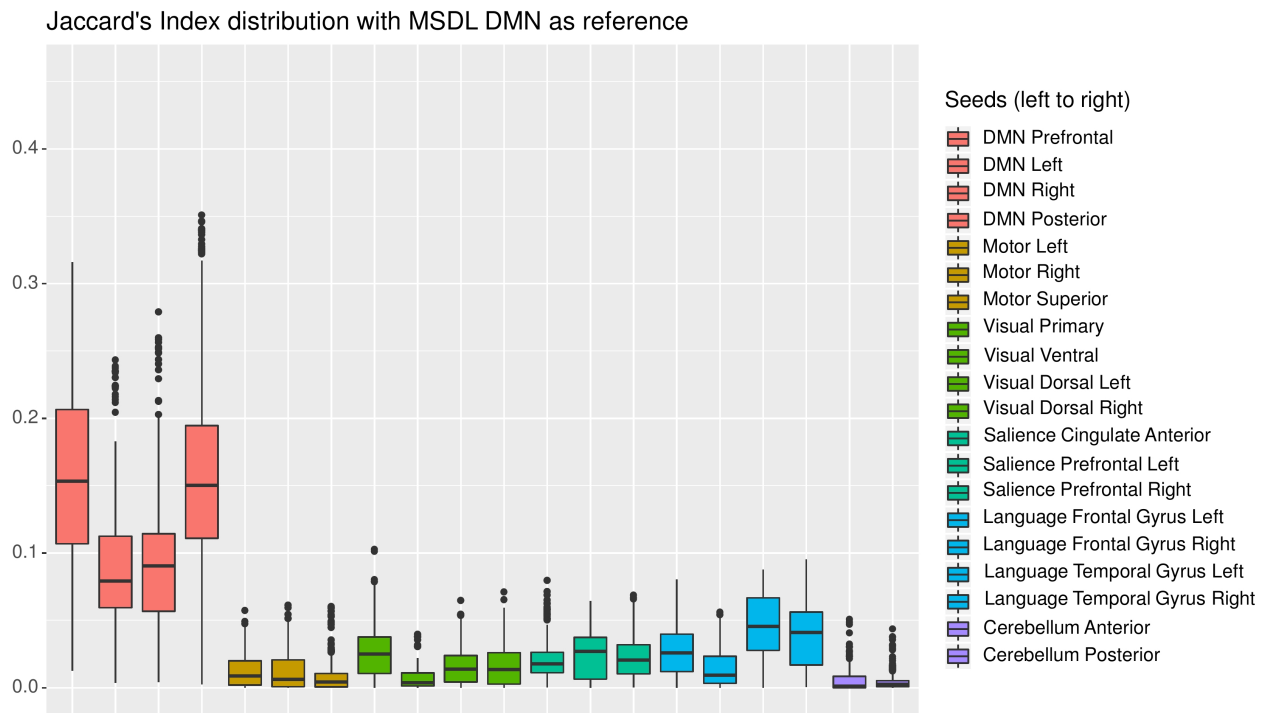


Figure 3. Each boxplot corresponds to one seed and shows the distribution of Jaccard's index between the estimated functional area corresponding to the considered seed on the one hand, and the MSDL DMN reference on the other hand, for all subjects and all durations. The seeds are grouped by color, each corresponding to one of the six functional areas considered. As expected, the seeds located in the expected DMN location (in pink) give the best results.

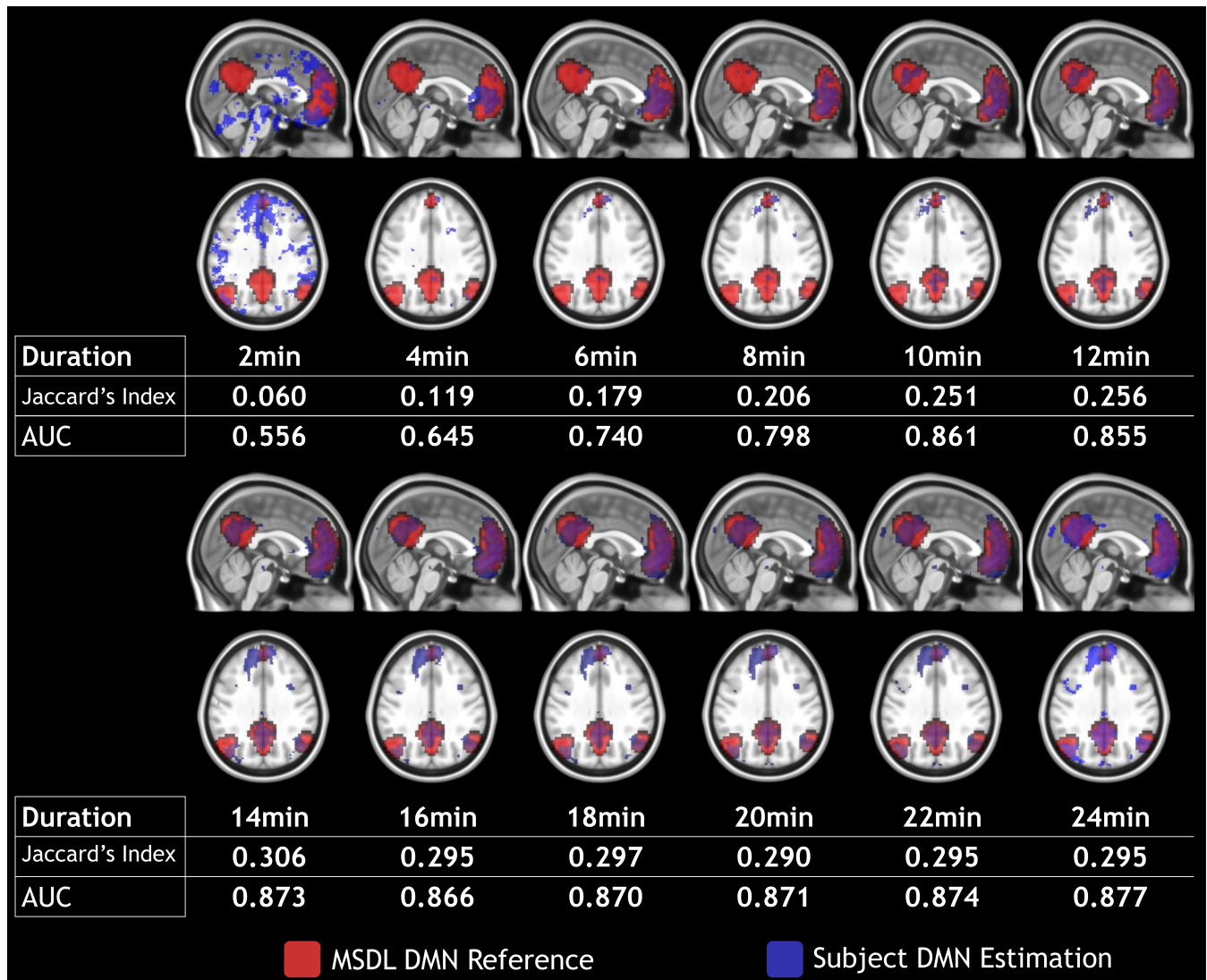


Figure 4. Subject 4 DMN detection (in blue) with prefrontal seed and MDSL DMN reference (in red) over a 2 min to 24 min duration with 2 min steps. Maps are shown in MNI152 space.

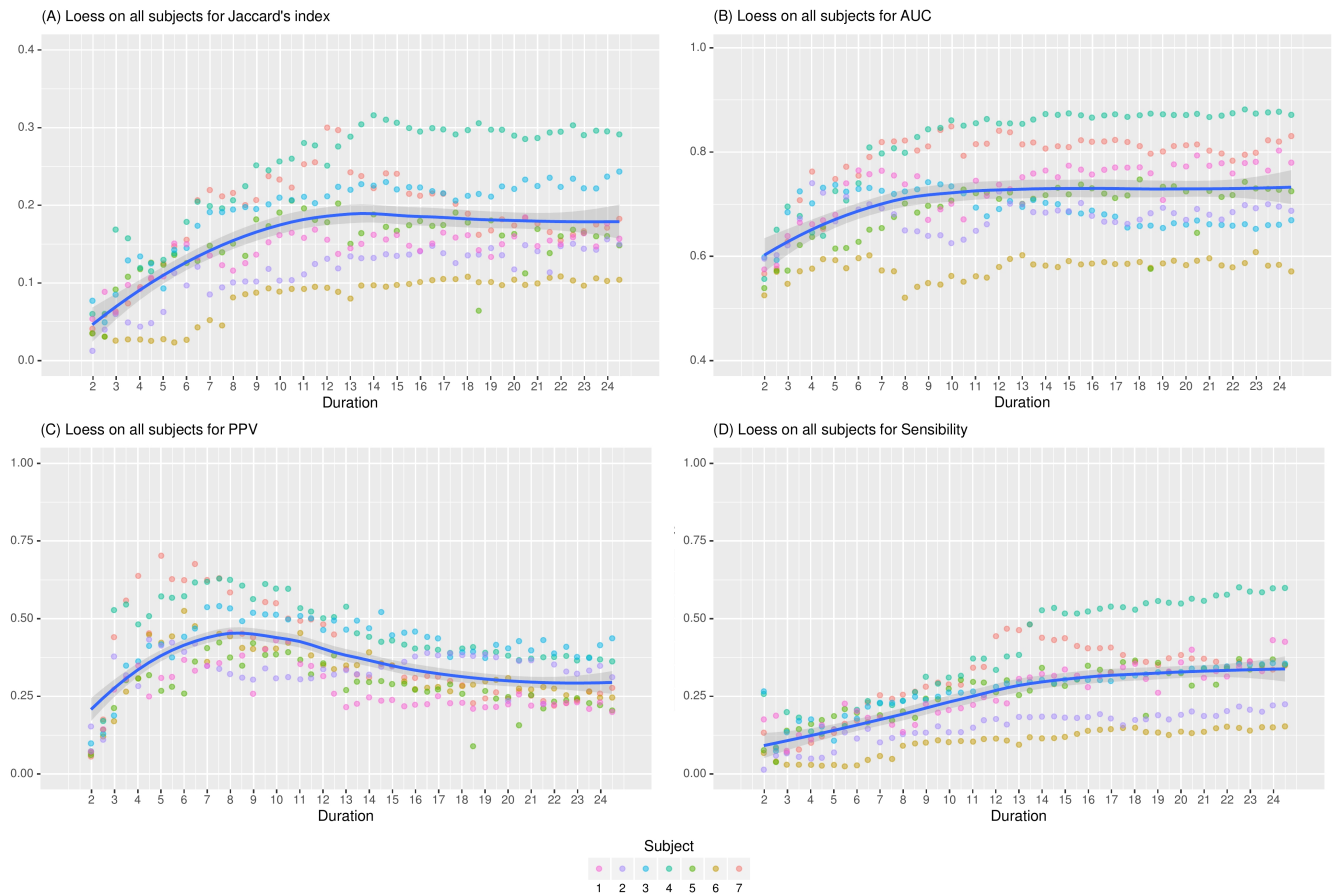


Figure 5. Jaccard's indices (A), AUC (B), Positive Predicted Value (C) and Sensibility (D) evolution with time with their associated Loess for the seed associated with prefrontal DMN. On all subjects, Jaccard's index increases with duration before 10-12 min, then stabilizes. The AUC shows the same trend with an earlier stabilization, around 9-10 min. PPV grows rapidly, reaches a peak at 8 min, then decreases slowly. Finally, Sensitivity increases with time, but more slowly for longer durations. Subjects show different level of response but good correlations.

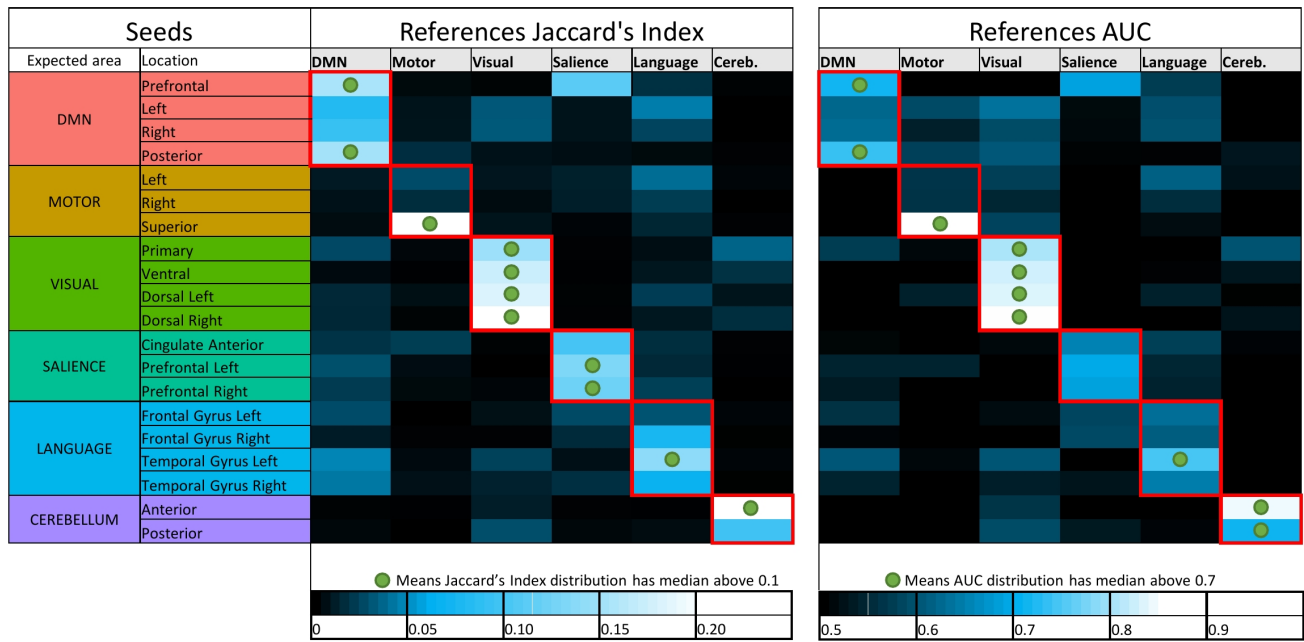


Figure 6. Median values of Jaccard’s indices and AUC for all combinations of seeds/references. Green circles show where seeds/reference combinations are selected with respect to our thresholding rules (0.1 for the Jaccard median and 0.7 for the AUC median).

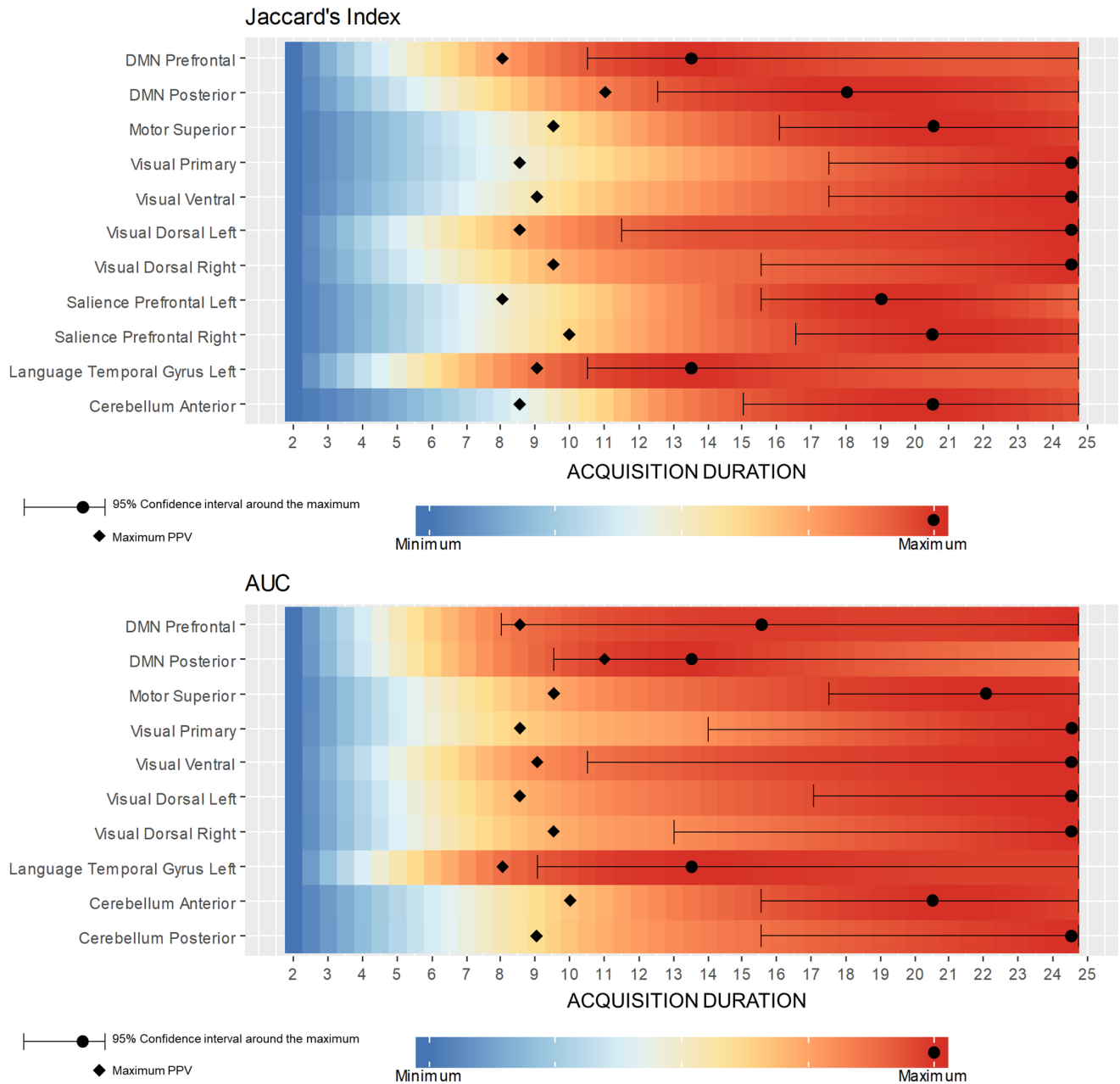


Figure 7. Color maps of Jaccard’s Index and AUC Loess value with respect to the acquisition duration for all selected reference/seed combination. Every combination has a fast increasing score followed by a stabilization stage. The PPV peak shows on all combinations around 9 min and appears always just before score start stabilizing. Maximum and its 95% confidence interval may be unstable since score variations are often low after PPV peak.

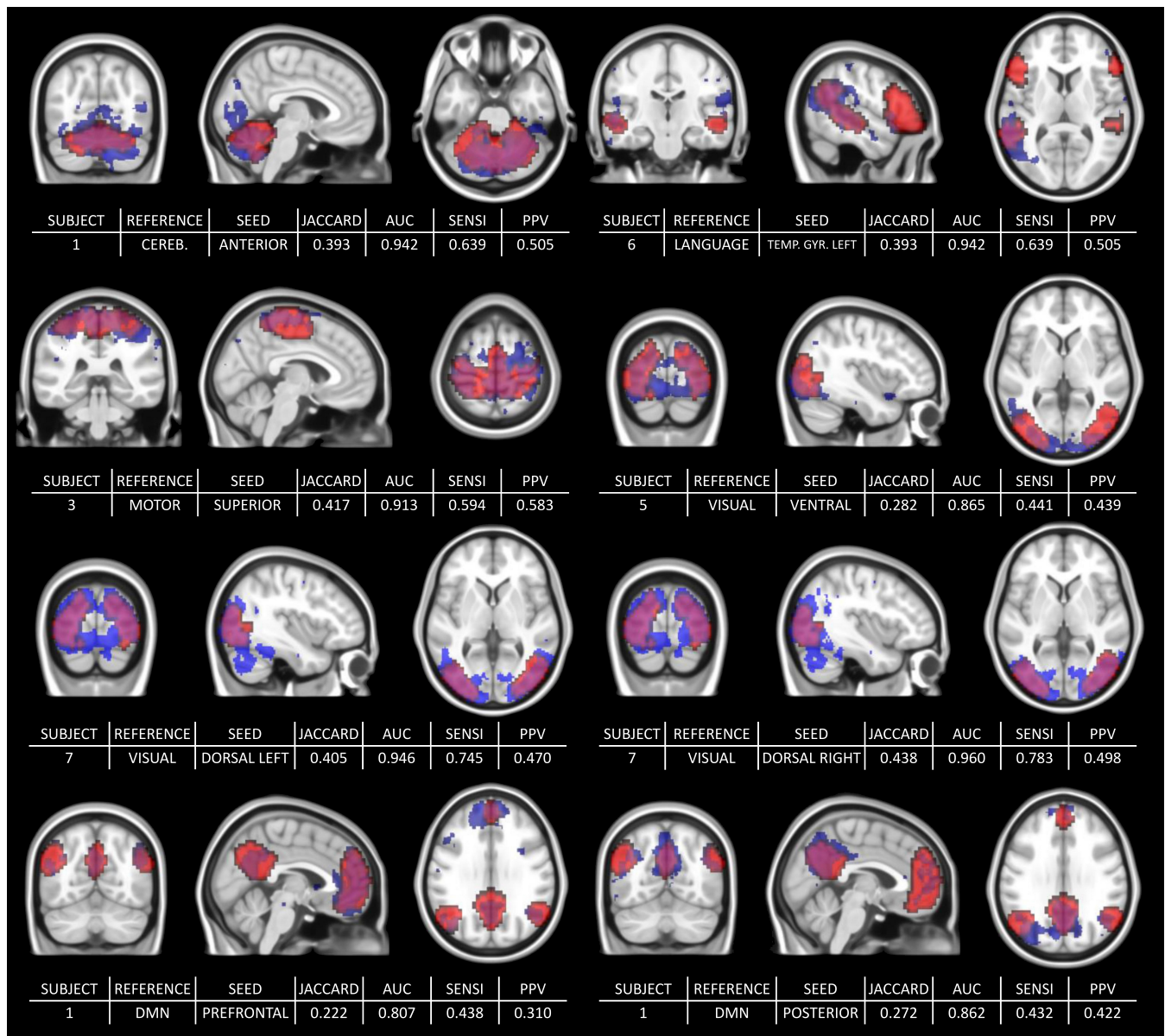


Figure 8. Collection of functional areas at 14 min with the corresponding scores: Jaccard’s Index, Area Under the Curve, Sensitivity and PPV. The two bottom rows show the same subjects and the same reference but with different seeds. The third row shows the estimated (blue) and reference (red) visual network for the same subject (subject 7) but different seeds (“dorsal left” seed on the left and “dorsal right” seed on the right). Similarly, the bottom row shows the same subject (subject 1) with different seeds (“Prefrontal” and “Posterior”).