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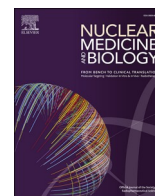
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Preclinical investigation of the effect of stress on the binding of [^{18}F]F13640, a 5-HT $_1\text{A}$ radiopharmaceutical

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ABSTRACT

Background: [^{18}F]F13640 is a new PET radiopharmaceutical for brain molecular imaging of serotonin 5-HT $_1\text{A}$ receptors. Since we intend to use this radiopharmaceutical in psychiatric studies, it is crucial to establish possible sensitivity modification of 5-HT $_1\text{A}$ receptors availability during an acute stress exposure. In this study, we first assessed the cerebrometabolic effects of a new animal model of stress with [^{18}F]FDG and then proceeded to test for effects of this model on the cerebral binding of [^{18}F]F13640, a 5-HT $_1\text{A}$ receptors PET radiopharmaceutical. **Methods:** Four groups of male Sprague-Dawley were used to identify the optimal model: “stressed group” ($n = 10$), “post-traumatic stress disorder (PTSD) group” ($n = 9$) and “restraint group” ($n = 8$), compared with a control group ($n = 8$). All rats performed neuroimaging [^{18}F]FDG $\mu\text{PET-CT}$ to decipher which model was the most appropriate to test effects of stress on radiotracer binding. Subsequently, a group of rats ($n = 10$) underwent two PET imaging acquisitions (baseline and PTSD condition) using the PET radiopharmaceutical [^{18}F]F13640 to assess influence of stress on its binding. Voxel-based analysis was performed to assess [^{18}F]FDG or [^{18}F]F13640 changes.

Results: In [^{18}F]FDG experiments, the PTSD group showed a pattern of cerebrometabolic activation in various brain regions previously implicated in stress (amygdala, perirhinal cortex, olfactory bulb and caudate). [^{18}F]F13640 PET scans showed increased radiotracer binding in the PTSD condition in caudate nucleus and brainstem.

Conclusions: The present study demonstrated stress-induced cerebrometabolic activation or inhibition of various brain regions involved in stress model. Applying this model to our radiotracer, [^{18}F]F13640 showed few influence of stress on its binding. This will enable to rule out any confounding effect of stress during imaging studies.

1. Introduction

During medical imaging studies, episodes of anxiety may occur in patients due to apprehension about the procedure, injection of a radioactive substance, or installation conditions (claustrophobia) [1,2]. The administration of a radiopharmaceutical generally requires placement of a venous catheter, which can also be stressful for the patient. Validation of new PET radiopharmaceuticals involves testing the repeatability of the method. A bias is observed in test-retest studies which can be attributed to pre-scan stress that affects the first scan more than the second, due to familiarization.

This stress-related bias is especially strong if the

radiopharmaceutical is exploring a receptor linked to cognitive and mood processes, such as certain serotonin receptors, or if the radiopharmaceutical has a binding sensitivity to neurotransmitter fluctuations.

In this context, [^{18}F]F13640 is a new radiopharmaceutical developed by our laboratory that enables imaging of the functional part of 5-HT $_1\text{A}$ receptors thanks to its pure agonist properties [3]. This radiotracer, recently used in humans for the first time, can be applied in numerous neurological and psychiatric indications [4]. As 5-HT $_1\text{A}$ receptors are strongly involved in stress and anxiety processes [5], it is important to check whether an episode of stress or anxiety in the run-up to an imaging acquisition could modify brain uptake of the radiopharmaceutical.

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We therefore sought to explore the influence of a stress episode on the binding of [¹⁸F]F13640 in preclinical studies, using a stress model in rodents. The model of post-traumatic stress disorder (PTSD) with predator odor and restraint, described in the literature, is frequently used to explore the effect of stress in rodents [6]. MRI studies have validated the model’s ability to detect brain activation in stress-related brain regions [7]. The objective of the present study was, therefore: (i) to validate the stress model by PET imaging using [¹⁸F]fluorodeoxyglucose (FDG), demonstrating cerebrometabolic activation of stress-related brain areas; and (ii) to use the same model to assess the influence of stress on the binding of the radiopharmaceutical [¹⁸F]F13640.

2. Material and methods

2.1. Animals

Male Sprague-Dawley rats (Charles River Laboratories; 250 ± 50 g) were used. All experiments were conducted following a protocol approved by the local ethics committee (“Comité d’éthique pour l’Expérimentation Animale Neurosciences Lyon,” registration code: C2EA - 42), authorized by the French Ministry of Higher Education and Research (authorization number: 2018072617334618), and in accordance with the European guidelines for the protection and use of laboratory animals (Council Directive 2010/63/EU; French decree 2013-118). The animals had a minimum 7 days to acclimatize to the conventional housing facility, under controlled temperature conditions (range, 20–24 °C), and a 12 h–12 h light-dark cycle with lights on at 07:00 and off at 19:00.

2.2. Validation of models in PET imaging using [¹⁸F]FDG

Four groups were used to identify the optimal model. Three types of model were assessed: “PTSD group”, “stressed group”, and “restraint group”, compared with a control group. All the groups followed a protocol derived from the PTSD model.

The PTSD group (n = 9) was based on stress conditioning. Rodents were placed under restraint in a plexiglass tube and exposed to a predator odor (cat feces) for 30 min on 2 days (D-2 and D-1) before the imaging day. On the day of imaging, [¹⁸F]FDG was injected into the animals. During uptake, the rats were restrained for 30 min, without the predator odor.

The stressed group (n = 10) underwent restraint conditioning for 30 min on the 2 days before imaging, but without exposure to predator

odor. On the imaging day, injection and 30-min uptake were performed under restraint, with the predator odor.

The restraint group (n = 8) did not receive any conditioning on the days preceding imaging. 30-min uptake was monitored with the rats in restraint, without predator odor.

The control group (n = 8) also did not receive any conditioning. 30-min uptake was monitored in an awake, free-vigilance state, in a cage.

For each group, a 30-min PET/CT acquisition was performed at the end of uptake. Fig. 1 summarizes the conditions for each group.

2.3. PTSD protocol using [¹⁸F]F13640

Based on the results from these initial experimental groups, a group of rats (n = 10) underwent two PET imaging acquisitions. The first scan was conducted under the restraint protocol described above and served as a control of restraint condition. The second scan was performed under the PTSD protocol as described above. The radiotracer used for this group was [¹⁸F]F13640, and acquisition was performed for 30 min in static mode 30 min after injection.

2.4. PET/CT imaging protocol

For [¹⁸F]FDG scans, rats were fasted for 4 h before injection. [¹⁸F]FDG or [¹⁸F]F13640 (37 kBq/g) was administered into the caudal vein of the awake rat, with a volume of 0.5 mL. Static brain PET acquisitions were then performed for 30 min, 30 min after radiotracer injection, using an Inveon PET/CT scanner (Siemens), with the animals in prone position, heads centered in the field of view. Real-time monitoring of respiratory frequency and temperature was conducted during the imaging session. A computed tomography (CT) scan was performed for attenuation and scatter correction. The images were reconstructed with attenuation and scatter correction using OP-OSEM3D (3D Ordinary Poisson Ordered Subsets Expectation-Maximization) with 4 iterations and a zoom factor of 2. The reconstructed volume consisted of 159 slices of 128 × 128 voxels within a bounding box of 49.7 × 49.7 × 126 mm³ and a voxel size of 0.388 × 0.388 × 0.796 mm³.

2.5. Statistical analysis

SPM12 (Wellcome Trust Centre for Neuroimaging, London, United Kingdom) was used for voxel-to-voxel comparisons of PET scans by statistical parametric mapping. For the groups that underwent [¹⁸F]FDG scans, PTSD, stressed, and restraint groups were compared to the control

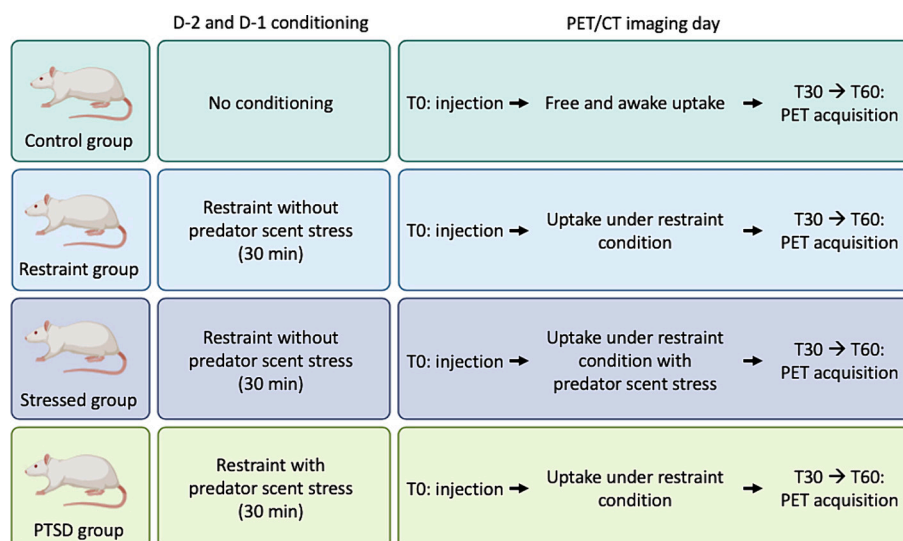


Fig. 1. Study design for validation of the stress model.

group. Parametric images of standardized uptake value ratio (SUVR), normalized with the whole-brain, were compared voxel-by-voxel on Student *t*-test. For [¹⁸F]F13640 scans, each rat's control restraint condition was compared to its PTSD condition. The paired Student *t*-test was used for voxel-to-voxel comparison.

The significance threshold for clusters was set at $p < 0.05$ (family-wise error (FWE) corrected). Clusters with extent ≥ 60 voxels (corresponding to the raw size of PET voxels) and $p < 0.05$ FWE corrected were considered significant.

3. Results

3.1. Validation of model in PET imaging using [¹⁸F]FDG

In the PTSD group, rats showed increased [¹⁸F]FDG uptake in the temporal cortex (perirhinal), caudate nucleus, olfactory bulb, hippocampus and amygdala compared to the control group, and decreased uptake in the occipital cortex and cerebellum. Statistical characteristics of the different clusters are summarized in Table 1. Fig. 2 shows cluster differences for the PTSD group compared to the control group.

In the stressed group, rats showed increased [¹⁸F]FDG uptake compared to the control group in the temporal cortex (perirhinal) and sensory trigeminal nucleus, and decreased uptake in the thalamus and cerebellum. Statistical characteristics of the different clusters are summarized in Table 1.

In the restraint group, rats showed increased [¹⁸F]FDG uptake compared to control group in the sensory trigeminal nucleus and olfactory bulb, and decreased uptake in the occipital cortex and cerebellum. Statistical characteristics of the different clusters are summarized in Table 1.

Table 2 summarizes significant differences between each group and control the group.

3.2. Influence of stress on [¹⁸F]F13640 binding using the PTSD model

[¹⁸F]F13640 PET scans showed increased radiotracer binding in the PTSD condition compared to the restraint condition. The differences were in two clusters within the caudate nucleus and brainstem.

Table 1

Statistics of clusters differences for [¹⁸F]FDG PET experiments. + = increase uptake, - = decrease uptake.

Cluster (uptake)	Cluster <i>p</i> -value	Size (number of voxels)	Peak level <i>p</i> -value	Regions associated in the cluster
PTSD vs. control				
1 (+)	<0.0001	6553	<0.0001	Caudate nucleus
2 (+)	<0.0001	7553	<0.0001	Temporal cortex (perirhinal)
3 (+)	0.01	898	<0.0001	Olfactory bulb
4 (+)	<0.0001	2328	<0.0001	Hippocampus and amygdala
5 (-)				Occipital cortex
6 (-)	0.01	637	<0.0001	Cerebellum
Stressed vs. control				
1 (+)	0.001	1371	<0.0001	Cortex temporal (perirhinal)
2 (+)	0.008	792	<0.0001	Sensory trigeminal nucleus
3 (-)	0.002	1221	<0.0001	Thalamus
4 (-)	0.01	612	<0.0001	Cerebellum
Restraint vs. control				
1 (+)	0.005	834	<0.0001	Sensory trigeminal cortex
2 (+)	0.002	1146	<0.0001	Olfactory bulb
3 (-)	0.009	717	<0.0001	Occipital cortex
4 (-)	0.01	585	<0.0001	Cerebellum

Radiotracer binding was lower in the PTSD condition than in the restraint condition in the olfactory bulb. Fig. 3 shows cluster differences between the PTSD and the restraint condition in the rats that underwent [¹⁸F]F13640. Table 3 summarized clusters differences characteristics.

4. Discussion

In this study, we first assessed three stress models in rats: PTSD model, stress model, and a restraint model. [¹⁸F]FDG PET/CT imaging demonstrated that the PTSD model could induce cerebrometabolic effects in various brain regions known to be involved in stress and anxiety mechanisms. Other studies explored stress-related cerebrometabolic effects using [¹⁸F]FDG PET [8–10]. However, our study is the first to evaluate multiple stress models. Subsequently, using the PTSD model, we investigate the influence of stress on our radiotracer binding, [¹⁸F]F13640, a new radiopharmaceutical to image functional 5-HT_{1A}. Once again, differences in binding were observed in the PTSD condition.

4.1. Validation of the PTSD model with [¹⁸F]FDG

In the PTSD model, increased [¹⁸F]FDG uptake was found in the amygdala, hippocampus, perirhinal cortex, caudate nucleus and olfactory bulb. The most interesting results concern the amygdala and hippocampus, which are known for their involvement in stress phenomena [11–13]. The increased uptake of [¹⁸F]FDG highlights heightened neuronal activity in these regions, confirming the stress response induced in rats. The perirhinal cortex is located in the temporal cortex surrounding the hippocampus and amygdala [14]. It is involved in various functions and contributes significantly to recognition memory [15]. Recognition involves two components: recall memory and familiarity. Studies showed that the perirhinal cortex is involved in the familiarity process [16–18], defined by recognition, without contextual information, of an item that has been previously presented. Thus, the cerebrometabolic changes of the perirhinal cortex in our situation could involve the conditioning on days D-1 and D-2. This observation is particularly interesting, as this region was not activated in the restraint group, which did not undergo any conditioning. The involvement of the perirhinal cortex was also suggested, by Kent and Brown in 2012, to be responsible for fear conditioning [19]. Additionally, close interactions were shown between the perirhinal cortex and the amygdala, a region known to be involved in stress phenomena [20]. Finally, studies showed direct involvement of the perirhinal cortex in stress and anxiety phenomena [21,22].

The caudate nucleus is less well described, but also appears to be involved in stress phenomena and, more recently, in PTSD [23]. The increased uptake in the olfactory bulb may correspond to cerebrometabolic activation of the olfactory bulb by “sniffing” [24]. Olfactory bulb activity was also increased in the restraint group, whereas increased activity was not found in the stressed group. The PTSD and restraint groups were not exposed to predator odor during [¹⁸F]FDG uptake, unlike in the stressed group. This leads us to believe that “sniffing” activity is triggered only in the absence of odor, as the rats are searching for its presence.

Decreased [¹⁸F]FDG uptake was also observed in the cerebellum and occipital cortex. The cerebellum is responsible for motor activity in rats [25]. In the restraint condition, with motor activity incapacitated, decreased activity in the cerebellum can be expected. Similarly, the restraint group showed decreased [¹⁸F]FDG uptake in the cerebellum, confirming the involvement of restraint in this decrease. The occipital cortex has also been reported to be involved in post-traumatic stress phenomena. Most studies of the occipital lobe reported decreased volume and functional activity in the occipital cortex in patients with post-traumatic stress disorder [26]. These results are consistent with the decreased activity found on [¹⁸F]FDG PET, indicating a decrease in brain activity.

In the stressed model, increased [¹⁸F]FDG uptake was observed in

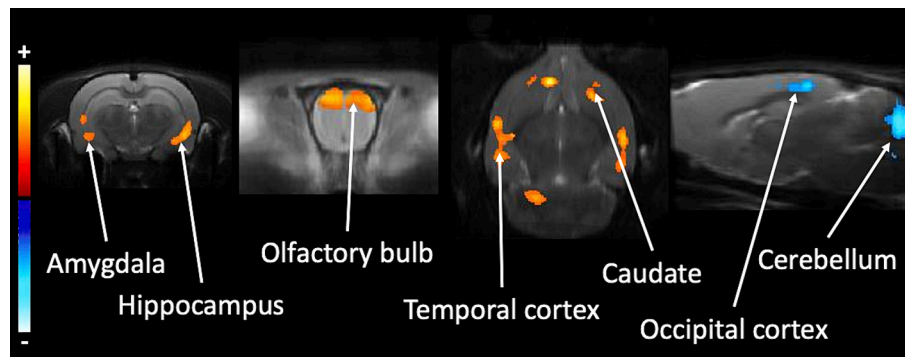


Fig. 2. Cluster differences in $[^{18}\text{F}]\text{FDG}$ uptake between the PTSD and control groups projected on an MRI template. Amygdala, hippocampus, olfactory bulb, temporal cortex and caudate show increased uptake (“hot” color clusters). Occipital cortex and cerebellum show decreased uptake (“cold” color clusters).

Table 2

Cluster differences in $[^{18}\text{F}]\text{FDG}$ uptake for each group compared to the control group. + = increased uptake, - = decreased uptake and 0 = no difference.

Cluster regions of differences	PTSD	Stressed	Restraint
Temporal cortex (perirhinal)	+	+	0
Sensory trigeminal nucleus	0	+	+
Caudate	+	0	0
Olfactory bulb	+	0	+
Hippocampus	+	0	0
Amygdala	+	0	0
Thalamus	0	-	0
Occipital cortex	-	0	-
Cerebellum	-	-	-

the perirhinal cortex and sensory trigeminal nucleus. The perirhinal cortex, as mentioned above, seems to be involved in stress conditioning. As this group also underwent conditioning, this result is consistent with that of the PTSD group. The sensory trigeminal nucleus integrates sensory information from the face, including vibrissae input from the trigeminal nerve [27]. Recent studies demonstrated the involvement of these nuclei in stress phenomena related to pain under restraint [28,29]. The restraint group also showed increased $[^{18}\text{F}]\text{FDG}$ uptake in the sensory trigeminal nucleus, suggesting that these differences were not due to stress induced by the predator’s odor, but rather to the restraint itself.

Decrease uptake was also observed in the thalamus and cerebellum in the stressed group. The thalamus was reported to be involved in stress episodes, including PTSD [30]. A decrease in volume and atrophy were shown in the thalamus, related to PTSD [31–33]. PTSD patients also showed reduced functional activity in the thalamus on functional MRI [34,35]. These results confirm that the decrease in activity observed in our study was linked to the stress episode. Again, since uptake occurred under restraint, decreased uptake in the cerebellum, known for its

involvement in motor control, confirms the results obtained with the PTSD rats.

Finally, we chose the PTSD model to test the influence of stress on our radiotracer, due to the increased uptake in several brain regions known to be involved in stress, such as the amygdala and hippocampus. Additionally, the perirhinal cortex, which was strongly activated in the stress model, was also activated in the PTSD model. Furthermore, the effect of restraint on the sensory trigeminal nerve was not found in the PTSD model, unlike in the stress model. Moreover, the restraint group only showed significant differences in regions known to be involved in locomotion or pain. Thus, this group seemed to be an adequate control group for the second step of the study, avoiding specific effects of restraint.

4.2. Influence of stress on $[^{18}\text{F}]\text{F13640}$ binding

The PTSD condition showed increased radiotracer binding in the brainstem and decreased binding in the olfactory bulb. 5-HT_{1A} receptors are found in the olfactory bulb [36], and the decrease in $[^{18}\text{F}]\text{F13640}$ binding can be interpreted in two different ways: (i) reduction in 5-HT_{1A} receptor expression, or (ii) by competition with endogenous serotonin release. The second hypothesis is more likely, as studies in cats

Table 3

Statistics of clusters differences for $[^{18}\text{F}]\text{F13640}$ PET experiments, PTSD vs. restraint condition. + = increase uptake, - = decrease uptake.

Cluster (uptake)	Cluster p-value	Size (number of voxels)	Peak level p-value	Regions associated in the cluster
PTSD vs. restraint				
1 (+)	0.04	848	<0.0001	Caudate nucleus
2 (+)	0.01	1174	<0.0001	Brainstem
3 (-)	0.01	1218	<0.0001	Olfactory bulb

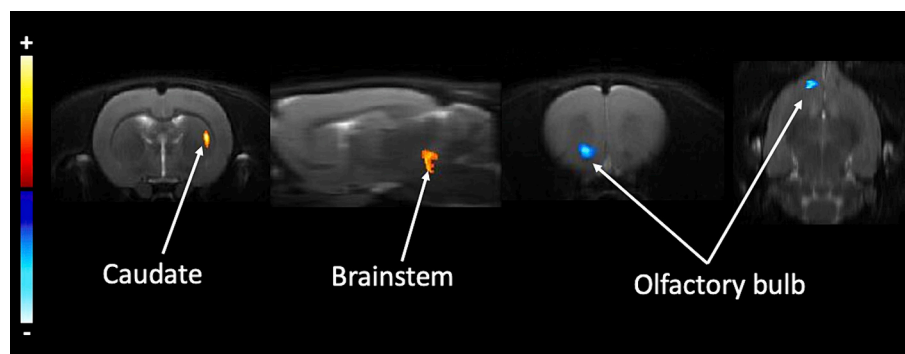


Fig. 3. Cluster differences in $[^{18}\text{F}]\text{F13640}$ uptake between PTSD condition and restraint condition projected on an MRI template. Caudate and brainstem show increased uptake (“hot” color clusters). Olfactory bulb shows decreased uptake (“cold” color cluster).

demonstrated the ability of [¹⁸F]F13640 to measure serotonin release in the synaptic cleft [37]. Moreover, this correlates with cerebrometabolic activation of the olfactory bulb observed with [¹⁸F]FDG during model validation. Cerebrometabolic activation of the olfactory bulb could thus induce serotonin release, resulting in a decrease in [¹⁸F]F13640 binding to postsynaptic 5-HT_{1A} receptors.

A region of the brainstem also showed increased radiotracer binding. The brainstem, and particularly the raphe nuclei, is known to express 5-HT_{1A} receptors, for which [¹⁸F]F13640 showed strong affinity [3,4]. Additionally, serotonergic neurons send direct projections regulating the olfactory bulb [38]. The 5-HT_{1A} receptors in the raphe nuclei are autoreceptors located on the soma of neurons, responsible for regulating the serotonergic axis [39]. Thus, increased activity in the olfactory bulb following sniffing during stress can lead to negative feedback on serotonin release, resulting in increased radiotracer binding.

Other 5-HT_{1A} radiotracers also explored 5-HT_{1A} availability in stress episode. [¹⁸F]MPPF showed, in a model restraint stress in twelve consecutive days, higher binding in hippocampus compared to control [9]. Also, [¹⁸F]Mefway showed a significant binding reduction after acute physical stress on hippocampus [40]. Our study did not show binding differences in hippocampus. In fact, the main goal of our study was not to determine the implication of 5-HT_{1A} receptor in stress episode but to determine whether a stress episode could influence [¹⁸F]F13640 binding after its injection which could contribute to be a confounding factor. Based on this hypothesis our study design differs from several points to the other radiotracers studies cited above. For example, on the type and duration of stress episode or on the radiotracer timeline administration (before vs after stress episode).

Altogether, a stress episode after radiotracer injection and during uptake does not appear to modify [¹⁸F]F13640 binding to 5-HT_{1A} receptors. In our opinion, the differences observed in rats were primarily due to activation of the olfactory bulb associated with sniffing activity during stressful situations.

In conclusion, the present study showed, [¹⁸F]FDG PET cerebrometabolic activation or inhibition of various brain regions previously involved in stress. The PTSD model, combined with a restraint control condition, was the most suitable design for molecular imaging of the influence of stress on radiotracer brain uptake in PET imaging. Applying this model to our radiotracer, [¹⁸F]F13640 showed few influence of stress on its binding. This will enable to rule out any confounding effect of stress during imaging studies. In a translational research approach, these data will be valuable for interpreting clinical images in patients in the future.

Disclosures

The authors have no relevant financial or non-financial interests to disclose.

CRedit authorship contribution statement

Pierre Courault: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sandrine Bouvard:** Writing – review & editing, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Caroline Bouillot:** Writing – review & editing, Project administration, Investigation, Data curation, Conceptualization. **Luc Zimmer:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Conceptualization. **Sophie Lancelot:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization.

Declaration of competing interest

Nothing to disclose.

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