BEHAVIORAL AND BRAIN FUNCTIONS

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Acute reversible inactivation of the bed nucleus of stria terminalis induces antidepressant-like effect in the rat forced swimming test

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Abstract

Background: The bed nucleus of stria terminalis (BNST) is a limbic forebrain structure involved in hypothalamo-pituitary-adrenal axis regulation and stress adaptation. Inappropriate adaptation to stress is thought to compromise the organism's coping mechanisms, which have been implicated in the neurobiology of depression. However, the studies aimed at investigating BNST involvement in depression pathophysiology have yielded contradictory results. Therefore, the objective of the present study was to investigate the effects of temporary acute inactivation of synaptic transmission in the BNST by local microinjection of cobalt chloride ($CoCl_2$) in rats subjected to the forced swimming test (FST).

Methods: Rats implanted with cannulae aimed at the BNST were submitted to 15 min of forced swimming (pretest). Twenty-four hours later immobility time was registered in a new 5 min forced swimming session (test). Independent groups of rats received bilateral microinjections of $CoCl_2$ (1 mM/100 nL) before or immediately after pretest or before the test session. Additional groups received the same treatment and were submitted to the open field test to control for unspecific effects on locomotor behavior.

Results: $CoCl_2$ injection into the BNST before either the pretest or test sessions reduced immobility in the FST, suggesting an antidepressant-like effect. No significant effect of $CoCl_2$ was observed when it was injected into the BNST immediately after pretest. In addition, no effect of BNST inactivation was observed in the open field test.

Conclusion: These results suggest that acute reversible inactivation of synaptic transmission in the BNST facilitates adaptation to stress and induces antidepressant-like effects.

Background

The bed nucleus of stria terminalis (BNST) is a limbic forebrain structure situated ventrally to the lateral septal nucleus and dorsally to the preoptic area of the hypothalamus [1,2]. It has extensive reciprocal connections with other limbic structures as well as with brainstem autonomic nuclei [2-5], and it is an import relay station for the integration of information from brain regions associated with the control of emotional, cognitive, autonomic, endocrine and behavioral responses [2,6-13].

Several studies have suggested that the BNST mediates behavioral responses to acute and chronic aversive stimuli [5,14]. This is supported by reports that the BNST is activated in response to stress [15-18] and modulates anxiety-related behaviors in several animal models [5,10,19,20]. Moreover, the BNST could also mediate behavioral adaptation to chronic stress exposure [21-24]. Inappropriate adaptation to stress is thought to compromise the organism's coping mechanisms, which have been implicated in the etiology of stress-related disorders, such as posttraumatic stress disorder (PTSD) and depression [25-27].

BNST involvement in the activation and termination of the hypothalamo-pituitary-adrenal (HPA) axis response to stress has been well documented in the literature [6,28-30]. Activation of the HPA axis is a primary mechanism

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for maintaining homeostasis in response to stress. Although adaptative in nature, glucocorticoids secretion is tightly regulated since prolonged exposure to their effects can lead to serious metabolic, immune, and psychological dysfunction. Dysfunction in forebrain limbic regions that exert control over the HPA axis, such as the amygdala, hippocampus and medial prefrontal cortex (MPFC) [31,32], has been implicated in the etiology of stress-related disorders, including PTSD and depression, which often exhibit HPA axis abnormalities [33,34]. Substantial information from these forebrain regions are integrated in the BNST that could either excite or inhibit HPA activity depending on the region of the BNST targeted [6].

Failure of coping mechanisms has been recognized as a major factor precipitating depressive episodes in humans [27,35,36]. BNST role in stress adaptation and its connections with other limbic structures traditionally related to depression, such as the hippocampus and the MPFC, has made it a subject of study in different behavioral paradigms aimed at investigating the neurobiology of depression. In fact, the BNST is activated by stressful stimuli that induce depressive-like behavior in rodents [16,37,38] and this can be attenuated by systemic antidepressant-treatment [37], corroborating the idea that BNST dysfunction could contribute to the pathophysiology of depression. However, the studies aimed at investigating this hypothesis through local inactivation of BNST have yielded contradictory results. For example, while chemical lesions of the BNST induced antidepressant-like effects in the rat learned helplessness model [16,19], electrolytic lesions of the BNST increased depressive-like behavior in the rat forced swimming test (FST) [39-41]. The reasons for these contradictory results are not clear, but could involve the different animal models used or methodological differences in the lesions employed (size, time of recovery or nature of the lesion - chemical versus electrical). In addition, irreversible lesions can also destroy fibers of passage and induce local plastic changes [42]. Another disadvantage of lesion techniques is that they do not allow the identification of the precise moment when the disruption of BNST activity affects the development of the depressive-like behavior (during the pretest or during the test). Considering that BNST is interconnected with brain structures implicated in learning and memory of aversive events, such as the MPFC, the hippocampus and the amygdale [43], it would be interesting to investigate the participation of such cognitive mechanisms in the development of stress-induced behavioral consequences in the FST mediated by BNST.

Therefore, considering the contradictory results regarding the role of the BNST in the modulation of depressive-like behavior, and the fact that the time point of BNST influence in the FST has never been evaluated, the objective of the present study was to investigate the effects of temporary acute inactivation of synaptic transmission in the BNST, at different time points (before pretest, after pretest or before test), by local microinjection of cobalt chloride ($CoCl_2$) in rats submitted the FST. This drug reduces calcium pre-synaptic influx [44] and causes a reversible inhibition of neurotransmitter release with a consequent synaptic blockage, without affecting passage fibers.

Methods

Animals

Male Wistar rats weighing 230-250 g at the beginning of each experiment were housed in pairs in a temperature-controlled room (24 ± 1°C) under standard laboratory conditions with free access to food and water and a 12 h light/12 h dark cycle (lights on at 06:30 h a.m.). Procedures were conduct in conformity with the Brazilian Society of Neuroscience and Behavior guidelines for the care and use of laboratory animals, which are in compliance with international laws and politics. The protocols described herein have been approved by the local Ethical Committee and all efforts were made to minimize animal suffering.

Drugs

The following drugs were used: cobalt chloride ($CoCl_2$; Sigma, St Louis, Missouri, USA), tribromoethanol (Aldrich, St Louis, Missouri, USA) and urethane (Sigma, St Louis, Missouri, USA). $CoCl_2$ was dissolved in sterile artificial cerebrospinal fluid (ACSF: 100 mM NaCl; 2 mM Na3PO4; 2.5 mM KCl; 1 mM MgCl2; 27 mM NaHCO3; 2.5 mM CaCl2; pH = 7.4). Tribromoethanol and urethane were dissolved in saline 0.9%.

Stereotaxic surgery and intracerebral drug administration

Seven days before the experiment, animals were anaesthetized with tribromoethanol (250 mg/kg, i.p.) and fixed in a stereotaxic frame. After scalp anesthesia with 2% lidocaine, the skull was surgically exposed and stainless steel guide cannulae (26 G) were implanted bilaterally in the BNST using a stereotaxic apparatus (Stoelting, Wood Dale, Illinois, USA). Coordinates for cannula implantation (AP = +8.6 mm from interaural coordinate; L = +4 mm from the medial suture, V = -5.8 mm from the skull with a lateral inclination of 23°) were selected from the rat brain atlas of Paxinos and Watson [44]. The cannulae tips were 1 mm above the site of injection and the cannulae were attached to the skull bone with stainless steel screws and acrylic cement. An obturator inside the guide cannulae prevented obstruction. After surgery, the animals received a poly-antibiotic (Pentabiotico®, Fort Dodge, Brazil), with streptomycins and penicillins, to prevent infection and a nonsteroidal anti-inflammatory, flunixine

meglumine (Banamine®, Schering Plough, Brazil), for post- operation analgesia.

The needles (33G, Small Parts, Miami Lakes, FL, USA) used for microinjection into the BNST were 1 mm longer than the guide cannulae and were connected to a 2 μL syringe (7002-H, Hamilton Co., Reno, NV, USA) through PE-10 tubing. A volume of 100 nL/side was injected in 1 minute using an infusion pump (Kd Scientific, Holliston, MA, USA). The movement of an air bubble inside the polyethylene catheter confirmed drug flow.

Forced swimming test (FST)

The procedures for the FST, a widely used behavioral test for the detection of antidepressant-like effects, were similar to those described earlier [45-48]. Animals were initially placed individually to swim in plastic cylinders (30 cm of diameter by 40 cm in height containing 25 cm of water at 24 ± 1°C [49] for 15 min (pretest). They were then removed and allowed to dry in a separate cage before returning to their home cages. Twenty-four hours later the animals were submitted to a 5 min session of forced swimming session (test). During this session the total amount of time in which animals remained immobile (except for small limb movements necessary for floating) were recorded by an observer that was blind to the treatments. The water was changed after each trial to avoid the influence of alarm substances.

Open field test

Independent groups of animals were submitted to the open field test in order to investigate if the treatments used induced any significant motor effect, which would interfere in the FST results. The animals were placed individually in the center of an open circular arena (72 cm in diameter with a 50 cm high Plexiglas wall) located in a sound-attenuated, temperature-controlled room, illuminated with three 40W fluorescent bulbs. The animals were left in the arena for 10 minutes. Their exploratory activity was videotaped and the behavioral analysis was blindly performed with the help of the Ethovision software (version 1.9; Noldus, the Netherlands). This software detects the position of the animal in the open arena and calculates the distance moved.

Histological analysis

After the behavioral tests, animals were anesthetized with urethane (1.25 g/kg, i.p.) and then 100 nl of 1% Evan's blue dye was injected into the BST as a marker of injection site. Following that, they were perfused through the left ventricle of the heart with isotonic saline followed by 10% formalin solution. The brains were removed and after a minimum period of 3 days immersed in a 10% formalin solution, 40 μm sections were obtained in a Cryostat (Cryocut 1800). The injection sites were identified on diagrams from the Paxinos and Watson's atlas [50]. Rats that had received injections outside the aimed area were excluded from analysis.

Experimental design

Experiment 1: effects of $CoCl_2$ injection into the BNST of rats submitted to the FST

Animals were randomly assigned to three independent groups which received bilateral injection into the BNST of either 100 nL of vehicle (ACSF) [51] or 1 mM/100 nL of $CoCl_2$ [10,51] and were submitted to FST. The first group of animals received the microinjections into the BNST 10 minutes before the pretest session (ACSF: n = 5 and $CoCl_2$: n = 6). The second group received the microinjections into the BNST immediately after the end of pretest session (ACSF: n = 6 and $CoCl_2$: n = 6). Finally, the third group received the microinjections into the BNST 10 minutes before the test session (ACSF: n = 6 and $CoCl_2$: n = 7). Additional groups received the microinjections into structures surrounding the BNST before the pretest (ACSF: n = 4 and $CoCl_2$: n = 3) or before the test (ACSF: n = 3 and $CoCl_2$: n = 5).

Experiment 2: effects of $CoCl_2$ injection into the BNST of rats submitted to the open field test

Animals received the microinjections of either 100 nL of vehicle (ACSF, n = 6) or 1 mM/100 nL of $CoCl_2$ (n = 6) [10,12] into the BNST and were submitted to the open field test 10 min later.

Statistical analysis

The results of the FST and from open-field test were analyzed using unpaired *Student*-t test. Probability less than 0.05 was accepted as significant.

Results

Determination of microinjection sites

A representative photomicrograph of a coronal brain section depicting bilateral microinjection sites in the BST of one representative animal is presented in Figure 1. Moreover, diagrammatic representation showing microinjection sites of ACSF and $CoCl_2$ into the BNST and in structures surrounding the BNST are also shown in Figure 1.

Experiment one: effects of $CoCl_2$ injection into the BNST of rats submitted to the FST

Injection of $CoCl_2$ into the BNST before the pretest (170 ± 21 vs 46 ± 13 s, $t_9 = 5.024$, $P < 0.001$) or the test (182 ± 20 vs 111 ± 17 s, $t_{10} = 2.627$, $P < 0.05$) sessions induced a significant reduction of immobility time in the FST (Figure 2). There was no significant statistical difference between vehicle and $CoCl_2$-treated groups that received the injection into the BNST immediately after the pretest

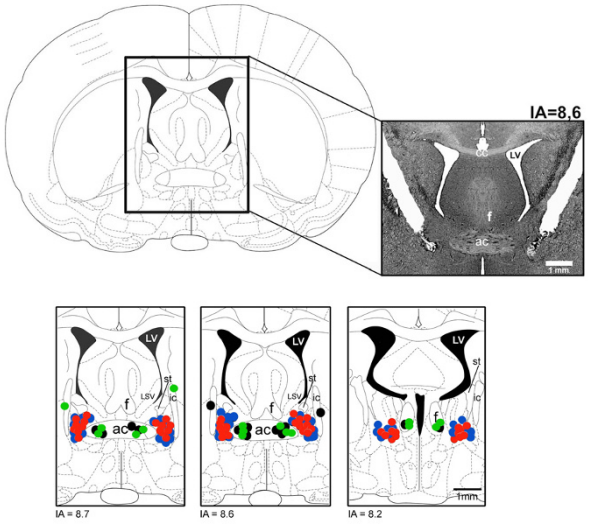

Figure 1 Photomicrograph of a coronal brain section from a representative rat showing bilateral injection sites in the BNST and a diagrammatic representation based on the rat brain atlas of Paxinos and Watson [50]**, indicating injection sites of ACSF (blue circles) and $CoCl_2$ (red circles) into the BNST and ACSF (black circles) and $CoCl_2$ (green circles) into structures surrounding the BNST**. IA - Interaural coordinate, ac - anterior commissure, cc - corpus callosum, ic - internal capsule, LSV - lateral septal ventral, LV - lateral ventricle, st - stria terminalis and f - fornix.

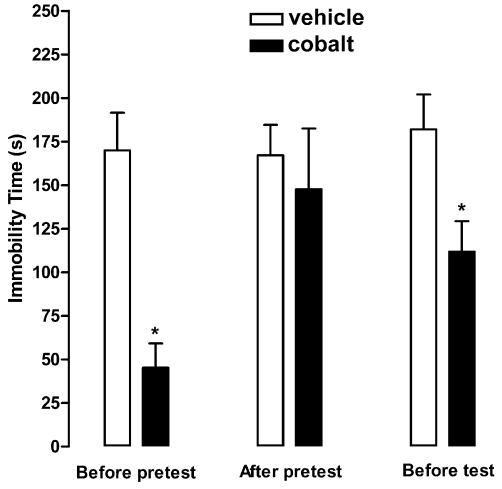

Figure 2 Total immobility time during the 5 min session of the forced swimming test (test) shown by animals treated with vehicle (ACSF, open columns) or $CoCl_2$ (cobalt, filled columns) into the BNST. Intra-BNST injection of $CoCl_2$ (1 mM/100 nL) before pretest or before test reduced immobility time in the rat forced swimming test. Data are expressed as mean ± S.E.M. (n = 5-7/group). * indicates significant difference from respective vehicle-treated group ($P < 0.05$, *Student*-t test).

(167 ± 17 vs 148 ± 33 s, $t_{10} = 2.627$, $P < 0.05$) (Figure 2). Immobility time obtained in animals that received $CoCl_2$ before the pretest or before the test was significantly different ($t_{11} = 2.9$, $P < 0.05$). Injection of $CoCl_2$ into structures surrounding the BNST, such as anterior commissure, internal capsule or fornix, before the pretest (173 ± 11 vs 182 ± 23 s, $t_5 = 0.385$, $P > 0.05$) or the test (189 ± 19 vs 162 ± 12 s, $t_6 = 1.244$, $P > 0.05$) did not affect immobility time in the FST.

Experiment two: effects of $CoCl_2$ injection into the BNST of rats submitted to the open field test

Analysis of total distance travelled in the open-field test did not show a significant effect of BNST treatment with $CoCl_2$ (14 ± 2 vs 15 ± 3 m, $t_{10} = 0.27$, $P > 0.05$), when compared with animals treated with vehicle (Figure 3).

Discussion

The FST is probably the most frequently used animal model predictive of antidepressant activity [45]. It is based on the observation that rodents exposed to an enclosed cylinder filled with water perform escape oriented behaviors for few minutes and, afterwards, assume a posture of immobility which is of shorter duration in animals that had received antidepressant treatment [45,47,48]. In the present study, the results showed that intra-BNST injection of $CoCl_2$ decreased the immobility time in this model when microinjected before pretest or before test, whereas treatment after pretest did not have any effect. These effects do not rely on unspecific motor changes, since $CoCl_2$ administration into the BNST did

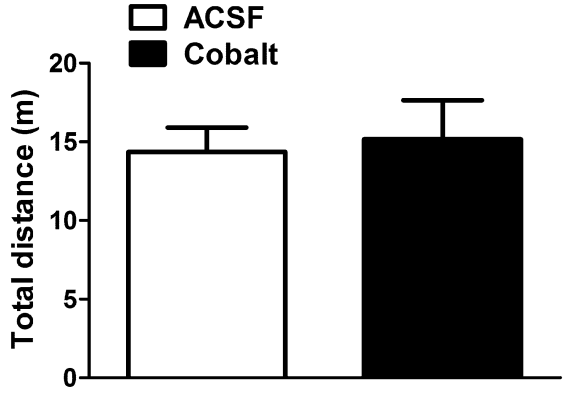

Figure 3 Total distance travelled by rats exposed to the open field test shown by animals treated with vehicle (ACSF, white bar, n = 6) or $CoCl_2$ (cobalt, black bar, n = 6) into the BNST. Intra-BNST injection of $CoCl_2$ (1 mM/100 nL) before test did not modify the rat locomotor activity in the open field test ($P > 0.05$, unpaired *Student*-t test).

not modify animal's locomotor activity in the open field test. Therefore, these results are indicative of an antidepressant-like effect induced by transient blockage of synaptic transmission in the BNST during the pretest or test sessions in the rats submitted to the FST.

Contradictory results regarding BNST role in animal models of depression have been previously described [39-41]. It has been reported, for example, that chronic electrolytic lesion of the BNST increases rather than decreases the behavioral consequences produced by the FST [38-40]. However, chronic electrolytic lesion destroy not only intrinsic BNST neurons, but also fibers of passage that project through it on their way to other structures. On the other hand, chemical lesions of the BNST, which spares fibers of passage, induced antidepressant-like effects in the rat learned helplessness model of depression [19]. The present study, by inducing a reversible inactivation of synaptic transmission that spares fibers of passage, corroborates the previous results in the learned helplessness model and suggests that this experimental approach ($CoCl_2$-induced inactivation) could be more useful to unveil the specific role of BNST neurons in FST model.

It should be noticed, however, that inconsistent findings regarding BNST participation in the modulation of depressive-like behaviors could be related to the fact that BNST is a cluster of 12 nuclei, which can be divided into anterior and posterior subdivisions, each containing several nuclei, which differ in their projection pattern and neurochemical identity [52]. In this regard, depending on the extension of the lesion or the drug distribution into BNST, different portions of it could have been affected in different studies, thus allowing the occurrence of contradictory behavioral effects in the FST.

The BNST has long been recognized as an important structure that integrates and mediates emotional, cognitive, autonomic, endocrine and behavioral responses to stress [2,6,9,10,14,20,51,53]. Lesions as well as pre-test infusions into the BNST of compounds that disrupt its function reduce behavioral and autonomic responses to stress [10,16,51,54,55]. This is especially evident in paradigms in which behavior is influenced by long-duration stimuli and in paradigms that assess the persistent behavioral effects of even a brief stressor, but that are severe and unpredictable, such inescapable shocks [5]. Corroborating this proposal, the BNST is activated in response to several aversive stimuli [15-18] including inescapable shock exposure [16]. BNST stimulation, on the other hand, produces behavioral consequences similar to those induced by forced restraint [14] whereas its chemical lesion prevents the development of behavioral deficits that characterize the "learned helplessness" phenomenon [19]. These deficits are thought to arise from increased fear and anxiety produced by the previous exposure to inescapable shocks [56,57]. Finally, blockade of BNST noradrenergic transmission attenuates immobilization stress-induced anxiogenic-like effects [54,55,58].

Taken together, these pieces of evidence indicate that activation of BNST during stress could contribute to the development of stress-induced behavioral consequences, thus impairing adaptation in a subsequent stressful situation. In this way, BNST activation during stress pre-exposure could facilitate a hyperanxiety state that would impair adaptation to a subsequent stress exposure. This is supported, for example, by the observation that the positive effects of BNST lesions in the learned helplessness model are due to a reduction in the anxiogenic effects of pre-exposure to the inescapable shocks [19]. Moreover, behavioral manipulation that prevents learned helplessness development also reduces the anxiogenic effect and the increased BNST Fos expression caused by the previous exposure to inescapable shocks [16]. Finally, stress-induced hyperanxiety have been shown to occur in association to structural and functional changes in BNST [21-24], thus supporting the involvement of this nucleus in mechanisms of stress-induced emotional consequences.

Failure to coping with stress is an important precipitating factor in depressive illnesses [27,35,36] and antidepressants promote behavioral adaptation to stress [59]. In this context, blockade of synaptic transmission within the BNST before pre-test could reduce the stress-induced behavioral outcomes (e.g. hyperanxiety) and, thus, facilitate adaptation to the subsequent stress section, inducing antidepressant-like effects [19]. Moreover, considering that BNST is uniquely positioned to receive emotional and learning associated informations and to integrate these into the reward/motivation circuitry [43], its inactivation before the test might have induced antidepressant-like effect by increasing motivation and goal-directed behavior to aimed at performing escape from the swimming stress.

It could also be speculated that the reduced immobility observed during the test could have been a consequence of learning and memory impairments induced by BSNT inactivation during pre-test and test, respectively. However, previous results from the literature showed that BNST lesions did not impair navigational learning and memory in the Morris water Maze [40], thus questioning the aforementioned suggestion. Despite that, the antidepressant-like effect reported after BNST blockage in the present study cannot be completely dissociated from effects in the cognitive performance. In fact, cognitive mechanisms have been implicated in the neurobiology of depression and antidepressant response, since they might interfere with stress adaptation and biases in the processing of negative affect [60].

Finally, it can be speculated that the deleterious effects of stress could also be mediated, at least in part, by dys-

regulation (e.g., overactivation) of the HPA axis [61]. Activation of the HPA axis is a primary mechanism for maintaining homeostasis in response to stress. Neurons in the paraventricular nucleus of the hypothalamus (PVN) synthesize corticotropin-releasing hormone (CRH), which is released into the hypophysial portal system and trigger adrenocorticotropin (ACTH) secretion from the anterior pituitary. ACTH stimulates the secretion of glucocorticoids from the adrenals into the circulation to mobilize energy stores, maintain blood pressure, and exert negative feedback at the HPA brain and pituitary sites (for review, see [9]). Glucocorticoids secretion needs to be tightly regulated since prolonged exposure to their effects can lead to serious metabolic, immune, and psychological dysfunction. The BNST has a central role in controlling HPA axis activity [6,28,30], which can be dysfunctional in depression [31-34]. Considering that glucocorticoids facilitate whereas adrenalectomy impairs the expression of the depressive-like behavior in the FST [62-64], it is also possible that BSNT inactivation might have attenuated HPA axis activation in response to stress, and the consequent reduction in the glucocorticoid levels could have contributed to the reduced expression of the depressive-like behavior in the FST. This hypothesis, however, warrants further investigation.

Limitations

Considering that distinct neurobiological mechanisms can be involved in the different experimental procedures used to study the neurobiology of depression, the hypothesis discussed herein should be further tested in other animal models of depression with higher face validity than the FST.

Conclusion

In conclusion, stress-induced BNST activation could promote a bias in the processing of threatening cues which could render the animal more susceptible to the development of behavioral consequences of stress. On the other hand, BNST inactivation before stress could protect animals against emotional changes caused by previous stressful stimuli presentation, perhaps by facilitating mechanisms involved in the ability to cope with a new stressful situation. Further studies are necessary to characterize the neurotransmitters involved in these effects.

List of abbreviations used

ACSF: artificial cerebrospinal fluid; ACTH: adrenocorticotropin hormone; BNST: bed nucleus of the stria terminalis; $CoCl_2$: cobalt chloride; CRH: corticotropin-releasing hormone; FST: forced swimming test; HPA: hypothalamo-pituitary-adrenal; MPFC: medial prefrontal córtex; PVN: paraventricular nucleus of the hypothalamus; PTSD: posttraumatic stress disorder.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

C.C.C. and S.R.L.J. contributed to the conception and design of the study. Moreover, S.R.L.J. was responsible by analysis and interpretation of data, drafted the manuscript and continuously supervised the study. C.C.C. and F.H.F.A. were responsible for data collection and helped to draft the manuscript. F.S.G. and F.M.A.C. helped to draft the manuscript and continuously supervised the study. All authors read and approved the final manuscript.

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