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Positive psychological effects of seated acupressure massage are associated with a rise in plasma oxytocin without affecting CGRP levels or circulating IL-6

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A R T I C L E I N F O

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A B S T R A C T

Work-related stress is a major public health issue. Given the relationship between acute stress responses and health, finding strategies to deal with the unpleasant symptoms brought on by stress is essential. Massage therapy is a popular stress-reduction technique, but its effectiveness has yet to be shown. In that matter, this study investigates the effects of a 17-minute session of seated Amma massage on young healthy people. Subjective stress perception, anxiety and self-confidence were assessed before and after the massage using the Spielberger State Anxiety Scale (STAI-Y, Spielberger et al., 1983) and the Competitive State Anxiety Inventory (EEAC, Cury et al., 1999), together with cardiovascular parameters. Cortisol, CGRP, IL-6, and oxytocin plasma levels were measured before and after the massage to investigate its possible mode of action. This study enrolled 59 people: 33 receiving the massage, and 26 controls only seated on the massage chair.

Interaction Time x Group demonstrates significant differences for all psychological measurements (STAI, EEAC) before and after the Amma massage, showing a beneficial effect of this treatment, in particular on perceived anxiety and self-confidence. No evidence was found of any correlation between cortisol plasma levels and psychological outcomes. No relationship was shown between the decrease of perceived stress and measured CGRP or IL-6 release, but the data demonstrated that heart frequency could be slightly decreased. The oxytocin plasma levels were significantly increased by the massage and could be responsible for the recovery of psychological outcomes.

We conclude that seated acupressure Amma massage could be a useful tool to ameliorate quality of life at work.

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1. Introduction

Quality of work life in our occidental societies is a major preoccupation for many companies. In fact, an often-stated goal is to reduce the feeling of stress at work, which causes tiredness, muscular tensions, lombalgia, sleeping problems, anxiety, decrease of alertness, and is linked with cardiovascular diseases incidence [1]. Stress at work in France is responsible for 50–60 % of the illness-caused absences and is the primary cause of work interruption. According to WHO, France is the country that rates third in the world for the number of recorded depressed employees. Stress also affects students who are more and more faced to organizational difficulties and apprehension of exams [2–4].

Given its detrimental effects on the human body, finding ways to deal with stress is crucial nowadays. In the wellness area, massages are a well-known means of achieving relaxation. However, although it was recently described that oxytocin (OT) is released upon hand administrated massage of feet [5] for example, only few evidence supporting the effect of massages on the stress physiological and psychological response exist. For this reason, we decided to test whether a massage session could improve self-confidence and decrease anxiety symptoms, while attempting to establish a relationship between psychological outcomes and some physiological parameters related to the stress response. The Amma massage was chosen because it fits well with our scientific purpose. In fact, an Amma massage session consists in a precise protocol of sequential pressure on specific points at different places on the upper body. These points correspond to the traditional acupuncture points of the Chinese medicine; some of them being situated above autonomic nervous system ganglions. These ganglions and nerve terminals contain the neuropeptide CGRP that could be released upon pressure of the skin. As stress is known to influence physiological as well as psychological parameters, different indexes of the stress response can be investigated. The hypothalamic-pituitary-adrenal (HPA) axis is involved in the regulation of neuroendocrine responses to stress. Its activation triggers via CRF and ACTH the production of the "stress hormone" – cortisol by adrenal gland, but also involved the inflammatory cytokine IL-6 that is produced both by muscles, brain and adrenal cortex [6]. To examine the first line of response to stress, namely the cardiovascular system, measurements of heart frequency and systolic/diastolic pressure can be obtained. The plasmatic level of the neuropeptide CGRP is also interesting as it is released by sympathetic and parasympathetic nervous system activity, and is also involved in vasodilatation [7] and heart rate control. Indeed, end nerves containing GGRP are largely distributed around veins [8,9] and arteries, together with internal ducts [10] and skin [11], and is released in peripheral blood [12]. Thus, it could be hypothesized that pressure of specific acupoints could stimulate ganglions and nerves terminals to release this neuropeptide involved in pain together with cardiovascular modulation (see Fig. 1) Another way of investigating the stress response is through variations of plasmatic levels of oxytocin (OT) and interleukine 6 (IL-6). Indeed, OT is a cyclic nonapeptide involved in many social behaviors and is associated with trust, attachment, and wellbeing in humans [13], and IL-6 is a pleiotropic inflammatory cytokine, secreted upon infections [14], implicated in inflammation [15], but also recently reported to play a role in psychological stress [16–18] and possibly driving a central inflammation inducing depression [19].

In this study, to determine whether an Amma massage could improve physiological and psychological parameters, Heart Rate (HR) together with diastolic and systolic pressure, and plasma levels of CGRP, Oxytocin (OT), cortisol, and IL-6 (see Fig. 1), were measured before and after a 17-min Amma massage in a group of young healthy people. All volunteers also filled different forms for stress evaluation before and after the massage session. We hypothesized that due to its benefits on wellness, the group who received the Amma massage would present better recovery pattern than the control group, such as better score on the stress questionnaires, lower cortisol and IL-6 concentration levels, higher CGRP and oxytocin levels, and better heart rate parameters.

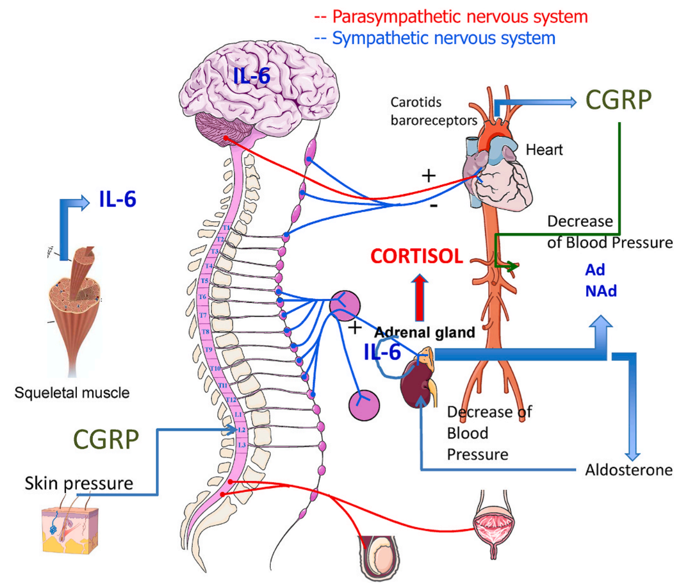

Fig. 1. Interplay between the different physiological parameters and biomarkers investigated. We hypothesized that massage could modify these different circulating mediators through its stimulatory action of the autonomic nervous system.

2. Material and methods

2.1. Participants

Volunteers were recruited between December 1st, 2019, and February 28th, 2020, principally among students from Biological Sciences section of the Université de Bourgogne. All participants were informed about the study and signed an agreement form according to the ethics procedure. This study was part of a research option offered to bachelor student enrolled at the Université de Bourgogne. The Université de Bourgogne scientific council approved the research. The study was submitted to the French authorities ANSM and received the N° ID RCB 2022-A01282-41 (MACBET).

A questionnaire was used to collect socio-demographic data such as age, education, medicine intake, nicotine use, psychoactive substance use, alcohol use, medical history, and diseases (for more information see Table 1). Due to their confounding influence on the human body in general, and the HPA axis in particular, exclusion criteria for the current study were the following: psychiatric diseases, heart disease, epilepsy, corticoid treatments and medical treatments for anxiety and depression.

Table 1
Sample characteristics (number of people) based on their response on the socio-demographics questionnaire.

Socio-demographics characteristics	AMMA group (n = 33)	Control group (n = 26)	Total (n = 59)
Sexe:			
Female	28	17	45
Male	5	9	14
Consumption:			
Tabacco	10	4	14
Alcohol	2	1	3
Sport:			
<1 h/week	10	12	22
1–3 h/week	14	12	26
>3 h/week	9	2	11
Contraception use:			
Hormonal	18	10	28

In the end, 33 participants in the AMMA massage group (28 female, M = 22.03 years old ± 2.19) and 26 in the control group (17 female, M = 22.46 years old ± 3.49) were included in this study.

2.2. Massages

Massages were all practiced by the same Amma massage professional, teacher of FEDEFMA. Massages were performed on a LUDION chair in an individual room. For 17 minutes, the back, the neck and the arms were repetitively pressed using a precise sequence according to the David Palmer method. In the control group, the participant sat in the same position as the massaged volunteers, doing nothing and awaiting 17 minutes.

2.3. Experimental design

Upon arrival and prior to physiological recording, participants were informed about the course of the study but were not given detailed information about the experimental paradigm nor the detailed conditions in the recovery phase. Then, they signed an informed consent in duplicate explaining the general procedure and filled out the socio-demographic questionnaire. Afterward, each participant had his cardiovascular parameters taken and blood drawn for the first phase (before the experimental intervention). Once the physiological pre-test measures were taken, participants filled out the different questionnaires to assess psychological parameters (STAI-Y and the EEAC). They were then randomized and dispatched to one of the rooms to receive or not receive the Amma session. After 17 minutes, they again completed the psychological forms (STAI-Y and the EEAC) for post-test measures, got a physiological assessment of their cardiovascular parameters, and a second tube of blood was drawn. When every form was completed and measures taken, participants were thanked and received a 15€-gift voucher.

2.4. Psychological tests

We evaluated Profile of Mood States for each participant using two different psychological measures: The State and Trait Anxiety Inventory (STAI-Y) and the Competitive State Anxiety Inventory – 2 (EEAC).

2.5. The State and Trait Anxiety Inventory (STAI-Y)

The State and Trait Anxiety Inventory (STAI-Y) [20] Form A was used to control for the effect of anxiety as a personality trait in our group. Meanwhile, Form B of this questionnaire was used to assess possible changes in anxiety during the experiment. The STAI consists of two 20-items questionnaires which measure respectively state and trait levels of anxiety in clinical and non-clinical populations. Each scale consists of 20 questions and is based on a 4-point Likert scale. Scores for both scales range between 20 (low anxiety) and 80 (high anxiety). The intensity of the anxiety state increases with the score. Thresholds used in the French literature are as follows: low, less than 46; moderate, between 46 and 55; and high, greater than 55.

2.6. The Competitive State Anxiety Inventory – 2

A French version of the Competitive State Anxiety Inventory – 2 (CSAI-2, [21]), the EEAC from Cury et al. [22] was adapted to our situation (i.e., used without competitive goal). The EEAC consists of a 23-item questionnaire that assess the intensity of cognitive anxiety (i.e., negative expectations and concerns about one performance), somatic anxiety (i.e., physiological manifestation of anxiety), and self-confidence (i.e., one's belief in their ability to be successful). Scores range from 7 to 28 for the two first subscales and from 9 to 36 for the self-confidence one. High scores are indicative of high anxiety and high self-confidence.

2.7. Cardiovascular parameters

Heart rate (HR) together with diastolic and systolic pressures were measured electronically using an arm blood pressure monitor (Terraillon®). Three measurements of HR and Pressures were conducted within 6 min (every 2 min) before the first and the last blood sampling.

2.8. Blood collection and plasma CGRP, IL-6 and OT assays

Blood was collected from a cubital vein using EDTA-K tubes (S-Monovetten, Sarstedt, Germany) refrigerated and centrifuged at 4 °C for 5 min at 3000 rpm within 1 h after the sampling. Supernatant (plasma) was immediately aliquoted (by several 50, 100 and 500 μL volumes) in eppendorfs and stored to -20 °C until analysis.

CGRP levels were measured using sandwich ELISA (Bertin Bioreagents, France). 100 μL plasma samples did not allowed detection in the range of the reference curve. Thus we used the assay following extraction of larger amount of plasma. Briefly, 500 μL extemporaneously defrosted plasma was mixed with 500 μL acetone and serum proteins precipitated at −20 °C overnight. Extraction was performed by a 5 min 15 000 rpm centrifugation of the eppendorfs. Supernatants were concentrated under a vacuum evaporator and suspended again in 100 μL of EIA buffer to perform the assay. The theory sensitivity for the assay is 10 pg/mL. The quantities measured in the different samples were all comprised between 20 and 50 pg/mL, and thus considered to be under the limit to be correctly interpreted.

Oxytocin (OT) levels were measured using Caiman® EIA kit for human plasma, a competitive assay between Oxytocin and Oxytocin-acetylcholinesterase conjugate with a sensitivity of 20 pg/mL (distributed by Bertin Technologies, France). 100 μL plasma samples were found out of range of the reference curve. Thus 700 μL were mixed with 700 μL acetone and serum proteins precipitated at −20 °C overnight, then centrifuged 5 min, 15 000 rpm for OT extraction. Supernatants were concentrated under a vacuum evaporator and suspended again in 100 μL of EIA buffer to perform the assay.

IL-6 levels were estimated in 50 μL plasma using Caiman® human Interleukin-6 ELISA kit distributed by Bertin Technologies, France. The sensitivity for the assay is 7.8 pg/mL. All samples were found in the range of the reference curve.

2.9. Plasma cortisol measurements

For cortisol measurements, blood was collected in BD Vacutainer®SST (TM) II Advance tubes. Cortisol analyses were carried out by BioMed 21 Laboratories (Dijon, France) using enzymatic chimioluminescence on a DXI 800 Beckman Coulter and a standard curve with 6 calibrates from 2 μg/dL to 60 μg/dL. The sensitivity of the assay was 0.4 μg/dL (11 nmol/L). All measurements were in the range of the assay.

2.10. Statistical analyses

We checked whether the experimental groups were homogenous with respect to possible confounding variables (sex, age, BMI, smoking, alcohol consumption, physical activity, use of hormonal contraceptives, and anxiety-trait scores) using χ^2 for class variables and one-way ANOVAs for continuous variables. One-way ANOVAs were also used to make sure that the initial values (at t0) of the variables of interest did not differ between experimental groups.

The distributions of cortisol and IL-6 concentration in plasma were highly right-skewed; therefore, these two variables were log-transformed to reduce skewness. Covariation among traits (both physiological and psychological variables) at t0 was assessed using Pearson's correlation coefficients (to check any possible redundancy between the measured variables).

The effect of massage on the different response variables was assessed using general linear mixed models with a normal distribution of errors. For each model, the fixed effects included the experimental group (control *vs* massage), time (t0 *vs* t1), and the two-way interaction. The interaction between time and experimental group addresses the main question under investigation here (whether massage has an effect on changes in physiological and psychological outcomes over time). The models also included a random intercept for each individual included in the experiment, which allows taking into account the repeated measurements for each subject (pre- and post-massage). Post-hoc comparisons of LS-means were also done with a Bonferroni adjustment for multiple tests. Degrees of freedom were estimated using the Satterthwaite approximation. The analyses were performed using SAS (9.4).

3. Results

3.1. Confounding factors

The distribution of potentially confounding factors was homogenous between experimental groups. In particular, the proportion of males and females, smokers, alcohol consumption, physical activity, and contraceptive use did not differ between experimental groups. Groups were also homogenous in terms of age, BMI and anxiety-trait scores.

The initial values (at t0) of the physiological and psychological outcomes did not differ between experimental groups, confirming that the subjects were randomly allocated to the groups.

3.2. Correlations among response variables

Except for diastolic and systolic blood pressures which were strongly correlated (r = 0.708, n = 59), the covariation between the other physiological traits was weak (ranging from −0.131 to 0.361). Overall, the psychological outcomes showed a strong pattern of covariation. In particular, anxiety-state scores were highly correlated with EEAC sub-scores, respectively on the self-confidence sub-scores (r = −0.782, n = 59) and somatic anxiety sub-scores (r = 0.757, n = 59), suggesting that these variables somehow carry redundant information.

3.3. Changes in physiological traits in response to massage

We used general linear mixed models to assess the effect of massage on each of the physiological and psychological outcomes measured. In each model, we tested the interaction between time and treatment (massage vs control) as evidence of an effect of massage on the response variable. Among the six physiological traits analyzed, only heart rate and plasma oxytocin changed over time differently for subjects in the massage compared to the control group (Table 2, Fig. 2). In particular, while heart rate overall declined between the two measurements the decrease was more pronounced for the control group (Fig. 2A). Massage had a clear effect on changes in oxytocin, since the plasma concentration increased in subjects included in the massage group, whereas the concentration decreased in the control group, resulting in a statistically significant interaction between time and treatment (Fig. 2B). However, note that post-hoc comparison of LS-means did not show any significant difference between groups.

3.4. Changes in psychological outcomes in response to massage

Concerning the four psychological outcomes, the models returned statistically significant Time x Experimental group for three of them (only for somatic anxiety scores on the EEAC the interaction was not significant). In all cases, the changes over time were consistent with the prediction that the massage reduced the level of perceived stress and anxiety and increased self-confidence (Table 3, Fig. 3). None of these interactions are due to initial differences between groups, since subjects included in the two experimental conditions had similar initial values for all psychological outcomes (all p's > .05) .

Table 2
Effect of experimental group on six physiological traits. For each response variable, we report the effect of time (t0 vs t1), experimental group (massage vs control), and the two-way interaction (fixed effects) and subject ID (random effect). For each fixed effect we report the F statistic, the degrees of freedom, the p value (***$p < 0.001$; **$p < 0.01$; *$p < 0.05$) and the parameters estimate (with standard errors). Time *1* and group *Massage* were set as reference, therefore positive estimate of Time indicates that the response variable decreased between the two measurements, and positive estimate of experimental group indicates that control have higher values of the response variable.

Response variable				
Systolic blood pressure (N = 59 subjects and 118 observations)				
Fixed effects	*F*	*df*	*p*	*estimate (SE)*
Time	11.25	1,57	0.0014**	4.21 (1.80)
Experimental group	0.00	1,57	0.9879	−0.38 (3.31)
time x experimental group	0.06	1,57	0.8050	0.67 (2.71)
Random effect	*Z*	*p*		
Subject ID	4.17	<0.0001***		
Diastolic blood pressure (N = 59 subjects and 118 observations)				
Fixed effects	*F*	*df*	*p*	*estimate (SE)*
Time	7.14	1,57	0.0098**	2.97 (1.24)
Experimental group	0.49	1,57	0.4863	−0.87 (0.69)
time x experimental group	0.27	1,57	0.6042	−0.97 (0.60)
Random effect	*Z*	*p*		
Subject ID	4.00	<0.0001***		
Heart rate (N = 59 subjects and 118 observations)				
Fixed effects	*F*	*df*	*p*	*estimate (SE)*
Time	25.89	1,57	<0.001***	3.21 (1.50)
Experimental group	0.88	1,57	0.3530	−0.39 (2.56)
Time x Experimental group	5.07	1,57	0.0283*	5.10 (2.26)
Random effect	*Z*	*p*		
Subject ID	3.94	<0.0001***		
Cortisol (N = 59 subjects and 116 observations)				
Fixed effects	*F*	*df*	*p*	*estimate (SE)*
Time	9.65	1,54.9	0.0030**	0.08 (0.04)
Experimental group	0.00	1,56.7	0.9972	−0.02 (0.13)
Time x experimental group	0.42	1,54.9	0.5211	0.04 (0.06)
Random effect	*Z*	*p*		
Subject ID	4.93	<0.0001***		
IL-6 (N = 59 subjects and 115 observations)				
Fixed effects	*F*	*df*	*p*	*estimate (SE)*
Time	0.32	1,54.5	0.5722	0.14 (0.09)
Experimental group	0.15	1,57.1	0.7009	0.20 (0.26)
time x experimental group	2.37	1,54.5	0.1292	−0.20 (0.13)
Random effect	*Z*	*p*		
Subject ID	4.96	<0.0001***		
Oxytocin (N = 59 subjects and 116 observations)				
Fixed effects	*F*	*df*	*p*	*estimate (SE)*
Time	0.20	1,55.8	0.6603	−1.82 (0.94)
Experimental group	0.42	1,57.3	0.5203	−2.68 (1.94)
Time x Experimental group	4.44	1,55.8	0.0396*	3.00 (1.43)
Random effect	*Z*	*p*		
Subject ID	4.48	<0.0001***		

On the State-Trait Anxiety Scale, the mean trait anxiety score was 46.3 ± 10.4 for the control group and 44.0 ± 10.1 for the Amma group. Results are consistent with normed values for French college students (45 ± 11) and confirmed that all participants were in an emotionally stable state [23]. With regards to the STAI-state scores, results showed

Table 3
Effect of experimental group on four psychological outcomes. For each response variable, we report the effect of time (t0 vs t1), experimental group (massage vs control), and the two-way interaction (fixed effects) and subject ID (random effect). For each fixed effect, we report the F statistic, the degrees of freedom, the *p* value (****p* < 0.001; ***p* < 0.01; **p* < 0.05) and the parameter estimate (with standard errors). Time *1*, and group *Massage* were set as reference, therefore positive estimate of Time indicates that the response variable decreased between the two measurements, positive estimate of experimental group indicates that control have higher values of the response variable.

Response variable				
STAI-status scores (N = 59 subjects and 118 observations)				
Fixed effects	*F*	*df*	*p*	*estimate (SE)*
Time	85.50	1,57	<0.0001***	9.70 (1.14)
Experimental group	1.22	1,57	0.2746	4.19 (2.39)
Time x Experimental group	4.05	1,57	0.0489*	−3.47 (1.72)
Random effect	*Z*	*p*		
Subject ID	4.49	<0.0001***		
Self-confidence (EEAC) (N = 59 subjects and 117 observations)				
Fixed effects	*F*	*df*	*p*	*estimate (SE)*
Time	25.76	1,56.4	<0.0001***	−3.55 (0.64)
Experimental group	0.00	1,57.2	0.9972	−1.08 (1.52)
Time x Experimental group	5.05	1,56.4	0.0286*	2.18 (0.97)
Random effect	*Z*	*p*		
Subject ID	4.71	<0.0001***		
Cognitive anxiety (EEAC) (N = 59 subjects and 117 observations)				
Fixed effects	*F*	*df*	*p*	*estimate (SE)*
Time	6.46	1,56.3	0.0138*	2.27 (0.54)
Experimental group	0.27	1,57.2	0.6041	1.97 (1.49)
Time x Experimental group	8.56	1,56.3	0.0047**	−2.44 (0.83)
Random effect	*Z*	*p*		
Subject ID	4.88	<0.0001***		
Somatic anxiety (EEAC) (N = 59 and 117 observations)				
Fixed effects	*F*	*df*	*p*	*estimate (SE)*
Time	47.92	1,56.7	<0.0001***	4.24 (0.74)
Experimental group	0.64	1,57.1	0.4257	1.02 (1.02)
time x experimental group	0.37	1,56.7	0.5468	−0.68 (1.13)
Random effect	*Z*	*p*		
Subject ID	2.73	0.0029*		

that when considering the Time x Experimental group interaction (Table 3, Fig. 3A), the difference between pre- and post-scores was significant ($F_{1,57} = 4.05$, $p = 0.0489$) indicating a positive effect of Amma massage on well-being.

On the EEAC, the mean pre-test cognitive anxiety score was 14.2 ± 6.3 for the control group, and 14.7 ± 5.2 for the Amma group. The post-test cognitive anxiety score was respectively 14.4 ± 6.3 for the control group and 12.4 ± 6.3 for the Amma group. With regards to the Time x Experimental group interaction, the difference between pre- and post-scores was significant ($F_{1,56.3} = 8.56$, $p = 0.005$) indicating a positive effect of Amma massage on subjective feeling of anxiety (Table 3, Fig. 3C). We found similar results (Table 3, Fig. 3B) with self-confidence, indicating a significant difference between pre- and post-test scores for the interaction Time x Experimental group ($F_{1,56.4} = 5.05$, $p = 0.028$), which reflected a positive effect of AMMA massage on boosting self-confidence.

4. Discussion

In this study, we investigated the short-term psychological and physiological effects of one 17-minute session of Amma massage, originated from traditional Asiatic medicine. In this experiment we selected a precise sequence of pressured points corresponding to the neck, the back and the arms of the patients, that are associated to traditional meridians of Chinese acupuncture technique. Although our study took place in Dijon (Burgundy) during 2020 winter and one of COVID-19 confinement period, the mean level of stress of the population studied here was 45.15 on the Spielberger scale Y-A (Trait Scale), corresponding to a moderately stressed population. Nevertheless, using a single 17-minute session of Amma massage, we succeeded in significantly reducing perception of stress, anxiety and in increasing self-confidence in volunteers. Previously, another study already demonstrated that eight sessions of 15-minute Amma massages during four weeks had a positive effect with regard to the reduction of pain in the lower and upper spine, and the arms of polish workers [24]. The present data indicates that this positive effect upon somatic anxiety is already present after a single session. Our findings corroborate the results of Meier and collaborators [25] in showing that massages significantly increase subjective relaxation while decreasing subjective stress. These results highlight the potential of short relaxation periods to reduce psychological tension and increase subjective relaxation.

In addition, we also investigated the effect of an Amma massage session on cardiovascular parameters. A significant decrease of heart rate, together with systolic pressure, was observed in both groups. Although the decrease was more pronounced for the control group, that unfortunately had a higher initial heart rate compared to subjects included in the massage group, which could explain our observation. In the same vein, the pilot study by Day and collaborators [26] investigating long term massage therapy on 14 people in their working place also demonstrated decrease of blood pressure. Heart frequency, as well as diastolic and systolic pressure, are mainly regulated by the autonomic nervous system (ANS), and more precisely by the interplay between the sympathetic and parasympathetic nervous systems. In view that these systems were probably stimulated by the massage, the effects are consistent with the physiological rules. However we were not able to evidence release of CGRP upon the massage session, possibly due to methodological limitations.

We did not observe significant modifications of the measured cortisol plasma levels induced by the massage, similarly, to several other papers

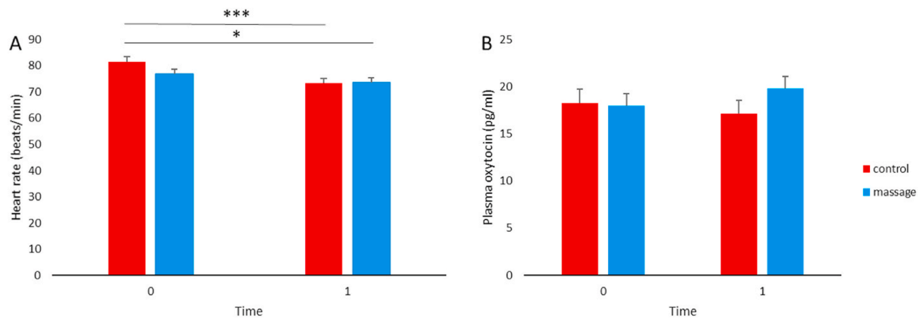

Fig. 2. Changes in heart rate (beats/min) (A) and oxytocin concentration in plasma (pg/ml) (B) as a function of time and experimental group. Time *0* refers to the pre-manipulation value and time *1* to the post-manipulation value. Red and blue bars refer to control and massage, respectively. We report the Least Squares Means (±SE) of the general linear mixed models. LS-means connected by bars are statistically different after a Bonferroni correction for multiple tests (***$p < 0.001$; **$p < 0.01$; *$p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

dealing with other massage protocols [27,28]. Furthermore, a previous preliminary study on Amma therapy performed on 15 healthy women already demonstrated that a 40-minute massage session was more efficient than a 40-minute rest in reversing perceived stress scores for muscle stiffness in the neck and shoulder and the state anxiety score but did not affect salivary cortisol secretion levels [29]. The positive effect of massage on cortisol levels reported by Rapaport and collaborators [30] could be due to the fact that measurements were performed after five weeks of massage rather than in a brief span of time as in our study. However, our data report a correlation between decrease of cortisol levels and decrease of heart frequency. This has apparently not yet been reported and must be probably further investigated. The only mentioned fact was that heart rate and cortisol level were significantly higher in men with high systolic blood pressure [31]. None of the more recent papers indicates a link between cortisol and cardiovascular parameters at our knowledge except that increased hair cortisol concentration has been linked with established cardiometabolic risk factors for cardiovascular disease including high blood pressure [32]. Even so, a linear relationship with measures of plasma ACTH and cortisol in blood or urine does not necessarily exist [33], because the different instances that control HPAA reactivity (hippocampus, hypothalamus, pituitary, adrenals) and their respective modulators, receptors, or binding proteins, may all affect salivary cortisol measures.

It is reported that acute mental stress in humans elicits increase in interleukin 6 (IL-6) serum concentration [18,34]. More recent works linked IL-6 levels and depressed mood to an inflammation at the brain level. Our data do not report any significant decrease in the IL-6 plasma levels after the massage. The half-life of circulating IL-6 in rats is around 20 minutes [35]. Thus, fluctuations of plasmatic levels should be rapid. In a normal context, IL-6 is mainly produced by muscular exercise and thus the more active the workers, the more IL-6 is produced [36,37]. It could be hypothesized that muscle cramping occurring during stress could be a stimulator for this inflammatory cytokine release. A 10-minute massage was described to attenuate the production of IL-6, together with TNF-α, released by injured myofibers after exercise-induced muscle damage for 2.5 h [38]. It was also shown that acupuncture or electro-acupuncture decreased blood inflammatory cytokine, including IL-6 [39,40] in rheumatoid arthritis patients.

With regard to oxytocin, our study demonstrates that a single 17-minute Amma massage significantly increased its plasma levels. First described for its role in lactation [41,42], OT receptors were then found in the brain of young rats with a changing pattern according to their developmental state [43]. More recently, OT was found to be involved in human social behaviour [44], with OT basal plasma levels being reduced in disorders with social dysfunction [45,46]. Other evidence show that basal oxytocin plasma concentrations are reduced in people with social cognition deficit and that OT nasal sprays can reduce anxiety, autism, and schizophrenia [44]. Our results come in line with previous literature. In 2008, Bello and collaborators [47] showed that a 20-minute massage, as well as a 20-minute reading session (control), elicited an OT release. Morhenn and collaborators [48] also showed an OT increase after a 15-min moderate pressure massage of the upper back. Our data together with those of Morhenn indicate that OT release is rapidly induced after the massage. This neuropeptide is the only biological parameter investigated here that was clearly modified by the massage. Hence, we hypothesize that this slight OT increase could act at the brain level to promote the well-being sensation, as suggested by Uvnäs-Moberg in her previous works [49,50], and could be responsible for the observed recovery of psychological outcomes in our study.

5. Limitations

While our study provides valuable insights into the potential benefits of seated Amma massage on stress in young healthy individuals, it is essential to acknowledge certain limitations that stem from practical constraints, and may impact the interpretation of our findings.

First, with regards to the cardiovascular parameters, it might be argued that the differences evidenced in our study at the HR level are low. In fact, as suggested by Mulcahy and collaborators [51], Heart Rate Variability (HRV) indices also regulated by the ANS, could be a more accurate measure than HR to understand emotional states experienced by individuals. Unfortunately, we were not equipped for these measurements. Further research should take into account this measurement. Furthermore, CGRP plasma levels are critical to measure due to possible rapid degradation of the peptide [52] and could be also reinvestigated using other approach.

Although cortisol is very often proposed as a stress marker [33,53], we did not find any evidence of a relationship between the stress level estimated by the Spielberg questionnaire (Trait or State scale) or with the cortisol level upon arrival of the volunteers. Cortisol plasma levels were between 100 and 650 pmol/L corresponding to a broad dispersion among participants at the beginning of the experiment. This could be due to the pulsatile secretion of cortisol. Although it didn't show on the statistical analyses, it was also described that cortisol levels in women with oral contraceptives were twice those of women without [54]. Therefore, more consideration to the sample characteristics should be done.

Levels of IL-6 apparently fluctuate rapidly. Upon infection, plasma IL-6 levels increase within 1 h, correlating with the fever increase [14]. Furthermore, half-life is short [35]. Our data report the measurement of IL-6 plasma levels within a 45–60 minute period and did not evidence significant differences. However, the basal level of IL-6 was probably too low to clearly demonstrate a decrease. It was probably easier to evidence an effect on patients with rheumatoid arthritis or muscular damages in whom IL-6 basal levels are certainly much higher than in healthier people.

Lastly, a significant limitation in our study was the design choices due to financial constraints. The lack of an active control group engaged

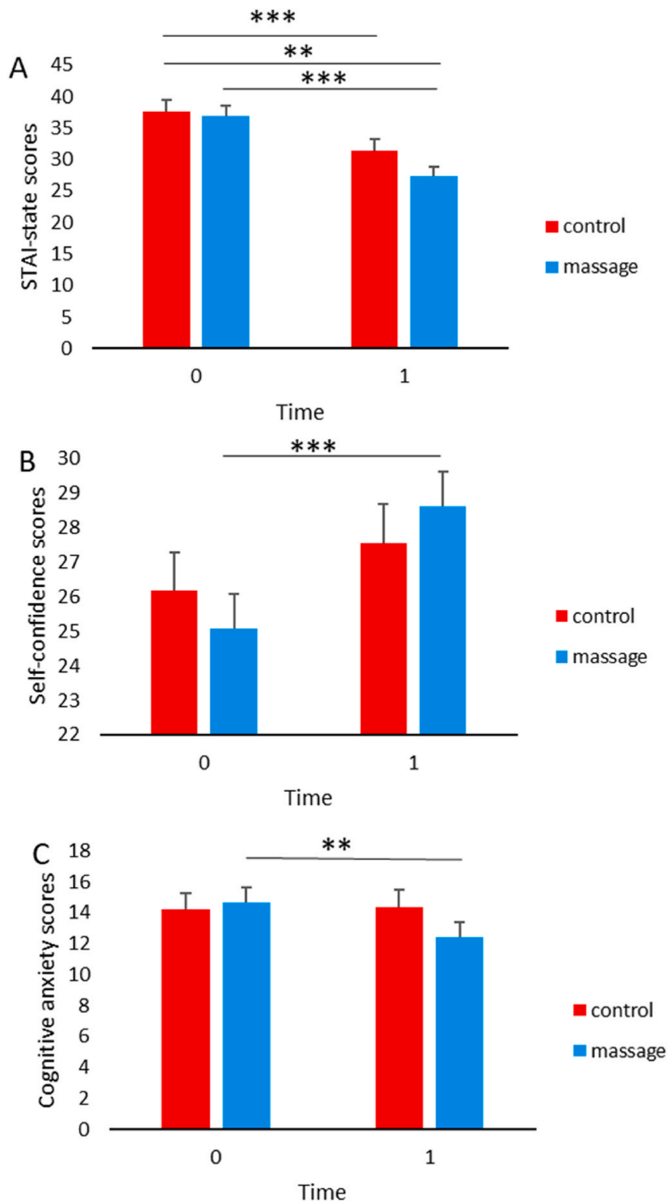

Fig. 3. Changes in STAI-status scores (A), Self-confidence score on the EEAC (B), and Cognitive anxiety scores on the EEAC (C) as a function of time and experimental group. Time *0* refers to the pre-manipulation value and time *1* to the post-manipulation value. Red and blue bars refer to control and massage, respectively. We report the Least Squares Means (±SE) of the general linear mixed models. LS-means connected by bars are statistically different after a Bonferroni correction for multiple tests (***$p < 0.001$; **$p < 0.01$; *$p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

in an alternative stress-reduction technique limits our ability to attribute the observed effects solely to seated Amma massage. Future research incorporating active control groups (e.g., another stress-reducing technique such as music listening) is warranted to better differentiate the specific effects of Amma massage from generalized stress reduction activities.

While these limitations temper the scope of our conclusions, they also highlight opportunities for future research to build upon our findings and further refine our understanding of the role of seated Amma massage in stress management.

6. Conclusion

In summary, taken together our results suggest a beneficial effect of a single 17-minute Amma massage on psychological parameters, showing perceived stress and anxiety scores decreased, and self-confidence scores improved after the massage. These results could be explained by the observed OT increase in the massage group. However, these beneficial psychological effects do not correlate with cortisol, CGRP and IL-6 plasma levels. In light of these observations, further consideration should be given to physiologically explain the benefits of a brief Amma massage, especially as part of corporate wellness initiatives to help employees deal with work-related stress.

CRediT authorship contribution statement

Florentine Fricker: Formal analysis, Investigation, Supervision, Writing – review & editing. **Marie-Virginie Barbotte:** Investigation, Project administration, Data curation. **Gaétan Pallot:** Investigation. **Nouhaila Radoua:** Investigation, Project administration. **Gabriele Sorci:** Data curation, Formal analysis, Writing – original draft. **Marie Heitz:** Investigation, Project administration, Supervision, Data curation. **Grégory Brison:** Conceptualization, Validation. **Edith Sales-Vuillemin:** Conceptualization. **Jean-Louis Connat:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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