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Is salivary chromogranin A a valid psychological stress biomarker during sensory stimulation in people with advanced dementia?

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Abstract
Salivary chromogranin A (sCgA) is gaining attention as a biomarker of psychological stress. The objective of this work was to determine whether individualized music intervention and multisensory stimulation environment (MSSE) in a Snoezelen room produce changes in sCgA in severely demented older patients, and to assess the possible existence of differences in sCgA levels between the two types of interventions. Older adults with severe dementia (*n* = 22) were randomly assigned to two intervention groups. They participated in MSSE or individualized music interventions in 30-min weekly sessions for 16 weeks. Levels of sCgA were evaluated before and after a session, or 30-min interval, at four different time points: before starting the trial, in the middle and end of the intervention period, and two months later. Comparison of sCgA values obtained after each session with those obtained before (or at the same hour in before trial and follow-up samplings) showed no significant differences either in the individualized music or in the MSSE group at any sampling time. Comparison between the two types of interventions, both before and after each session, in the four sampling times, did not produce any significant difference either. Furthermore, no significant correlation was obtained between agitation, anxiety, cognitive function, and dementia severity with sCgA levels. In conclusion, despite beneficial effects of both individualized music and MSSE interventions being previously reported on neuropsychiatric outcomes for older patients with dementia, sCgA seems to not be a good indicator of these benefits.

Keywords:
Chromogranin A, dementia, individualized music, multisensory stimulation, older adults, Snoezelen

INTRODUCTION

Chromogranin A (CgA) is an acidic phosphorylated secretory protein belonging to the granin family. It is co-stored and co-released with catecholamines into the circulation from secretory vesicles in the sympathetic nerve endings and adrenal medulla [1]. In clinical medicine, CgA is routinely used as a diagnostic marker of cancer and neurodegenerative diseases [2, 3]. It has also been demonstrated that CgA is secreted into saliva under autonomic control from the granular convoluted tubule of the submandibular gland [4]. Salivary CgA (sCgA) is a sensitive and quantitative index of the activity of the sympathetic nervous system [1, 5]. It has received attention in the last decade as a marker of psychological stress, as alternative to catecholamines, which are considered to be poor markers of acute changes in sympathoadrenal medullary activity [6, 7]. When a stressor occurs, sympathetic activation is immediate; thus, CgA is degranulated and secreted into saliva simultaneously and immediately. Increases in sCgA had been reported in response to different stressors, e.g., noise [8], living in microgravity [9], sport competition [10], and social or emotional stressful situations such as speaking in public [11, 12], taking an academic exam [13], a venipuncture intervention [14], or car driving [12].

Multisensory Stimulation Environment (MSSE) is one of the most popular sensory interventions for older adults with severe dementia, which typically occurs in a room known as a "Snoezelen" room [15]. Snoezelen intervention stimulates the primary senses, allowing the patient to freely explore a variety of stimulation devices. On the other hand, individualized music, defined as music that was integrated into the individual's life based on personal preferences, may have a soothing effect and alleviate or decrease agitated behaviors [16, 17]. Previous results from our research group showed that MSSE interventions were beneficial for improving agitation scores (Cohen- Mansfield Agitation Inventory, CMAI) in older adults with severe dementia. MSSE intervention also produced significant improvements in anxiety (Rating Anxiety in Dementia, RAID) and overall severity of dementia (Bedford Alzheimer Nursing Severity Scale, BANS-S) scores as compared with music intervention, revealing that MSSE could have better effects than music intervention on anxiety symptoms and dementia severity in demented older patients [18]. Nevertheless, the development of a biomarker which may effectively reflect the improvement in neuropsychological parameters in demented older adults subjected to MSSE or individualized music interventions would be very useful to assess rapidly and objectively the valuable effects of these therapies.

A previous study carried out in a small group of older patients with dementia treated with music intervention and compared with controls showed changes in the Gottfries-Brane-Steen Scale (GBS) and the Behavioral Pathology in Alzheimer's Disease Rating Scale (BEHAVE-AD), together with a statistically significant decrease in stress index as measured by sCgA concentration at the end of 25 1-h sessions conducted twice weekly for three months, comparing samples taken immediately before and after the sessions [19].

On the basis of these previously reported results, the objective of the current study was to determine whether individualized music intervention and MSSE in a Snoezelen room produce changes in the stress biomarker sCgA in severely demented older patients, and to assess the possible existence of differences in sCgA levels between the two types of interventions.

MATERIALS AND METHODS

Study sample

A non-probabilistic sample was selected from among the residents of a specialized dementia elderly center in A Coruña (Spain). The inclusion criteria were having a diagnosis of dementia and the presence of severe or very severe cognitive decline (Global Deterioration Scale, GDS 6–7) [20]. Dementia diagnosis was noted on the medical history, provided by a neurologist before placement in the gerontological complex, and corroborated by the elder care center's medical doctor. A clinical psychologist with wide experience in assessing demented patients administered the GDS to determine their level of severity: severe (GDS 6) or very severe (GDS 7) cognitive decline. The exclusion criteria

were the presence of a hearing impairment or other sensory disorder that would adversely affect interactions with the multisensory stimulation objects (e.g., severe vision impairment) and being bedridden.

After this evaluation, the clinical psychologist checked the participants' eligibility based on the inclusion and exclusion criteria, using an initial sample of 22 participants. A computer-based random number generator was used to randomly divide the sample into 2 groups of 11 subjects according to GDS score. Although a total of 22 subjects were initially involved in the study, only 18 individuals remained until the end: one was lost after the pretrial (MSSE group), two subjects after the mid-trial (MSSE and individualized music groups), and one was lost after the post-trial (individualized music group). The final sample consisted of 18 participants, with 9 participants in each group. A previous article with the same sample size as this study includes a Consolidated Standards of Reporting Trial (CONSORT) diagram, describing the sample selection and the MSSE and individualized music sessions in detail [18]. Data on the individual differences in sensorial preferences and interests were previously assessed to design each individualized session and minimize the behavioral problems that some participants could present within the MSSE and individualized music context.

The study protocol was approved by the Ethics Committee at the University of A Coruña, and it conformed to the principles embodied in the Declaration of Helsinki. Before data collection began, all participants' proxies were informed about the study, and the proxies were used as legally authorized representatives to provide informed consent for the older patients with dementia to participate in the research.

Procedure and measures

Participants from both groups took part in two weekly sessions for a period of 16 weeks, until they completed 32 sessions (MSSE or music intervention). Sessions lasted 30 min unless the participant expressed a desire to leave (no attrition occurred during the study). Evaluations were carried out at baseline (pre-trial), in the middle (mid-trial), at the end of the interventions (post-trial), and 2 months after the intervention (follow-up).

Agitation (CMAI), anxiety (RAID), cognitive function (Severe Mini Mental State Examination) and dementia severity (BANS-S) were assessed. A detailed description of these instruments is found in our previous article with this study sample [18].

Psychophysiological stress was evaluated by determining sCgA concentration in saliva samples collected immediately before and after each 30- min MSSE or music intervention. Evaluations were carried out at pre-trial, mid-trial, post-trial, and follow-up. Since in the pre-trial and follow-up assessments there was no intervention, two samples were also collected in a 30-min interval, at the same hour than the MSSE or music interventions for each individual. The first sampling time in this study was 10 : 00 am, approximately 1.5–2 h after patients awakening, and each subject was sampled always at the same half an hour interval throughout the study.

Saliva samples were collected using cotton rolls (Salimetrics children's swabs) and salivary collection tubes (Salimetrics swab storage tubes), and maintained refrigerated until processing (less than 4 h). Samples were centrifuged at 1500×g 5 min at 4ºC, aliquoted, and stored at –80º Cuntil analysis. All samples were coded at the moment of collection to ensure a blind study.

Before starting the study, in order to try to avoid additional stress as far as possible, a fake saliva sample collection was carried out with all participants so that they were familiarized with the procedure.

Evaluation of sCgA was carried out by enzymelinked immunosorbent assay (ELISA) using a commercial kit (manufactured by Yanaihara Institute Inc., distributed by BioVendor Laboratories Ltd.). Intra-assay and inter-assay coefficients of variation were <10.5% and <14.0%, respectively (sensitivity, 0.14 pmol/ml). All analyses were performed in duplicate.

Statistical analysis

A general description of the study population was performed through univariate analysis. The distribution within the study groups of sociodemographic factors was evaluated with the Student's *t*-test for continuous variables and the Pearson's Chi-square test for categorical variables.

Normal distribution for sCgA data was verified using the Shapiro-Wilk test. As the assumption of normality was not met and the sample size was not large, non-parametric tests were considered appropriate for further analysis. Differences in sCgA before and after interventions were assessed using the Wilcoxon matched-pairs signed-ranks test. Differences between MSSE and individualized music interventions were evaluated by Mann-Whitney *U*test. Associations between variables were analyzed by Spearman's rank correlation.

Statistical significance was set at a *p*-value below 0.05. Analyses were carried out using the SPSS software package V. 20.0 (SPSS, Inc.).

RESULTS

Table 1 presents the sociodemographic characteristics of the study population at the pre-trial. Females (68.2%) were slightly more abundant than males, age ranged between 77 and 102 years, most subjects (63.7%) had completed secondary or primary education, and most were widowed (68.2%), but distributions of gender, age, educational level, and marital status were not significantly different between the MSSE and the individualized music groups.

The possible influence of gender, age, educational level, and marital status on sCgA was assessed, and no significant result was found. Comparison of sCgA values obtained after each session with those obtained before (or at the same hour in pre-trial and follow-up samplings) showed no significant differences either in the individualized music or in the MSSE group at any of the four different sampling times (Fig. 1).

Table 1. Description of the study population at the beginning of the study (pre-trial)

	Total		MSSE		Music		*p*
	n	%	*n*	%	*n*	%	
Total individuals	22	100.0%	11	50.0%	11	50.0%	
Gender							0.170[b]
Females	15	68.2%	6	54.5%	9	81.8	
Males	7	31.8%	5	45.5%	2	18.2	
Age years)[a]		88.4±6.9 (77-102)		88.1±6.8 (78-102)		88.7±7.4 (77-97)	0.835[c]
Educational level							0.912[b]
No formal education	5	22.7%	2	18.2%	3	27.3%	
Primary	6	27.3%	3	27.3%	3	27.3%	
Secondary	8	36.4%	4	36.3%	4	36.3%	
College or higher degree	3	13.6%	2	18.2%	1	9.1%	
Marital status							0.308[b]
Single	4	18.2%	2	18.2%	2	18.2%	
Married/partner	2	9.1%	2	18.2%			
Separated/divorced	1	4.5%	1	9.1%			
Widowed	15	68.2%	6	54.5%	9	81.8%	

[a] mean±standard deviation (range); [b] Chi-square test (bilateral); [c] Student's *t*-test (bilateral).

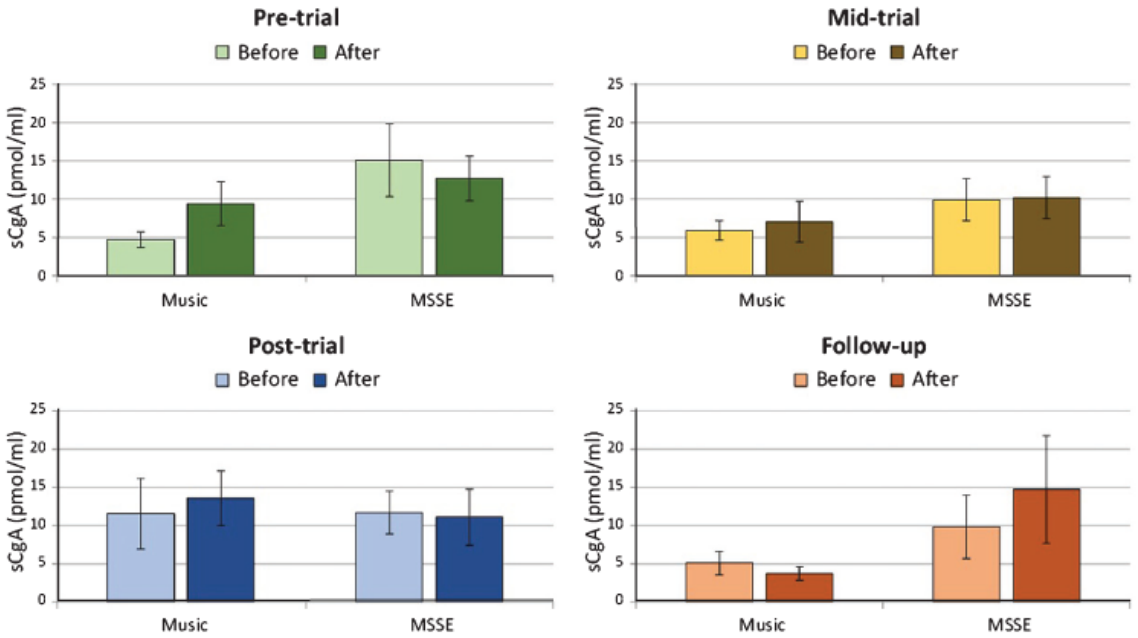

Fig. 1. Results of salivary chromogranin A (sCgA) determinations in the individualized music sessions and the multisensory stimulation environment (MSSE) groups, comparing values obtained before and after each session (or 30-min period in pre-trial and follow-up). Bars indicate mean standard error.

Moreover, as shown in Fig. 2, comparison between the two types of interventions, both before and after each session, in the four sampling times did not produce any significant difference either.

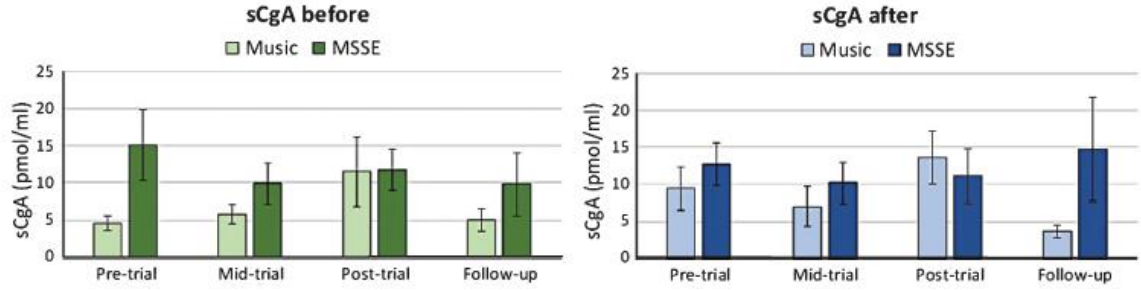

Fig. 2. Results of salivary chromogranin A (sCgA) determinations before and after each session (or 30-min period in pre-trial and follow-up), comparing values obtained in the individualized music sessions and the multisensory stimulation environment (MSSE) groups. Bars indicate mean standard error.

When sCgA values from each subject were considered separately (Figs. 3 and 4 for individualized music and MSSE group, respectively), a marked variation was observed at all sampling times, and no clear or regular pattern could be obtained for any of the two intervention groups.

Since assessment of agitation, anxiety, cognitive function, and dementia severity was carried out for these subjects at the four sampling times (pre-trial, mid-trial, post-trial, and follow-up) (results published in a previous paper [18]), the possible existence of associations with sCgA levels was explored, but no significant correlation could be obtained at any sampling time.

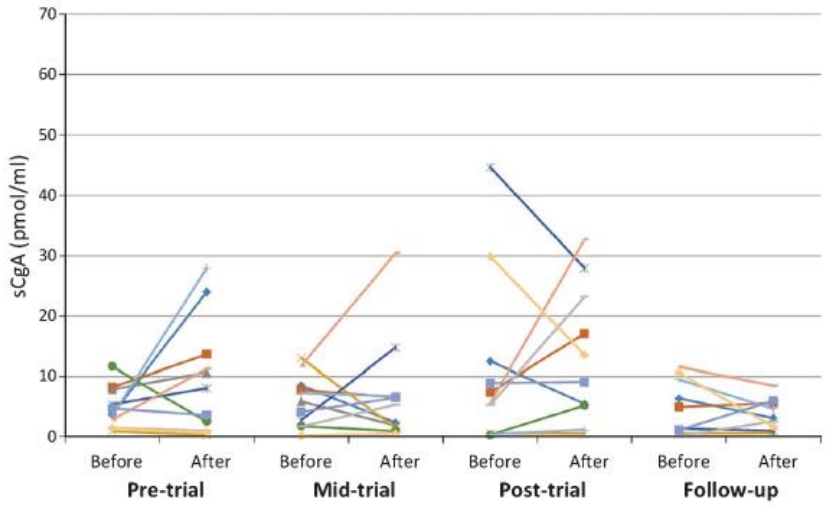

Fig. 3. Individual salivary chromogranin A (sCgA) determinations obtained in the individualized music sessions group.

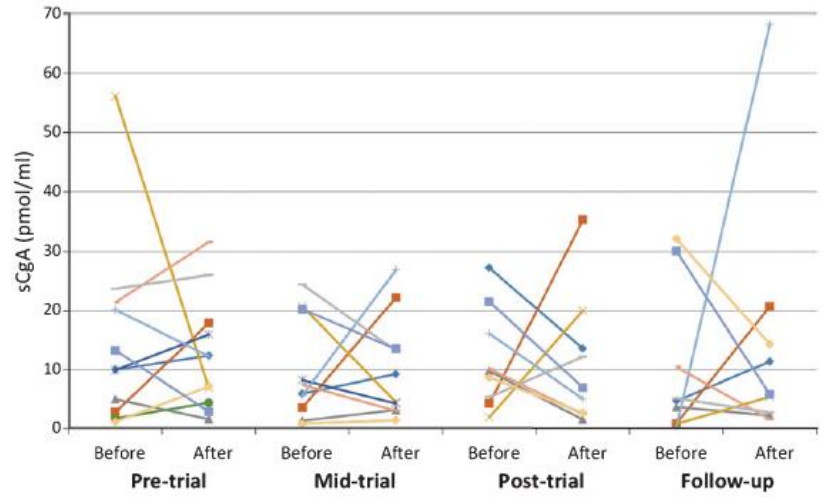

Fig. 4. Individual salivary chromogranin A (sCgA) determinations obtained in the multisensory stimulation environment group.

DISCUSSION

sCgA is a psychophysiological stress biomarker, reflecting sympathetic nervous system activity. Collecting saliva samples is an easy and non-invasive procedure that requires minimal manipulation. Saliva samples can be obtained without difficulty from children, individuals with physical or mental handicaps, and in situations of regular monitoring with repeated samplings. A significant advantage of sCgA with respect to other salivary biomarkers of stress, such as cortisol [21], alpha-amylase [22], and IgA [23], is that it is not influenced by circadian variation during a wide interval of time. sCgA levels were shown to peak upon awakening and then quickly decrease within 1 h, to remain at a low level throughout the day [24]. The first sampling time in this study was 10 : 00 am, approximately 1.5–2 h after patients awakening; thus sCgA variations due to circadian rhythm were avoided; besides, each subject was sampled always at the same half an hour interval throughout the study.

sCgA has been used to evaluate psychophysiological changes in stressful situations or activities already mentioned [8, 9, 11–14], usually reporting fast increases in this biomarker concentration. sCgA has been also employed to assess improvement resulting from healthcare modalities—such as aromatherapy [25–27], consumption of green tea [28], ingestion of enzyme-treated asparagus extract [29], and *Cimifuga racemosa* extract administration [30]—or from stress-relieving activities, such as laughter [31, 32], gum chewing [33], woodland walking [34, 35], short leisure trips [36], spa bathing [37], or exposure to negative air ions [38]. Nevertheless, results obtained in some of these studies are controversial. Whereas expected decreases in sCgA [25, 26, 29, 32, 33, 38], or attenuation of sCgA increases [28, 30] were observed in some studies, others report raises in sCgA concentrations [27, 31, 34–36], and mostly explain the increase as related to positive eustress or stress relieving characteristics of the performed activity. Moreover, the effect of a relaxing activity such as spa bathing on sCgA has been described as dependent on the subjects' background stress level: the high-stress group showed lower sCgA levels after spa bathing while the low-stress group presented higher sCgA levels in the same conditions [37].

The influence of individualized music intervention on sCgA concentrations in older patients with dementia was evaluated by Suzuki et al. [19]. They found a significant decrease in this stress biomarker after three months of 1-h session series (total sessions = 25) by comparing samples taken immediately before and after the intervention, with no differences in the control group. This decrease was neither observed before the intervention period nor maintained one month later (period without interventions). Nevertheless, the size of both music and control groups was limited (n = 6) and, according to the individual data reported, marked decreases in sCgA were obtained only in two subjects. Contrary to these results, no significant differences were observed in the current study when comparing values of sCgA obtained before and after each 30-min intervals, either in the absence of intervention (pre-trial and follow-up) or in the presence of intervention (mid-trial and post-trial), regardless the type of intervention (individualized music or MSSE). Therefore, our data do not support previous results by Suzuki et al. [19], and indicate that sCgA does not seem to reflect the previously demonstrated beneficial effects of individualized music or MSSE interventions on neuropsychiatric outcomes in these older patients with severe or very severe dementia [18, 39]. MSSE could be an appropriate intervention in these patients because its positive effects on neuropsychiatric symptoms [40, 41], improvement in the physiological rates—heart rate decrease and peripheral capillary oxygen saturation (SpO2) increase [42] —and also on dementia severity [41]. Individualized music has also shown benefits in decreasing agitation [43] and anxiety [17, 44] in people with moderate to severe dementia.

However, it is noteworthy that our sample size was larger (n = 11 at the beginning and 9 at the end), and current intervention programs were longer (total of 32 sessions carried out in 16 weeks). Besides, a possible influence of the severe dementia status on physiological mechanisms of sympathetic nervous system activation and consequent sCgA secretion, which might contribute to the absence/presence of effect of individualized music or MSSE interventions on sCgA, cannot be ruled out.

Different potentially favorable interventions carried out in older patients were previously investigated for their effect on sCgA levels. Toda and Ichikawa [32] evaluated the consequence of watching a comedy video after performing a stress task (an arithmetic test), and found significantly higher salivary flow rates after watching the video but no influence on sCgA. Shimizu et al. [45] did not observe significant variations in sCgA when assessing the effects of movement music intervention in female older adults. And, similarly, no effect of hand massage prompted relaxation was obtained in sCgA from female geriatric facility residents [46]. No indication was given in these mentioned studies on dementia status of participants. All these results, together with the ones in the current study, indicate that sCgA is not a suitable sign to detect positive relaxing/comforting effects in the older population.

Even though previous results obtained with MSSE suggest that this type of intervention could be a better option than music intervention for improving anxiety symptoms and dementia severity in demented older adults [18], significant differences in sCgA were not obtained in this study when comparing MSSE and individualized music groups; thus sCgA is not a good indicator to discriminate between the different effects of the two types of interventions. Moreover, no significant associations were observed between sCgA concentrations and agitation, anxiety, cognitive function, or dementia severity scores.

Levels of sCgA obtained throughout this study were highly variable. However, gender, age, educational level, and marital status were homogeneously distributed in the two groups, and no significant effect of any of these factors on sCgA was obtained, agreeing with previous studies [47–49]. Additionally, it has been reported that human sCgA is not influenced by food intake [36].

On the contrary, personal patterns of behavior were found to influence sCgA concentrations evaluated during stress relieving activities, such as woodland walking [34], and recently two polymorphisms in CgA gene (rs9658635 in the promoter region, and rs9658655 in exon 6, Glu264Asp) were also reported to modify sCgA [50]. A possible influence of all these factors on our results cannot be completely excluded, since patterns of behavior and genetic polymorphisms were not assessed. Furthermore, sCgA concentrations were described to be affected by several pathologies, such as aggressive [48] or chronic periodontitis [51], bruxism [52], type 2 diabetes [53], or xerostomia (dry mouth) [54]; in general the presence of these pathologies was associated with higher sCgA level. In addition, as our study participants were older subjects, they are administrated a wide variety of medications which may also affect the sCgA secretion.

Another possible source of variation in sCgA levels is the methodology used, as it has been reported that measuring sCgA with ELISA technique is strongly investigator-dependent [55]. Still, in this study, all analyses were carried out by the same well-trained investigator and the accuracy of the ELISA technique used is adequate, according to its intra- and inter-assay variation. Besides, the range of sCgA concentrations obtained in the current work (0–40 pmol/ml, excluding two extremely high values) was wider than the ones reported in most studies determining sCgA carried out in Japanese populations (highest values usually lower than 5 pmol/ml) [19, 24, 26, 31, 49]. Similar lower values were reported in Brazilian [50, 53] and Indian subjects [51]. And contrarily, concentrations analogous to the ones in the present study were described in Italians [56], Austrians [48, 55], French [11], and Koreans [57]. Nevertheless, most of these works used the same ELISA kit manufactured by a single commercial company to carry out sCgA analyses. This may be an indication that different socio-cultural features may influence sCgA values in some way.

In conclusion, despite beneficial effects of both individualized music and MSSE interventions being previously reported on neuropsychiatric outcomes for older patients with dementia, sCgA seems not to be a good indicator of these benefits. In fact, sCgA did not detect any difference between the two types of interventions tested.

This study has some limitations. First, the population size explored was relatively modest, but subjects were selected on a rigorous basis and randomly assigned to the corresponding intervention group ensuring their homogeneity; therefore, we consider that results reported are valuable, particularly considering the difficulty in recruiting this specific type of older individuals with severe or very severe dementia.

A second limitation may be related to the technique used for saliva sampling collection. This non-invasive technique is easy and perfectly valid for psychologically healthy subjects. However, although a simulated sample collection was carried out to familiarize patients with the procedure, it may introduce an additional stressful element in severely demented patients (who may not understand the safe nature of the technique), which may modify the basal level of stress biomarker to be evaluated. Moreover, in studies like the current one getting severe demented subjects' collaboration for obtaining the samples is sometimes difficult and takes several minutes. This involves a delay the moment for sample collection and possibly influences the level of sCgA (rapid response biomarker) in the particular sample.

And thirdly, high inter- and intra-individual variability in sCgA levels may have been influenced by different factors not controlled in this study, such as background levels of stress, individual patterns of behavior, genetic factors, and certain pathologies or medications. Nevertheless, most of these factors are not feasible to be controlled in patients with severe or very severe dementia.

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