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ORIGINAL PAPER

Explicit and Implicit Emotional Expression in Gambling Disorder Measured by a Serious Game: A Pilot Study

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Abstract
Behavioral addictions have been related with biased emotional reactions to risky choices. However, few studies have analyzed the role of both explicit and implicit emotional expression in gambling disorder (GD). This pilot study aims to examine emotion regulation in treatment-seeking patients with GD. The sample included $n = 35$ participants classified into three groups: patients with current GD, patients with GD in remission, and a control group without GD. Implicit emotional expressions were evaluated through a serious videogame (Playmancer) and explicit emotions were measured through self-reports. Patients in the current GD group had, compared to the remission and control groups, lower levels of implicit emotion expression and higher levels of explicit emotion expression. The patients in GD remission group endorsed better emotion regulation capacity in comparison to patients with current GD. We conclude that differences in emotion expression profiles (such as anger and anxiety) should be considered both in the development of screening and diagnostic measures and in the planning of prevention and treatment programs.

Keywords Anger · Anxiety · Emotion regulation · Gambling disorder · Serious videogames

Introduction

Serious videogames (SVG) have aroused considerable scientific interest in recent years as an alternative and complementary method to facilitate learning processes and as platforms with potential to provide self-reinforcement during psycho-therapeutics interventions (van der Kuil et al. 2018; Yahyaoui and Menelas 2017). Although SVG present a structure similar to games used for entertainment purposes, they are designed to address a specific trait

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(Serret et al. 2017; Stieler-Hunt et al. 2014) and have proved to be effective for disorders into the impulsive-compulsive spectrum (Giner-Bartolomé et al. 2015; Savazzi et al. 2018).

Playmancer is a SVG used as a complementary tool for the cognitive-behavioral therapy (Conconi et al. 2008) with the aim to modify attitudinal and emotional problems characteristic of psychiatric disorders, and it has demonstrated therapeutic effectiveness in different mental health conditions including bulimia nervosa and gambling disorder (Fernández-Aranda et al. 2012); Fagundo et al. 2013, 2014; Jiménez-Murcia et al. 2009a). This platform has also proven to be effective in identifying and assessing aspects related to emotion regulation during the course of these treatments (Claes et al. 2012a, b).

Emotion regulation has been defined as a goal directed processes to influence the type, intensity or duration of experienced emotions (Gross and Thompson 2007). This requires adequate flexibility when facing affective stimuli and on the long-term goals of the subjects. Different classification systems for emotion regulatory processes exist, with the explicit (also called effortful) versus implicit (also called automatic) dual-process conceptualization being the most common (Gyurak et al. 2011). This dual model conceives explicit emotion regulation as the processes required to consciously monitor and alter arousal levels. In contrast, implicit emotion regulation an automatic process evoked by the stimulus that is carried out largely without much insight or awareness. This dual model does not consider explicit and implicit regulation as mutually exclusive processes, and although a relationship between the two categories is supported, it allows for each process to vary over time and across situations.

The study of emotion regulation has led to many studies in the field of addictions and some conditions included along the impulse-control spectrum, such as bulimia nervosa, binge eating disorder or gambling disorder (Fernández-Aranda et al. 2012; Nikolaidou et al. 2016; Tárrega et al. 2014). Concretely, the explicit component of emotional regulation has received notably scientific interest, while implicit emotion regulation has generated less empirical evidences. This is particularly notably in the study of gambling disorder (GD).

Explicit Emotion Expression in GD

Different explicit emotion expression processes have been studied in GD, particularly those relating to negative emotions. It has been observed that a high proportion of patients with GD endorse difficulty in controlling anger (Aymamí et al. 2014). It has also been observed that, in patients with problematic gambling, anger (expressed both verbally and physically) is accompanied by other negative emotions (such as envy, resentment, hatred and disgust), and that high scores in the expression of the anger correlate with greater GD severity (Maniaci et al. 2017). Anger levels in pathological gamblers have also been related with more dysfunctional scores in certain personality dimensions (particularly novelty seeking) (Schwebel et al. 2006).

Anxiety is also a commonly studied dimension of emotion expression in GD. Some studies posit that gambling behaviors may act as a mechanism to reduce or avoid the expression of anxiety, and that high levels of anxiety are associated with greater GD behavior (Stewart et al. 2008). Other studies conclude that patients who express greater aversion to losses linked to gambling behaviors are those with higher levels of anxiety (Takeuchi et al. 2016). Finally, it has also been observed that the pathological gamblers with the highest degree of severity also present higher levels of anxiety during the gambling episodes and also after finishing those episodes (Barrault and Varescon 2013).

This evidence has led some researchers to postulate that the anxiety expressed by GD patients could even be part of the group of measures used to estimate the severity of the disorder itself (Ciccarelli et al. 2017).

Studies that simultaneously measure different components of negative emotional expression in GD outline that patients tend to present high levels of stress, anxiety and/or depression (Jonsson et al. 2017). It has also been observed that emotional negative states in patients include multiple components such as disgust, contempt, guilt, fear, sadness or low sensitivity to punishment (Goudriaan et al. 2004; Matthews et al. 2009; Navas et al. 2015). Relatedly, it has been concluded that some patients with high emotional vulnerability use gambling to alleviate negative affective states linked to stressful live events (Poole et al. 2017). Finally, negative mood has been found to have an inhibitory effect on gambling persistence only for non-regular gamblers, while regular gamblers seem to gamble regardless of their mood (Hills et al. 2001). And since mood after gambling episodes has been related with winnings only for regular gamblers, it has been suggested that GD patients may be condition to use gambling as an escape from distress (Hills and Dickerson 2002).

It is also known that explicit emotion regulation process in GD are closely related to the appearance and maintenance of cognitive biases associated with gambling behavior (Raylu and Oei 2004). Models of emotion regulation postulate that when gamblers use adaptive cognitive strategies to reduce the impact of negative emotions, they have a rebound effect increasing emotions such as fear, guilt or anger (Garnefski and Kraaij 2007; Navas et al. 2016). It has also been observed that pathological gamblers, in comparison with control groups without gambling related problems, have limited access to emotion regulation strategies (Williams et al. 2012), and also express less use of cognitive reassessment during emotion regulation processes (Poole et al. 2017; Williams et al. 2012).

Implicit Emotion Expression in GD

Regarding implicit emotion regulation, little research has clarified the role of these processes on an individuals' psychopathological state (partly as a consequence of the absence of reliable and valid measures for the assessment of this component). There is evidence of a relationship between failure to engage implicit emotion regulatory processes and symptom severity in anxiety disorders (Etkin et al. 2010), which suggests that implicit emotional processes are related to adaptive functional behaviors.

It has also been postulated that the origin of the emotion regulation difficulties found in the anxiety and mood disorders could be related with more spontaneous/implicit forms of emotion regulation (Egloff et al. 2006; Ehring et al. 2010; Phillips et al. 2008).

Finally, previous researches have provided evidence that emotion regulation modulates physiological correlates in decision-making tasks under risk (Grecucci et al. 2013; Martin and Delgado 2011; Sokol-Hessner et al. 2013), as well as subjective emotional experience to both gains and losses (Yang et al. 2015). Studies have also postulated that implicit emotion regulation could be more efficient than deliberate emotion regulation in modulating emotional reactions to gains and losses (Fenton-O'Creevy et al. 2012), as well as reducing emotional responses to emotional pictures (Christou-Champi et al. 2015). A current analysis of studies in the GD area has leaded to the link between executive processes related to attention, learning, planning and cognition to punishments or rewards that may generate positive or negative emotional states (Mestre-Bach et al. 2020).

But studies focused in the implicit emotion expression in the GD area are scarce. The lack of evidence and the partly contradictory results obtained highlight the need for new empirical research about implicit emotion regulation for this disorder.

Objectives

Although it is known that the expression of the emotions plays a relevant role in the onset of GD, few studies have evaluated the explicit and implicit components of emotion regulation in treatment-seeking GD patients. The aim of this study was to examine implicit and explicit emotion expression in currently ill GD patients, GD patients in remission and healthy controls (HC). We hypothesized that: (a) patients with GD would show lower emotion regulation functioning than HC, that is reduced implicit emotional expression, measured by facial expression measurement technology in response to a therapeutic videogame, and incongruent and dysfunctional explicit emotional expression, measured by self-report measures of anxiety and anger; and (b) GD patients in remission would display an improved emotion expression in comparison with currently ill patients.

Materials and Methods

Participants

The sample included N = 35 men, distributed in three independent groups: (a) n = 11 GD patients (currently meeting diagnostic criteria for GD, before treatment); (b) n = 12 GD patients after finishing a standardized cognitive behavioral therapy (CBT) program (Jiménez-Murcia et al. 2006, 2007), in remission state (Remission-GD, defined as the absence of gambling episodes during the last 12 weeks); and (c) n = 12 HC. Patients into the GD groups were consecutive referrals for outpatient treatment at a Hospital Unit specialized in pathological gambling, and the HC group included volunteers from the same geographical area.

Exclusion criteria were primary psychiatric or neurological disorders that could interfere with game performance (psychotic disorders, bipolar disorders, major depressive disorders and substance abuse-disorders) and active pharmacological therapy that might influence autonomic functioning or interfere with game performance. All participants were also assessed to guarantee the absence of current of lifetime Internet Gaming Disorder, following the criteria proposed in Section III of the DSM-5 (American Psychiatric Association 2013).

Measures

South Oaks Gambling Screen (SOGS) (Lesieur and Blume 1987). This diagnostic questionnaire uses 20 items to ascertain gambling disorder severity. This screening tool discriminates between probable pathological, problem and non-problem gamblers. The Spanish validation of this questionnaire shows high reliability and validity (Echeburúa et al. 1994). Cronbach's alpha in the sample was very good ($\alpha = 0.89$).

Stinchfield's Diagnostic questionnaire for pathological gambling according to DSM-IV criteria (Stinchfield 2003); *Spanish validation* (Jiménez-Murcia et al. 2009b). This 19-item

questionnaire measures the DSM-IV-R diagnostic criteria for pathological gambling (American Psychiatric Association 2000). Convergent validity in comparison to the SOGS questionnaire was estimated as r = 0.77 ($p < 0.01$) for the general population and r = 0.75 ($p < 0.01$) for a gambling treatment group. Cronbach's alpha in the sample was good (α = 0.74).

State-Trait Anger Expression Inventory 2 (STAXI-2) (Spielberger 1999). It is a 44-item self-report instrument that examines the experience and expression of anger. Items are rated on four-point Likert scales assessing either the intensity of the angry feelings or the frequency with which anger is experienced, expressed, suppressed, or controlled. The Spanish version of the tool was used in this study, which has reported adequate reliability indices ranging between 0.64 and 0.89 (Miguel-Tobal et al. 2001). Internal Consistency for the three scales analyzed in this work was excellent: α = 0.98 for anger-state, α = 0.94 for anger-trait and α = 0.90 for the general index of anger.

State-Trait Anxiety Inventory (STAI) (Spielberger et al. 1970) *Spanish adaptation* (Spielberger et al. 1982). This 40-item self-report questionnaire is answered on a 1–4 response scale which evaluates the temporary condition of "state anxiety" and the more long-standing condition of "trait anxiety". The questions assess feelings of anxiety and depression in the areas of worry, tension and apprehension. The psychometrical studies in the Spanish population achieved good reliability indices, ranging between 0.90 and 0.94 (Guillén-Riquelme and Buela-Casal 2011). Cronbach's alpha reliability in sample was excellent (0.92 for Trait and 0.90 for State Anxiety).

Implicit emotional expression It was measured with Playmancer. This platform includes three mini-games: Treasures of the Sea, The Face of Cronos and Sign of the Magupta. In these mini-games the player has to dive and collect different artifacts and fish, climb up a cliff avoiding obstacles, and connect a constellation of stars through breathing. The difficulty of the videogame depends on the arousal levels of the player. The overall goal of this SVG is to improve self-control skills and to also train arousal regulation skills in negative situations such as frustration, anxiety and time pressure. This SVG has been used as an add-on therapeutic tool for eating disorders with promising results (Fagundo et al. 2013, 2014) as well as in GD (Tárrega et al. 2015). Biofeedback and a focus on breathing to produce relaxation have been used to train emotion regulation in several impulse-related disorders (Claes et al. 2012a, b; Tárrega et al. 2014). Playmancer also includes a facial recognition software with an external camera which detects the individuals' facial expression during the videogame performance (processed by a facial tracking component) [previous experiments addressed to calibrate the facial emotion recognition software have obtained evidence guaranteeing its reliability (Claes et al. 2012a, b; Fernández-Aranda et al. 2012)]. The physiological reactivity and emotional state of the patient are continuously being monitored, which allows having a measure of the total time that emotions are identified during each session with the videogame. The times in seconds expressing anger and joy has been used as main outcomes in previous studies using Playmancer and have been considered as measures of the implicit emotional expression in this work.

Sociodemographic variables and other clinical measures Additional clinical and demographic and social/family variables were measured using a semi-structured face-to-face clinical interview (Jiménez-Murcia et al. 2006).

Procedure

The study was carried out according to the latest version of the Declaration of Helsinki and it was approved by the Ethics Committee of the University Hospital. Written informed

consent was obtained from all participants. For both clinical groups and HC, experienced psychologists/psychiatrists conducted face-to-face structured interviews. Participants completed the self-report questionnaires (STAI and STAXI-2). For GD patients, the videogame session took place before starting CBT. For the Remission-GD group, the session was recorded in a follow-up session after finishing the standard CBT program.

Statistical Analysis

Analyses were carried out with Stata16 for Windows (Stata-Corp 2019). The comparison of mean scores in emotional expression measures (facial expression, STAI and STAXI-2 scales) between the groups was carried out with Poisson regression, a log-linear model useful for count data that uses the logarithm as the link function and the Poisson distribution function. Finner's correction (a procedure included into the Familywise error rate stepwise procedures which offers more powerful test than the classical Bonferroni's correction) was used to control Type-I error due to multiple statistical comparisons (Finner 1993). The effect size for the pairwise comparisons was estimated through the Cohen's-*d* coefficient (low effect size was considered for $|d| > 0.20$, moderate effect size for $|d| > 0.50$ and good effect size for $|d| > 0.80$) (Kelley and Preacher 2012).

Results

Characteristic of the Sample

Table 1 shows the sociodemographic characteristics of the sample, and it shows no statistical differences between the groups for chronological age, civil status and education levels. All of the patients in the GD patient group were slot machine gamblers and did not report any other gambling preference. Eight patients (66.7%) in the Remission-GD group were also slot machines gamblers, and one patient in this cohort reported more than one preferred type of gambling. GD and remission-GD groups reported statistically equal clinical profiles at intake (before the CBT) in the GD related measures (second panel of Table 1).

Comparison of the Videogame Performance Between Groups

In order to control effects of playing success on the expression of emotions, the outcome of the diving performance on the mini-game "treasures of the sea" was calculated as a number of errors (Number of times out of breath) divided by the minutes playing the diving mini-game. No statistical differences were found between groups when videogame performance was compared ($p = 0.843$) [GD: mean = 0.25 (SD = 0.18); Remission-GD: mean = 0.25 (SD = 0.11); and HC: mean = 0.22 (SD = 0.16)].

Comparison of the of Implicit Emotional Expression Measures

The ANOVA for the outcomes joy and anger measures (Table 2) showed that GD group expressed both joy and anger during the shortest mean time, followed by Remission-GD and HC. All pairwise comparisons achieved significant results, but effect sizes were low ($|d| < 0.50$).

Table 1 Characteristics of the sample

Sociodemographic	GD ($n=11$)		Remission-GD ($n=12$)		HC ($n=12$)		*p*
Age (years); *mean and SD*	36.0	6.34	37.5	8.25	34.6	8.12	.651
Marital status; n%							
Single	6	54.5%	3	25.0%	5	41.7%	.442
Married-partner	3	27.3%	8	66.7%	5	41.7%	
Divorced-separated	2	18.2%	1	8.33%	2	16.7%	
Education; n%							
Primary	7	63.6%	6	50.0%	9	75.0%	.586
Secondary	4	36.4%	5	41.7%	2	16.7%	
University	0	0%	1	8.33%	1	8.33%	

Clinical profile (pre-treatment)	GD ($n=11$)		Remission-GD ($n=12$)		*p*
Onset of GD (years-old); *mean and SD*	31.13	9.27	30.92	7.70	.954
Duration of GD (years); *mean and SD*	14.09	5.26	13.56	9.67	.873
SOGS-total score; *mean and SD*	11.00	2.97	9.17	4.86	.293
DSM-V: total criteria; *mean and SD*	7.55	2.58	7.58	2.75	.973
[a]Maximum bets (euros); *median and SD*	500.0	803.9	650.0	791.2	.201
[a]Mean bets (euros); *median and SD*	100.0	159.9	100.0	70.8	.152
[a]Cumulate debts (euros); *median and SD*	1000.0	10,737.4	1000.0	66,151.8	.561

GD gambling disorder, *HC* healthy controls, *SD* standard deviation

[a]Median is reported due to high asymmetry. [a]Mann-Whitney test *U* due to high asymmetry

Comparison of the Explicit Emotional Expression Measures

The explicit emotional expression measures analyzed in the study (STAI and STAXI-2 scores) reached significance when comparing between groups, except for STAXI-2 anger trait scale (Table 2). As a rule, GD patient obtain the highest mean scores, followed by Remission-GD and HC. Excluding STAXI-2 anger-trait, Acute-GD statistically differed from Remission-GD and HC groups, and mean differences obtained effect sizes into the moderate to good range. Comparing Remission-GD versus HC, only STAI anxiety-state obtained significant differences (effect size for the pairwise comparison was moderate).

As a summary of the results of this study, Fig. 1 contains the radar-chart with the z-standardized mean scores obtained in the three groups compared in the study.

Discussion

This pilot study is aimed to examine emotion (dys)regulation in a sample of treatment-seeking patients with GD, and compare the implicit and explicit measures of emotion regulation between GD patients in different clinical states (acute vs. remission) and a healthy control group.

In this work, GD patients presented lower level of implicit expression of anger but higher level of explicit expression of anger. This result is consistent with studies concluding

Table 2 Comparison between groups in implicit facial emotion expression and explicit emotion expression (STAI and STAXI-2)

	Mean (standard deviation)						Factor	GD versus		GD versus		R-GD versus	
	GD		R-GD		HC		Group	R-GD		HC		HC	
	(N = 11)		(N = 12)		(N = 12)		*p*	*p*	|*d*|	*p*	|*d*|	*p*	|*d*|
Implicit													
Joy (sec.)	974.5	494.3	1025.8	545.0	1060.8	413.9	**< .001**[a]	**< .001**[a]	0.10	**< .001**[a]	0.19	**.008**[a]	0.07
Anger (sec.)	86.4	124.0	116.7	219.3	178.3	342.6	**< .001**[a]	**< .001**[a]	0.17	**< .001**[a]	0.36	**< .001**[a]	0.21
Explicit													
STAI: state	25.20	12.25	16.75	12.04	11.58	6.46	**< .001**[a]	**< .001**[a]	**0.70**[b]	**< .001**[a]	**1.39**[b]	**.001**[a]	**0.53**[b]
STAI: trait	25.40	11.54	18.50	13.22	18.25	5.10	**.001**[a]	**.001**[a]	**0.56**[b]	**< .001**[a]	**0.80**[b]	.886	0.02
STAXI-2: state	21.50	13.92	17.17	4.61	15.92	1.38	**.014**[a]	**.022**[a]	**0.51**[b]	**.003**[a]	**0.56**[b]	.452	0.37
STAXI-2: trait	20.60	8.86	17.25	7.84	19.75	4.75	.156	.073	0.40	.659	0.12	.155	0.39
STAXI-2: total	33.80	12.05	24.45	15.91	28.25	9.38	**.001**[a]	**< .001**[a]	**0.66**[b]	**.020**[a]	**0.51**[b]	.076	0.29

GD gambling disorder, *R-GD* remission gambling disorder, *HC* healthy controls, \|*d*\| Cohen's d

[a]Bold: significant comparison (including Bonferroni's-Finner correction)

[b]Bold: moderate to high effect size (\|*d*\| > 0.5)

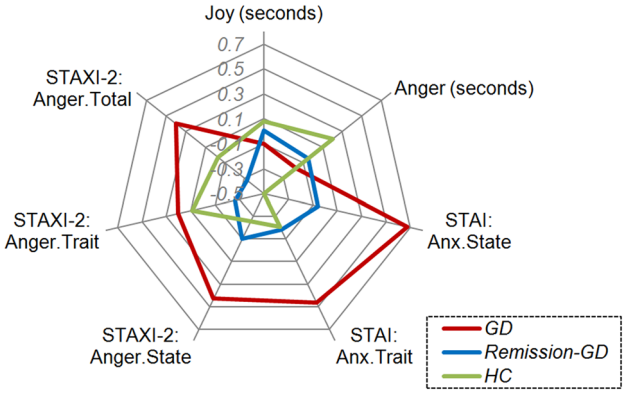

Fig. 1 Radar-chart comparing emotional measures between groups (z-standardized means)

that GD often co-occurs with emotions of anger (Maniaci et al. 2017; Schreiber et al. 2012; Williams et al. 2012). However, important gender differences have been described in samples of GD patients (Fernández and Scott 2009). Thus, while women with gambling problems have intense internal feelings of anger, in men expressions of anger tend to manifest externally (verbally or behaviorally, towards objects or people) (Aymamí et al. 2014). Given that the sample analyzed in this study consisted only of males, it is not possible to establish gender differences. Still, our findings confirm the results of previous research in which males with GD presented high levels of explicit anger (Delfabbro et al. 2018).

It is also known that deficits in inhibitory control contribute to increased anger, when facing negative events/stimuli, and that this difficulty in controlling anger is maintained over time (Jauregui et al. 2016; Maniaci et al. 2017). Some studies focusing on the recognition of emotions through the presence of different stimuli (such as music, voices or faces) have observed that there exist a clear deficit in emotional processing that causes pathological gamblers to exhibit higher levels of anxiety and fear that hinder the identification of emotions (Kornreich et al. 2016). In fact, this egodystonic effect in the control of negative emotions is not only present in GD, but also in other disorders characterized by impulsive behaviors such as bulimia nervosa (Tárrega et al. 2014). This inconsistency in the control of emotions has been related to other emotion alterations which are also highly comorbid with GD, such as depression, anxiety or stress (Aïte et al. 2014; Nigro et al. 2017). Therefore, in our study the incongruence between the implicit and explicit emotional expression of anger could be due to the fact that patients may be suppressing part of this emotion during the videogame session, and they do not have the adequate tools to regulate controlling their explicit expression. On the other hand, it has been observed that GD patients use gambling episodes as a means to alleviate negative emotional states (which would explain the lower score in implicit expression of anger), but since they feel worse after these episodes, the difficulty in regulating the mechanisms of negative emotional expression is increased in a long-term (Aymamí et al. 2014).

In our study, expression of positive emotions (joy) was the lowest for GD patients, followed by GD patients in remission and controls. This result is also consistent with the typical emotional dysregulation processes that accompany to the disorder. Several studies have even concluded that the patients' emotional profile have high discriminative capacity in identifying subjects with gambling problems and in classifying different states of this

disorder, since this pattern seems to be a powerful marker for the problem (Jonsson et al. 2017).

Compared to the GD group, GD patients in remission presented better emotion regulation (their levels of implicit and explicit emotional expression are more similar to those of the control group). Some studies have confirmed a relevant change in the emotional regulation of patients who finish psychological treatments or who are in a remission state. In fact, a close association between levels of anxiety (negative emotions) and GD severity has been described (Medeiros et al. 2016; Navas et al. 2016), as well as a strong relationship between levels of anger and the severity of GD (Ciccarelli et al. 2017; Maniaci et al. 2017). This result has also been obtained in studies using the Playmancer platform (Tárrega et al. 2015).

Limitations, Strengths and Implications

The most noteworthy limitation of this study is the sample size, which decreases statistical power and external validity. However, it should be argued that in spite of the low size of the groups, significant relationships have emerged, and that coefficients used to estimate the effect size (Cohen's-*d* coefficient) are not dependent on sample size. The inclusion of only male patients also affects the external validity of the investigation. In any case, this work is presented as a pilot study, whose results should be reviewed based on what is obtained in future research.

The strengths of this research include the analysis of GD patients in different clinical states and the simultaneous inclusion of explicit and implicit measures of emotions.

Conclusion

The results of this study shows that patients with GD have more dysfunctional emotion regulation levels than HC, and that implicit and explicit emotional regulation do not appear in the same direction depending on the patients' clinical state: while the GD patients had lower scores in implicit emotional expression and higher in explicit emotional expression, this relationship is reversed in GD patients in remission. Explicit and implicit emotion expression scores in the HC were more similar to the GD patients in remission than to GD patients.

These results have clinical implications in the areas of diagnostic evaluation and in the development of new therapeutic intervention tools. Cognitive behavioral therapy currently constitutes the most widely intervention procedure for GD, but it has been shown to have non-compliance issues and high dropout and relapses rates which have been related to changing core characteristics such as emotion regulation abnormalities (Challet-Bouju et al. 2017). Assessing the therapeutic effectiveness of new approaches such as SVG is a key challenge that must be taken into account when considering the implicit and explicit emotions profile of GD.

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Author's Contribution RG, DG-V, FF-A, JMM and SJ-M designed the experiment based on previous results and the clinical experience of NM-B, GM-B, NA, MG-P, AP-G, MB and LM. RG, VM-R GM-B, TM-M,

ZA, CV-A, ML-M, ST, FF-A and SJ-M conducted the experiment, analyzed the data, and wrote a first draft of the manuscript. RG, GM-B, FF-A, JMM, and SJ-M further modified the manuscript.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval The study was carried out according to the latest version of the Declaration of Helsinki and it was approved by the Ethics Committee of the University Hospital. Written informed consent was obtained from all participants.

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