TITLE: Autonomic reactivity in blood-injection-injury and snake phobia

RUNNING HEAD: Autonomic reactivity in blood phobia

Department: Human Anatomy and Psychobiology. School of Psychology, University of Murcia. Murcia (Spain)

Authors: Juan P. Sánchez-Navarro, Ph.D.[a,b], José M. Martínez-Selva, Ph.D. [a,b], Enrique F. Maldonado, Ph.D.[c], Eduvigis Carrillo-Verdejo, Ph.D. [a,b], Sara Pineda, M.A.[a,d], Ginesa Torrente, Ph.D.[a,b]

[a]School of Psychology, University of Murcia, Murcia, Spain

[b]Institute of Biomedical Research of Murcia (IMIB-Arrixaca), Spain

[c]AndaluciaTECH, School of Psychology, University of Malaga, Malaga, Spain

[d]Hospital General Universitario Morales Meseguer, Murcia, Spain

Corresponding author: Juan P. Sánchez-Navarro. Department of Human Anatomy and Psychobiology. School of Psychology. University of Murcia. 30100 Murcia, Spain. Phone: +34 868 887 707. FAX: +34 868 884 111. E-mail: jpedro@um.es

Abstract

Objective: This research aimed to study the salivary flow and other autonomic reactions -heart rate (HR) and skin conductance response (SCR)- in blood-injection-injury (BII) phobia and snake phobia participants, under the assumption that exposure to blood-related pictures in BII phobia will provoke an increase in parasympathetic activity that, in turn, will lead to a greater saliva production than other affective contents.

Methods: We selected 18 BII phobia and 14 snake phobia participants along with 22 non-phobia individuals. All participants were exposed to 3 blocks of pictures (12 pictures per block) depicting either mutilations, snakes or neutral, household objects. Saliva samples were taken in the 2-min interval before and after each block.

Results: In comparison to other contents, blood-related pictures provoked an increase in salivary flow in BII phobia participants, as well as an increase in the number of SCRs. In the snake phobia group, snake pictures provoked HR acceleration, but the SCRs they elicited did not differ from the SCRs provoked by the blood-related pictures.

Conclusion: BII phobia individuals react to their phobic object with a series of physiological changes resulting from a sympathetic-parasympathetic co-activation. This is in contrast with other specific phobias (e.g., small animal phobias) that usually show a sympathetically mediated, defensive reactivity when exposed to their disorder-relevant stimuli. These data support the use of therapeutic interventions in BII phobia that may differ in some respect from those used in other specific phobias.

Keywords: Blood-injection-injury phobia; Snake phobia; Salivary flow; Skin conductance; Heart rate

1. Introduction

Individuals suffering from specific phobia usually respond with a feeling of fear accompanied by a variety of behavioural, physiological, endocrine and neural changes when they are exposed to their phobic object (1). They typically show a defence response (i.e., a complex and varied set of responses provoked by potentially threatening stimuli, whose function is to protect the integrity of the organism (2)), characterized by a feeling of fear accompanied by a fight-or-flight reaction and an increase in the activity of the sympathetic branch of the autonomic nervous system (ANS) (e.g. (3, 4)). This strong sympathetic reactivity provokes changes in different body systems and glands, including a short-latency heart rate (HR) acceleration, increases in blood pressure, cephalic vasoconstriction, increased amplitude and slow habituation of the skin conductance response (SCR), as well as a potentiation of defensive reflexes, such as the startle blink reflex (4-8).

A different pattern of responses characterises patients suffering from blood-injection-injury (BII) phobia, who do not usually display the defensive reaction described above when exposed to their feared objects. For example, some authors have found a diphasic cardiovascular response composed of an initial increase in heart rate and blood pressure, followed immediately by a marked decrease in both reactions, which may lead, in addition, to fainting as a consequence of the subsequent reduction in cerebral blood flow (9). Several explanations have been suggested for this atypical reaction. Graham et al. (9) proposed that the sustained decrease of HR and blood pressure is the result of an increase in parasympathetic activity initiated to counteract the initial abrupt sympathetic reaction provoked by the phobic stimulus. Following this proposal, Page (10) has attributed a key role to the parasympathetic activity associated to the disgust promoted by blood-related stimuli. Elsewhere, Engel (11) proposed that

the cardiovascular pattern found in BII phobia would be the result of a conflicting sympathetic and parasympathetic co-activation, or activation in rapid alternation of both branches of the ANS. Several studies, however, have failed to find data that support either a diphasic cardiovascular response in BII phobia or a key role of the parasympathetic activity (e.g. (12-15); for a review, see (16)). Rather, some findings show a sympathetic dysregulation (15) or even a lack of sympathetic activity, i.e., sympathetic withdrawal (17-19). In this line, some works have found no increase in other responses controlled by the sympathetic nervous system (e.g., SCR) when BII phobic participants were exposed to pictures related to blood and injuries (e.g. (6, 8, 20)).

Since research on this topic has not reconciled the data obtained in previous studies, measurements of some responses, other than cardiovascular reactions, under parasympathetic control would be helpful to study the involvement of vagal activity in this phobia. One index of parasympathetic activity is saliva production. Saliva is related to a variety of functions, including digestive, protective and trophic ones (for a review, see (21)). Salivary flow is mainly under parasympathetic control and allows the exploration of the vagal activity in isolation (21-23). Previous research has found that anxiety and emotional reactivity provoke changes in saliva production. Gemba et al. (24) found that the largest salivary flow rates were promoted by arousing emotions. Other studies have revealed that acute mental stress (25, 26) and the induction of emotional states, such as sadness (27), increase salivary flow rate. Viewing unpleasant stimuli has also been proved to provoke saliva production. Bosch et al. (28) demonstrated that viewing a surgical video increased salivary flow, and Ritz & Thoens (29) found that unpleasant pictures provoke increases in swallowing in comparison to pleasant and neutral pictures. The only work that has measured this index in BII fear

was conducted to study the physiological response related to disgust in BII fearful participants (30). For this purpose, authors collected saliva samples, in addition to other physiological measures, while participants imagined disgust-related and neutral scenes. Their results did not reveal differences between high- and low-fearful individuals, probably because no experimental condition related to blood or injury was included. It is worth mentioning that, taken together, these results do not support the dry-mouth effect related to stressful and aversive stimuli described in early studies (31).

Following previous research demonstrating the increase of salivary flow rate promoted by the exposition to unpleasant conditions, as well as the relationship of this index with parasympathetic activity, we measured this response in BII phobia participants exposed to phobia-relevant and non-phobia relevant pictures. The rationale behind this research was that if blood-related pictures provoke an increase in parasympathetic activity in BII phobic participants, then this would be reflected in a greater increase in salivary flow provoked by these pictures in comparison to other pictures, although this effect would not appear in other non-BII phobia participants. Saliva production was measured, therefore, after sustained exposure to phobic-related and non-phobic pictures. Previous research has also shown that a sustained aversive affective state can lead to the maintenance or even increase of defensive responses (32, 33). This effect is enhanced in subjects suffering from high state anxiety, suggesting that sustained exposure to an aversive context leads to a defensive mood state (34), characterized by an increase in sympathetic activity, which leads to a greater number of SCRs and HR acceleration. Thus, we also recorded these autonomic measures, in order to check whether phobic-related pictures provoked an increase in these indices and, therefore, a concurrent increase in sympathetic activity, as shown particularly by SCR. Although other autonomic indices can be used to study the influence of sympathetic

activity (e.g., peripheral vasomotor response), we decided to employ SCR in order to compare our data with those of previous studies that have used the continuous exposure to affective pictures (e.g., (32, 33, 35)). No specific prediction was made regarding the monophasic or diphasic HR response.

Since, as noted above, the BII phobia is a specific phobia that differs from other phobias in the reactions provoked by their feared objects, we also selected individuals suffering from other specific phobia (snake phobia), as a control phobic group, together with a non-phobia control group. We hypothesized that blood-related pictures would provoke in BII participants an augmented salivary production (e.g. (28)), as pointed above, accompanied by an increase in HR deceleration, in comparison with other picture contents (20), and an increase in SCRs related to the concurrent sympathetic activation. This pattern of responses would be in contrast to the defence response expected in snake phobia participants exposed to pictures related to their fear, which is characterised by increases in both HR -or lower deceleration- and SCR (6) and also in contrast to control participants, showing decreased HR and increases in SCRs provoked by the most arousing, unpleasant pictures, in comparison to neutral ones (e.g. (32, 34)).

2. Material and Methods

2.1 Participants

The study was advertised to students of the two first years of the studies in Psychology of the University of Murcia through both class and virtual platform advertisements. We included a statement in the advertisement indicating that we were looking for volunteers to study the physiological reactivity promoted by both unpleasant and fear-related pictures in fearful and non-fearful individuals. The Spanish versions of the Mutilation Questionnaire (MQ) and the Snake Questionnaire (SNAQ) (36) were

administered to 332 undergraduate students. Participants were initially included in the high blood-fear group (N = 22) if their scores in the MQ were above the 85th percentile (≥ 17) of the scoring distribution of the whole sample, and their SNAQ scores were below the 50th percentile (≤ 9). High snake-fear subjects (N = 18) were initially selected if their SNAQ scores were above the 85th percentile (≥ 17) of the scoring distribution of the whole sample, and their MQ scores were below the 50th percentile (≤ 8). Subjects were then screened with a semi-structured interview by a clinical psychologist in order to check whether they fulfilled the DSM-IV-TR criteria for specific phobia (37). Exclusion criteria were: a) suffering any other medical or psychopathological condition, or b) under prescribed medication.

The final phobic samples comprised 18 women who fulfilled the criteria for BII phobia –but not the criteria for other disorders, including snake phobia – and 14 women who fulfilled the criteria for snake phobia –but not for other disorders, including BII phobia (Table 1 summarizes the sociodemographic and clinical characteristics of the selected participants). The BII phobia group showed a MQ score mean = 19.84 and a SNAQ score mean = 5.56, while the snake phobia group showed a SNAQ score mean = 20.43 and a MQ score mean = 3.86. Since the resulting phobic groups were exclusively composed of women -an expected result according to previous studies on the prevalence of specific phobias (e.g. (38-42))- we selected only females for the non-phobic control group. This group, therefore, was composed of 22 participants randomly selected from the women who scored below the 50th percentile of the whole distribution in both questionnaires (MQ and SNAQ; see Table 1). Participants in this control group did not have any specific fear, as assessed by a 72-item form from the Fear Survey Schedule (FSS-III, (43)).

	BII phobia	Snake phobia	Non-phobia	Significance (*p*)
Group characteristics				
Number of participants	18	14	22	-
Age (years)	19.94 (1.80)	22.64 (9.72)	21.82 (6.91)	.497 (*n.s*)
Education (years)	14	14	14	-
Psychiatric comorbidities	0	0	0	-
Neurological disease	0	0	0	-
MQ Total	19.84 (2.12)	3.86 (2.45)	3.82 (2.79)	< .001
MQ range	17-24	1-8	0-8	-
SNAQ Total	5.56 (2.09)	20.43 (2.28)	5.59 (2.30)	<. 001
SNAQ range	2-9	17-25	0-9	-
FSS-III	-	-	122.02 (17.54)	-
Fainters	7	0	0	-
Experimental conditions				
Temperature (ºC)	18.28 (1.32)	18.50 (2.02)	19.21 (1.61)	.244 (*n.s.*)
Humidity (%)	66.67 (7.81)	66.82 (8.18)	64.36 (6.13)	.598 (*n.s.*)

Note: FSS = Fear Survey Schedule; MQ = Mutilation Questionnaire; SNAQ = Snake Questionnaire;

Table 1. Sociodemographic and clinical characterization of the groups of participants included in the study.

All participants under study signed a written informed consent, and all procedures were conducted in accordance with the Declaration of Helsinki and were approved by the Ethics Committee of the University of Murcia.

2.2 Materials and Design

We selected 36 affective pictures[1] from the International Affective Picture System (IAPS; (44)), according to the normative ratings for the Spanish population (45, 46). Pictures comprised three specific contents: blood-related (e.g., mutilations, illness,

and blood), snakes and household objects (neutral). The three categories of pictures differed in affective valence (a continuous affective dimension representing the hedonic tone, ranging from high unpleasantness to high pleasantness), $F(2,22) = 105.79$, $p < .001$ (blood-related < snakes < neutral; $p < .001$ for all comparisons). Arousal (a continuous affective dimension representing the level of activation, ranging from low arousal to high arousal) was also different between categories, $F(4,44) = 531.89$, $p < .001$. Blood-related and snake pictures did not differ in arousal level, and both were more arousing than neutral pictures (both $Ps < .001$).

We constructed three different blocks of pictures. Each block consisted of 12 pictures of the same content. Pictures were presented consecutively for 10 s each, with no inter-picture interval. To avoid any effect of the order of presentation of the blocks on the responses measured, we constructed three different presentation orders, and each participant was randomly assigned to one of them. Within each block, pictures were presented in the same order for all subjects. Samples of saliva were collected before and after each block of pictures. The pictures, as well as all the task instructions, were presented on a 19-inch computer monitor placed in front of the subject.

2.3 Saliva Collection and Data Reduction

For saliva collection, the passive drooling method was modified from the original procedure suggested by Navazesh (47) as described by Rohleder et al. (48). Participants were instructed first to void their mouths of saliva by swallowing. In contrast to the original method, saliva was then allowed to accumulate passively (without tongue movements) for 2 min. (instead of 5 min.). Participants then spat out all the saliva into 10-ml polystyrene tubes. This procedure was rehearsed during a pre-study session for all the participants, who received a written description of the full

process.

Immediately after collection, saliva samples were frozen and stored at -20ºC until analysis. After thawing, saliva obtained after 2 min of passive drooling was weighed and flow rate was expressed in ml/min (g/min) in accordance with the gravimetric method (49). For each block of pictures, the salivary flow deviated from the salivary flow obtained in the 2 min baseline interval preceding the block onset. Three participants (one per group) were discarded because of problems in saliva collection.

2.4 Psychophysiological Recording and Data Reduction

The physiological signals were acquired, amplified, and filtered by a Biopac MP150 data acquisition system (16-bit A/D converter). The electrocardiographic and respiratory signals were sampled at 1,000 Hz, while skin conductance was sampled at 250 Hz.

Lead-3 electrocardiogram was recorded by means of 7-mm Ag/AgCl surface electrodes filled with a hypertonic electrolyte paste. Heart rate was obtained by the offline transformation of the R-wave, using an automatic procedure to detect R-waves and to make corrections for false R-waves detection (heart periods shorter than 300 ms), providing a weighted mean of the beat-to-beat HR. The data of 3 participants (a non-phobic participant and 2 BII phobia participants) were discarded because of artefacts in their recordings, as revealed by visual inspection. For each picture within a block, we obtained the average HR for each second of picture duration. Each value was then deviated from the HR baseline defined as the HR average obtained in the 30s previous to the block onset. Lastly, for each picture within a block we calculated the minimum HR in the first 3 seconds after picture onset. These data were then averaged for each block.

Skin conductance was recorded by the bipolar placement of 7-mm Ag/AgCl standard surface electrodes filled with an isotonic electrolyte paste on the thenar (C6) and hypothenar (C8) eminences of the non-dominant hand surface. Following previous research (e.g. (32, 33)), we obtained the number of skin conductance responses higher than 0.05 μSiemens during each block of pictures. Finally, a square root transformation was used to normalize the SCR data.

2.5 Procedure

All the experimental sessions were conducted between 10:00 a.m. and 13:00 p.m. Participants were previously instructed that they should not eat or drink (except water) for at least one hour before the experimental session. On arrival at the laboratory, participants were accommodated in an armchair located 0.5 m in front of a computer screen and all the sensors were attached. Subjects were informed that a series of pictures would be displayed consecutively on the screen and that they should pay attention to each picture for its entire display length. In addition, they were also informed that all the instructions concerning each step of the task (e.g., accumulate saliva, dribble saliva, etc.) would be displayed on the screen. The task comprised a 3 min rest period followed by the presentation of the three blocks of pictures. Before each block, subjects were required, via a message on the screen, to accumulate saliva in their mouths for 2 min. After this period, a new message appeared indicating that they should dribble the saliva into a polystyrene tube. The pictures of the first block were then started. After the picture block offset, subjects were again required to accumulate saliva in their mouths for 2 min., and at the end of this interval a new message indicated that they should dribble the saliva into another polystyrene tube. A 270s rest period followed, after

which the sequence began again until the completion of all three blocks of pictures (see Figure 1).

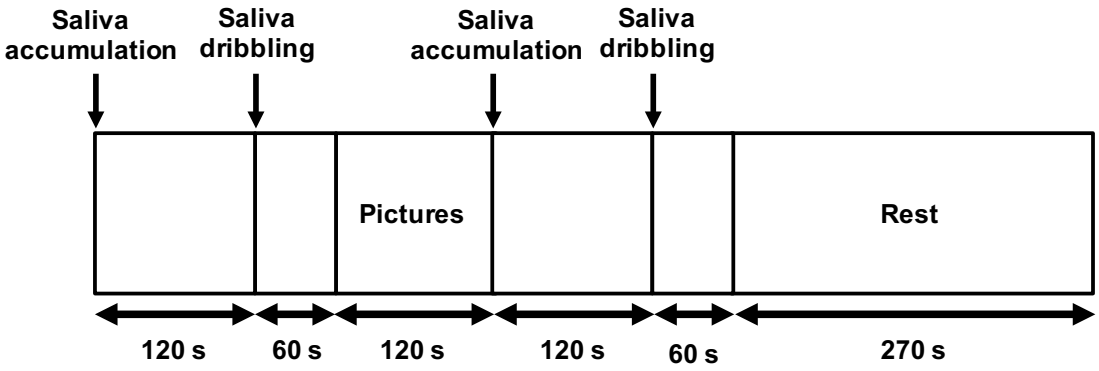

Figure 1. Sequence of events in a block (the three blocks followed the same sequence).

After the psychophysiological task, electrodes were removed and the saliva samples were placed inside the freezer. Subjects were then asked to view each picture again in a free viewing time setting and to rate the affective valence and arousal of each picture using a computerized version of the Self-Assessment Manikin, based on the original paper and pencil version (50). This computerized version presents, after each picture, a 9-point Likert scale for each dimension. For affective valence, 1 represents high unpleasantness and 9 high pleasantness, with 5 representing a neutral point between both, while for arousal, 1 represents very low arousal and 9 high arousal. The presentation order of the pictures was the same as in the psychophysiological task. The rating data from two participants were lost because of problems with the computers.

2.6 Data Analysis

The effect of the 3 blocks of pictures on each dependent variable was analysed by a mixed model ANOVA, 3 (Group: BII phobia, snake phobia and non-phobia participants) x 3 (Picture content: blood-related, snakes, and neutral), with the Group as between-subject variable, and Picture content as within-subject factor.

All the statistical analyses were performed with the PASW package (version 19; Chicago, IL). A measure of the effect size, partial eta-squared (η_p^2), was obtained for the main statistical tests. When appropriate, a Greenhouse-Geisser adjustment to the degrees of freedom was used in repeated measures tests in order to correct any potential inflation of the reported probability values, and epsilon values (ε) are reported (51). Post-hoc comparisons, as well as correlational analyses, were performed with a Bonferroni correction to control the overall level of significance (52). Lastly, the sample sizes needed to achieve a 0.80 power and an effect size $f = 0.14$ ($\eta_p^2 = 0.14$) were calculated with the G*Power software (version 3.1.9.2).

3. Results

3.1 Salivary Flow

The salivary flow varied depending on the Picture content, $F(2, 96) = 4.65$, $p = .01$, $\eta_p^2 = .088$. Pairwise comparisons showed that blood-related pictures provoked higher salivary flow (*Mean* = .107, *SD* = .26) than neutral pictures (*Mean* = -.014, *SD* = .25) ($p = .020$). A significant effect of Group was also found, $F(2, 48) = 4.74$, $p = .01$, $\eta_p^2 = .165$, revealing higher salivary flow in snake phobia participants (*Mean* = .16, *SD* = .15) than in non-phobia participants (*Mean* = .021, *SD* = .095) ($p = .01$).

However, these effects were qualified by a Group x Picture content significant interaction, $F(4, 96) = 2.56$, $p = .04$, $\eta_p^2 = .096$ (see Figure 2). Separate analyses conducted in each group revealed a significant effect of Picture content in only the BII

phobia group, $F(2, 32) = 3.89$, $p = .03$, $\eta_p^2 = .195$. Pairwise comparisons conducted in this group showed that blood-related pictures promoted higher salivary flow than neutral pictures ($p = .049$).

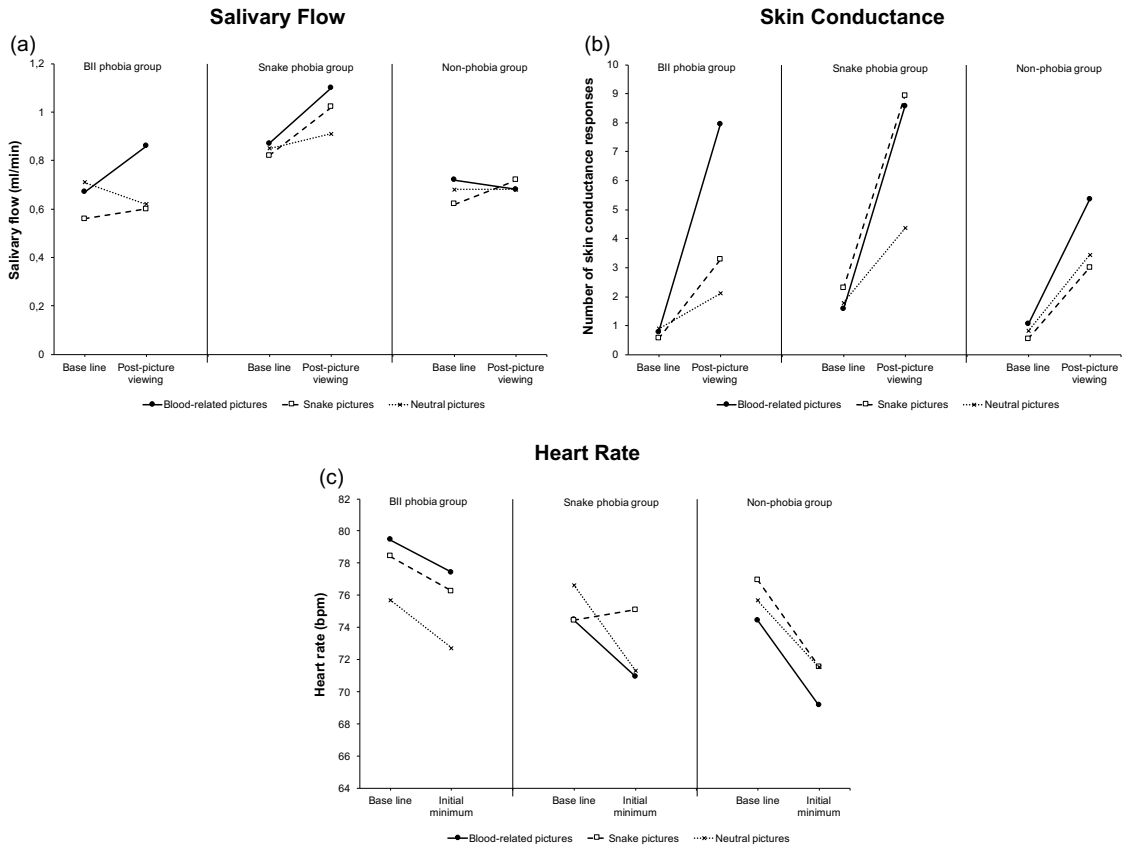

Figure 2. Physiological responses provoked by the block of pictures. (a) Salivary flow provoked by blood-related pictures was greater than that elicited by other contents in BII phobia. (b) Blood-related pictures elicited more SCRs than other contents in BII phobia, while in snake phobia both snake pictures and blood-related pictures provoked more SCRs than neutral pictures (for descriptive purposes, the figure shows the number of SCRs in the 30s previous to each block as baseline). (c) Heart rate showed an accelerative change only in snake phobia participants during the exposure to snake pictures.

3.2 Skin Conductance Response

The number of SCRs varied depending on the Picture content, $F(2,102) = 31.28$, $p < .001$, $\eta_p^2 = .380$. Blood-related pictures provoked more responses (Mean = 7.06, *SD* = 5.08) than snake pictures (*Mean* = 4.63, *SD* = 5.2) and neutral pictures (*Mean* = 3.24, *SD* = 3.53) ($p < .001$ for both comparisons), and, in turn, snake pictures provoked more SCRs than neutral pictures ($p = .046$). A significant main effect of Group was also found, $F(2,51) = 3.23$, $p = .048$, $\eta_p^2 = .112$. Paired comparison revealed that snake phobia participants showed more responses (*Mean* = 7.29, *SD* = 5.60) than control participants (*Mean* = 3.51, *SD* = 0.75) ($p = .04$).

This effect was qualified, however, by a Group x Picture content significant interaction, $F(4, 102) = 7.05$, $p < .001$, $\eta^2 = .217$ (see Figure 2). Separate analyses showed an effect of Picture content in all the groups, control group, $F(2,42) = 6.39$, $p = .004$, $\eta_p^2 = .233$, BII phobia, $F(2, 34) = 30.03$, $p < .001$, $\eta_p^2 = .639$, and snake phobia, $F(2, 26) = 10.85$, $p < .001$, $\eta_p^2 = .455$. In the BII group, blood-related pictures promoted more SCRs than both snake and neutral pictures ($p < .001$ for both comparisons), whereas these two picture categories did not differ between each other. In the snake phobia group, blood-related and snake pictures provoked more responses than neutral pictures ($p < .001$ and $p = .01$, respectively), whereas blood-related and snake pictures did not differ in the number of SCRs they elicited. Finally, in the control group, blood-related pictures provoked more responses than snake pictures ($p = .009$).

3.3 Heart Rate

Statistical analyses showed a significant Group x Picture content interaction, $F(4, 96) = 2.48$, $p = .049$, $\eta_p^2 = .094$. Analyses conducted separately in each group revealed a marginal effect of Picture content on HR in only the snake phobia group, $F(2,26) = 3.04$, $p < .07$, $\eta_p^2 = .190$ (see Figure 2). Snake pictures provoked lower HR deceleration than the blood-related and neutral contents, significant quadratic trend, $F(1,13) = 4.70$, $p = .05$, $\eta_p^2 = .265$, but paired comparisons showed only that snake pictures provoked lower HR deceleration than neutral pictures, $p = .05$.

3.4 Affective Judgments

Affective valence. A significant main effect of Picture content, $F(2, 98) = 108.15$, $p < .001$, $\eta_p^2 = .69$, revealed that blood-related pictures were rated as the most unpleasant, followed by snake pictures and neutral pictures ($p < .001$ for all comparisons; see Table 2). This effect was qualified by a significant Group x Picture content interaction, $F(4, 98) = 12.04$, $p < .001$, $\eta_p^2 = .239$ (see Table 2). In the BII phobia and non-phobia groups, $F(2, 30) = 82.60$, $p < .001$, $\varepsilon = .675$, $\eta_p^2 = .846$, and $F(2, 42) = 22.13$, $p < .001$, $\varepsilon = .724$, $\eta_p^2 = .513$, respectively, blood-related pictures were rated with lower affective valence than snakes and neutral pictures ($p < .001$ for both comparisons), while snake pictures and neutral pictures did not differ. In the snake phobia group, an effect of Picture valence was also found, $F(2, 26) = 47.99$, $p < .001$, $\eta_p^2 = .787$, but, in contrast with the other groups, blood-related pictures and snake pictures received similar affective valence ratings, and both were lower than the ratings of neutral pictures ($p < .001$ for both comparisons).

Participants	Pictures	Affective Valence Ratings (1-9)	Arousal Ratings (1-9)
BII phobia	Blood-related	1.80[a] (0.49)	7.24[d,e] (1.51)
	Snakes	5.13[b] (1.42)	3.37[f] (1.79)
	Neutral	5.39 (0.75)	1.19 (0.42)
Snake phobia	Blood-related	2.48 (0.88)	5.39[d] (2.08)
	Snakes	2.95[b,c] (1.61)	5.34[f,g] (2.37)
	Neutral	6.56 (1.68)	1.52 (0.93)
Non-phobia	Blood-related	2.94[a] (1.15)	4.77[e] (2.04)
	Snakes	4.85[c] (1.06)	3.10[g] (1.69)
	Neutral	5.39 (1.81)	1.40 (0.87)

Note: Shared superscripts indicate group differences ($p < .05$)

Table 2. Means (and standard deviations) for the affective ratings of the pictures

Arousal ratings. A significant main effect of Picture content was found, $F(2, 98) = 126.74$, $p < .001$, $\eta_p^2 = .721$, and paired comparisons revealed that blood-related pictures received the highest arousal ratings, followed by snake pictures, and by neutral ones ($p < .001$ for all comparisons; see Table 2). We also found a significant main effect of Group, $F(2,49) = 4.02$, $p = .02$, $\eta_p^2 = .141$, showing that snake phobia participants rated the pictures with higher arousal than non-phobia participants ($p = .045$). These effects were qualified by a significant interaction between Group and Picture content, $F(4, 98) = 9.48$, $p < .001$, $\eta_p^2 = .279$ (see Table 2). Analyses conducted separately showed an effect of Picture content in each group, BII phobia, $F(2, 30) = 101.79$, $p <$

.001, η_p^2 = .872, snake phobia, $F(2, 26) = 21.77$, $p < .001$, η_p^2 = .626, and non-phobia participants, $F(2, 42) = 40.22$, $p < .001$, η_p^2 = .657. Whereas BII phobia and non-phobia participants rated blood-related pictures the highest, followed by snake pictures and neutral ones ($p < .001$ for all comparisons), snake phobia participants rated blood-related and snake pictures with similar arousal ratings that were higher than those of neutral pictures ($p < .001$ for both comparisons).

3.5 Correlation analyses between the physiological indices

We conducted correlation analyses in order to check whether peripheral activity jointly varied in each group. Whereas in both control and snake phobia groups we did not find any significant correlation between variables, in the BII phobia group we only found a significant positive correlation between salivary flow and number of SCRs, r = .305, $p = .03$, $d = 0.641$.

4. Discussion

The main aim of this work was to study the salivary flow in BII participants exposed to pictures related to their phobia - as well as other autonomic responses including HR and SCR. We expected exposure to blood-related pictures would provoke a greater increase in BII participants' saliva production, as a result of an increase in parasympathetic activity in comparison to the salivary flow provoked by other affective contents. In addition, we also included a snake phobia group and a non-phobic group as control groups.

Overall, salivary flow was related to the content of the pictures, with blood-related pictures provoking greater saliva production than neutral ones. These findings are in agreement with past research that has revealed that mutilation pictures are among

the most threatening emotional stimuli and promote both strong physiological and somatic responses and high negative affect and arousal (e.g. (35, 53-55)). Our results also give support to previous studies that have related saliva production and emotion. For example, salivary flow has been found to increase as a result of acute stress and emotional arousal (e.g. (24-26)), and viewing unpleasant stimuli (e.g., videos and pictures) increases saliva production (28, 29). Moreover, the data obtained in this study give support to the work conducted by Bosch et al. (28), who found an increase in salivary flow provoked by the passive viewing of a surgical video and proposed that the mechanism behind this increase relies on parasympathetic activity. Accordingly, saliva flow could be a good measure of the emotional response provoked by affective stimuli, given the convergence of results from different studies.

In agreement with our main hypothesis, salivary flow increased in BII phobia participants following the presentation of blood-related pictures as compared to neutral ones. By contrast, salivary flow did not differ between phobia-related pictures and other contents in snake phobia. Neither did we find differences in salivary flow depending on the affective contents in non-phobic participants. These findings suggest, therefore, that BII phobic participants showed a salivary response more specifically related to their phobic stimuli than snake phobic or control participants. Our results do not agree with those of van Overveld et al. (30), who did not find greater saliva production in high blood-fear individuals in comparison to low blood-fear ones, probably because of the absence of an experimental condition related to blood, injection or injuries in their study. By including this condition, our research revealed that salivary production is greater in BII phobia after exposure to blood-related pictures, although we could not relate this response to disgust, since we did not include a disgust condition or obtain a subjective measure of this emotion.

Blood phobia participants, together with non-phobic individuals, rated blood-related pictures with the highest arousal values. This increase in the subjective arousal was also reflected in the higher number of SCRs provoked by these pictures in BII phobia in comparison with the other two affective contents. In both control and snake phobia groups, however, blood-related pictures did not evoke more SCRs than either neutral pictures (in the case of the non-phobia group) or snake pictures (in the case of the snake phobia group). Past research has not always found larger SCRs provoked by disorder-relevant pictures in BII fearful individuals (e.g. (6, 8, 20)). These contrasting data might be related to the continuous exposure to the affective contents used in our study. Thus, it has been found that continuous exposure to unpleasant stimuli increases or maintains defensive response mobilization, provoking larger and more frequent SCRs (e.g. (32, 33, 56)). Hence, the continuous exposure to blood-related pictures may have produced an increase in the number of SCRs in our BII phobia group. Snake pictures also provoked an increase in the number of SCRs in snake phobia individuals, although these responses did not differ from those provoked by blood-related pictures, probably because snake and BII pictures elicited the same subjective arousal in snake phobics.

The increase of SCRs provoked by blood-related pictures in BII phobic participants was not accompanied by HR acceleration, but by a cardiac deceleration similar to that elicited by the other picture contents. This is in contrast to the HR response found in snake phobia participants, who showed HR acceleration during the exposure to snake pictures and HR deceleration provoked by the other picture contents –that is, snake phobia participants exhibited a HR defence response when viewing pictures related to their phobia while BII phobia participants did not. In this regard, the absence of HR reactivity provoked by the blood-related pictures in BII phobia is in

agreement with past research reporting a HR drop in these phobic individuals, although some studies have also found increased HR and blood pressure, resembling a sympathetic response similar to the fight-and-flight response reported in other specific phobias (e.g. (15)). The decrease of HR in BII phobia during the exposure to blood-related stimuli may be related to a combination of several factors, including an increase in parasympathetic activity (9), a decrease in sympathetic activity (15), and/or an increase in ventilation by deep breathing (13, 57). It would be necessary, therefore, to include some additional sympathetic index related to cardiovascular activity, such as the vasomotor response or pre-ejection contractility, in order to characterize better the autonomic activity supporting cardiac response.

Our data support Engel's proposal of a sympathetic-parasympathetic imbalance in BII phobia, as shown by the increase in both salivary flow and number of SCRs provoked by the exposure to blood-related pictures -and the positive correlation found between these two indices- accompanied by HR deceleration. This is in contrast to the responses displayed by snake phobia participants exposed to snake pictures, who showed HR acceleration and increased SCRs, which have been previously related to the sympathetic reactivity associated to a defence response (4-8), whereas the salivary flow did not show any difference depending on the content of the pictures. These findings were supported by medium and large effect sizes (η_p^2), ranging from 0.088 to 0.872, according to Richardson (58) and Cohen (59). The origin of the sympathetic-parasympathetic imbalance in BII phobia might rely upon the uncertainty of the outcome of a potential threat (11). This uncertainty would perhaps provoke a dysfunction of the mechanisms of emotion regulation supported by structures of the prefrontal cortex, resulting in non-adaptive physiological responses (60, 61). Convergent neuroimaging data in BII phobia and dental phobia reveal an increase of

brain activity in the medial prefrontal cortex and the lateral orbitofrontal cortex, in contrast to other phobia and non-phobia participants, which would support this interpretation ((62, 63); see (64) for contrasting results). In this regard, a recent ERP study using a go/no-go task in BII phobia also found an increase in right frontal alpha power for no-go mutilation picture trials, which the authors interpret as a mechanism of passive avoidance from the phobogenic stimuli (65). The activity of these prefrontal regions would increase the parasympathetic activity via cortical-subcortical circuits (66) and would provoke, among other effects, an increase in salivary flow (e.g. (28)). Further research is needed, however, to confirm this hypothesis.

We must note some limitations related to our study. First, although the participants were instructed to look at the monitor during the picture viewing, we did not use any experimental procedure to control their gaze averting (e.g., through an eye tracker or video recording). Future research should control for participant's gaze, particularly when exposure times are as long as those used in this research. Second, some measure related to the participants is missing, such as the body mass index (BMI); though previous research has not related this index to BII phobia, it is always desirable that studies obtaining autonomic measures in patients make a complete characterization of their sample, as BMI. Third, a measure of heart rate variability (HRV) would have been desirable because of the relationship between this index (high-frequencies) and parasympathetic activity. However, we did not analyse HRV in our study because we had 2 min of continuous exposure to pictures and only 30s previous to the block onset free of participants' movements (they spat out saliva in the 1min before block onset), which did not allow us to obtain a reliable measurement. And fourth, we only examined female participants, as a result of our selection procedure. Although this is in accordance with previous research showing a greater prevalence of BII phobia in

females, it would be desirable to include men suffering from BII phobia in order to increase the generalization of our results.

In summary, our research reveals that BII phobia participants exhibit an increase in salivary flow, accompanied by increases in the number of SCRs, provoked by their feared objects, whereas snake phobia and non-phobic groups do not. In accordance with Engel's proposal (11), our findings show that phobic response in BII phobia is characterised by an imbalance in sympathetic and parasympathetic activity. From a clinical approach, these results are of importance because they imply that the reaction of these individuals is different from other specific phobias and, therefore, their treatment will differ in some respects from those used in other specific phobias. In this line, our data justify the treatment of BII phobia by using some procedures aimed at initially increasing their sympathetic reactivity (e.g., by means of the applied tension technique), which will result in HR and SCR increases, will avoid the fainting observed in some BII phobia patients and will thus allow application of treatments based on exposure to their feared object (67).

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Footnotes

[1]IAPS code for the pictures employed in the study. Blood-related: 3000, 3051, 3053, 3060, 3064, 3071, 3102, 3250, 3400, 3550, 9405, 9594. Snakes: 1019, 1022, 1030, 1040, 1050, 1052, 1070, 1080, 1101, 1110, 1113, 1114. Neutral: 7004, 7006, 7009, 7010, 7020, 7025, 7040, 7080, 7175, 7185, 7187, 7217.