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The role of testosterone and cortisol levels in nonsuicidal selfinjury in adolescents

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Abstract

Introduction: Nonsuicidal selfinjury (NSSI) is an important problem in adolescence, which is thought to serve several reinforcement functions (positive vs. negative, automatic vs. social). While the psychological mechanisms involved in NSSI are relatively well known, there is an important gap in the knowledge regarding the underlying biological mechanisms. This study examined the role of testosterone (*T*) and cortisol (*C*) in the frequency and reinforcement functions of NSSI.

Methods: A total of 423 adolescents (age range = 13–17; 54.4% girls) from Basque Country (Spain) provided saliva samples to determine *T* and *C* levels and completed measures of NSSI 6 months later in 2017–2018.

Results: The results showed that *T* but not *C* was significantly associated with higher NSSI frequency and the four types of NSSI functions. In addition, *C* moderated the predictive association between *T* and NSSI for automatic negative reinforcement, such that this association was significant only when *C* was high. Participant sex did not moderate any association between hormones and NSSI.

Conclusions: These preliminary results suggest that testosterone levels can affect NSSI behaviors in adolescence, thus helping to explain the increase in NSSI during this stage. Moreover, in situations in which NSSI serves to alleviate negative internal states, high levels of cortisol can be involved.

KEYWORDS

adolescents; nonsuicidal selfinjury; testosterone, cortisol

1 | INTRODUCTION

During adolescence, there is a notable increase in rates of nonsuicidal selfinjury (NSSI; Brown & Plener, 2017), defined as "the direct and deliberate destruction of one's own body tissue in the absence of lethal intent" (Nock, 2010; p. 340). Selfinjury involves risky behaviors like cutting, scratching, burning, hitting, and interfering with wound healing (Klonsky, 2011). Moreover, it has been proposed that NSSI represents a preliminary step in the development of suicidality in adolescents (Grandclerc et al., 2016). It has been proposed that the increase in risk-taking behaviors during this developmental stage could be partially explained by a mismatch between the incentive processing system, which matures earlier, and the capacity for selfregulation, which matures later (Casey et al., 2016).

Most of the theoretical models developed to explain NSSI propose that selfinjury may be performed as a dysfunctional emotional regulation function in situations in which the adolescent experiences considerable distress (Klonsky, 2011). This would explain why NSSI is more frequent in adolescents exposed to adverse circumstances, such as family maltreatment and peer victimization (Faura-Garcia et al., 2021b; Lanzillo et al., 2023; Liu et al., 2018). The four-function model of NSSI (Nock & Prinstein, 2004, 2005) completes this perspective by adding behavioral principles to explain NSSI. According to this model, NSSI behavior is maintained through four reinforcement functions, hierarchically organized along two dimensions: positive

versus negative reinforcement and automatic versus social reinforcement. Combining both dimensions, the four resulting functions are: (1) automatic positive reinforcement, when NSSI serves to generate positive feelings or selfstimulation; (2) automatic negative reinforcement, when it serves to reduce or eliminate negative feelings or thoughts; (3) social positive reinforcement, when it serves to obtain attention, resources, or help from other people; and (4) social negative reinforcement, when it serves as a way to escape or avoid social situations or interpersonal demands (Bentley et al., 2014). Previous research has found that automatic positive reinforcement is one of the most common functions of NSSI in adolescents (for reviews, see Edmondson et al., 2016; Klonsky, 2007). For example, Edmondson et al. (2016) found that 93% of the studies using questionnaires endorsed items including content like relieving distress or emotional pain and calming down. Moreover, in a study on adolescents, the most frequent functions were related to stopping bad feelings, relieving "numb" or "empty" feelings, and selfpunishment (Zetterqvist et al., 2013). However, in their review, Kaess et al. (2021) concluded that the evidence for the reward system involved in NSSI is mixed and that further research is needed.

While knowledge about the psychosocial mechanisms involved in the development of NSSI has advanced considerably, the identification of biological mechanisms is still at an early stage (Kaess et al., 2021). One biological factor that could be involved in NSSI behaviors is testosterone (*T*) levels. *T* is a hormone that is an end product of the hypothalamic–pituitary–gonadal (HPG) axis (Terburg et al., 2009). *T* levels increase considerably during adolescence, especially in boys (Konforte et al., 2013), and have been studied for decades in relation to risk-taking behaviors (i.e., engagement in behaviors that are associated with some probability of negative consequences; Boyer, 2006). However, several reviews and meta-analyses have failed to clearly establish these associations, and, overall, the associations found have been relatively weak (Geniole et al., 2020; Kurath & Mata, 2018).

The lack of consistency in the results concerning the role of *T* in risk-taking behavior led to the proposal that its action may be moderated by other variables, such as cortisol (*C*). *C* is an end product of the hypothalamic–pituitary–adrenal (HPA) axis, which is activated when an individual is confronted with stressors (Terburg et al., 2009). Whereas *T* levels have been associated with the behavioral activation system, which leads to an increase in the probability of performing behaviors that lead to rewards, *C* levels have been related to behavioral inhibition and punishment sensitivity (Arnett, 1997; Terburg et al., 2009). Although the HPA and HPG axes can modulate each other, the way in which this mutual modulation occurs is an ongoing debate in the literature (Grebe et al., 2019). A model that has received considerable attention is the dual hormone hypothesis (DHH), which proposes that high *T* is associated with risk taking only when *C* is low. Conversely, when *C* levels are high, the DHH predicts that the association between *T* and risk taking is blocked or inhibited (Mehta & Josephs, 2010; Mehta et al., 2015). Mehta et al. (2015) found a positive association between *T* and several indicators of risk-taking behavior (selfreported, informant report, and behavior) among participants who were low in *C* but not among participants who were high in *C*. Moreover, their results were consistent in both men and women.

Despite the research interest in DHH, posterior empirical support for it remains modest. One meta-analysis found only marginal support for the DHH, with very small effect sizes for the interaction between *T* and *C* on several behaviors, including risk taking (Dekkers et al., 2019). Moreover, some studies have found statistically significant $T \times C$ interactions, but with different patterns than proposed by the DHH. For instance, Barel et al. (2017) found that the association between *T* and selfreported risk-taking behavior was strengthened with high levels of *C*. Other studies focused on aggressive reactions have also found that they were associated with different combinations of *T* and *C*. For example, in one study, *T* was associated with more aggressive reactions only when *C* was high (Denson et al., 2013). In another study, the combinations of both low *C* and *T* and both high *C* and *T* were associated with more aggressive reactions (Armstrong et al., 2021).

While the DHH has been used to examine several risk-taking behaviors, there is an important gap in its application to NSSI. Surprisingly, the combined role of *T* and *C* in NSSI has not been examined. Moreover, the only available evidence about the association between *T* and NSSI was obtained in a study on male adolescents with depression, in which higher levels of *T* were found in those who selfinjured than in those who did not (Ma et al., 2022). In contrast, several studies have evaluated the role of *C* in NSSI. Most of these studies have found an attenuated salivary *C* response to social stress. In a sample of adolescent girls, those with NSSI compared to those without NSSI showed an attenuated salivary *C* response to a standardized psychosocial stress procedure (Kaess et al., 2012). In another study on adolescent girls, those with severe NSSI showed a blunted *C* response (Başgöze et al., 2021). In samples including both boys and girls, adolescents with both depression and NSSI showed an attenuated salivary *C* response compared with both adolescents with only depression and healthy adolescents (Klimes et al., 2019; Klimes-Dougan et al., 2018). In a different stressful situation, adolescents with NSSI showed reduced salivary *C* levels compared to their siblings during a stressful interview in which they discussed adverse experiences (Reichl et al., 2019).

Although these findings generally support the conclusion that lower salivary *C* may be involved in NSSI, there are also mixed results. For example, Carosella et al. (2023) studied adolescent girls, finding that a higher prepandemic *C* response to stress was associated with the persistence of NSSI. Koenig et al. (2017) found that adolescents with NSSI exhibited a greater increase in *C* levels during nociceptive stimulation than healthy adolescents. The authors suggested that the *C* response to pain may be different from that observed in social stress situations. Finally, results based on other methodologies have been mixed. For example, Plener et al. (2017) examined plasma *C* levels in a sample of adolescent girls and reported that those with

NSSI showed lower *C* levels in response to a social stressor but no differences in emotional reactivity to the task. Likewise, NSSI has been associated with higher levels of hair *C* (Reichl et al., 2019). However, in a sample of mostly adolescent females, Reichl et al. (2016) found no differences in hair *C* levels between the NSSI group and the healthy group. Instead, the authors found that participants with NSSI exhibited a greater *C* awakening response (Reichl et al., 2016). The authors interpreted this result as indicating that adolescents in the NSSI group were able to anticipate more difficulties and stressors throughout the coming day. Interestingly, this increase in *C* awakening was not maintained throughout the day, which they interpreted as implying that NSSI acted as a maladaptive strategy to manage daily stressors, such that high *C* levels were not necessary, and even protected against excessive activation of the HPA axis (i.e., allostatic load theory; McEwen, 2004). Thus, the elevated *C* awakening response is not incompatible with lower salivary *C* response to acute stressors.

The role of *T* and *C* in NSSI could be different for boys and girls. Previous research has reported mixed results regarding possible sex differences in NSSI. Bresin and Schoenleber (2015) found that females were significantly more likely to report a history of NSSI than males, while other meta-analyses found no statistically significant differences according to sex (Lang & Yao, 2018; Swannell et al., 2014). Regarding the functions of NSSI, women reported significantly higher use of NSSI for automatic negative and positive reinforcement than males in samples of adolescents (Faura-Garcia et al., 2022), young adults (Whitlock et al., 2011), and adult patients (Claes et al., 2007), while adolescent boys reported more use of NSSI for social negative and positive reinforcement than girls (Calvete et al., 2015). Regarding hormone levels, *T* levels are higher in boys than in girls (Konforte et al., 2013). Moreover, although some studies have found that the association between *T* and risk behaviors is higher in boys than in girls (Costello et al., 2007; de Water et al., 2013; Susman et al., 2017), the only study on the association between *T* and NSSI involved a small sample of boys (Ma et al., 2022). Finally, most of the previous studies on the role of *C* in NSSI were based on samples of adolescent girls (Başgöze et al., 2021; Carosella et al., 2023; Kaess et al., 2012; Reichl et al., 2019), and those that included both boys and girls did not examine differences in the role of *C* in NSSI by sex (Reichl et al., 2016). For all these reasons, it is necessary to evaluate the role of *T* and *C* levels in NSSI in samples of both adolescent boys and adolescent girls.

2 | THE CURRENT STUDY

The aim of this study was to examine the joint role of *T* and *C* in NSSI in adolescents. Despite the gap in studies on the DHH in the context of NSSI, there are reasons to predict that both hormones (*T* and *C*) may play a relevant role. Adolescence is a stage in which NSSI (Brown & Plener, 2017), *C* (van den Bos et al., 2014), and *T* (Konforte et al., 2013) levels increase, the latter in boys especially. In addition, as mentioned above, NSSI serves different immediate reinforcement functions (Nock & Prinstein, 2004), and it has been proposed that both *T* and *C* may play an important role in the incentive processing system (Herbert, 2018). Thus, *C* has been associated with the behavioral inhibition system such that adolescents with high levels of *C* show greater sensitivity to punishment, whereas *T* has been associated with the behavioral activation system, which can inhibit punishment sensitivity (Terburg et al., 2009).

However, the role of *T* and *C* in the different functions of NSSI is uncertain. In principle, *T* might be expected to be particularly involved in functions involving positive reinforcement (i.e., the possibility of obtaining pleasurable sensations or social benefits). As for *C*, previous research suggests that adolescents with NSSI show an attenuated response to stressors (Kaess et al., 2012; Klimes-Dougan et al., 2018, 2019). However, it has also been found that the role of *C* may be different in the event of painful stimulation (Koenig et al., 2017), which is what occurs in NSSI behaviors. Moreover, as *C* increases in stressful situations (Kurath & Mata, 2018; Law & Clow, 2020), elevated *C* levels are expected to be involved in NSSI behaviors that serve to reduce distress. Therefore, we adopted an exploratory perspective and did not propose any specific hypothesis about the associations between *T*, *C*, and NSSI.

Finally, as mentioned, an important gap in previous research is the lack of studies comparing the effect of *T* and *C* on NSSI according to sex. Therefore, a secondary objective was to examine whether the associations between these hormones and the frequency and functions of NSSI were similar in boys and girls.

3 | MATERIALS AND METHODS

3.1 | Participants

The sample for this study came from a larger longitudinal project, in which the role of *T* and *C* in aggressive behaviors was assessed (Calvete & Orue, 2024). Measures of NSSI were included in the third wave of the study (November 2017–January 2018). Although the measures of *T* and *C* were collected in all waves of the broader project, because the NSSI measure employed in this study retrospectively assessed the occurrence of selfinjury during the past 6 months, the hormone measures in the second wave (May–June 2017) were chosen in this study to assess whether they predicted NSSI behaviors during the next 6 months. The sample for this study

included 423 adolescents who completed both hormone measures and NSSI selfreport (Age range = 13–17; mean age = 15.05, SD = .92; 54.4% girls). The participants came from seven schools in Basque Country (Spain) and were in ninth grade (31%), tenth grade (50.1%), and high school (18.9%). According to the parents' occupational data, the family socioeconomic levels were as follows: 16.4% low, 20.5% medium–low, 29.7% medium, 20.2% medium–high, and 13.2% high.

3.2 | Measures

Saliva samples for measuring *T* and *C* levels were collected in the morning, approximately 3 h after the adolescents woke up (*M* = 11.02 a.m.; SD = 1.25). This collection took place in the adolescents' classrooms, and the participants were asked to spit a minimum of 2 mL into a plastic tube. Before that day, the participants were given instructions to follow for the saliva sample to be valid: They should not brush their teeth or ingest food or drink for at least 1 h before the saliva collection. They were also asked not to perform any strenuous exercise in the previous 8 h. The researchers checked that the instructions had been followed and that the samples were not contaminated with blood. After collection, the samples were frozen at −20°C until they were analyzed.

Saliva samples were assayed for *T* (pg/mL) and *C* (nmol/L) levels in duplicate determinations. A Salimetrics® Testosterone Enzyme Immunoassay Kit (Item No.1-2402, 96-Well Kit) was used to analyze T. This is a competitive immunoassay specifically designed to standardize the detection of *T* in saliva samples. *T* values from samples with a pH < 4.0 or >9.0 may be inaccurate, and thus they were discarded. Interassay coefficients range between 1.9% and 6.7%. The minimum concentration of *T* that can be differentiated from 0 is 0.458 pg/mL, and the functional sensitivity of the salivary ER *T* assay is 0.68 pg/mL. C was measured with an electro-chemiluminescence immunoassay (ECLIA; Elecsys Cortisol II, F. Hoffmann-La Roche AG) with Cobas e 602 (Roche, Switzerland). Inter-assay coefficients range between 1.7% and 9.3%, and the minimum concentration that can be detected is 1.5 nmol/L. In total, 31 samples (1%) were eliminated because they did not meet the required criteria (e.g., participants' disregard for the rules).

The Functional Assessment of Selfmutilation (FASM) Scale (Lloyd-Richardson et al., 2007; Spanish version: Calvete et al., 2015) was used 6 month later to assess the frequency of NSSI behaviors and the use of NSSI with automatic and social reinforcement functions in the last 6 months. In the first part of the FASM, adolescents indicated the frequency with which they had engaged in selfinjurious behaviors (e.g., cutting, picking a wound, selfinjury, burning their skin) during the past 6 months using a scale from 0 to 4 (0 = never; 1 = 1–2 times; 2 = 2–5 times; 3 = 6–10 times; 4 = 11 or more times). Lloyd et al. (1997) differentiated between methods of NSSI that could be more severe from a clinical perspective (cutting, burning, selftattooing, scraping, and erasing skin) and less severe methods (hitting self, pulling hair, biting self, inserting objects under nails or skin, and picking at a wound). Responses were recoded to 0, 1.5, 3.5, 8, and 15, respectively, to more precisely indicate the frequency of the behaviors. In the second part of the FASM, the adolescents answered questions about how often they had engaged in NSSI for each of the different reasons, with responses ranging from 0 (*never*) to 3 (*very often*). The questions in the second part assess four combinations of reinforcement: automatic negative reinforcement (ANR; two items; e.g., using NSSI to relieve feeling numb or empty), automatic positive reinforcement (APR; two items; e.g., to feel something, even if it was pain), social negative reinforcement (SNR; 12 items; e.g., to avoid punishment or paying the consequences), and social positive reinforcement (SPR; four items; e.g., to receive more attention from your parents or friends). The FASM has been validated internationally (Faura-Garcia et al., 2021a) and adapted for use with a large sample of adolescents in Spain (Calvete et al., 2015). In the validation study, the factor structure of the reasons section was confirmed, and the items showed excellent factor loadings on their corresponding subscales (ANR, APR, SNR, SPR). The alpha coefficients obtained in previous studies for the reasons subscales were adequate (Calvete et al., 2017). In the current study, the alpha coefficients were 0.67, 0.65, 0.91, and 0.74, respectively, for the four subscales.

3.3 | Procedure

The ethical committee of University of Deusto approved the study protocol. Adolescents and their parents received information about the study and were required to provide informed active consent. The acceptance rate was 74% for the parents and 100% for the adolescents. Only adolescents whose parents agreed to their participation were allowed to participate (*N* = 423). The participants provided the saliva samples and responded the selfreport questionnaires in the classroom. The adolescents without parental permission used this time for academic activities.

3.4 | Statistical analysis

Little's test of Missing Completely at Random (MCAR) was not statistically significant ($\chi 2$ (14) = 12.39, $p = .574$). IBM-SPSS-28 was used to test the study hypotheses. Ten outliers were identified in *T* values, which were winsorized at three SDs above

the mean. *C* values were logarithmically transformed because they were not normally distributed. With this operation, the distribution was normal. *T* and *C* values were transformed into *z* scores to create the $T \times C$ interaction term.

We conducted separate regressive models for each NSSI indicator (frequency of NSSI behaviors, SPR, SNR, APR, and ANR). In all models, *T* and *C* levels were included in the first step, and the $T \times C$ interaction term was included in the second step. In addition, as differences in hormones are expected to depend on age, the age of the participants was included as a covariate in the first step.

Finally, we performed a series of secondary analyses to assess whether the sex of the participants moderated the associations between hormones and NSSI. In the first step, the models included the sex variable along with age and hormone levels. In the second step, the second-order interaction terms ($T \times C$, $T \times$ sex, $C \times$ sex) were included, and in the third step, the $T \times C \times$ Sex interaction term was included.

Post hoc power estimated with G*Power.3.9.7 was 0.92 for the main models ($N = 423$, four predictors, $\alpha = .05$, $\chi^2 = .04$) and 0.96 for the secondary models that included the moderating effect of sex ($N = 423$, eight predictors, $\alpha = .05$, $\chi^2 = .06$). Data are available at osf (https://osf.io/wy94k).

4 | RESULTS

NSSI behaviors were frequent: 48.2% of the adolescents in the sample reported engaging in at least one NSSI behavior in the last 6 months, and 13.9% reported engaging in at least five severe NSSI behaviors (e.g., burning skin, cutting). The percentages of adolescents who indicated having performed each behavior at least once were as follows: cut or carved your skin (15.1%), hit yourself on purpose (14.3%), pulled your hair out (10.5%), gave yourself a tattoo (5.9%), picked at a wound (27.9%), burned your skin (11.4%), inserted objects under your nails or skin (10.6%), bit yourself (18.3%), scraped your skin (19.6%), and erased your skin to the point of drawing blood (11.1%). The average number of methods used was 1.44 (SD = 2.23, range: 0–10).

Table 1 displays the correlation coefficients of the study variables. As can be seen in the table, *T* was related to *C* and also to the rest of the study variables: frequency of NSSI, SPR, SNR, APR, and ANR. On the contrary, *C* was not related to any of them. As expected, the frequency of NSSI and the four functions were all interrelated. Finally, age was only positively related to *T*.

A series of *t*-tests were performed to examine differences in the study variables by sex. The results are shown in Table 2. Boys scored higher than girls on *T*, SPR, SNR, APR, and ANR, while girls scored higher on *C*. There were no differences in the frequency of NSSI. Effect sizes were large for *T* and small for the rest of the variables.

Table 3 displays the results for the regression models for *T*, *C*, and $T \times C$ explaining indicators of NSSI. *T* was significantly associated with all NSSI indicators. In addition, the $T \times C$ interaction was statistically significant for ANR. Figure 1 presents the form of the interaction for ANR for low (−1 SD) and high (+1 SD) levels of *T* and *C*. *T* was associated with higher use of NSSI for ANR when *C* was high ($B = .17$, $t = 3.78$, $p < .001$) and was not associated when *C* was low ($B = .01$, $t = .29$, $p = .771$).

TABLE 1 Correlation coefficients between the study variables and descriptive statistics.

	1	2	3	4	5	6	7	8	Range
1. *T*	1								5.5–173.30
2. Log(*C*)	0.19**	1							−0.65–1.95
3. NSSI Frequency	0.12*	0.04	1						0–90
4. SPR	0.17**	0.01	0.52**	1					0–2
5. SNR	0.16**	0.00	0.51**	0.88**	1				0–2.17
6. APR	0.14**	0.04	0.58**	0.74**	0.73**	1			0–3
7. ANR	0.16**	0.09	0.61**	0.63**	0.67**	0.62**	1		0–3
8. Age	0.26**	0.09	−0.01	−0.05	−0.07	−0.06	−0.04	1	13.44–17.86
Mean	60.72	0.41	6.28	0.19	0.22	0.24	0.22	15.05	
SD	33	0.54	1.33	0.43	0.45	0.56	0.54	0.92	
N	423	420	423	423	422	422	422	423	

Abbreviations: ANR, automatic negative reinforcement; APR, automatic positive reinforcement; *C*, cortisol; NNSI, nonsuicidal selfinjury; SNR, social negative reinforcement; SPR, social positive reinforcement; *T*, testosterone.

**$p < .01$

TABLE 2 Sex differences in the study variables.

	Boys ($N = 193$)		Girls ($N = 230$)					
	M	SD	*M*	SD	*t*	*df*	*p*	*d*
T	80.59	33.03	44.04	21.89	13.14	423	<.001	1.33
Log (C)	0.32	0.54	0.49	0.53	−3.29	420	<.001	0.32
NSSI frequency	7.31	1.51	5.41	11.45	1.43	423	.153	0.14
SPR	0.28	0.51	0.12	0.32	−3.75	423	<.001	0.38
SNR	0.32	0.54	0.14	0.34	4.03	422	<.001	0.41
APR	0.30	0.60	0.20	0.51	1.75	422	.080	0.17
ANR	0.29	0.62	0.16	0.46	2.34	422	.020	0.23

Abbreviations: ANR, automatic negative reinforcement; APR, automatic positive reinforcement; *C*, cortisol; NNSI, nonsuicidal selfinjury; SNR, social negative reinforcement; SPR, social positive reinforcement; *T*, testosterone.

TABLE 3 *T* and *C* as explanatory variables of NSSI indicators.

	B	SD	*β*	*t*	*p*	95%CI	
NSSI frequency							
Age	−0.59	.72	−.04	−0.82	.411	−2.01	0.82
T	1.67	.70	.12	2.41	.017	0.31	3.04
C	0.26	.65	.02	0.41	.684	−1.01	1.54
T × C	1.04	.66	.08	1.57	.117	−0.26	2.34
SPR							
Age	−0.04	.02	−.10	−1.98	.048	−0.09	0.00
T	0.09	.02	.21	4.07	.000	0.05	0.13
C	−0.01	.02	−.03	−0.52	.606	−0.05	0.03
T × C	0.01	.02	.02	0.31	.754	−0.03	0.05
SNR							
Age	−0.06	.02	−.13	−2.60	.010	−0.11	−0.02
T	0.10	.02	.21	4.19	.000	0.05	0.14
C	−0.01	.02	−.03	−0.60	.550	−0.06	0.03
T × *C*	0.00	.02	.01	0.19	.850	−0.04	0.05
APR							
Age	−0.06	.03	−.10	−1.90	.058	−0.12	0.00
T	0.10	.03	.17	3.35	.001	0.04	0.15
C	0.01	.03	.01	0.28	.783	−0.05	0.06
T × *C*	−0.02	.03	−.03	−.60	.551	−0.07	0.04
ANR							
Age	−0.06	.03	−.10	−1.92	.055	−0.11	0.00
T	0.09	.03	.17	3.26	.001	0.04	0.15
C	0.03	.03	.06	1.32	.189	−0.02	0.09
T × *C*	0.08	.03	.14	2.94	.003	0.03	0.13

Abbreviations: ANR, automatic negative reinforcement, APR, automatic positive reinforcement; *C*, cortisol; NNSI, nonsuicidal selfinjury; SNR, social positive reinforcement; SPR, social positive reinforcement; *T*, testosterone.

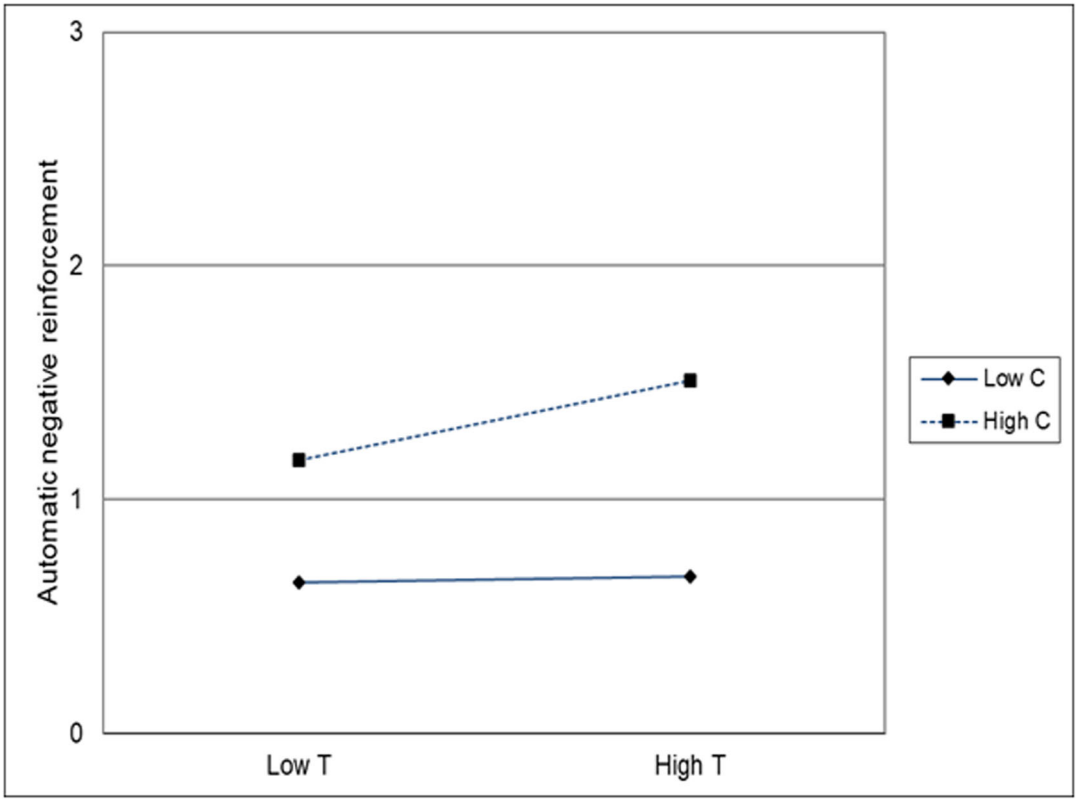

FIGURE 1 $T \times C$ Interaction for automatic negative reinforcement. *C*, cortisol; *T*, testosterone.

Table 4 displays the results of the models including sex as moderator of the associations between hormones and NSSI. None of the interactions with sex was statistically significant, suggesting that the associations between hormones and NSSI are similar in boys and girls. The rest of the associations were very similar to those obtained in the main models that did not include sex as a predictor. The only difference was that the associations between *T* and NSSI frequency and SNR became marginally significant.

5 | DISCUSSION AND CONCLUSION

NSSI is a clinical problem with great relevance in adolescence, a developmental stage accompanied by numerous psychosocial and biological changes. It has been proposed that some hormones that increase in adolescence, such as *T* and *C*, may play an important role in the incentive processing system that is involved in risk-taking behaviors (Terburg et al., 2009). Consequently, the main objective of this study was to evaluate the joint role of salivary *T* and *C* levels in the behavior and functions of NSSI in adolescents. In doing so, we contributed to filling a knowledge gap regarding the biological mechanisms involved in NSSI during the adolescence.

The main finding of the study is that *T*, but not *C*, is associated with both a higher frequency of NSSI behaviors and higher levels of all the assessed functions. The association between *T* and NSSI is consistent with the results of the only previous study that examined such an association (Ma et al., 2022). In that study, higher levels of *T* were found in individuals who selfinjured than in those who did not, but the study was limited to a small sample of male adolescents with depression. Thus, the current study extends this finding to a larger sample of both boys and girls and to several indicators of NSSI. Moreover, the association of *T* with the frequency and functions of NSSI is consistent with the motivational imbalance model (Arnett, 1997), which implies that *T* would be associated with the behavioral activation system and inhibit punishment sensitivity (Terburg et al., 2009).

C, on the other hand, was not associated with any indicator of NSSI. This result differs somewhat from those obtained in several previous studies in which adolescents who selfinjured showed an attenuated salivary *C* response compared to those who did not selfinjure (Kaess et al., 2012; Klimes-Dougan et al., 2018; Klimes-Dougan et al., 2019). The different results can likely be attributed to differences in the methodologies employed, as most previous studies assessed the change in *C* in

TABLE 4 T, C, and sex as explanatory variables of NSSI indicators.

	B	SD	*β*	*t*	*p*	95%CI	
NSSI frequency							
Age	−0.61	0.78	−.04	−0.79	.430	−2.14	0.91
T	1.91	1.14	.14	1.67	.096	−0.34	4.16
C	−0.17	1.18	−.01	−0.14	.887	−2.50	2.16
Sex (1 = female)	−0.27	1.90	−.01	−0.14	.887	−4.01	3.47
T × *C*	1.09	0.99	.08	1.11	.269	−0.85	3.03
T × sex	−0.81	1.85	−.03	−0.44	.662	−4.44	2.83
C × sex	1.12	1.66	.06	0.68	.499	−2.13	4.37
T × *C* × sex	0.60	1.63	.03	0.37	.712	−2.61	3.81
SPR							
Age	−0.03	0.02	−.06	−1.10	.272	−0.07	0.02
T	0.08	0.04	.18	2.14	.033	0.01	0.15
C	0.01	0.04	.03	0.33	.742	−0.06	0.09
Sex (1 = female)	−0.13	0.06	−.16	−2.21	.027	−0.25	−0.02
T × *C*	−0.01	0.03	−.02	−0.26	.794	−0.07	0.05
T × sex	−0.09	0.06	−.11	−1.48	.141	−0.20	0.03
C × sex	0.01	0.05	.01	0.15	.879	−0.09	0.11
T × *C* × sex	0.05	0.05	.07	0.88	.379	−0.06	0.15
SNR							
Age	−0.05	0.03	−.09	−1.74	.082	−0.10	0.01
T	0.07	0.04	.16	1.92	.056	−0.00	0.15
C	−0.01	0.04	−.01	−0.14	.889	−0.08	0.07
Sex (1 = female)	−0.12	0.06	−.13	−1.84	.067	−0.24	0.01
T × *C*	0.01	0.03	.02	0.22	.827	−0.06	0.07
T × sex	−0.04	0.06	−.05	−0.69	.492	−0.16	0.05
C × sex	0.02	0.06	.03	0.29	.770	−0.09	0.12
T × *C* × sex	0.00	0.05	.01	0.08	.936	−0.10	0.11
APR							
Age	−0.06	0.03	−.09	−1.70	.091	−0.12	0.01
T	0.13	0.05	.22	2.65	.008	0.03	0.22
C	0.01	0.05	.03	0.31	.758	−0.08	0.11
Sex (1 = female)	0.01	0.08	.01	0.09	.925	−0.15	0.16
T × *C*	−0.01	0.04	−.03	−0.35	.730	−0.10	0.07
T × sex	−0.07	0.08	−.07	−0.90	.371	−0.22	0.08
C × sex	−0.03	0.07	−.03	−0.36	.720	−0.16	0.11
T × *C* × sex	−0.02	0.07	−.02	−0.27	.785	−0.15	0.12
ANR							
Age	−0.05	0.03	−.08	−1.44	.151	−0.11	0.02

TABLE 4 (Continued)

	B	SD	*β*	*t*	*p*	95%CI	
T	0.12	0.05	.22	2.70	.007	0.03	0.21
C	0.02	0.05	.04	0.43	.669	−0.07	0.11
Sex (1 = female)	−0.03	0.08	−.02	−0.33	.742	−0.17	0.12
T × *C*	0.11	0.04	.20	2.78	.006	0.03	0.19
T × sex	−0.11	0.07	−.11	−1.45	.149	−0.25	0.04
C × sex	0.00	0.07	.00	0.01	.995	−0.13	0.13
T × *C* x Sex	−0.07	0.07	−.09	−1.13	.257	−0.20	0.05

Abbreviations: ANR, automatic negative reinforcement; APR, automatic positive reinforcement; *C*, cortisol; NNSI, nonsuicidal selfinjury; SNR, social positive reinforcement; SPR, social positive reinforcement; *T*, testosterone.

response to a standardized laboratory social stressor, whereas the present study assessed *C* levels in a pretest conducted 6 months before selfreporting NSSI.

The second finding is that support for the DHH was weak, as *C* only moderated the association between *T* and ANR. Specifically, *T* was associated with more NSSI for ANR only when *C* levels were high. This combination of high *T* and *C* is different from that predicted by the DHH, which states that T is more strongly associated with risk-taking behaviors when *C* is low (Mehta et al., 2015), as *C* is assumed to exert an inhibitory effect on risk behavior due to a fear of negative consequences. However, in the context of NSSI, the results may make sense. ANR, which is considered a main function of NSSI (Zetterqvist et al., 2013), involves selfinjury with the goal of reducing negative feelings and thoughts. These negative internal states are often the result of stressful experiences, such as rejection by others, which can lead to elevated cortisol levels (Kurath & Mata, 2018; Law & Clow, 2020). Moreover, other studies have also found combinations of *T* and *C* other than that proposed by the DHH to be more relevant for some risk behaviors. For example, Barel et al. (2017) found that the association between *T* and risk-taking was greater when *C* levels were high.

A secondary objective of the study was to examine whether the role of the two hormones (*T* and *C*) in NSSI was moderated by the sex of the adolescents. Although sex differences were observed in T levels and several indicators of NSSI, with boys showing higher levels than girls, sex did not moderate the associations between hormones and NSSI. These results contrast with those of previous studies in which *T* was more strongly associated with other risk-taking behaviors in boys than in girls (Johnson et al., 2020; Susman et al., 2017). However, they are consistent with the meta-analysis of Kurath and Mata (2018), who found that the significant association between risk taking and *T* was not influenced by sex.

The results of the study also provide information on the high prevalence of NSSI behaviors in a nonclinical sample of adolescents. A high percentage of the adolescents reported having engaged in at least one NSSI behavior in the 6-month period evaluated (48.2%), and the percentage of adolescents who had frequently used severe methods like cutting or burning their skin was high (13.9%). Although comparison of these data with those obtained in other studies is challenging due to the differences in NSSI assessment methods (Faura-Garcia et al., 2021a), they alert us to the relevance of this problem in adolescence. Beyond the physical risk of injury, these behaviors are concerning because they are often related to experiences of family or school victimization (Lanzillo et al., 2023; Liu et al., 2018) and lead to an increased risk of suicidality (Grandclerc et al., 2016).

6 | LIMITATIONS

One of the most important limitations of the study is that, although the data were collected in two waves separated by 6 months, the NSSI measure was only taken in the second wave. Although the adolescents answered the NSSI measure in reference to their behaviors during the last 6 months, the study would be improved by the inclusion of previous NSSI levels. Another limitation is that hormone levels were measured on a specific day, and they may fluctuate over time. Hormone levels are expected to act as biological states that directly precede or follow NSSI (Kaess et al., 2021). Therefore, a more rigorous assessment of the predictive role of *T* and *C* in NSSI behaviors would involve measuring levels of these hormones that proximally precede NSSI behaviors. Although such an assessment is difficult to conduct for procedural as well as ethical reasons, future studies could include measures not only of *T* and *C* levels but also of changes in these variables in response to NSSI-relevant stressors, such as interpersonal rejection situations. Finally, the measure employed in this study to assess NSSI (FASM) assesses selfharm behaviors but does not expressly indicate that they are nonsuicidal. Although in general the behaviors included in the FASM are nonsuicidal in nature (e.g., pulling out hair, scraping skin, picking at a wound), future

studies should explicitly differentiate between selfharm with and without suicidal intent to examine whether the roles of *T* and *C* are different. In fact, previous studies using the FASM with samples of Spanish adolescents indicated that 6.4% had engaged in selfinjurious behavior in an attempt to kill themselves (Calvete et al., 2015). Moreover, some of the behaviors included in the FASM could be considered unrepresentative of NSSI (i.e., picking at a wound), so future studies should employ more current NSSI assessment methods.

Due to these limitations, the results of this study should be considered preliminary. However, despite these limitations, the study also has strengths, such as the inclusion of a relatively large sample of male and female adolescents, the collection of data in two waves, and the inclusion of both biological and selfreported measures.

7 | CONCLUSIONS

The study contributes to filling the knowledge gap regarding the role of two hormones (*T* and *C*) in NSSI. Furthermore, the results suggest that *T* levels may contribute to an increased risk for NSSI behaviors with all types of reinforcement assessed: automatic and social, positive and negative. Although *C* only moderated the association of *T* with ANR, this finding is relevant since ANR is considered the most common function of NSSI (Calvete et al., 2017; Edmondson et al., 2016; Zetterqvist et al., 2013). Thus, in adolescents experiencing distress, a combination of both high *T* and high *C* levels may promote selfinjurious behaviors.

AUTHOR CONTRIBUTIONS

Esther Calvete conceived of the study, participated in its design, performed the analyses and prepared the initial draft of introduction and discussion; Angel Prieto-Fildalgo, Juan Faura-García, and Izaskun Orue participated in the review of the literature and wrote subsections of the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data of this study are available at OSF: https://osf.io/wy94k/

ETHICS STATEMENT

Participation was voluntary and participants were informed that their responses were confidential and would only be read by the research team. The procedure always followed the standards of the Declaration of Helsinki. The Ethics Committee of University of Deusto approved this study (ETK-28/22-23). Active informed consent by parents was required to participate in the study.

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