

# Modulation of neuropeptide Y levels is impaired in crack withdrawal patients

Fabiana Galland,<sup>1\*</sup> Jaqueline B. Schuch,<sup>1,2\*</sup> Daiane Silvello,<sup>1</sup> Karina Ligabue,<sup>1</sup> Fernanda Hansen,<sup>1,3</sup> Juliana N. Scherer,<sup>1</sup> Anne O. Sordi,<sup>1</sup> Lisia von Diemen<sup>1,2</sup>

## Abstract

**Introduction:** The dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis has a key role in drug addiction susceptibility. In addition to the well-known relationship between cortisol and the HPA axis, other molecules are involved with stress response and could modify the HPA activation, such as the neuropeptide Y (NPY), which has anxiolytic properties. There are few studies evaluating the effect of NPY levels on addiction, especially in crack cocaine dependence.

**Objective:** To evaluate NPY in crack users during early withdrawal to determine its relationship with drug use and cortisol levels.

**Methods:** We analyzed 25 male inpatient crack users. Serum NPY levels were measured at admission and discharge (mean of 24 days). Morning salivary cortisol was measured at admission.

**Results:** Serum NPY levels at admission and discharge were very similar. Lower NPY levels at discharge were associated with higher lifetime crack use. Also, a negative correlation was found between morning cortisol and delta NPY (NPY discharge – NPY admission).

**Conclusion:** These preliminary findings indicate that crack use influences the modulation of NPY levels and modifies stress response. The NPY pathway may play an important role in the pathophysiology of crack addiction, and the anxiolytic effect of NPY may be impaired in crack users. Future studies should consider NPY as a measurable indicator of the biological state in addiction.

**Keywords:** Neuropeptide Y, stress, cocaine, crack, addiction.

## Introduction

Drug addiction is associated with several biological dysfunctions. Chronic exposure to drugs of abuse affects the reward system, but evidence also showed that other brain systems are deeply involved with addictive behaviors.<sup>1</sup> Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis plays a key role in drug susceptibility, addiction severity and craving,<sup>2,3</sup> and is also involved in stress response. In animal studies, it has been shown that the HPA axis is activated after administration of

cocaine, which promotes the release of corticotropin-releasing hormone (CRH), adrenocorticotrophic hormone (ACTH) and cortisol.<sup>4</sup> Nevertheless, chronic exposure to drugs is also associated with attenuated HPA response.<sup>5</sup> Animal models have demonstrated the essential influence of stress on addiction-related outcomes, being associated with increased self-administration of drugs and the reestablishment of drug seeking behavior.<sup>6</sup> Moreover, the inability to tolerate stress is an important factor that affects drug consumption, relapse and treatment dropout.<sup>7,8</sup>

<sup>1</sup> Centro de Pesquisa em Álcool e Drogas (CPAD), Hospital de Clínicas de Porto Alegre (HCPA), Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil. <sup>2</sup> Programa de Pós-Graduação em Psiquiatria e Ciências do Comportamento, Faculdade de Medicina, UFRGS, Porto Alegre, RS, Brazil. <sup>3</sup> Laboratório de Nutrição Experimental, Departamento de Nutrição, Centro de Ciência da Saúde, Universidade Federal de Santa Catarina (UFSC), Florianópolis, SC, Brazil. \* These authors contributed equally to this study.

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Several studies have shown that neuropeptide Y (NPY) administration can modify the activation of the HPA axis.<sup>9,10</sup> In fact, NPY is associated with anxiolytic effects and with modulation of stress response by inhibiting ACTH and cortisol release in humans and animals.<sup>9,10</sup> NPY is a 36-amino acid peptide widely expressed in the brain and could affect the reward-seeking behavior related to drug addiction.<sup>11</sup> Animal studies showed that acute and chronic alcohol intake decrease NPY expression in brain areas related to reward behavior, such as the parietal cortex, amygdala and hypothalamus.<sup>12,13</sup> Interestingly, NPY administration in the brain reduces alcohol intake in ethanol-abstinent rats<sup>14</sup> and anxiety behavior in alcohol-preferring rats.<sup>15</sup> Conversely, NPY administration in the brain has also been involved with increased cocaine self-administration and hyperactivity.<sup>16</sup>

Only few clinical studies have assessed the influence of peripheral NPY levels on addiction to different substances. For instance, Meng et al. found no differences in NPY plasma levels between alcohol-dependent and non-dependent individuals.<sup>17</sup> To the best of our knowledge, no studies have evaluated NPY levels in crack addiction. Variations in NPY levels during drug withdrawal have also not been evaluated, which precludes understanding the influence of NPY on the pathophysiology of addiction. Thus, our aim was to evaluate NPY levels among crack cocaine users during early drug withdrawal to determine their relationship with cortisol levels, time of drug use, days of hospitalization and addiction severity.

## Methods

### Sample

This study included 25 crack cocaine-addicted patients who had been hospitalized in an inpatient addiction treatment unit at a public hospital in southern Brazil between September 2015 and August 2016. During this period, 83 patients were hospitalized with crack use disorder and presented a positive urine test for cocaine. However, 58 patients met our exclusion criteria and were therefore excluded from the study. Exclusion criteria were having a severe cognitive deficit that could impair the patient's capacity to respond to the instruments and presenting an incomplete clinical research protocol. All patients were  $\geq$  18 years old and male. The research protocol was carried out independently from clinical care, with patient detoxification treatment conducted as usual, i.e., following the routine established at the hospital.

Clinical diagnoses were based on criteria from the Diagnostic and Statistical Manual of Mental Disorders,

4th edition (DSM-IV) and defined by a clinical committee supervised by a senior psychiatrist. The consent form was given to the patients one day after admission, and the research instruments were applied between the 3th and 7th day. The Addiction Severity Index - 6 (ASI-6) was used to evaluate addiction severity and history of drug use.<sup>18</sup> The Childhood Trauma Questionnaire was applied to investigate history of childhood maltreatment, since several studies have highlighted that early trauma affects stress response and the HPA axis.<sup>19</sup> Presence of childhood maltreatment was considered if the patient reported at least one moderate or severe type of childhood abuse (emotional neglect, emotional abuse, sexual abuse, physical neglect and/or physical abuse). The consent form signed by all subjects included had been previously approved by the hospital's research ethics committee. This project was conducted in accordance with Declaration of Helsinki guidelines.

### Laboratory analyses

Blood samples were collected at two points during the treatment period: admission ( $n = 25$ , during the first 24 hours of hospitalization) and discharge ( $n = 25$ ). All samples were collected after a 10-hour fast by venipuncture into a free vacuum tube. Immediately after collection, the samples were centrifuged at 3,000 rpm for 10 minutes and the serum was aliquoted, labeled and stored at  $-80\text{ }^{\circ}\text{C}$  until assay testing. Serum NPY levels were measured by competitive enzyme immunoassay, using a commercial kit according to the manufacturer's instructions (EZHNPY-25K, Millipore, Burlington, USA). The samples were diluted 1:2 and read at 450 nm. The intensity of the colorimetric signal was directly proportional to the amount of captured biotinylated NPY peptide and inversely proportional to the amount of endogenous NPY in the standard or samples. Delta NPY levels were defined as the difference between NPY levels at admission and discharge ( $\text{delta NPY} = \text{NPY discharge} - \text{NPY admission}$ ).

Salivary samples were obtained using the SaliCap Set (RE69995, IBL International, Hamburg, Germany) and cortisol was measured through electrochemiluminescence (Elecsys and Cobas analyzers®, Roche, Basel, Switzerland), following the manufacturer's instructions. Samples were collected on the second day after admission at morning time, between 8 and 9:30 AM, avoiding the period of cortisol awakening response (CAR), which represents the maximum cortisol levels in plasma according to the circadian rhythm, occurring about 30-45 minutes after wakening. Participants were asked to be fasting for at least 30 minutes and not to have brushed their teeth for at least two hours before sample collection; they were also asked to wash their mouth

with water so as to avoid the collection of substances other than saliva components (i.e., food, medicines). Samples were centrifuged at 1,000 g for 2 minutes, and the supernatant was aliquoted and stored in -80 °C until assay testing.

### Statistical analyses

For continuous data, normality of distribution was assessed with a histogram and the Shapiro-Wilk test. A generalized estimating equation (GEE) was used to compare serum NPY levels at admission and discharge. Gamma regression with log link function was applied in this longitudinal analysis. In order to assess the relationship of NPY levels (admission, discharge and delta) with morning cortisol, days of hospitalization and addiction severity (ASI drug score), Spearman's correlations were performed. The influence of recent crack use (frequency of use in the last 30 days) and lifetime crack use (years of frequent use, i.e., at least three or more days/week) on NPY levels was analyzed using the Mann-Whitney test, with NPY levels dichotomized according to their respective median values (admission = 3.21 ng/mL, discharge = 3.40 ng/mL, delta = 0.04 ng/mL). In order to evaluate possible

clinical confounders in our analyses, the effect of psychiatric comorbidities and childhood maltreatment on NPY levels was investigated using Student's *t*-test or the Mann-Whitney test. No influence on NPY levels was found for psychiatric comorbidities or childhood maltreatment (Table S1, available as online-only supplementary material). The analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows version 18.0.

## Results

The clinical characteristics of the sample are shown in Table 1. Serum NPY levels at admission and discharge were similar: 3.21 ng/mL (interquartile range [IQR] 2.78-4.32) and 3.40 ng/mL (IQR 3.10-3.90), with  $p = 0.793$ . Low levels of NPY at discharge were associated with higher lifetime crack use ( $p = 0.030$ ; Table 2). No influence of recent crack use was detected, although a trend towards association was observed between NPY levels at admission and recent crack use ( $p = 0.057$ ; Table 2). These results indicate that lower NPY levels are associated with crack use. No association was

**Table 1** - Clinical characteristics of the sample (n = 25)

	<b>Crack users</b>
Age	39.43 ± 10.73
Caucasian	16 (64)
Schooling (years)	8.86 ± 2.80
Days of hospitalization	24.27 ± 3.73
Years of crack use	10.25 ± 7.92
Age at first crack use	29.25 ± 10.25
Years of alcohol use	12.35 ± 2.65
Years of marijuana use	14.00 ± 3.16
Years of tobacco use	20.78 ± 2.75
ASI-6 drug score	52.21 ± 8.07
Lifetime drug use	
Alcohol dependence	12 (48)
Tobacco use	17 (68)
Marijuana use	16 (64)
Number of lifetime inpatient psychiatric treatments	3.50 ± 1.00
Presence of psychiatric comorbidities	
Major depression episode	16 (64)
Social phobia	11 (44)
Suicide attempt	7 (28)
Generalized anxiety disorder	5 (20)
Childhood maltreatment	15 (60)

Values are presented as mean ± standard deviation or n (%).  
ASI-6 = Addiction Severity Index - 6.

found between days of hospitalization or addiction severity and NPY levels at admission ( $p = 0.744$  and  $p = 0.065$ , respectively), discharge ( $p = 0.719$  and  $p = 0.075$ , respectively) or delta ( $p = 0.751$  and  $p = 0.657$ , respectively) (data not shown).

In order to evaluate NPY levels and stress response, we analyzed morning cortisol levels. Even though we found no correlation between cortisol and NPY levels at discharge ( $p = 0.579$ ), there was a trend regarding NPY levels at admission ( $r = 0.493$ ,  $p = 0.052$ ). A negative correlation was also found between delta NPY and cortisol ( $r = -0.651$ ;  $p = 0.005$ ). This analysis reveals that increased cortisol levels correlate with a smaller difference between NPY levels at discharge and admission (Figure 1).

## Discussion

The present study shows, for the first time, the influence of crack addiction on NPY levels during early withdrawal. These findings were strengthened by the two collection time points, which demonstrated that crack users, even without current drug consumption, could exhibit a disruptive stress response.

Our results indicate that lifetime crack use (years of frequent use) has a negative impact on NPY levels during early withdrawal. These findings suggest that crack use is associated with a putative down-regulation of peripheral NPY levels. This occurs specifically during discharge, when stress and craving could be elevated due to abstinence symptoms.<sup>20</sup> This could explain the increase in anxiety symptoms that may lead to early relapse.<sup>21</sup> The variation in NPY levels may be affected primarily by drug use, since NPY levels were not associated with psychiatric comorbidities or childhood maltreatment in our sample (Table S1, available as online-only supplementary material).

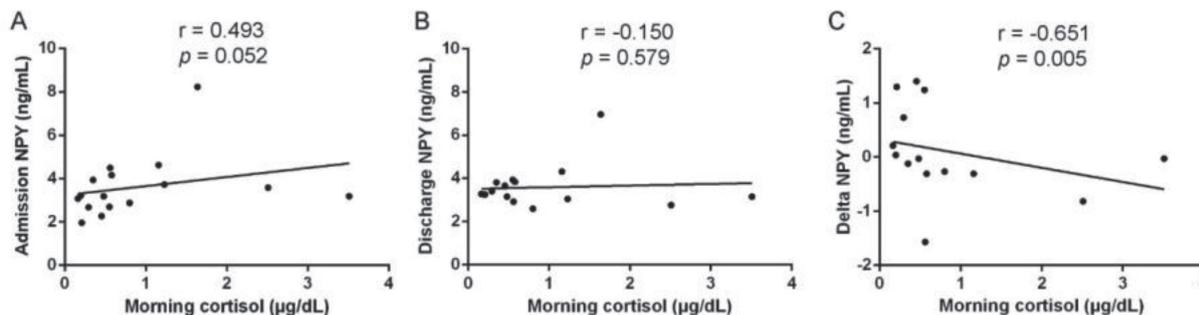
Corroborating our findings, animal studies have shown a reduction of NPY levels in the brain during early withdrawal of alcohol and cocaine abuse. Interestingly, this reduction of NPY levels was observed in specific brain areas related to the HPA axis, such as the arcuate nucleus and paraventricular regions of the hypothalamus and the central and medial nucleus of the amygdale.<sup>12,22</sup> It is also known that these brain areas play a key role in addiction severity and craving.<sup>3</sup> Moreover, NPY infusion was associated with decreased activity of pyramidal output neurons in the basolateral amygdala, which was associated with stress resilience.<sup>23,24</sup>

**Table 2** - The influence of lifetime crack use on NPY levels at discharge

	NPY at admission			NPY at discharge			Delta NPY		
	< median	> median	p-value	< median	> median	p-value	< median	> median	p-value
<b>Lifetime crack use</b>	10 (7-13)	6 (3.50-13)	0.093	10 (9-16)	6 (4-7.5)	<b>0.030</b>	7 (4.5-16.5)	9 (5-11.5)	0.902
<b>Recent crack use</b>	30 (20.7-30)	10 (0.25-30)	0.057	30 (10.5-30)	25.5 (7-30)	0.845	17 (0.75-30)	30 (21-30)	0.113

NPY = neuropeptide Y.

NPY levels were dichotomized according to median values (admission = 3.21 ng/mL, discharge = 3.40 ng/mL, delta = 0.04 ng/mL). Lifetime drug use was characterized by years of frequent use, i.e., at least three or more days/week. Recent drug use was characterized by frequency of use in the last 30 days. Results are expressed as median (interquartile range).



**Figure 1** - Delta NPY levels were negatively correlated with morning cortisol. Correlation of morning cortisol with A) NPY at admission, B) NPY at discharge and C) delta NPY. NPY = neuropeptide Y.

Withdrawal symptoms have been associated with increased stress response, modifying cortisol levels.<sup>20</sup> In this sense, increased salivary cortisol levels have been correlated with short abstinence time and treatment dropout in addicted individuals.<sup>2,7</sup> NPY levels have been positively correlated with cortisol following acute stress exposure in humans,<sup>25</sup> and we observed a trend towards association between cortisol and NPY at admission. This specific response could be an attempt to balance the stress mechanism, since NPY has anxiolytic effects. In a homeostatic mechanism, increased NPY levels could aid to reestablish the negative feedback and thus prevent the dysregulation of the HPA axis. In our study, the relationship between cortisol and delta NPY levels suggests that, in crack users, during early withdrawal, there is an increased stress response leading to a physiological increase in cortisol. Nonetheless, during this period a decrease in the variation of NPY levels is also observed, which may indicate little influence of NPY on cortisol levels and on HPA axis regulation. This impaired feedback could be related to a dysfunction in the HPA axis in addictive patients. These findings are supported by Xu et al., who observed a disruptive NPY stress response in substance-dependent individuals regardless of genetic variation.<sup>26</sup> Furthermore, rat models of cocaine addiction presented lower NPY levels and increased NPY mRNA expression.<sup>27,28</sup> NPY infusion in the basolateral nucleus of the amygdala was found to reduce behavioral stress and increase social interaction.<sup>23</sup> In addition, NPY neutralizes the action of CRH released in the hypothalamus in response to stress, therefore preventing stress effects.<sup>29</sup>

Some limitations of this study must be considered. First, our sample size was relatively small, which may have prevented us from detecting more robust associations. In this sense, correlations between addiction severity and NPY levels may have been missed due to our sample size. Nevertheless, similar approaches to other biological measures have used sample sizes comparable with the one in our study.<sup>26</sup> Also, this study involved only male patients who were enrolled in a public drug treatment program. We also did not have a control group. Finally, the cortisol assessment was cross-sectional, precluding additional and more complex comparisons with variations in NPY levels. Conversely, our study is strengthened by the follow-up approach.

It remains to be known why NPY is an understudied peptide in the clinical setting, considering the biological mechanisms of addiction. Scientific literature has deeply explored the reward system related to addiction, but there appear to be other valuable neurological

pathways that may enhance the substrate for the discovery of new medications. There is a lack of studies evaluating serum NPY levels in humans with addictive disorder, and therefore the present findings suggesting that the NPY pathway may play an important role in the pathophysiology of crack addiction are a valid contribution. In future studies, NPY levels could be considered a measurable indicator of the biological state in addiction.

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## Disclosure

No conflicts of interest declared concerning the publication of this article.

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#### Correspondence:

Lisia von Diemen  
Centro de Pesquisa em Álcool e Drogas (CPAD)  
Hospital de Clínicas de Porto Alegre (HCPA)  
Rua Professor Álvaro Alvim, 400  
90420-020 - Porto Alegre, RS - Brazil  
E-mail: lisiavd@gmail.com