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Cocaine use severity and cerebellar gray matter are associated with reversal learning deficits in cocaine-dependent individuals

Laura Moreno-López[1], José C. Perales[2], Dana van Son[3], Natalia Albein-Urios[1], Carles Soriano-Mas[4,5], Jose M. Martinez-Gonzalez[6,7], Reinout W. Wiers[3] & Antonio Verdejo-García[1,6,7,8]

Department of Personality, Evaluation and Psychological Treatment, Universidad de Granada, Spain[1], Mind, Brain and Behavior Research Center (Centro de Investigación Mente, Cerebro y Comportamiento-CIMCYC), Universidad de Granada, Spain[2], Addiction, Development and Psychopathology (ADAPT) Lab, Department of Psychology, University of Amsterdam, The Netherlands[3], Department of Psychiatry, Bellvitge University Hospital-IDIBELL, Spain[4], Carlos III Health Institute, Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), Spain[5], Institute of Neurosciences Federico Olóriz, Universidad de Granada, Spain[6], Red de Trastornos Adictivos, RETICS Program, Instituto de Salud Carlos III, Universidad de Granada, Spain[7] and School of Psychological Sciences, Monash University, Australia[8]

ABSTRACT

Cocaine addiction involves persistent deficits to unlearn previously rewarded response options, potentially due to neuroadaptations in learning-sensitive regions. Cocaine-targeted prefrontal systems have been consistently associated with reinforcement learning and reversal deficits, but more recent interspecies research has raised awareness about the contribution of the cerebellum to cocaine addiction and reversal. We aimed at investigating the link between cocaine use, reversal learning and prefrontal, insula and cerebellar gray matter in cocaine-dependent individuals (CDIs) varying on levels of cocaine exposure in comparison with healthy controls (HCs). Twenty CDIs and 21 HCs performed a probabilistic reversal learning task (PRLT) and were subsequently scanned in a 3-Tesla magnetic resonance imaging scanner. In the PRLT, subjects progressively learn to respond to one predominantly reinforced stimulus, and then must learn to respond according to the opposite, previously irrelevant, stimulus-reward pairing. Performance measures were errors after reversal (reversal cost), and probability of maintaining response after errors. Voxel-based morphometry was conducted to investigate the association between gray matter volume in the regions of interest and cocaine use and PRLT performance. Severity of cocaine use correlated with gray matter volume reduction in the left cerebellum (lobule VIII), while greater reversal cost was correlated with gray matter volume reduction in a partially overlapping cluster (lobules VIIb and VIII). Right insula/inferior frontal gyrus correlated with probability of maintaining response after errors. Severity of cocaine use detrimentally impacted reversal learning and cerebellar gray matter.

Keywords Cerebellum, cocaine, inferior frontal gyrus, insula, reversal learning.

Correspondence to: Antonio Verdejo-García, School of Psychological Sciences, Faculty of Medicine, Nursing and Health Sciences, Monash University, 3800 Wellington Road, Clayton, Bldg 17, Room 536, Melbourne, Vic. 3800, Australia. E-mail: antonio.verdejo@monash.edu

INTRODUCTION

Cocaine addiction is characterized by neuroadaptations leading to persistence of habitual behaviors in spite of profound changes in the interoceptive milieu (punishment progressively prevails over reward) (Baker *et al.* 2004) and in the social domain (rise of interpersonal problems or legal issues) (Volkow, Baler & Goldstein 2011; Hulka *et al.* 2013; Preller *et al.* 2013, 2014). This perseverative behavior has been elegantly modeled by experimental reversal learning paradigms, exemplified by simple discrimination tasks in which subjects first learn to respond to one predominantly reinforced stimulus, and then must learn to respond according to the opposite, previously irrelevant, stimulus-reward pairing (Clark, Cools & Robbins 2004; Duka, Crombag & Stephens 2011). Research in non-human primates has demonstrated that extended cocaine self-administration induces reversal learning deficits (Jentsch *et al.* 2002; Porter *et al.* 2011). Similarly, in humans, heavy cocaine use

is associated with reversal learning deficits: stimulus-reward learning and reversal patterns discriminate cocaine-dependent individuals (CDIs) from recreational users (Verdejo-García *et al.* 2010; Fernández-Serrano *et al.* 2012) and CDIs exhibit substantially higher rates of perseveration errors than users of other stimulant or opiate drugs (Ersche *et al.* 2008). In cocaine-dependent users, perseveration is often observed following changes in response-outcome contingencies and during tracking of probabilistic errors (i.e. in reversal learning tasks involving probabilistic wins and losses) (Fernández-Serrano, Pérez-García & Verdejo-García 2011); however, response flexibility is relatively preserved in non-rewarded stimulus-discrimination tasks involving fixed schedules (Vonmoos *et al.* 2013). These response perseveration-related deficits are dosage sensitive, and are thought to index the transition from impulsive (goal-driven) behavior to compulsive (repetitive, outcome-detached) behavior characterizing stimulant addiction (Everitt & Robbins 2005; Ersche *et al.* 2011). Accordingly, reversal learning deficits linger in CDI that have been abstinent for several months (Verdejo-García *et al.* 2007; Hanlon *et al.* 2011) and these deficits are associated with poorer prognosis of addiction (Turner *et al.* 2009).

Reversal learning is underpinned by a dopaminergic neural circuitry comprising ventromedial and ventrolateral prefrontal cortex, insula, and their connections with the striatum (Cools *et al.* 2002; Cohen *et al.* 2007; Gläscher, Hampton & O'Doherty 2009). The ventromedial prefrontal aspect is primarily involved in flexible tracking of response-outcome values (Gläscher *et al.* 2009; Tsuchida, Doll & Fellows 2010), whereas the ventrolateral aspect [i.e. inferior frontal gyrus (IFG) and insula] is essential for behavioral shifting following perseveration and response accuracy after reversal (Cools *et al.* 2002; Ghahremani *et al.* 2010). Both brain regions are detrimentally impacted by cocaine regimens (Jentsch *et al.* 2002) and display enduring gray matter abnormalities in populations of CDIs (Mackey & Paulus 2013). In addition, interspecies neuroanatomical research has recently raised awareness about the involvement of cerebellar networks in reward/punishment reversal learning and in cocaine addiction (Miquel *et al.* 2009; Bostan, Dum & Strick 2013; Carbo-Gas *et al.* 2013). In humans, neuropharmacological evidence indicates that specific cerebellar regions (lobules VIII–IX) are rich in dopamine transporters and sensitive to cocaine-induced sensitization (Anderson *et al.* 2006). Moreover, human neuropsychological studies have demonstrated that focal cerebellar lesions are specifically associated with impaired reversal learning following optimal stimulus-reward acquisition (i.e. the trade-off between reward learning and adjustment to changing contingencies) (Thoma *et al.* 2008). These findings suggest that cerebellar structures are sensitive to cocaine direct detrimental effects and that these neuroadaptations likely contribute to cocaine users' response perseveration and difficulty to adjust to changing environments (Izquierdo & Jentsch 2012). The link among cocaine use, cerebellar neuroadaptations and response perseveration is consistent with the association between length of cocaine exposure and cerebellar gray matter attrition in CDI (Sim *et al.* 2007), and with the finding that cerebellar gray matter deficits are one of the most persistent brain dysfunctions in long-term abstinent CDI (Mackey & Paulus 2013).

In this study, we tested whether cocaine exposure impacts on both behavioral perseveration and reversal learning-related brain regions by examining the performance in a probabilistic reversal learning task (PRLT) and gray matter content in targeted regions of interest (i.e. ventromedial and ventrolateral aspects of prefrontal cortex, insula and cerebellum) in a group of abstinent CDI with different levels of cocaine exposure and a group of non-cocaine-exposed comparison participants. We examined two specific aspects of response perseveration: (1) the difficulty to switch behavioral responses after reversal (i.e. reversal cost); and (2) the probability of maintaining response after loss during reversal phases. We hypothesized that severity of cocaine use would detrimentally impact on reversal performance and neural circuitry. Moreover, we hypothesized that cerebellar gray matter would be associated with acquisition of stimulus-reward contingencies and subsequent reversal cost, whereas prefrontal gray matter would be associated with dynamic tracking of response-outcome feedback during reversal phases.

METHODS

Participants

Twenty CDIs and 21 healthy controls (HCs) participated in this study. Cocaine users were recruited through consecutive admissions to the clinic 'Centro Provincial de Drogodependencias (CPD)' in Granada (Spain), which provides psychosocial treatment for substance use disorders in an outpatient setting. HCs were recruited from local employment agencies, matching them to the clinical group in the main socio-demographic characteristics.

The inclusion criteria for the cocaine group were defined as follows: (1) age range between 18 and 45 years old; (2) IQ levels above 80—as measured by the Kaufman Brief Intelligence Test (Kaufman & Kaufman 1990); (3) meeting DSM-IV criteria for cocaine dependence—as assessed by the Structured Clinical Interview for DSM-IV Disorders Clinician Version (SCID) (First *et al.* 1997); (4) having started treatment in the 2 weeks preceding assessments; and (5) abstinence duration >15 days. Abstinence was confirmed twice weekly through urine tests plus an

Table 1 Socio-demographic and drug use characteristics of the sample.

	CDI (n = 20)		*HC (n = 21)*			
Demographics	*Mean*	*SD*	*Mean*	*SD*	*t*	*P*
Age	34.60	6.81	31	4.6	1.992	.053
Years of education	9.7	1.62	10.38	1.96	−1.207	.235
Verbal IQ	101.75	7.57	105.76	8.75	−1.566	.125
Patterns of drug use	*Median*	*IQR*	*Median*	*IQR*	t	P
Cocaine (20 CDI/0 HC)						
Cocaine grams per month	7.5	11			3.139	.005
Cocaine duration of use (months)	34.5	55.5			4.318	.000
Cocaine duration of abstinence (months)	1	1.33				
MDMA (4 CDI/0 HC)						
MDMA pills per month	10	13			1.868	.077
MDMA duration of use (months)	18	21			1.926	.069
Hallucinogens (2 CDI/0 HC)						
Hallucinogen units per month	6	4			1.371	.186
Hallucinogen duration of use (months)	24	24			1.285	.214
Alcohol (18 CDI/14 HC)						
Alcohol standard units per month	20	24	7	16.25	3.008	.006
Alcohol duration of use (months)	60	105	114	99.63	1.049	.301
Tobacco (15 CDI/8 HC)						
Tobacco cigarettes per month	600	600	300	457.5	3.313	.003
Tobacco duration of use (months)	84	192	18	124.5	2.219	.034
Cannabis (6 CDI/4 HC)						
Cannabis joints per month	64	227.75	.75	1.38	1.755	.095
Cannabis duration of use (months)	54	144	19.5	33.75	1.490	.152

CDI = cocaine-dependent individual; HC = healthy control; IQR = interquartile range; MDMA = 3,4-methylenedioxymethamphetamine.

ad hoc test on testing days. The exclusion criteria were: (1) current Axis I or II disorders—as measured by the SCID, with the exceptions of alcohol abuse, nicotine dependence, and attention deficit and hyperactivity disorder (ADHD)—as measured by the Conners Adult ADHD Diagnostic Interview for DSM-IV (Conners 1999); (2) current treatment with antipsychotic or benzodiazepine pharmacotherapies; (3) history of head injury or neurological, infectious, systemic or any other diseases affecting the central nervous system; (4) having followed other treatments within the 2 years preceding the study onset; and (5) having entered treatment by court request. In addition to the former exclusion criteria, HC could not meet any diagnosis of substance-related disorders—with the exception of nicotine dependence. All the diagnoses were conducted by a registered clinical psychologist, whereas all subsequent tests were administered by an independent assessor.

Instruments

Patterns of drug use

Data regarding lifetime amount and duration of drug use were self-reported by participants and collected using the Interview for Research on Addictive Behavior (see Verdejo-García *et al.* 2006 for a detailed rationale of this measure). This interview provides an estimation of monthly use of each substance during regular use (e.g. grams for cocaine, standard alcohol units for alcohol, number of cigarettes for tobacco) and total duration of use of each substance (in months). The descriptive scores for these variables can be found in Table 1. For statistical analyses, the amount and duration measures were combined in a composite measure of severity of drug/s use, defined as the sum of the standardized (Z-score) monthly amount and the standardized (Z-score) number of months of use.

PRLT

The reversal learning task used here is based on the PRLT described in Swainson *et al.* (2000). A graphical depiction of the task can be found in Verdejo-García *et al.* (2010). In each trial, participants were required to choose (mouse clicking) between two similar stimuli (two squares of different colors) located in the left and right sides of the screen (locations randomized). Participants were instructed that, according to a predefined rule, one of the stimuli was correct and the other was incorrect 'most of the times'. Actually, the 'correct' stimulus was

rewarded in 70–80% of the trials. Participants were also warned of the possibility that the rule changed throughout the task: the previously 'correct' stimulus could become 'incorrect' and vice versa. Both negative and positive feedbacks were presented visually, and involved winning or losing five points. The total amount of points accrued was continuously viewed just below the centre of the screen.

The task was composed of four phases of 40 trials each (160 choices). In each phase, one stimulus was 'correct' (rewarded in most cases) and the other was 'wrong' (punished in most cases). The correct response shifted after every phase, i.e. the stimulus that was previously correct became incorrect and vice versa. Hence, the task is composed of an initial discrimination phase (trials 1–40) and three reversal phases: trials 41–80, 81–120 and 121–160, respectively. In order to get a better appraisal of learning curves and problems to adjust after reversal points, we also analysed 10-trial blocks, with special interest in blocks 4–5, 9–10 and 12–13, immediately preceding and following reversal points.

Image acquisition and pre-processing

Participants were scanned on a 3T whole body magnetic resonance imaging (MRI) scanner (3T Achieva, Philips Medical Systems, Best, the Netherlands) operating with an eight-channel phased array head coil. For each participant, a 3D volume was acquired using a T1-weighted turbo-gradient-echo sequence (3D-TFE) in the sagittal plane, with a $0.94 \times 0.94 \times 1.0$ mm resolution (160 slices, FOV = 240×240 mm^2, matrix 256×256), TR = 8.3 ms, TE = 3.8 ms, TI = 1022.6264 ms and flip angle = 8°. This sequence was optimal for reducing motion sensitivity, susceptibility artifacts and field inhomogeneities. Structural imaging data were pre-processed and analyzed using statistical parametric mapping 8 (SPM8) (http://www.fil.ion.ucl.ac.uk/spm/software/spm8) implemented in Matlab R2007b (MathWorks, Natick, MA, USA). We used the VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm/) to segment raw images and extract probabilistic maps of gray matter, white matter and cerebrospinal fluid; normalize the gray matter segments (using DARTEL normalization) to a gray matter template in MNI space; modulate normalized gray matter images with the Jacobian determinants (from the flow fields derived from the normalization step) to restore volumetric information; and finally smooth images with a 3-D Gaussian filter of 8 mm full width at half maximum.

Statistical analyses

Background and clinical characteristics

Independent-sample *t*-tests were used to examine between-group differences in the main socio-demographic and clinical variables using SPSS 20 for Macintosh (SPSS Inc., Chicago, IL, USA). Significance threshold was set at $P < .05$. *t*-tests (with or without homogeneity of variances assumption, depending on the results of previous Levene tests) were carried out on raw duration and amount scores of each substance, assigning a 0 value to non-users. Severities of use of substances showing significant associations with group or severity of cocaine use (within the CDI group) were included as covariates in all subsequent analyses.

PRLT performance

The raw number of correct choices (selections of the most frequently rewarded option) per phase and block entered a General Linear Model (GLM) analysis across groups, with age, and severities of alcohol and tobacco use as potential confounders. Complementarily, we carried out a within-group GLM analysis in the CDI group, with cocaine use severity as the main independent variable; age, alcohol and tobacco use severities as potential confounders; and the raw number of correct choices per phase and block as dependent variable. Changes across phases reflect the effect of reversal and reinstatement of reward contingencies. Changes across blocks, on the other hand, reflect acquisition and reacquisition curves within phases. The main aim of these analyses was to examine the influence of group (CDI, HC) and cocaine severity on such changes across phases and blocks.

In order to capture the behavioral shifting aspect of PRLT performance, we computed the difference between the number of correct choices in phases 1 and 3 (phases with the original reward contingency signs), and phases 2 and 4 (phases with reversed-reward contingency signs). Henceforth, we will refer to this measure as *reversal cost*, which can be interpreted as a specific difficulty to learn the new (reversed) contingency, after acquisition with the original one. Reversal cost was compared across groups, and entered within-group correlational analyses both with cocaine severity and gray matter volume in selected region of interests (ROIs) (see the following section for details).

In order to capture the feedback-tracking aspect of PRLT performance and its possible contribution to reversal cost, we computed the adjusted probability of maintaining the same response after loss following reversal points. To compute this dynamic tracking measure, we divided the task into eight 20-trial blocks, and the probability of maintaining the same response after negative feedback for that choice, $P(\text{stay} \mid \text{loss})$, was computed and averaged across blocks 3 and 7, i.e. for blocks subsequent to reversals, in inverted contingency phases. $P(\text{stay} \mid \text{win})$ in those blocks, and $P(\text{stay} \mid \text{loss})$ and $P(\text{stay} \mid \text{win})$ in preceding ones (2 and 6) were used as predictors in a linear

regression model, with post-reversal *P*(stay|loss) as the variable to be predicted. Standardized residuals from that regression analysis (henceforth, standardized *loss-stay scores*) were compared across groups, and entered within-group correlational analyses both with cocaine severity and gray matter volume in selected ROIs. The correlation between the two PRLT measures was .15 ($P = .35$), indicating that they capture dissociable aspects of perseveration response.

Imaging analyses

Gray matter differences between CDI and HC. The GLM was used to conduct voxel-wise comparisons between groups using SPM8. Group differences in gray matter volume were tested using an ROI approach. Specifically, we created four ROIs (ventromedial prefrontal cortex, IFG, insula and cerebellum) using the Wake Forrest University PickAtlas (Maldjian *et al*. 2003). Age, severity of alcohol and tobacco use, and total gray matter volume (TGMV) were modeled as linear confounds, and significance threshold was set at $P < .05$ after family-wise correction for multiple comparisons across each ROI [i.e. using small volume correction (SVC) procedures] (*pFWE-SVC* < .05).

Associations between severity of cocaine use and reversal learning performance and gray matter in reversal-related ROIs. The associations of severity of cocaine use, behavioral measures of reversal learning (i.e. PRLT reversal cost and loss-stay measures) and gray matter volumes in targeted ROIs were tested using three multiple regression models in SPM8, with severity of cocaine use, reversal cost and loss-stay measures as predictors; and age, severity of alcohol and tobacco use and TGMV as confounding covariates. Significance threshold was set at $P < .05$ after family-wise correction for multiple tests across our ROIs (*pFWE-SVC* < .05). These analyses were carried out to find out in which clusters (if any) gray matter volume was significantly predicted by cocaine use severity, PRLT reversal cost or loss-stay scores.

RESULTS

Background and clinical characteristics

Both groups had statistically equivalent distributions for years of education and verbal IQ. They were also homogeneous in terms of sex, ethnicity and language (Table 1). The two groups, however, were close to differ in age ($P = .053$), so that this variable was considered a potential confounder in subsequent analyses. Table 1 also shows that most cocaine users exhibited limited exposure to other substances [none of the CDI participants had used methamphetamine or opiates and less than 20% had used 3,4-methylenedioxymethamphetamine (MDMA) or hallucinogens] with the exception of alcohol and tobacco, which were also covaried as relevant confounders. Within the CDI group, cocaine use severity did not significantly correlate with duration, amount or severity of use of any other substance ($r_{max} = .26$; $p_{min} = .278$). Only one of the participants (in the CDI group) met the criteria for diagnosis of ADHD, and therefore this variable was not further controlled.

Relationships between severity of cocaine use and PRLT performance

A GLM analysis with group as between-subject factor; number of correct choices per phase and block as dependent variables; and age, and tobacco and alcohol use severities as confounders yielded significant effects of block [$F(3, 108) = 7.71$, $MSE = 2.86$, $P < .05$, $\eta^2 = .07$] and the block × group interaction [$F(3, 108) = 2.88$, $MSE = 2.86$, $P < .04$, $\eta^2 = .07$]. There was no significant main effect of group on the number of correct choices across phases and blocks ($P = .17$). Corrected mean (SE) values across blocks were 5.16 (.20), 6.33 (.26), 5.92 (.30) and 6.82 (.30) for the CDI group, and 5.18 (.20), 6.47 (.25), 7.12 (.29) and 7.15 (.29) for the HC group. Bonferroni-corrected ($\alpha/4$) block-by-block *post hoc* comparisons yielded a single significant difference between groups, restricted to block 3.

Similar between-group GLM analyses, with group as independent factor, age, alcohol and tobacco use severities as potential confounders, and reversal cost and loss-stay measures as dependent variables, did not yield any group effects ($P = .61$ and $P = .25$ for reversal cost and loss-stay measures, respectively).

A final GLM analysis, restricted to the CDI group, with cocaine use severity as a continuous predictor, age and alcohol and tobacco use severities as potential confounders, phase and block as within-subject variables, and the number of correct choices as dependent variable, yielded a reliable phase × cocaine use severity effect [$F(3, 45) = 4.31$, $MSE = 4.46$, $P < .01$, $\eta^2 = .22$; for other effects involving cocaine use severity $p_{min} = .29$]. Figure 1 (black bars, left panel) shows correlations between severity and the number of correct choices in phases 1–4 ($r = .49, -.44, .40$ and $-.32$, respectively). As a consequence of this, severity of cocaine use strongly correlated with reversal cost in the CDI group ($r = .68$; $P < .01$; Fig. 1, right panel, shallow markers), even after controlling for age and severity of alcohol and tobacco use (partial $r = .59$; $P = .01$; black markers).

The loss-stay score, however, did not correlate with severity of cocaine use (partial $r = .11$; $P = .66$). This measure was nonetheless correlated with estimated duration of cocaine use (partial $r = .51$, $P < .04$).

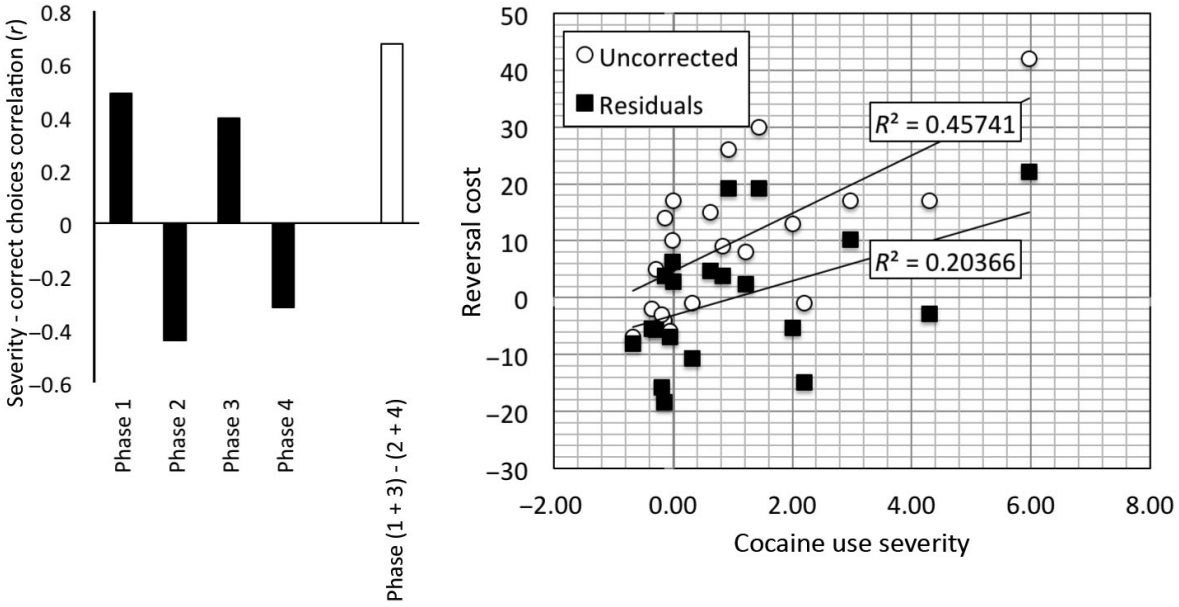

Figure 1 Left panel: *r* coefficients for the bivariate correlation between severity of cocaine use and number of correct choices in phases 1–4 ($r = .49, -.44, .40$ and $-.32$, respectively, $P < .01$) (black bars) and between severity of cocaine use and reversal cost ($r = .68$, $P < .01$) (shallow bar). Right panel: Scatterplot of the correlation between severity of cocaine use and reversal cost. Shallow markers: uncorrected reversal cost scores; black markers: non-standardized reversal cost residuals after regressing them upon age, alcohol use severity and tobacco use severity

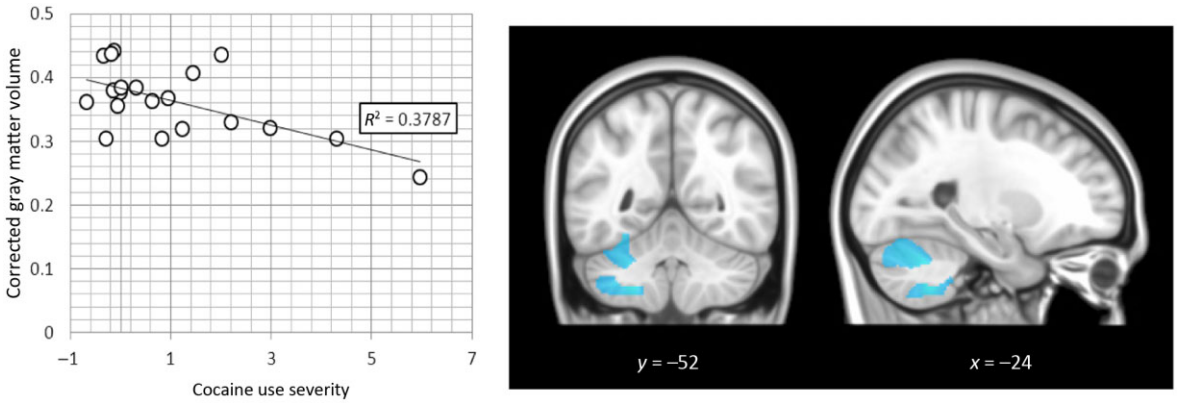

Figure 2 Scatterplot (left) and cluster (right) of correlation between cerebellar gray matter volume and severity of cocaine use in cocaine-dependent individuals. Peak coordinates were located in the left posterior lobe of the cerebellar cortex (peak at *x*, *y*, $z = -24, -52, -48$; $t = 6.94$; *pFWE-SVC* < .05). Results are overlaid on coronal and sagittal sections of a normalized brain, and the numbers correspond to the '*y*' and '*x*' coordinates in MNI space

Relationship between severity of cocaine use and gray matter volumes

There were no significant group effects on gray matter volumes in the targeted ROIs (at *pFWE-SVC* < .05). However, within-group analyses for the CDI group showed that severity of cocaine use significantly correlated with regional gray matter volume within the cerebellum. Specifically, we observed a cluster of significant negative correlation located in the left lobe of the cerebellar cortex (lobule VIII, peak at *x*, *y*, $z = -24, -52, -48$; $t = 6.94$; *pFWE-SVC* < .05; Fig. 2).

Using a more lenient significance threshold, we observed an additional cluster of significant negative correlation located in the right IFG (peak at *x*, *y*, $z = 50, 41, -6$; $t = 3.33$; $P < .005$ uncorrected; Supporting Information Fig. S1). We did not find any other significant effects on the ventromedial prefrontal or ventrolateral prefrontal ROIs.

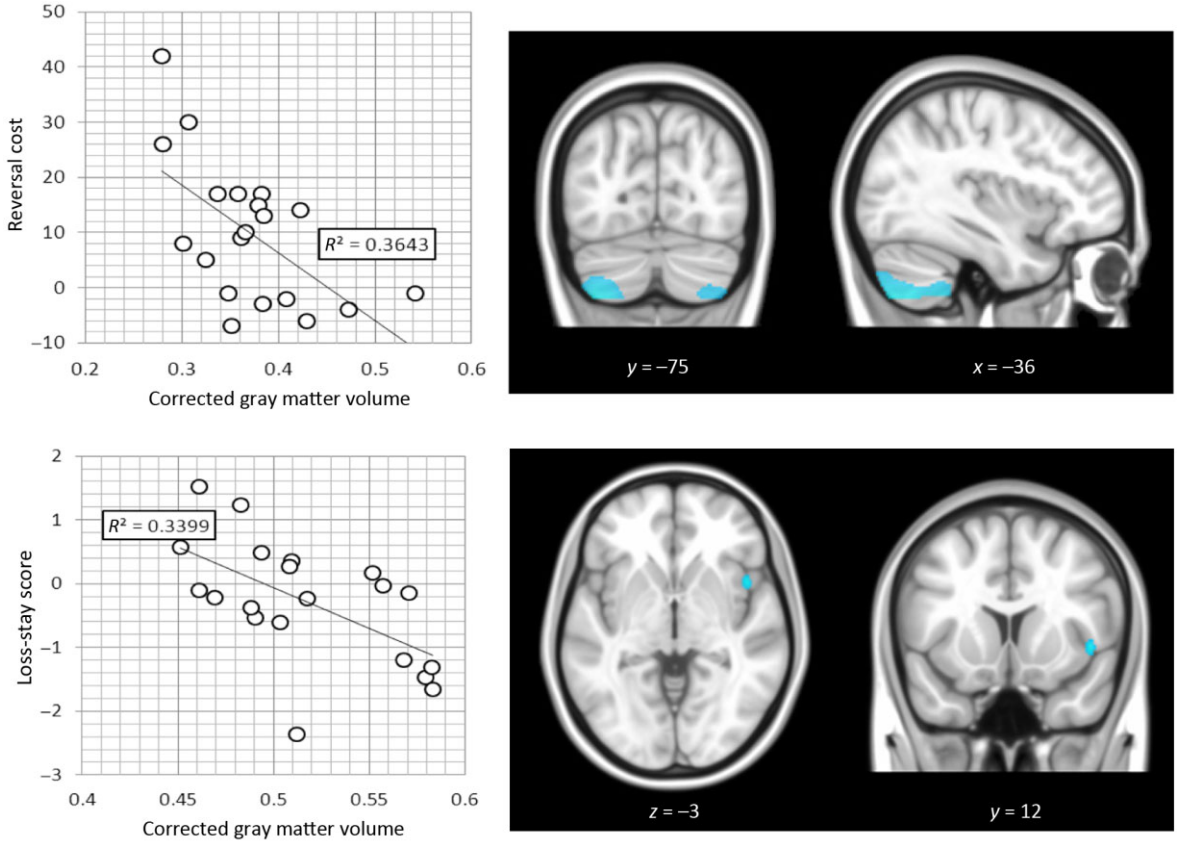

Figure 3 Top panel: scatterplot (left) and cluster (right) of correlation between cerebellar gray matter volume and reversal cost in cocaine-dependent individuals. Peak coordinates were located in the left posterior lobe of the cerebellar cortex (peak at *x*, *y*, *z* = −36, −75, −54; *t* = 5.02; *pFWE-SVC* < .05). Results are overlaid on coronal and sagittal sections of a normalized brain, and the numbers correspond to the '*y*' and '*x*' coordinates in MNI space. Bottom panel: scatterplot (left) and cluster (right) of correlation between insula gray matter volume and loss-stay score in cocaine-dependent individuals. Peak coordinates were located in the right insula (peak at *x*, *y*, *z* = 45, 12, −3; *t* = 5.19; *pFWE-SVC* < .05). Results are overlaid on axial and coronal sections of a normalized brain, and the numbers correspond to the '*z*' and '*y*' coordinates in MNI space

Relationship between PRLT performance and gray matter volumes

A larger reversal cost was significantly predicted by gray matter volume loss in a left cerebellar hemisphere location (lobule VIIb–lobule VIII, peak at *x*, *y*, *z* = −36, −75, −54; *t* = 5.02; *pFWE-SVC* < .05 Fig. 3—top panel). The loss-stay measure was significantly predicted by gray matter volume loss in the right insula (peak at *x*, *y*, *z* = 45, 12, −3; *t* = 5.19; *pFWE-SVC* < .05, Fig. 3—bottom panel). The cerebellar cluster associated with reversal cost showed an overlap of 26.8% with the cerebellar cluster associated with cocaine use (Fig. 4). We did not find significant effects on the ventromedial prefrontal or ventrolateral prefrontal ROIs.

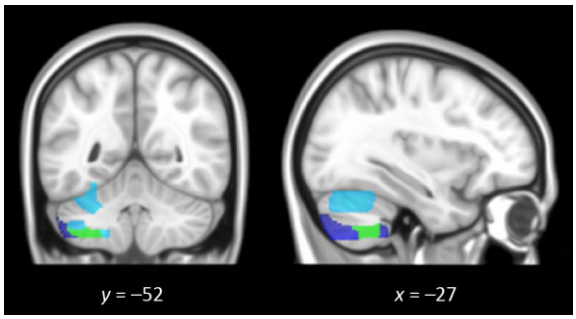

Figure 4 Clusters of correlation between cerebellar gray matter volume and severity of cocaine use and reversal cost in cocaine-dependent individuals. The figure shows the clusters of correlation of cerebellar gray matter volume with severity of cocaine use (light blue) (peak coordinates at *x*, *y*, *z* = −24, −52, −48; *t* = 6.94; *pFWE-SVC* < .05), and with the measure of reversal cost (dark blue) (peak coordinates at *x*, *y*, *z* = −36, −75, −54; *t* = 5.02; *pFWE-SVC* < .05), as well as the overlap of these two clusters (green). Results are overlaid on coronal and sagittal sections of a normalized brain, and the numbers correspond to the '*y*' and '*x*' coordinates in MNI space

DISCUSSION

This study was aimed at examining the association between cocaine use and reversal learning performance

and brain circuitry (i.e. gray matter volume in a set of brain regions associated with cocaine use and reversal learning). In agreement with our hypotheses, we found that greater severity of cocaine use was significantly associated with greater perseveration after reversal shift, and with lower gray matter in the cerebellum. Moreover, gray matter volume in the right insula was associated with the probability of shifting response after negative feedback during post-reversal phases.

CDI showed only subtle differences with HC in PRLT performance. The block × group interaction in the task was of similar shape and size than the one reported by Fernández-Serrano *et al.* (2012), although in-depth analysis of that interaction did not reveal significant differences across groups in any block/phase of the task. However, in agreement with previous findings (Torres *et al.* 2013), we reason that difficulties to find clear-cut differences between groups are explained by within-group heterogeneity in the CDI sample. The degree to which PRLT performance is hampered by cocaine abuse strongly depends on the cumulative effect of cocaine. In accordance with this notion, the GLM analysis (restricted to the CDI sample) with cocaine abuse severity as a continuous predictor, and phase and block as within-subject variables yielded a strong phase × severity effect. The performance difference between phases with reversed-sign reward contingencies (2 and 4), and phases with the original sign (1 and 3) was found to be a function of severity. In other words, the difficulty to learn inversed-sign contingencies increased with cocaine abuse severity (Fig. 1).

Severity of cocaine use is also a similarly strong predictor of gray matter abnormalities in the cerebellum. Interestingly, the affected area is located in the lobule VIII of the left hemisphere (Fig. 2), which is thought to be richly innervated by dopamine (Anderson *et al.* 2006). However, the direct correlation between reversal learning performance (i.e. reversal cost) and regional cerebellar gray matter revealed a significant finding in an only partially overlapping area, extending through left lobules VIIb and VIII. Moreover, severity of cocaine use showed a dose-related detrimental impact on brain volume in the right IFG. Although this result did not survive correction of multiple comparisons, a functionally related right insula cluster showed significant correlations with the probability of maintaining response after losses (tracked in a trial by trial basis) during reversal phases. Therefore, our data concur with animal and human evidence suggesting that the cerebellum is involved in steady acquisition and reversal of stimulus-reward representations (Thoma *et al.* 2008; Miquel *et al.* 2009; Carbo-Gas *et al.* 2013), whereas the IFG/insula regions have a more dynamic role in tracking and predicting response-related outcomes (Ghahremani *et al.* 2010; Rygula *et al.* 2010). Cocaine use has previously been associated with gray matter deficits in these reversal learning-related regions (Sim *et al.* 2007; Moreno-López *et al.* 2012; Mackey & Paulus 2013); however, the small overlap between cocaine use ROIs and reversal learning ROI correlations suggest that reversal learning deficits are at least partially independent of cocaine neurotoxic effects, and may be part of a broader addiction phenotype (Izquierdo & Jentsch 2012). For example, pathological gamblers (i.e. a toxicity-free addiction) similarly display reduced right inferior frontal cortex and cerebellar brain activations during reversal learning (de Ruiter *et al.* 2009).

The contribution of lateral prefrontal/insula regions to executive control and cognitive flexibility is well known (Hampshire & Owen 2006). However, the contribution of cerebellar regions to these functions and their relevance to addiction has been more recently unfolded (Moulton *et al.* 2013). Lobule VIII is principally involved in motor and sensorimotor tasks (Stoodley & Schmahmann 2009), but it is also engaged in working memory and other executive function tasks (Stoodley & Schmahmann 2010). Interestingly, both sensorimotor gating and working memory are significantly impaired in cocaine-dependent users (Preller *et al.* 2013; Vonmoos *et al.* 2013). Moreover, functional imaging studies have shown that during executive tasks, lobule VIII activation extends to more lateral regions such as the lobule VIIb (Stoodley & Schmahmann 2010). These results nicely match our findings, showing a lateral extension of the cerebellar cluster found to be associated with severity of cocaine use when the correlation was assessed with reversal cost measurements. Therefore, we reason that lobule VIII alterations are characteristic of cocaine addiction, and they may possibly relate to sensorimotor and executive deficits. Moreover, cocaine users with greater executive deficits may extend their area of alteration to more lateral regions of the cerebellar hemispheres. All in all, our pattern of findings is compatible with the observation that cocaine-induced PRLT abnormalities are partially mediated by cocaine neuroadaptations in the cerebellum. Moreover, the lateralization of such effects to the left hemisphere suggests that, given the crossed cerebro-cerebellar fiber pathways, severity of cocaine use will mainly relate to abnormalities in visual-spatial and non-linguistic tasks, such as the one assessed here.

Our findings show that severity of cocaine use detrimentally impacts reversal learning and cerebellar gray matter, but the cerebellar contribution to reversal learning is dissociable from cocaine-related effects. We also found an association between right insula gray matter content and trial by trial negative feedback response. We did not find significant associations between cocaine use

and ventromedial prefrontal and insula gray matter, or between reversal learning and ventromedial prefrontal gray matter. The former finding is in agreement with findings showing that ventromedial prefrontal and insula gray matter abnormalities are already present at very early stages of stimulant use (Mackey *et al.* 2014). The latter finding is consistent with human functional neuroimaging studies, suggesting that ventrolateral rather than ventromedial regions are essential to reversal learning (Cools *et al.* 2002; de Ruiter *et al.* 2009). Nonetheless, studies in larger samples are warranted to validate these notions. Strengths of the study include the strict recruitment (i.e. consecutive admissions to public center) and selection (i.e. eligibility) criteria, which excluded coexisting substance use disorders and psychiatric comorbidities, the monitoring of drug abstinence, and the precise characterization of self-reported cocaine use history, which yielded biologically plausible correlations with cognitive performance and brain measures. One limitation (common in addiction research) was that CDI scored higher on smoking and drinking than controls. Results were replicated after including these variables as covariates, although we acknowledge that this is not a full-proof exclusion of the possibility that these differences affected results (Meehl 1970). Another relevant limitation is that the sample size is limited and therefore more susceptible to reliability biases (Button *et al.* 2013). To address this limitation, we have strengthened the methodological control through the recruitment process and eligibility criteria, clear 'a priori' hypotheses and stringent correction of statistical effects. An additional limitation is the correlational design. The direct manipulation of reversal learning contingencies during 'in vivo' functional imaging experiments can provide a more precise and integrated approach to the fronto-striatal-cerebellar circuitry involved in reversal learning (including relevant subcortical regions; i.e. striatum and amygdala). Last, but not least, the cross-sectional design precludes us from drawing inferences about causality. Numerous preclinical studies have shown that cocaine regimens dose dependently impact reversal learning and frontal and cerebellar regions, but human longitudinal studies are warranted to address this issue.

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Authors Contribution

AVG was responsible for the study concept and design. NAU and JMM conducted the recruitment and clinical and neuropsychological assessments. LML, DVS and JCP performed the analyses. AVG, RWW and CSM assisted with data analysis and interpretation of findings. LML, JCP, CSM and AVG drafted the manuscript. All authors critically reviewed content and approved final version for publication.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1 Scatterplot (left) and cluster (right) of correlation between IFG gray matter volume and severity of cocaine use in cocaine-dependent individuals. Peak coordinates were located in the right IFG (peak at $x, y, z = 50, 41, -6$; $t = 3.33$; $P < .005$ uncorrected). Results are overlaid on axial and sagittal sections of a normalized brain, and the numbers correspond to the 'z' and 'x' coordinates in MNI space