

Appendix A. Supplementary material

A 1. Material and Methods

A 1.1. Participants

Hair samples were collected as part of the longitudinal *Zurich Cocaine Cognition Study* (ZuCo²St). From the previously included 166 ZuCo²St participants (Vonmoos et al., 2013), hair samples of 140 participants were available for the additional neuroendocrinological analyses reported here. These participants were divided into cocaine-naïve control group (n=67) and chronic cocaine users (n=73). The cocaine users were further subdivided into recreational cocaine users (RCU; n=48) and dependent cocaine users (DCU; n=25). Cocaine dependence was diagnosed following the DSM-IV criteria, with DCU fulfilling and RCU not meeting these criteria. Further inclusion criteria for the chronic cocaine users were cocaine use of at least 1 g per month, cocaine as primary used illegal substance, and a current abstinence duration <6 months. Exclusion criteria for the user groups were past or current use of opioids, a polytoxic substance use pattern, and an axis-I DSM-IV adult psychiatric disorder (American Psychiatric Association, 1994), with exception of cocaine and alcohol abuse/dependence, a history of a depression (acute major depression was excluded), and attention deficit hyperactivity disorder (ADHD). Control subjects were excluded when they displayed any axis-I DSM-IV psychiatric disorder and any form of addiction or regular substance use (lifetime use <15 occasions), with exception of cannabis. Exclusion criteria for all participants were a neurological disorder or head injury, clinically relevant medical diseases, family history of schizophrenia or bipolar disorder, or current use of prescription drugs affecting the central nervous system. All participants had to abstain from illegal substances for a minimum of 72 hours and from alcohol for at least 24 hours. Abstinence, and self-reported substance use was controlled via urine and 6-months hair analysis, respectively.

A 1.2. Procedure

Trained psychologists conducted a *Structured Clinical Interview* (SCID-I) according to DSM-IV procedures (Wittchen et al., 1997). Substance use was assessed by means of a structured and standardized *Interview for Psychotropic Drug Consumption* (IPCD) (Quednow et al.,

2004). For the estimation of verbal intellectual performance a German vocabulary test (*Mehrfachwahl-Wortschatz-Intelligenztest*, MWT-B) was conducted (Lehrl, 1999). The brief version of the *Cocaine Craving Questionnaire* (CCQ) was used to capture current cocaine craving (Sussner et al., 2006). The *Beck Depression Inventory* (BDI) (Beck et al., 1961) measured the current severity of depression and the *ADHD Self-Rating Scale* (ADHD-SR) (Rösler et al., 2005) assessed adult ADHD symptoms according to DSM-IV criteria. Severity of psychiatric symptoms and current psychological distress was assessed by the *Symptom Checklist-90-Revised* (SCL-90-R) (Derogatis and Cleary, 1977). The SCL-90-R includes 90 items related to psychological health and contains nine subscales (Aggressiveness, Anxiety, Depression, Obsessive-compulsive, Paranoid ideation, Phobic anxiety, Psychoticism, Somatization, and Uncertainty to social contact) and three indices of global wellness (Global Severity Index, Positive Symptoms Total, and Positive Symptom Distress Index, [PSDI]). Here, we only included the SCL-90-R subscales Anxiety and the Psychoticism in the analysis, as these symptoms have been associated with increased physiological stress markers in hair (Steudte et al., 2011; Romero-Gonzalez et al., 2019; Gao et al., 2020). Additionally, only the global index PSDI, indicating the average intensity of symptoms, was analyzed as an estimator for the total stress burden (Derogatis and Cleary, 1977). Urine toxicology analyses comprised the compounds: tetrahydrocannabinol (metabolite), cocaine (metabolite), amphetamines, benzodiazepines, opioids, and methadone and were assessed by a semi-quantitative enzyme multiplied immunoassay method using a Dimension RXL Max (Siemens, Erlangen, Germany).

Table A.1: Variables used in the data analysis, variable description, type of variable (numerical or binary), type of transformation.

Variable name	Variable description	type	transformation
2-AG	Conc. in pg/mg in proximal 3 cm hair segment	num	log
AEA	Conc. in pg/mg in proximal 3 cm hair segment	num	log
OEA	Conc. in pg/mg in proximal 3 cm hair segment	num	log
PEA	Conc. in pg/mg in proximal 3 cm hair segment	num	log
Cortisol	Conc. in pg/mg in proximal 3 cm hair segment	num	log
Cortisone	Conc. in pg/mg in proximal 3 cm hair segment	num	log
Cortisone/cortisol sum	Sum of cortisol and cortisone	num	log
Cocaine total hair concentration	Sum of cocaine, benzoylecgonine, and norcocaine	num	log
Cocaine grams per week	Cocaine use: grams/week	num	log
Cocaine years of use	Years of cocaine consumption	num	log
Cocaine lifetime grams	Lifetime cocaine use in grams	num	log
MDMA hair concentration	Conc. In pg/mg in proximal 3 cm hair segment	num	log
MDMA pills per week	MDMA use: pills/week	num	log
MDMA years of use	Years of MDMA consumption	num	log
MDMA lifetime pills	Lifetime MDMA use in pills	num	log
Cannabis grams per week	Cannabis use: grams/week	num	log
Cannabis years of use	Years of cannabis consumption	num	log
Cannabis lifetime grams	Lifetime cannabis use in grams	num	log
Cigarettes per week	Nicotine use: cigarettes/week	num	none
Alcohol grams per week	Alcohol use: grams/week	num	log
Years of school education	Years of school education	num	none
Estimated verbal IQ	Estimated verbal IQ	num	none
BDI sum score	Sumscore for depressive symptoms	num	none
ADHD sum score	Sumscore for ADHD symptoms, items 1 to 18	num	none
SCL-90-R anxiety scale	Scale for anxiety symptoms from the SCL-90	num	log
SCL-90-R psychoticism scale	Scale for psychoticism symptoms from the SCL-90	num	log
SCL-90-R positive symptom distress	Positive symptom distress index measures the intensity of symptoms in the SCL-90	num	none
Sex	Sex (male; female)	binary	none
Age	Age in years	num	none

1. Test whether age or sex should be included as covariates

Model: $Y_i \sim \text{age} + \text{sex}$, with type II SS

Subjects included: only controls

Y_i : dependent variable i

Outcome: if age/sex are significantly correlated to Y_i , include age/sex as co-variables in all models below that concern Y_i . Added as **[age/sex]** in the notation below.

Results in Table A.2.

2. Test for differences between experimental groups (controls/recreational/dependent users)

Model examples (all with type I SS):

all subjects

$Y_i \sim \text{[age/sex]} + \text{expGroup}$
 $Y_i \sim \text{[age/sex]} + \text{CvsU} + \text{RvsD}$

expGroup: factor with the three levels control/recreational/dependent. Can be split into multiple pairs of 1-degree-of-freedom contrasts. The example with CvsU and RvsD compares first controls with users (CvsU) and then recreational with dependent users (RvsD).

Results in Figure 1 and Table 2.

3. Search additional co-variables that may explain differences in glucocorticoids and eCBs

Model: $Y_i \sim \text{[age/sex]} + X_j$, with type I SS

only chronic users

X_j : regressor j

Outcome: if X_j is significantly correlated to Y_i , include it as co-variate in the models below that concern Y_i or a substance of the same class (i.e., if Y_i =cortisone, include X_j also in the models with cortisol and cortisone + cortisol). Added as **[co-variables]** in the notation below.

Results in Figure 2 and Table A.3.

4. Test whether cocaine and MDMA correlations are independent of co-variables and each other

Model: $Y_i \sim \text{[age/sex]} + \text{[co-variables]} + \text{MDMA} + \text{Cocaine}$, with type II SS

only chronic users

Results in Text.

Fig A.1: Schematic of the analysis process. Formula notation follows the programmatic notation used in R. ANOVA type I sum of squares (type I SS) means that all terms are fitted and tested sequentially as they are written in the formula. Hence, $y \sim a + b$ does not necessarily yield the same result as $y \sim b + a$ for a and b . ANOVA type II SS means that the program internally tests each variable as if it was fitted and tested last in a type I SS ANOVA. Hence, $y \sim a + b$ would be tested as $y \sim b + a$ for a and $y \sim a + b$ for b . For the list of all variables included in the analysis, see Table A.1.

Table A.2: Testing for age and sex as covariates using the controls.

	age (df = 1)		sex (df = 1)		Residual df
	%-SS	P-value	%-SS	P-value	
PEA	1.61	0.3048	2.14	0.2374	64
OEA	0.01	0.9408	4.92	0.0735	64
AEA	5.42	0.0524	10.11	0.0089	61
2-AG	1.57	0.2936	8.91	0.0141	64
CortSum	4.31	0.113	2.66	0.2106	56
Cortisol	1.84	0.3097	0.06	0.8509	56
Cortisone	4.41	0.0816	5.53	0.0517	64
Model: y ~ age + sex					
Test: Type II					

Table A.3: Covariates for direct effects

Residual Df (within users)	70	67	67	70	66	71	71	71	71
Residual Df (total)	137	126	126	137	130	138	138	not tested	not tested

F-values:	Cortisone	Cortisol	Cort Sum	2-AG	AEA	OEA	PEA	MDMA	Cocaine total
Cocaine total hair concentration	11.06	5.31	8.79	1.66	0.02	5.21	3.11	NA	NA
Cocaine grams per week	0.40	0.02	0.26	0.22	0.20	0.27	0.04	0.24	9.98
Cocaine years of use	3.50	1.92	2.85	0.50	0.02	3.42	6.70	0.40	8.87
Cocaine lifetime grams	3.38	1.48	2.40	0.35	2.74	2.78	3.50	2.13	16.67
MDMA hair concentration	5.11	1.07	3.46	2.69	4.96	11.91	9.48	NA	NA
MDMA pills per week	0.38	0.87	0.68	2.89	0.75	5.58	1.43	13.97	1.17
MDMA years of use	0.00	0.06	0.02	0.62	0.05	1.80	1.46	14.74	0.00
MDMA lifetime pills	0.27	1.32	0.66	0.28	0.47	2.58	2.08	16.28	0.06
Cannabis grams per week	2.46	0.91	1.26	0.87	0.21	1.51	1.15	0.74	0.26
Cannabis years of use	0.08	0.02	0.03	5.30	9.02	6.74	11.02	0.34	0.00
Cannabis lifetime grams	1.47	0.32	0.42	4.01	6.04	3.60	4.57	0.06	0.08
Cigarettes per week	5.10	2.63	4.28	0.18	0.10	0.01	0.14	0.01	3.04
Alcohol grams per week	0.22	0.37	0.33	2.84	1.99	1.29	0.89	2.51	0.35
Years of school education	4.49	1.75	2.89	0.14	1.20	0.37	0.00	0.12	6.89
Estimated verbal IQ	0.03	1.76	0.25	0.33	0.03	0.24	0.69	7.45	0.13
BDI sum score	0.80	2.53	2.09	0.12	0.01	2.71	0.67	1.47	0.38
ADHD sum score	2.05	1.67	2.00	1.20	0.35	5.00	0.82	0.82	0.63
SCL-90-R anxiety scale	2.34	3.84	3.94	1.48	0.56	2.34	1.14	0.08	1.65

SCL-90-R psychoticism scale	0.57	1.08	0.77	0.89	0.01	4.12	1.74	1.18	0.29
SCL-90-R positive symptom distress	2.37	5.48	4.46	0.92	0.20	4.51	1.41	0.83	1.93

P-values:	Cortisone	Cortisol	Cort Sum	2-AG	AEA	OEA	PEA	MDMA	Cocaine total
Cocaine total hair concentration	0.001	0.024	0.004	0.202	0.898	0.025	0.082	NA	NA
Cocaine grams per week	0.531	0.885	0.612	0.643	0.659	0.607	0.849	0.626	0.002
Cocaine years of use	0.066	0.170	0.096	0.482	0.875	0.068	0.012	0.530	0.004
Cocaine lifetime grams	0.070	0.227	0.126	0.553	0.102	0.100	0.066	0.149	0.000
MDMA hair concentration	0.027	0.304	0.067	0.106	0.029	0.001	0.003	NA	NA
MDMA pills per week	0.538	0.353	0.414	0.094	0.391	0.021	0.237	0.000	0.284
MDMA years of use	0.990	0.800	0.881	0.435	0.817	0.185	0.231	0.000	0.977
MDMA lifetime pills	0.604	0.255	0.420	0.597	0.496	0.113	0.154	0.000	0.802
Cannabis grams per week	0.119	0.342	0.265	0.352	0.650	0.221	0.284	0.393	0.610
Cannabis years of use	0.784	0.877	0.870	0.023	0.003	0.010	0.001	0.564	0.967
Cannabis lifetime grams	0.228	0.570	0.520	0.047	0.015	0.060	0.034	0.809	0.773
Cigarettes per week	0.025	0.107	0.041	0.675	0.747	0.913	0.708	0.922	0.085
Alcohol grams per week	0.638	0.543	0.566	0.094	0.160	0.258	0.346	0.117	0.554
Years of school education	0.036	0.188	0.091	0.708	0.276	0.544	0.946	0.729	0.011
Estimated verbal IQ	0.864	0.187	0.618	0.567	0.855	0.626	0.406	0.008	0.723
BDI sum score	0.373	0.114	0.151	0.727	0.914	0.102	0.414	0.229	0.539
ADHD sum score	0.155	0.198	0.160	0.275	0.557	0.027	0.367	0.369	0.431
SCL-90-R anxiety scale	0.128	0.052	0.049	0.225	0.456	0.128	0.288	0.777	0.203
SCL-90-R psychoticism scale	0.450	0.300	0.381	0.348	0.905	0.044	0.190	0.282	0.589
SCL-90-R positive symptom distress	0.126	0.021	0.037	0.338	0.654	0.035	0.238	0.365	0.169

% Sum of squares ($\eta^2 \times 100$):	Cortisone	Cortisol	Cort Sum	2-AG	AEA	OEA	PEA	MDMA	Cocaine total
Cocaine total hair concentration	13.64	7.34	11.6	2.31	0.02	6.84	4.19	0	0
Cocaine grams per week	0.56	0.03	0.39	0.31	0.27	0.37	0.05	0.34	12.32
Cocaine years of use	4.76	2.79	4.08	0.71	0.03	4.6	8.63	0.56	11.11
Cocaine lifetime grams	4.6	2.17	3.46	0.5	3.63	3.77	4.69	2.91	19.01
MDMA hair concentration	6.8	1.58	4.92	3.69	6.36	14.36	11.78	0	0
MDMA pills per week	0.54	1.29	1	3.96	1.02	7.28	1.97	16.44	1.62
MDMA years of use	0	0.1	0.03	0.87	0.07	2.47	2.01	17.19	0

MDMA lifetime pills	0.39	1.93	0.97	0.4	0.64	3.5	2.84	18.66	0.09
Cannabis grams per week	1.77	0.72	0.99	0.62	0.14	1.08	0.83	1.03	0.37
Cannabis years of use	0.05	0.02	0.02	3.64	5.88	4.66	7.39	0.47	0
Cannabis lifetime grams	1.06	0.26	0.33	2.78	4.03	2.54	3.21	0.08	0.12
Cigarettes per week	3.59	2.04	3.28	0.13	0.07	0.01	0.1	0.01	4.11
Alcohol grams per week	0.16	0.29	0.26	1.98	1.37	0.93	0.64	3.42	0.49
Years of school education	3.18	1.37	2.24	0.1	0.83	0.27	0	0.17	8.85
Estimated verbal IQ	0.02	1.38	0.2	0.23	0.02	0.17	0.5	9.49	0.18
BDI sum score	0.58	1.97	1.63	0.09	0.01	1.93	0.49	2.03	0.53
ADHD sum score	1.47	1.31	1.56	0.85	0.24	3.5	0.59	1.14	0.88
SCL-90-R anxiety scale	1.68	2.96	3.03	1.05	0.39	1.67	0.82	0.11	2.27
SCL-90-R psychoticism scale	0.42	0.85	0.61	0.63	0.01	2.9	1.24	1.63	0.41
SCL-90-R positive symptom distress	1.7	4.16	3.42	0.65	0.14	3.17	1.01	1.16	2.65

Standardized (β) coefficients:	Cortisone	Cortisol	Cort Sum	2-AG	AEA	OEA	PEA	MDMA	Cocaine total
Cocaine total hair concentration	0.402	0.284	0.365	-0.166	-0.015	-0.265	-0.218	0.000	0.000
Cocaine grams per week	0.080	0.019	0.068	0.060	0.050	-0.062	0.024	-0.058	0.351
Cocaine years of use	0.240	0.178	0.220	-0.091	0.019	-0.218	-0.313	-0.075	0.333
Cocaine lifetime grams	0.230	0.150	0.195	-0.077	-0.184	-0.197	-0.231	-0.171	0.436
MDMA hair concentration	0.281	0.130	0.235	0.206	0.237	0.384	0.366	0.000	0.000
MDMA pills per week	-0.079	-0.116	-0.104	0.215	0.093	0.274	0.150	0.405	-0.127
MDMA years of use	0.002	0.033	0.020	0.106	0.027	0.159	0.151	0.415	0.003
MDMA lifetime pills	0.067	0.143	0.104	0.069	0.077	0.190	0.180	0.432	0.030
Cannabis grams per week	0.132	0.082	0.097	-0.079	-0.040	-0.104	-0.091	0.092	-0.055
Cannabis years of use	0.024	-0.014	-0.015	-0.191	-0.242	-0.216	-0.272	-0.071	0.005
Cannabis lifetime grams	0.103	0.052	0.058	-0.167	-0.201	-0.159	-0.179	-0.030	-0.035
Cigarettes per week	0.189	0.147	0.187	-0.036	-0.028	0.009	-0.032	-0.012	0.210
Alcohol grams per week	0.040	0.053	0.050	0.142	0.117	0.096	0.080	0.171	-0.065
Years of school education	-0.178	-0.119	-0.153	-0.032	-0.091	0.052	0.006	-0.050	-0.361
Estimated verbal IQ	-0.015	-0.122	-0.046	0.049	0.015	0.042	0.071	-0.331	-0.045
BDI sum score	0.076	0.151	0.137	-0.030	-0.009	-0.139	-0.070	-0.128	0.066
ADHD sum score	0.121	0.113	0.123	-0.093	-0.049	-0.187	-0.077	0.093	0.082
SCL-90-R anxiety scale	0.129	0.183	0.185	-0.104	-0.065	-0.129	-0.090	0.030	0.133
SCL-90-R psychoticism scale	0.065	0.095	0.080	-0.080	-0.010	-0.170	-0.111	-0.112	0.056

SCL-90-R positive symptom distress	0.130	0.211	0.192	-0.083	-0.040	-0.178	-0.100	-0.105	0.158
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