Stress rapidly reorganizes the glutamate synapse in the prefrontal cortex of cocaine-withdrawn adolescent rats

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1. INTRODUCTION

Drug addiction is a major public health issue worldwide characterized by a compulsive drug-seeking and drug-taking behavior. Illicit use of drugs begins and escalates during adolescence, with long-term adverse consequences. The progression from first use to addiction appears to be shorter during adolescence (Clark et al., 1998). The adolescent brain is in a unique state of transition as it undergoes structural and synaptic changes (Crews and Hodge, 2007). The evidence that initiation of drug taking primarily occurs during adolescence suggests a greater addictive potential than at adulthood. The brake against compulsive behaviours is provided by prefrontal cortex that is still developing during adolescence. It is thus possible to hypothesize that if the brake is defective, the chances of risky behaviours become higher. For this reason, adolescence can be considered a crucial period for investigating the development of drug addiction.
Recent evidence has implicated, besides dopamine, a role for glutamate among the effects produced by cocaine (Schmidt and Pierce, 2010; Mameli et al., 2011). Using animal models of drug addiction, it has been shown that drug-induced craving, also after withdrawal periods, is accompanied by changes in extracellular dopamine and glutamate concentrations and in glutamatergic receptor expression and phosphorylation in a region- and time-dependent manner (Churchill et al. 1999; Ferrario et al. 2011).

The aim of the present work was 1) to investigate the short-term effects of repeated exposure to cocaine during adolescence on the glutamatergic synapse in prefrontal cortex and 2) to evaluate the dynamic response to a challenging event such as an acute stress as a potential indication of coping ability under a challenging condition.

2. MATERIALS AND METHODS

PROTEIN ANALYSIS

Prefrontal cortex was dissected, frozen on dry ice and stored at –80°C. Tissues were homogenized and membrane fraction was prepared. After being separated on a SDS-10% polyacrylamide gel, proteins (10 µg) were transferred onto nitrocellulose membrane and blocked 1 hour with 10% nonfat dry milk. Membranes were incubated with antibody against phosphorylated forms of GluN1 Ser896 (1:1000, O/N, 4°C), Pak1 Thr423 (1:1000, O/N, 4°C), αCaMKII T286 (1:2000, O/N, 4°C) and then stripped and reprobed with antibody against total proteins (GluN1 1:1000, O/N, 4°C; Pak1 1:1000, O/N, 4°C, 4°C; αCaMKII: 1:5000, 1h, RT; Arc 1:500, O/N, 4°C; β-actin 1:20000, 1h, RT). All immunocomplexes were visualized by chemiluminescence using the ECL Western Blotting kit using the Chemidoc MP imaging system (Biorad Laboratories). The activation of the proteins investigated were expressed as a ratio between the phosphorylated and the respective total forms and analyzed using the Image Lab software from Biorad.

PLASMA CORTICOSTERONE ANALYSIS

Plasma from each rat was separated by centrifugation and corticosterone was determined by an enzyme-linked immunosorbent assay (ELISA) using a commercial kit according to the manufacturers' instructions (IBL, Hamburg, Germany).

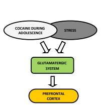
STATISTICAL ANALYSIS

Data are presented as mean ± SEM of several indipendent determinations. Changes were analyzed by two-way ANOVA, with treatment (saline- and cocaine-injected animals) and status (sham- and stressed animals). Status and treatment were considered as independent variables. When appropriate, further differences were analyzed by single contrast post hoc test (SCPHT). An unpaired Student's *t* test was used to analyze the immobility time, measured during the swim stress. Significance for all tests was assumed at p<0.05.

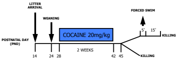
mRNA ANALYSIS

Total RNA was isolated from prefrontal cortex was isolated using PUREZOL reagent. The analysis of vGLUT1, vGAT, GAD67, EAAT1, EAAT2, GFAP, system X_c^-, GS, Cdc42 and cFOS mRNA levels were performed by TaqMan qRT-PCR instrument (CFX384 real time system, Bio-Rad Laboratories) using iScriptTM one-step RT-PCR kit for probes. All the samples were run in 384-well formats in triplicate with a normalizing internal control (36B4). The levels of target genes expression were calculated using a comparative cycle threshold (Ct).

AIM OF THE WORK



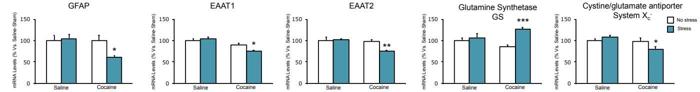
EXPERIMENTAL PARADIGM



3. RESULTS

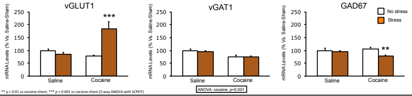
GLUTAMATERGIC TRANSMISSION

GLIAL MARKERS



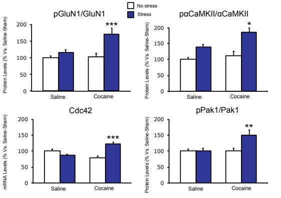
* $p < 0.05$ vs cocaine-sham; ** $p < 0.01$ vs cocaine-sham; *** $p < 0.001$ vs cocaine-sham (2-way ANOVA with SCPHT)

PRE-SYNAPTIC MARKERS



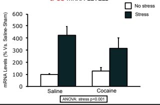
** $p < 0.01$ vs cocaine-sham; *** $p < 0.001$ vs cocaine-sham (2-way ANOVA with SCPHT)

POST-SYNAPTIC MARKERS

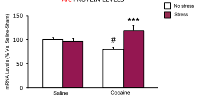


* $p < 0.05$ vs cocaine-sham; ** $p < 0.01$ vs cocaine-sham; *** $p < 0.001$ vs cocaine-sham (2-way ANOVA with SCPHT)

cFOS mRNA LEVELS



Arc PROTEIN LEVELS



# $p < 0.05$ vs saline-sham; *** $p < 0.001$ vs cocaine-sham (2-way ANOVA with SCPHT)

REFERENCES

Churchill L, Swanson CJ, Urbina M, Kalivas PW (1999) Repeated cocaine alters glutamate receptor subunit levels in the nucleus accumbens and ventral tegmental area of rats that develop behavioral sensitization. J Neurochem 72: 2397-403

Clark DB, Kirisci L, Tarter RE (1998) Adolescent versus adult onset and the development of substance use disorders. Drug Alcohol Dep 49: 115-121

Crews F, He J, Hodge C (2007) Adolescent cortical development: a critical period of vulnerability for addiction. Pharmacol Biochem Behav 86: 189-99

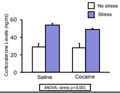
Ferrario CR, Li X, Wolf ME (2011) Effects of acute cocaine or dopamine receptor agonists on AMPA receptor distribution in the rat nucleus accumbens. Synapse 65: 54-63

Mameli M, Bellone C, Brown MT, Luscher C (2011) Cocaine inverts rules for synaptic plasticity of glutamate transmission in the ventral tegmental area. Nat Neurosci 14: 414-6

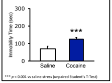
Murakoshi H, Wang H, Yasuda R (2011) Local, persistent activation of Rho GTPases during plasticity of single dendritic spines. Nature 472 (7341): 100-4

Schmidt HD, Pierce RC (2010) Cocaine-induced neuroadaptations in glutamate transmission: potential therapeutic targets for craving and addiction. Ann N Y Acad Sci 1187: 35-75

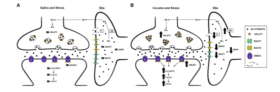
PLASMA CORTICOSTERONE LEVELS



IMMOBILITY TIME



*** $p < 0.001$ vs saline-stress (unpaired Student's T-Test)



4. CONCLUSIONS

Stress rapidly reorganizes the glutamate synapse in the prefrontal cortex of cocaine-withdrawn adolescent rats.

Hyper-reactive glutamatergic synapses in mPFC may contribute to explain the hypersensitivity to stress observed in abstinent cocaine users.

Dysregulation of the glutamate homeostasis may contribute to the negative emotional state and stress-induced reinstatment observed in animal models of cocaine abuse.

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