



In vivo microdialysis studies of brain dopamine, glutamate and GABA overflow during cocaine self-administration and its extinction

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INTRODUCTION

Cocaine abuse and addiction is the serious medical and social worldwide problem [1, 2]. Positive subjective effects of cocaine in humans (i.e., increased mood, euphoria) motivate to its repeated use what might lead to uncontrolled drug taking (drug craving) and/or relapses that can occur even after long period of abstinence [3, 4]. Current methods of treatment of cocaine addiction are not effective, probably due to the fact that the mechanisms underlying drug abuse/addiction are not yet fully understood. In laboratory animals, cocaine induces different kinds of behavioral effects associated mostly with the mesolimbic dopamine (DA) pathway, which starts in the ventral tegmental area (VTA) where the cell bodies of DA neurons are localized and ends in the nucleus accumbens (NAc) where DA terminals are present [5, 6]. This neurotransmission signaling is related to rewarding properties of cocaine, while other brain structures e.g. prefrontal cortex (PFC) and ventral pallidum (VP) are related to the extinction/relapses of the drug [7].

AIM

The present study was aimed to analyze the concentration of DA, glutamate (Glu) and γ – aminobutyric acid (GABA) during cocaine self-administration and its extinction training in the NAc and VP in rats.

METHODS

Animals

Male Wistar rats (260-310g; Charles River, Germany) were housed under standard laboratory conditions.

Self-administration and extinction training procedures

Under anesthesia, rats were implanted with a silastic catheter in the external jugular vein and the guide cannulae (MAB 4, AgnTho's, Sweden) into the NAc shell (AP: +1.7 mm; ML: +1.0 mm; DV: -5.8 mm) and the VP (AP: -0.5 mm; ML: +2.8 mm; DV: -6.2 mm) according to the atlas of Paxinos and Watson [8]. After recovery, animals were trained to self-administer cocaine (Sigma, USA) in a standard two-lever operant chambers (Med-Associates, Inc., USA) under a fixed ratio (FR) 1 schedule of reinforcement. Rats were given access to cocaine during 2-h daily sessions (performed 6 days/week for 8-10 days; maintenance phase). Each completion of a FR 1 schedule on the “active” lever resulted in an infusion of cocaine (0.5 mg/kg over 5 s). A tone and illumination of the stimulus light directly above the “active” lever (i.e., the drug-associated cue) was presented for 5 s, concurrent with a successful response for cocaine. Following each injection, there was a 20-s time-out period during which responding was recorded, but had no programmed consequences. During extinction training sessions, subjects had 2-h daily sessions with no delivery of cocaine or the presentation of the conditioned stimulus. Once they reached the extinction criteria (a minimum of 10 extinction days with the responding on the active lever below 20% of the lever observed during at least 3 consecutive maintenance days).

The use of a "yoked" procedure (in which rats were tested simultaneously in groups of three, with one rat actively self-administering cocaine and the other two receiving passively cocaine or its vehicle) enabled to separate the pharmacological effects of cocaine from the effects evoked by the motivational and cognitive processes associated with active cocaine administration.

Microdialysis procedure

On the last day of cocaine self-administration or its extinction training, the microdialysis probes (2 mm membrane, 6 kDa cut-off, 0.24 mm outer diameter, AgnTho's, Sweden) were inserted into the guide cannulae (with the internal cannulae extending 2 mm beyond the end of the guide cannulae). The rats were then left in the operant chambers and cannulae were rinsed (2 μ l/min) with an artificial cerebral fluid (2.7 mM KCl, 1.2 mM CaCl₂, 145 mM NaCl, 1.0 mM MgCl₂, pH 7.4) for 4 h. Then, every 22 min 4 fractions of dialysates were collected to determine spontaneous release, and subsequent 6 fractions were collected during 2-h self-administration session. The levels of DA (electrochemical detection), Glu (VIS detection after sample derivatization with dabsyl chloride) and GABA (electrochemical detection after sample derivatization with OPA/SO₃) were quantified with the use of high performance liquid chromatography (HPLC).

Histology

Rats were overdosed with pentobarbital (>100 mg/kg, i.p.; Biowet, Poland), brains were dissected and stored in 4% formaline for at least 24 h. Coronal sections (10- μ m) were cut to examine the placement of microdialysis probes according to the atlas of Paxinos and Watson.

Statistical analyses

The data are shown as means (SEM). Data from the behavioral experiments, i.e. the number of responses on the active and inactive lever, were analyzed using a one-way ANOVA for repeated measures and Dunnett's post hoc test. Microdialysis data were analysed using repeated measures ANOVA followed by Tukey's post hoc test. The criterion for statistically significant differences was set at $p < 0.05$.

RESULTS

Maintenance

➤Animals that self-administered cocaine showed stable response rates during the last three self-administration sessions, with less than 10% variability in daily cocaine intake (15-18 mg per day).

➤Repeated cocaine administration (either active or passive injections) reduced basal Glu levels in the NAc and VP, while basal DA levels in the NAc were left unchanged.

➤Only rats that self-administered cocaine had increased basal GABA levels both in the NAc and VP.

➤Cocaine i.v. injections changed accumbal DA levels in rats.

➤Rats self-administered cocaine showed decrease in extracellular GABA levels in the NAc, while Glu levels were not changed in both brain structures.

Withdrawal

➤Extinction training resulted in gradually decreasing responses on the active lever. During the last three sessions of extinction training active lever presses did not differ by more than 10%.

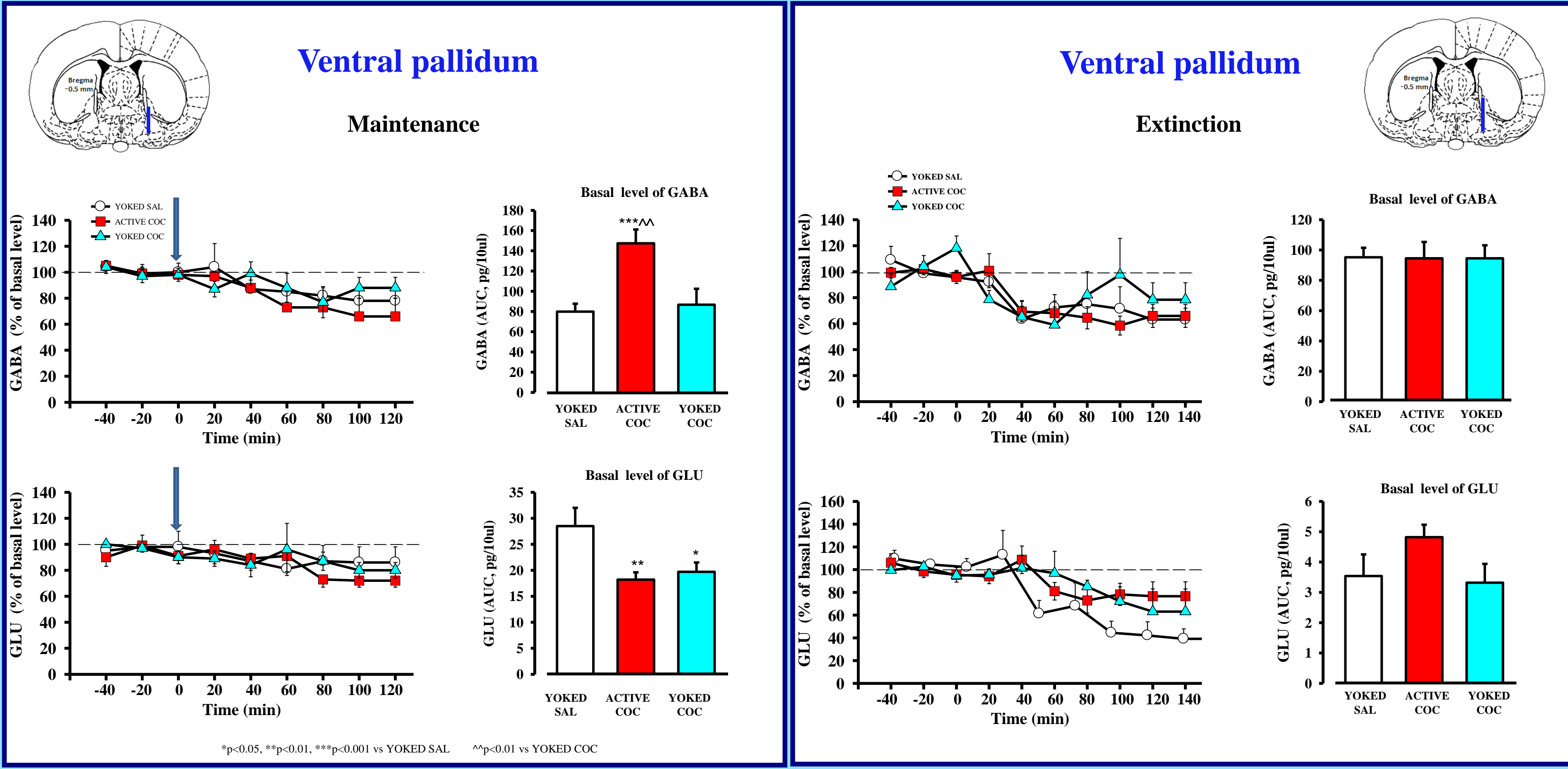
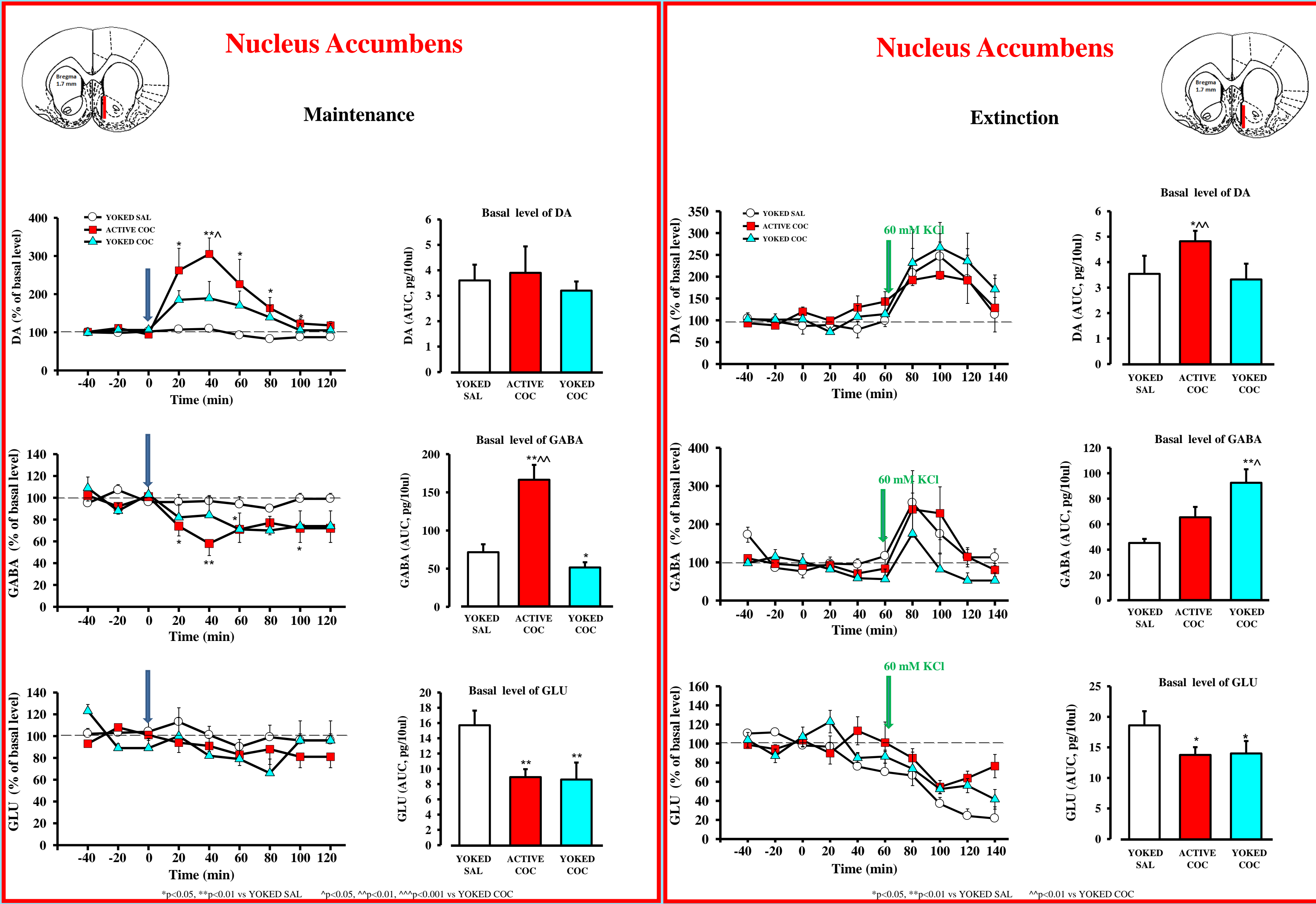
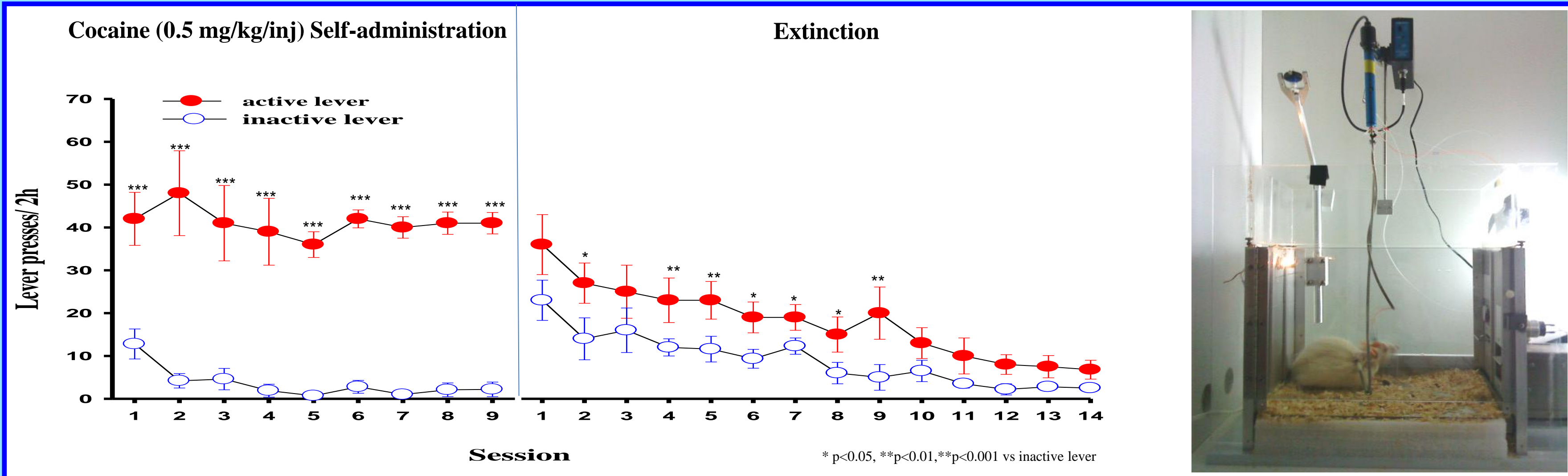
➤Basal DA levels in the NAc in rats actively self-administered cocaine exposed to extinction training were increased.

➤Rats actively and passively administered cocaine during maintenance phase showed decreases in basal level of Glu in the NAc following extinction training.

➤Rats passively exposed to cocaine during maintenance phase had an increase in basal level of GABA in the NAc during withdrawal.

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CONCLUSIONS

❑ Our study demonstrate that increased DA-ergic neurotransmission in the NAc during administration of cocaine (maintenance phase) and decreases in basal levels of Glu in the NAc and VP (maintenance and extinction phases) reflect pharmacological properties of the psychostimulant.

❑ Increased basal GABA levels in the NAc and VP may be linked with motivational aspects of cocaine intake [9].

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