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Supplemental Information

Behavioral Effects of Cocaine Mediated by Nitric Oxide-GAPDH Transcriptional Signaling

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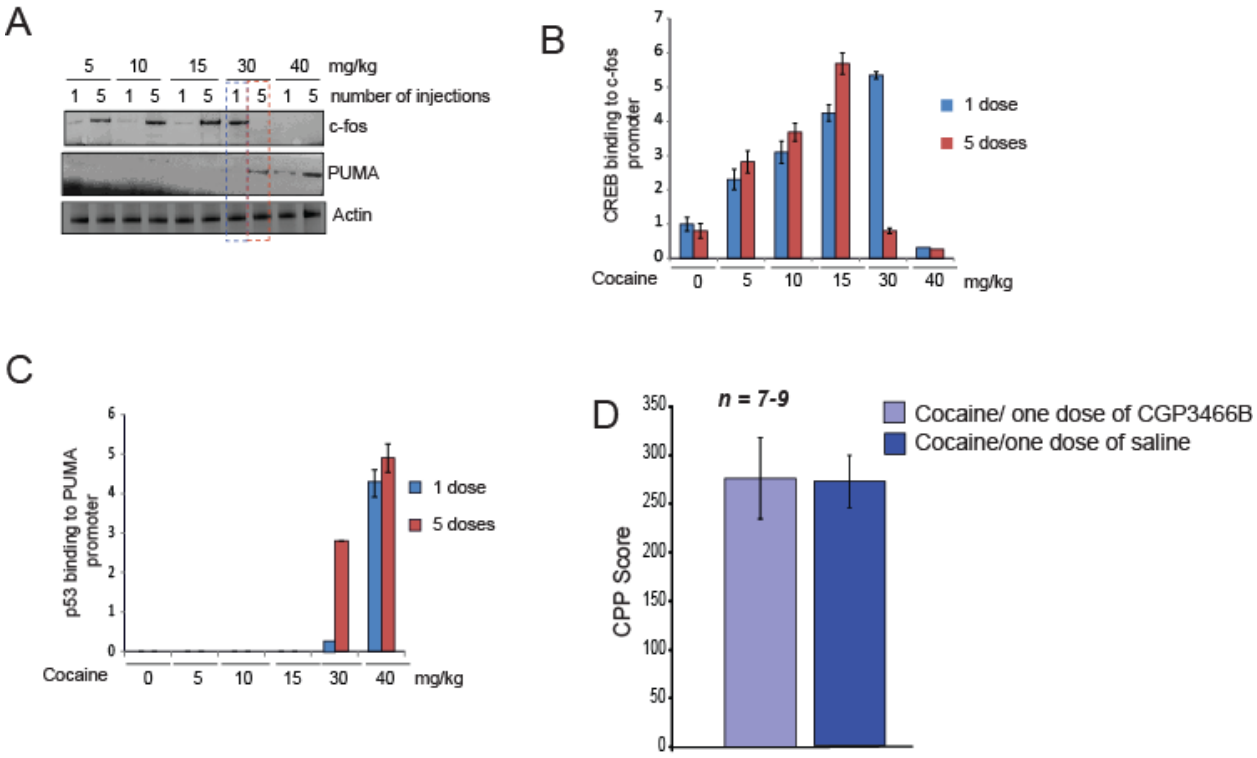
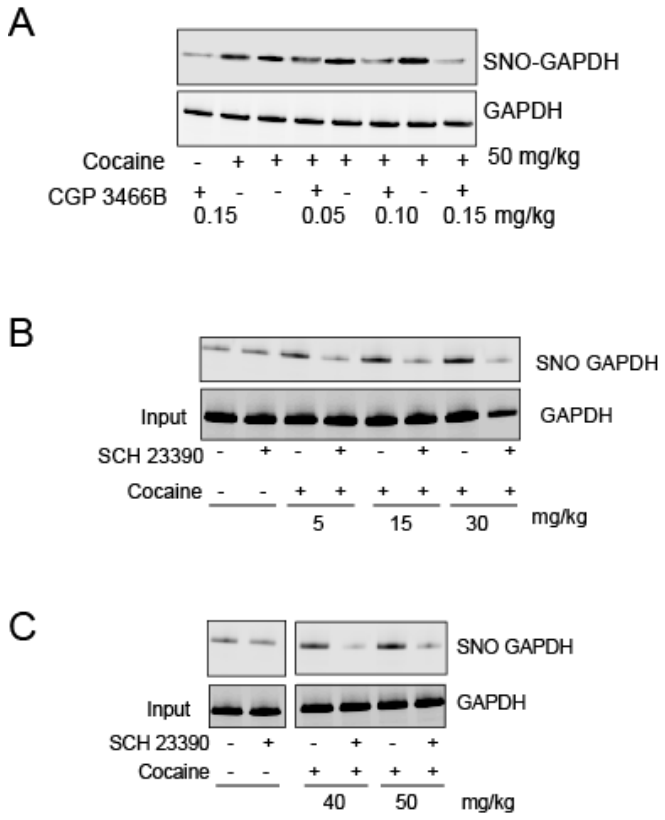
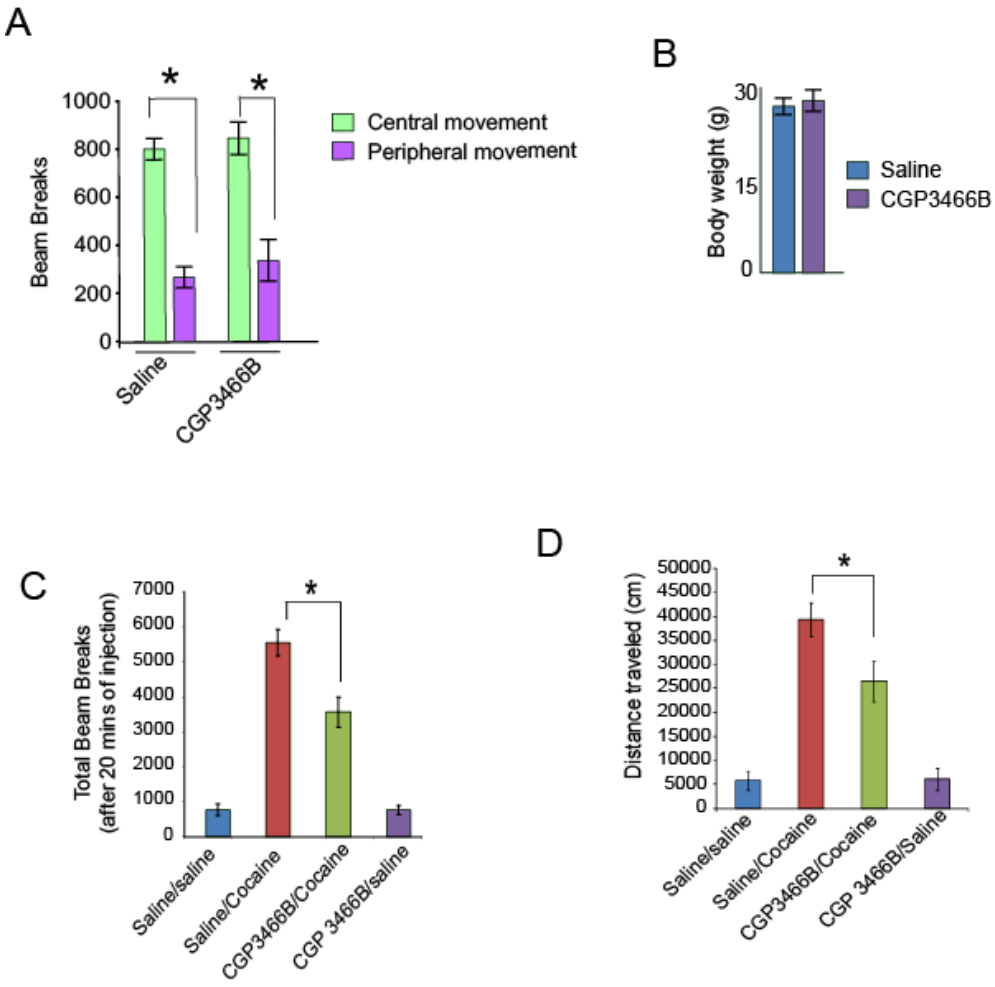
Supplementary Figure 1



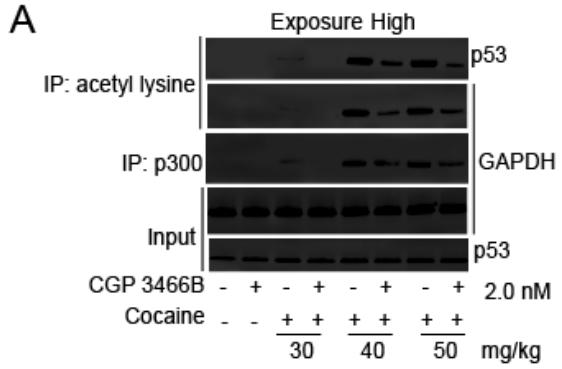
Supplementary Figure 2

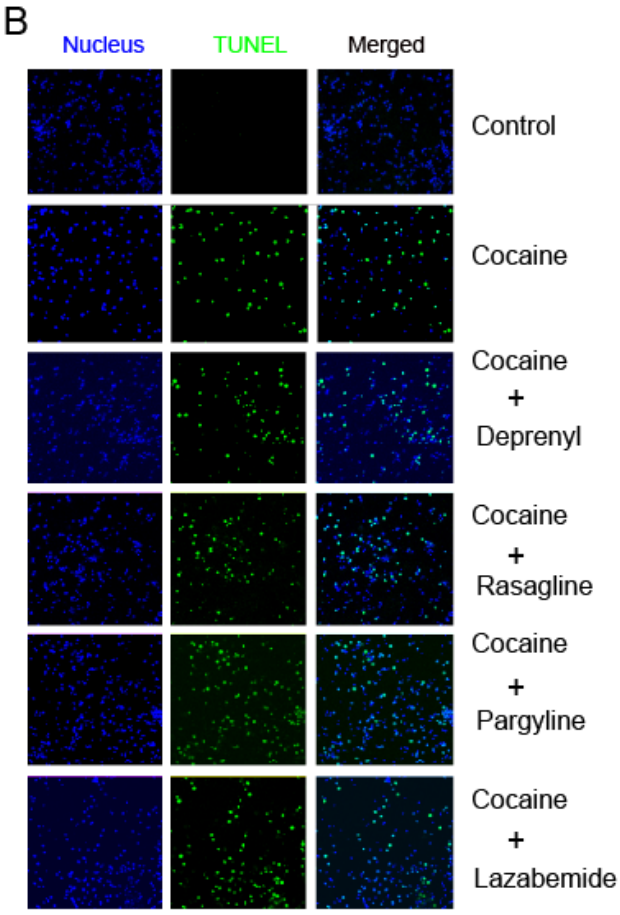


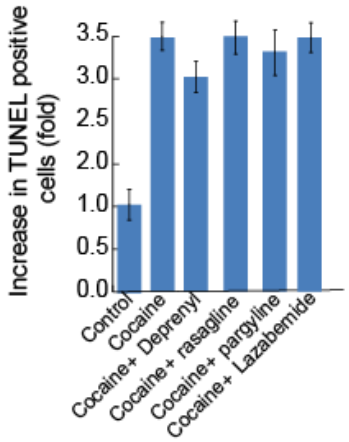
Supplementary Figure 3

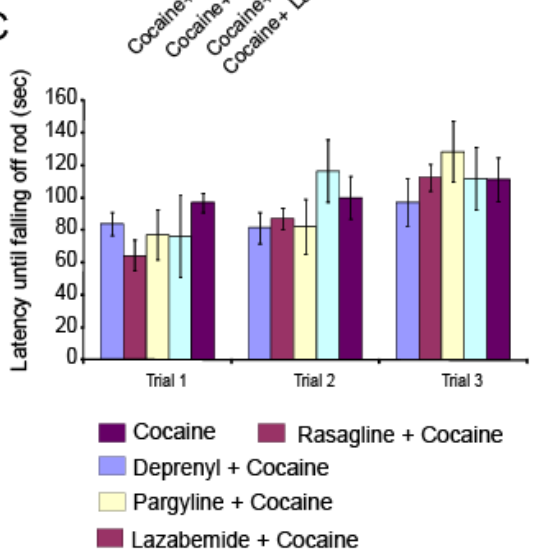


Supplementary Figure 4









Supplemental Figure Legends

Figure S1. Dose dependent responses of CREB and p53 upon treatment with cocaine. Related with Figure 1.

(**A**) Analysis of c-fos and PUMA levels with various doses of cocaine. (**B**) ChIP analysis to measure CREB binding to the c-fos promoter. (**C**) ChIP analysis to measure p53 binding to the PUMA promoter. (**D**) Treatment of cocaine-pretreated mice with one dose of CGP3466B on trial day does not alter the conditioned place preference score, which is calculated as the time spent in cocaine chamber minus time spent in saline/CGP3466B chamber.

Figure S2. Effects of D1 receptor antagonist on GAPDH nitrosylation . Related with Figure 3.

(**A**) Analysis of nitrosylation of GAPDH (SNO-GAPDH) in mice treated with cocaine alone or with cocaine and various doses of CGP3466B. (**B-C**) The D1 receptor antagonist (SCH23390) prevents the stimulation of GAPDH nitrosylation by cocaine, establishing that cocaine's effects reflect dopamine acting at D1 receptors.

Figure S3. Effect of CGP3466B on locomotor sensitization of mice. Related with Figure 3.

(**A**) CGP3466B has no effect on the distribution of central and peripheral movement by mice. Data were derived from open field test. *$p < 0.01$, $n = 12$, one-way ANOVA, mean ± SEM. (**B**) CGP3466B has no effect on body weight of mice. (**C-D**) Total beam breaks (**C**) and total distance traveled (**D**) were measured in mice treated with cocaine alone or with cocaine and CGP3466B treatment. *$p < 0.01$, $n = 12$, one-way ANOVA, mean ± SEM.

Figure S4. Effect of MAO-B inhibitors on cocaine induced cell death of mice. Related with Figure 5.

(**A**) Acetylation of both GAPDH and p53 and interaction between p300-GAPDH were monitored through western blot analysis. High exposure blot is shown here. (**B**) Analysis of cell death in striatum of mice treated with cocaine alone or with cocaine along with deprenyl, rasagiline, lazabemide or pargyline. (**C**) Balance and coordination were measured by rotarod analysis following treatment with cocaine alone or with cocaine along with deprenyl, rasagiline, lazabemide or pargyline.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Chromatin immunoprecipitation (ChIP) assay

This assay was performed as described previously (Enwright et al., 2010; Kumar et al., 2005; Tsankova et al., 2004). In brief, intact cells were treated with 2 mM disuccinimidyl glutarate (Pierce) to crosslink protein complexes, then were treated with formaldehyde to link protein to DNA covalently. Cells were lysed, the nucleoprotein complexes sonicated and the crosslinked DNA-protein complexes enriched by immunoprecipitation with specific antibodies. The retrieved complexes were analyzed by PCR amplification to detect and quantify specific DNA targets. For performing the ChIP assay using striatum section of brain we followed the protocol of Chromatin preparation from tissues for chromatin immunoprecipitation (ChIP), provided by Abcam. For real-time PCR we used Brilliant SYBR green master mix (Stratagene) according to the manufacturer's protocol.

Extraction of nuclear and cytoplasmic proteins

Nuclear and cytoplasmic extracts were prepared using Biovision Nuclear/cytosol extraction kit according to the manufacturer’s instructions.

Co-immunoprecipitation

Striatum tissue were lysed in lysis buffer (50 mM Tris pH 7.4, 150 mM NaCl, 0.1% Chaps, 100 μM deferoxamine and 1 mM EDTA) and homogenized by passing through a 26-gauge needle. Crude lysates were cleared of insoluble debris by centrifugation at 14,000g. IP buffer (50 mM Tris pH 7.4, 150 mM NaCl, 0.1% Chaps, 100 μM deferoxamine, 1 mM EDTA and 0.1 mg/ml BSA) was added to 100 μg of cell lysates to bring samples to a total volume of 1 ml. Anti-Siah and anti-SUV39H1 antibody and 30 μl of protein G agarose were added and incubated on a rotator at 4 °C. The protein G agarose was washed four times with lysis buffer and quenched with 30 μl of SDS sample buffer. Co-immunoprecipitates were resolved by SDS–PAGE and analyzed by western blotting with anti-GAPDH and anti-SUV39H1 antibodies.

Quantitative real-time PCR

This assay was performed as described before (Nott et al., 2008). PCR reactions (25μl) contained 12.5μl of PCR Sybr Green mix (NEB) with 0.3μM primers. At the end of the 35 cycles of

amplification, a dissociation curve was performed in which Sybr Green was measured at 1°C intervals between 50°C and 100°C. Results were normalized using total input DNA and expressed as bound/Input (percentage).

S-nitrosylation biotin switch assay

Primary neurons overexpressed with dopamine transporter (DAT) were treated with cocaine with or without treatment with deprenyl, rasagaline, lazabemide or pargyline for 16 hr. Then cells were lysed and lysates were used for biotin switch assay to detect nitrosylation of GAPDH (SNO-GAPDH). The assay was performed as described (Jaffrey et al., 2001). In brief, cells were lysed, and reduced cysteines blocked with 4 mM methyl methanethionsulphonate (MMTS). Subsequently, S-nitrosylated cysteines were reduced with 1 mM ascorbate and biotinylated with 1 mM Biotin-HPDP (Pierce, Rockford, IL). The biotinylated proteins were pulled down with streptavidin agarose and analyzed by western blotting.

TUNEL assay

Primary neurons were grown on coverslips with a density of 2×10^5 per 12-well. Cells were treated with deprenyl (2nM), rasagaline (10 μM), lazabemide (10μM) or pargyline (10μM) for 16 hr and cell death was measured by TUNEL assays using TUNEL enzyme (Roche) and TUNEL label (Roche) following the manufacturer's instructions (Kickstein et al., 2010)
To analysis cell death induced by neurotoxic dose of cocaine, we used TUNEL apoptosis detection kit (Millipore) using manufacturer's protocol (Lathia et al., 2010). Positive nuclei were counted in 30 fields (×400) for each slide.

Lentiviral injections in mice brain

The stereotaxic injections (David Kopf Instruments, Tujunga, CA; n=3 per group) were performed under pentobarbital anesthesia (45 mg/kg,i.p.) using a syringe (Hamilton, Reno, NV) with a 30 gauge blunt-tip needle (de Almeida et al., 2002). Lentiviral vectors expressing the wild-type GAPDH or mutant C150S GAPDH were injected in the left or the right striatum, respectively. The animal received 4 μl injections of lentiviral vectors in each side at the following coordinates: 1.0 and 0.0 rostral to bregma, 3.0 and 3.3 lateral tomid line, and 5.0 ventral from the skull surface, with the mouth bar set at3.3. The viruses were injected at 0.2 μl/min by means of an automatic injector (Stoelting Co.), and the needle was left in place for 5

min. The skin was closed using a 6-0 Vicryl suture (Ethicon; Johnson & Johnson, Brussels, Belgium). Three animals were killed, and the brains processed for both immunofluorescence and western blot hybridization 10 days after injection to confirm expression of HA-GAPDH.
Mice overexpressing either HA-GAPDH or HA-C150S GAPDH were treated with cocaine with both behavioral and neurotoxic doses as mentioned before. Then mice were sacrificed and striatum was isolated from each animal. Striatum lysates were used to perform various western blots and ChIP analyses.

Statistical analysis. Data are expressed as mean ± standard error (SE). Statistical significance of differences between 2 groups was determined by unpaired two tailed t-test, and between 3 or more groups by a one-way analysis of variance (ANOVA), two-way ANOVA or an ANOVA with repeated measures followed by the Tukey-Kramer post-hoc correction for multiple comparisons. $p<0.05$ was regarded as statistically significant.

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