

## Single exposure to cocaine or ecstasy induces DNA damage in brain and other organs of mice

Tathiana A. Alvarenga<sup>1</sup>, Monica L. Andersen<sup>1</sup>, Daniel A. Ribeiro<sup>2</sup>, Paula Araujo<sup>1</sup>, Camila Hirotsu<sup>1</sup>, José L. Costa<sup>3</sup>, Murilo C. Battisti<sup>1</sup> & Sergio Tufik<sup>1</sup>

Department of Psychobiology, Universidade Federal de São Paulo (UNIFESP), Brazil<sup>1</sup>, Biosciences, Universidade Federal de São Paulo (UNIFESP), Brazil<sup>2</sup> and Instrumental Analysis Laboratory, Criminalistic Institute, Brazil<sup>3</sup>

### ABSTRACT

We evaluated the overall genetic damage induced by different doses of cocaine and MDMA (3,4-Methylenedioxymethamphetamine) in several organs. One hour after intraperitoneal drug administration, mice were euthanized; peripheral blood, liver and brain were collected, and the cellular suspensions were used for the single cell gel (comet) assay. We determined that all doses of cocaine and MDMA tested were able to induce DNA damage in blood cells. Extensive genotoxic damage was induced by cocaine or MDMA at the highest doses used in liver cells. Brain cells were affected by all doses administered. These findings demonstrate that cocaine and MDMA are potent genotoxins.

**Keywords** Acute administration, cocaine, DNA damage, ecstasy, MDMA, single cell gel (comet) assay.

Correspondence to: Monica L. Andersen, Rua Napoleão de Barros, 925, Vila Clementino, SP 04021-002, São Paulo, Brazil. E-mail: mandersen@psicobio.epm.br

Cocaine is one of the most addictive illicit drugs in use today (Howell & Wilcox 2001). Ecstasy (3,4-methylenedioxymethamphetamine, MDMA) consumption is also widespread and increasing in many countries (Leung & Cottler 2008).

A number of studies suggest that the progression from intermittent to regular abuse, the transition from abuse to addiction and the propensity to relapse after many drug-free years result from complex interactions between genetic and environmental factors (Guindalini *et al.* 2008). Romieu *et al.* (2008) highlighted the regulation of gene transcription in neurons by chromatin remodeling, a process in which post-translational modifications of histones are suggested to play a major role. Taken together, these studies demonstrate a close relationship between drugs of abuse and genetic factors.

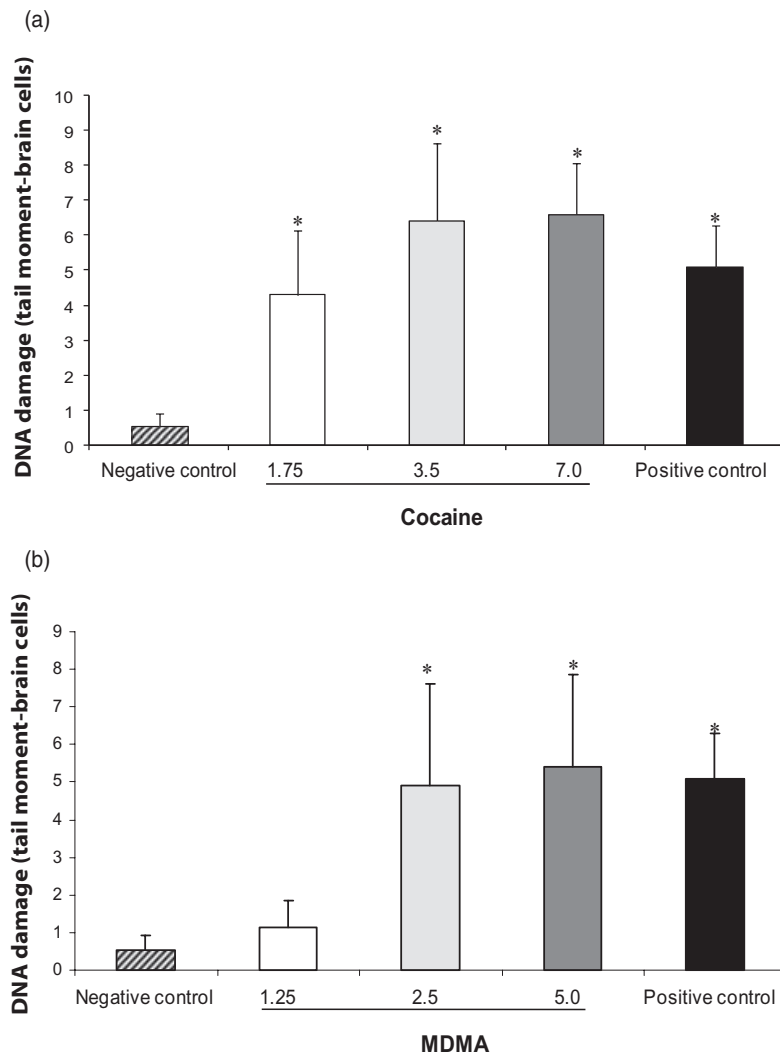
Our group has consistently demonstrated that the single cell gel (comet) assay is a useful tool for detecting DNA breaks in multiple organs and under different paradigms (Ribeiro *et al.* 2007). Thus, we used this assay to examine whether cocaine and MDMA are able to induce genetic damage in multiple organs of mice.

Male C57BL/6J mice were bred and raised in the animal facility of the CEDEME (Centro de Desenvolvimento de Modelos Animais de Medicina e Biologia). The

animals were housed in a colony maintained at 22°C with 12 : 12 hours light–dark cycle and allowed free access to food and water inside standard polypropylene cages. All procedures used in the present study complied with the Guide for the Care and Use of Laboratory Animals.

The mice were randomly distributed into seven independent groups ( $n = 10$ /group). The experimental groups were given cocaine (1.75, 3.5 and 7 mg/kg, Sigma) or MDMA (1.25, 2.5 and 5 mg/kg). The control group was given saline. All animals were euthanized by decapitation 1 hour after drug injection. MDMA used in this experiment were donated to us by the Criminalistic Institute (São Paulo).

Blood was collected in sterile tubes containing liquid ethylenediaminetetraacetic acid (EDTA). An aliquot of blood was removed, and cellular suspensions (~10  $\mu$ l) were used for the single cell gel (comet) assay. In addition, central fragments from the liver and brain were collected and minced in 0.9% NaCl. The supernatant was removed and the cellular suspensions (~10  $\mu$ l) were used for the single cell gel (comet) assay as well. For this purpose, a volume of 5  $\mu$ l of peripheral blood or cellular suspensions from the liver or brain were added to 120  $\mu$ l 0.5% low-melting-point agarose at 37°C, layered into a pre-coated slide with 1.5% regular agarose and were covered with a

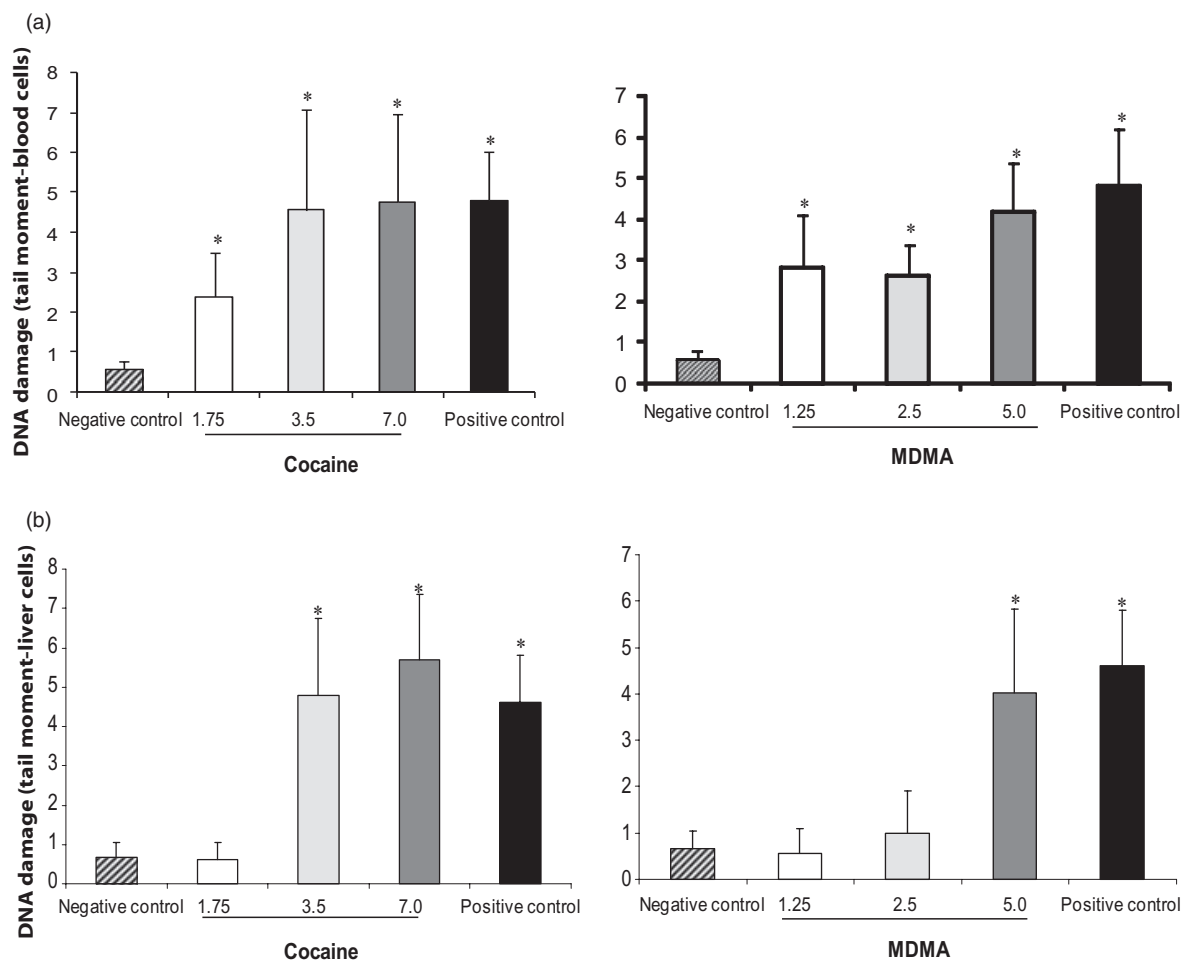


**Figure 1** DNA damage expressed as the mean tail moment in mice brain cells after cocaine (a) and MDMA (3,4-Methylenedioxymethamphetamine) (b) exposure. Values are expressed as mean  $\pm$  standard deviation. \* $P < 0.05$  as compared to negative control

coverslip. Prior to electrophoresis, the slides were left in alkaline buffer (pH > 13) for 20 minutes and then were electrophoresed for another 20 minutes at 0.7 V/cm and 300 mA. After electrophoresis, the slides were neutralized in 0.4 M Tris-HCl (pH 7.5), fixed in absolute ethanol and stored until analysis on a fluorescent microscope at 400 $\times$  magnification. Independent positive controls using cells from peripheral blood were treated in vitro with 10  $\mu$ M H<sub>2</sub>O<sub>2</sub> (hydrogen peroxide) for 10 minutes at 4 $^{\circ}$ C to ensure the reproducibility and sensitivity of assay. An automatized analysis system (Comet Assay II, Perceptive Instruments, Haverhill, Suffolk, UK) was used to determine DNA damage. Tail moment (product of tail DNA/total DNA by the center of gravity) was considered to estimate DNA damage from 50 cells per treatment (Tice *et al.* 2000). Because tail moment data are expressed in arbitrary units, these values were evaluated statistically with the Kruskal–Wallis non-parametric test followed by the post-hoc Dunn's test. Values are expressed as mean  $\pm$  standard deviation. The level of significance was set at  $P < 0.05$ .

Our findings revealed that cocaine induced expressive genotoxic damage in all doses, whereas MDMA induced DNA damage at the highest doses used on brain cells (Fig. 1). Some authors have pointed out that cocaine was able to induce apoptosis as a result of DNA fragmentation in cortical neurons of fetal mice (Nassogne *et al.* 1997). MDMA also induced alterations on brain mitochondria such as increased lipid peroxidation, protein carbonylation and decrease in the expression of the respiratory chain subunits II of reduced nicotinamide adenine dinucleotide dehydrogenase (NDII) and I of cytochrome oxidase (COXI) (Alves *et al.* 2009).

In the blood cells, cocaine all induced DNA damage as compared to negative controls ( $P < 0.05$ ), i.e. specimens not exposed to the chemical agent (Fig. 2a). An increased DNA migration rate was detected in all doses in the MDMA-treated groups. Taken together, cocaine and MDMA can induce genetic damage in peripheral blood cells, possibly as a result of oxidative DNA damage. It is important to stress that the single cell gel (comet) assay does not necessarily predict the mutagenic potential of



**Figure 2** DNA damage expressed as the mean tail moment in mice blood (a) and liver (b) cells after cocaine and MDMA (3,4-Methylenedioxymethamphetamine) exposure. Values are expressed as mean  $\pm$  standard deviation. \* $P < 0.05$  as compared to negative control

the test compound. Therefore, such findings should be interpreted cautiously.

Regarding the liver, our results demonstrated extensive genotoxic damage induced by cocaine at the highest dose used in this study (Fig. 2b). MDMA was able to induce DNA breakage at the highest dose only ( $P < 0.05$ , Fig. 2b). Wang *et al.* (2001) have assumed that cocaine is toxic to multiple organs, and at low dose can induce hepatic damage. MDMA elicits DNA damage, suggesting that nuclei as well, as mitochondria, are target sites of this compound on rat hepatocytes (Nakagawa *et al.* 2009).

In conclusion, the present study indicates that cocaine and MDMA are potent genotoxins in multiple organs of mice.

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#### Authors Contribution

TA, MA and ST were responsible for the conception and design of the manuscript. TA, MA, DR, PA, CH, JLC, MB collected the data. TA, MA and DR did the analysis and interpretation of the data. TA, MA, DR, PA, CH and ST gave the final approval of the article.

#### References

- Alves E, Summavielle T, Alves CJ, Custodio JB, Fernandes E, de Lourdes Bastos M, Tavares MA, Carvalho F (2009) Ecstasy-induced oxidative stress to adolescent rat brain mitochondria in vivo: influence of monoamine oxidase type a. *Addict Biol* 14:185–193.
- Guindalini C, Laranjeira R, Collier D, Messas G, Vallada H, Breen G (2008) Dopamine-beta hydroxylase polymorphism and cocaine addiction. *Behav Brain Funct* 4:1 [Online].

- Howell LL, Wilcox KM (2001) The dopamine transporter and cocaine medication development: drug self-administration in nonhuman primates. *J Pharmacol Exp Ther* 298:1–6.
- Leung KS, Cottler LB (2008) Ecstasy and other club drugs: a review of recent epidemiologic studies. *Curr Opin Psychiatry* 21:234–241.
- Nakagawa Y, Suzuki T, Tayama S, Ishii H, Ogata A (2009) Cytotoxic effects of 3,4-methylenedioxy-N-alkylamphetamines, MDMA and its analogues, on isolated rat hepatocytes. *Arch Toxicol* 83:69–80.
- Nassogne MC, Louahed J, Evrard P, Courtoy PJ (1997) Cocaine induces apoptosis in cortical neurons of fetal mice. *J Neurochem* 68:2442–2450.
- Ribeiro DA, Calvi SA, Picka MM, Persi E, de Carvalho TB, Caetano PK, Nagoshi LR, Lima CR, Machado JM, Salvadori DM (2007) DNA damage and nitric oxide synthesis in experimentally infected Balb/c mice with *Trypanosoma cruzi*. *Exp Parasitol* 116:296–301.
- Romieu P, Host L, Gobaille S, Sandner G, Aunis D, Zwiller J (2008) Histone deacetylase inhibitors decrease cocaine but not sucrose self-administration in rats. *J Neurosci* 28:9342–9348.
- Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC, Sasaki YF (2000) Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. *Environ Mol Mutagen* 35:206–221.
- Wang JF, Ren X, DeAngelis J, Min J, Zhang Y, Hampton TG, Amende I, Morgan JP (2001) Differential patterns of cocaine-induced organ toxicity in murine heart versus liver. *Exp Biol Med (Maywood)* 226:52–60.