M₁ and M₃ muscarinic receptors may play a role in the neurotoxicity of anhydroecgonine methyl ester, a cocaine pyrolysis product

Raphael Caio Tamborelli Garcia¹,²,⁶,⁷†, Livia Mendonça Munhoz Dati¹†, Larissa Helena Torres¹, Mariana Aguilera Alencar da Silva¹, Mariana Sayuri Berto Udo¹, Fernando Maurício Francis Abdalla³, José Luiz da Costa⁴, Renata Gorjão⁵, Solange Castro Afeche³, Mauricio Yonamine¹, Colleen M. Niswender⁶,⁷, P. Jeffrey Conn⁶,⁷, Rosana Camarini⁸, Maria Regina Lopes Sandoval³, Tania Marcourakis¹,*

¹Department of Clinical and Toxicological Analysis, School of Pharmaceutical Sciences, University of São Paulo, Av. Prof. Lineu Prestes, 580, Bl. 13B, 05508-000, São Paulo/SP, Brazil.
²Institute of Environmental, Chemical and Pharmaceutical Sciences, Federal University of São Paulo, Rua São Nicolau, 210, 1º andar, 09913-030, Diadema/SP, Brazil.
³Laboratory of Pharmacology, Butantan Institute, Av. Vital Brasil, 1500, 05503-900, São Paulo/SP, Brazil.
⁴Criminalistic Institute of São Paulo, Rua Moncorvo Filho, 410, 05507-060, São Paulo/SP, Brazil.
⁵Institute of Physical Activity Sciences and Sports, Post-Graduate Program in Human Movement Sciences, Cruzeiro do Sul University, São Paulo, Brazil.
⁶Department of Pharmacology, Vanderbilt University Medical Center.
⁷Vanderbilt Center for Neuroscience Drug Discovery, Vanderbilt University Medical Center, 2201 West End Avenue, 1205 Light Hall, 37232-0697, Nashville/TN, USA.
⁸Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo, Av. Prof. Lineu Prestes, 1524, Prédio 1, 05508-900, São Paulo/SP, Brazil.

Contact email: tmarcour@usp.br
*Corresponding author.
†These authors contributed equally to this work.

Address for correspondence
Faculdade de Ciências Farmacêuticas da Universidade de São Paulo, Av. Prof. Lineu Prestes, 580, Bl. 13B, CEP 05508-000, São Paulo/SP, Brazil.
Tel/Fax: +55-11-3091-1504
Email: tmarcour@usp.br (T.Marcourakis)
Saturation binding of $[^3\text{H}]$NMS for each mAChR subtype expressed in CHO cells.

**Supplementary Figure S1.** Saturation binding curves (data represent the mean of three independent experiment performed in triplicate) of $[^3\text{H}]$NMS for each mAChR subtype expressed in CHO-K1 cells. $B_{\text{max}}$ and $K_d$ values were determined for radioligand competition binding using *GraphPad Prism 5*. A. Rat M_1 ($B_{\text{max}}$= 1087±165 fmol/mg of protein; $K_d$=0.085 nM); B. Rat M_2 ($B_{\text{max}}$= 1470±53 fmol/mg of protein; $K_d$=0.159 nM); C. Rat M_3 ($B_{\text{max}}$= 1132±33 fmol/mg of protein; $K_d$=0.063 nM); D. Rat M_4 ($B_{\text{max}}$= 1835±127 fmol/mg of protein; $K_d$=0.038 nM) and E. Rat M_5 ($B_{\text{max}}$= 1564±27 fmol/mg of protein; $K_d$=0.224 nM. Data presented as mean±SEM.