Sex Dependent Long-Term Effects Of Adolescent Exposure To THC And/Or MDMA On Glial Reactivity And Serotoninergic And Cannabinoid Systems In Rats: An Immunohistochemical Study

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Young people typically consume ecstasy as a recreational drug during the week-ends and generally in combination with cannabis. However, few animal studies have analysed the interactions between the two drugs. In the present study we tried to mimic human consumption patterns and investigated the long-term effects of increasing doses of delta-9-tetrahydrocannabinol [THC; 2.5, 5 and 10 mg/kg in ethanol:cremophor:saline (1:1:18); i.p.] from postnatal day (pnd) 28 to 45 and/or ecstasy [3,4-methylenedioxymethamphetamine, MDMA; two daily doses of 10 mg/kg every 5 days in saline (0.9%); s.c.], from pnd 30 to 45 in male and female Wistar rats (Table 1).

Table 1. A total of 8 experimental groups (n=6).

THC	MDMA	Males	Females
Vehicle	Saline	Vh-Sal	
	MDMA	Vh-MDMA	
THC	Saline	THC-Sal	
	MDMA	THC-MDMA	

We have previously reported the behavioural and neuroendocrine effects of these treatments (Llorente-Berzal A et al. ICRS Congress, 2012), and here we show the effects on proteins involved in glial reactivity [glial fibrillary acidic protein (GFAP) and ionized calcium-binding adaptor molecule-1 (Iba-1)] as well as on the serotonin transporter (SERT) and the CB1 cannabinoid receptor (CB1R) by immunohistochemical analyses in the hippocampus or parietal cortex of adult rats (pnd 89-92) (Table 2).

Table 2: Protocols used for immunohistochemical analyses

Antibody	Reference / Antibody concentration
CB1 / GFAP	López-Gallardo M et al, Neuroscience 204:90, 2012
Iba-1	Diz-Chaves Y et al, J Neuroinflammation 9:71, 2012

SERT	Primary antibody concentration: 1:500

Post-hoc comparisons (Tukey test) performed after ANOVA showed that THC, induced a significant increase of hippocampal GFAP immunoreactivity in male rats treated with saline (17.38 ± 0.73 vs 21.94 ± 0.36; $p<0.001$) or MDMA (17.38 ± .73 vs 21.21 ± 0.60; $p<0.01$). However, in females, only THC-Sal animals showed a significant increase of GFAP expression (18.13 ± 0.44 vs 22.87 ± 0.77; $p<0.001$).

A sexual dimorphism was found among control animals when analysing Iba-1 immunoreactivity within the hippocampus, with females exhibiting higher levels than males ($p<0.001$). THC and MDMA either separately or in combination induced a significant increase of Iba-1 expression in males ($p<0.001$, $p<0.05$, $p<0.001$). However, in females both drugs, when administered separately, induced a decrease in this parameter ($p<0.05$) but the combined treatment (THC+MDMA) resulted in a recovery to control levels ($p<0.01$ versus THC-Sal and Vh-MDMA) (See Table 3).

Table 3. Effects of THC, MDMA or the double treatment on the percentage of reactive microglia cells in the hippocampus

	Vh-Sal	THC-Sal	Vh-MDMA	THC-MDMA
Males	38.67 ± 4.34	75.27 ± 2.91 *****	68.38 ± 3.40 *	77.09 ± 3.16 *****
Females	76.06 ± 2.76 *****	58.15 ± 5.72 #	57.99 ± 2.96 #	76.68 ± 1.45 % +

Data is presented as mean ± SEM. Tukey post-hoc comparisons ($p<0.05$), * vs males Vh-Sal; # vs females Vh-Sal; + vs females THC-Sal; % vs females Vh-MDMA

Regarding the serotoninergic system (Table 4), control females showed a higher number of SERT positive fibers in the parietal cortex than their male counterparts ($p<0.01$). MDMA per se induced a significant decrease of SERT expression in males ($p<0.01$) and females ($p<0.001$) whereas THC induced a significant increase of SERT immunoreactivity exclusively in males ($p<0.001$). In both sexes, the combination of THC+MDMA induced a normalization of this parameter to control values (males $p<0.001$ vs. THC-Sal and Vh-MDMA; females $p<0.001$ vs. THC-Sal and $p<0.05$ vs. Vh-MDMA).

Table 4. Effects of THC, MDMA or the double treatment on the number of serotonine transporter (SERT) in the parietal cortex

	Vh-Sal	THC-Sal	Vh-MDMA	THC-MDMA
Males	61.80 ± 1.17	92.11 ± 2.51 *****	45.83 ± 2.67 *****	65.25 ± 3.05 & $
Females	80.73 ± 2.75 *****	92.99 ± 3.83	55.63 ± 1.67 #	76.92 ± 5.72 % +

Data is presented as mean ± SEM. Tukey post-hoc comparisons ($p<0.05$), * vs males Vh-Sal; $ vs males THC-Sal; & vs males Vh-MDMA; # vs females Vh-Sal; + vs females THC-Sal; % vs females Vh-MDMA

CB1R immunoreactivity was studied in hilus, CA1 and CA3 areas of the hippocampus. A significant overall effect of TCH (a decrease of CB1R) was found in females in the three areas analysed (CA1, $F_{1,16}$=5.87; $p<0.05$; CA3, $F_{1,16}$=6.88; $p<0.05$; Dentate gyrus, $F_{1,16}$=6.03; $p<0.05$). In CA3, post hoc comparisons showed that MDMA intensified the THC-induced decrease of CB1R (137.62 ± 4.37 vs 123.76 ± 2.60; $p<0.05$), and the same trend was observed in CA1 and hilus. No significant differences were found among male groups.

The present results indicate that adolescent exposure to THC and/or MDMA induced long-term, sex-dependent alterations in glial reactivity and serotoninergic and endocannabinoid systems, and reveal interesting interactions between the two drugs.

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