336.3 **DIRECT INTRACEREBRAL ADMINISTRATION OF MDA AND MDMA DOES NOT PRODUCE SEROTONIN NEUROTOXICITY.** M.E. Molliver, E. O'Hearn*, G. Battaglia, and E.B. De Souza. (SPON: B. Kosofsky). Dept. of Neuroscience, Johns Hopkins Univ. School of Medicine; and ARC, National Institute on Drug Abuse, Baltimore, MD 21205

Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA, Ecstasy) are psychotropic substituted phenethylamines which are selectively neurotoxic to serotonin (5HT) axon terminals when administered systemically to rats (cf, O'Hearn et al; Yeh et al, this vol). In this study we microinjected MDA and MDMA directly into the cerebral cortex to determine the effects of these amphetamines on neuronal processes and to control local drug concentration. Single stereotaxic microinjections of (±)MDA (n=12) or (±)MDMA (n=13) [3, 10, or 12 ug in 0.3-0.5 ul] were made into right frontal cortex of male rats through 70 um tip glass pipettes. Vehicle control injections of 0.9% NaCl were placed in contralateral cortex (n=16). Microinjections included a pulse of rhodamine labeled latex microspheres to identify the injection site location. To suppress MDA or MDMA oxidation, ascorbic acid (0.1 mg/ml) was added to the drug vehicle in half of the rats (n=12). After survival times of 3 days to 3 weeks, 5-HT and catecholamine (CA) axons were visualized by immunocytochemistry in brain sections incubated with antibodies to 5-HT [courtesy of H. Lidov] and to tyrosine hydroxylase (TH) [courtesy of T.H. Joh].

5-HT and CA axon density surrounding MDA and MDMA injection sites could not be distinguished from that at NaCl control sites. Many fine 5-HT and TH axons could be traced directly up to and through the injection sites. This absence of a cytotoxic effect was found at all survival times and injection dosages. Addition of ascorbic acid to the drug vehicle had no effect. Nissl-stained sections revealed a small zone of gliosis at the injection sites. The cortex around the center of the injection sites (100-200 um zone) in MDA, MDMA, and NaCl injections exhibited a few 5-HT and TH axons with increased diameter and immunoreactivity and, rarely, a very subtle decrease in 5-HT and TH fiber density; more than 300 um away, the cortex was completely normal. The latter findings in MDA, MDMA, and NaCl injected rats are consistent with nonspecific effects induced by the trauma of microinjections. In conclusion, single large doses of MDA or MDMA, administered directly into brain tissue, have no effect on the density, morphology, or staining intensity of 5-HT or CA axon terminals. This indicates that the dramatic neurotoxicity demonstrated after subcutaneously administered MDA and MDMA depends upon systemic administration of these substances. These data suggest strongly that the neurotoxin is a peripheral metabolite of MDA and MDMA, a feature that may prove crucial to elucidate the mechanism of action and to prevent the neurotoxicity of MDA and MDMA.

[Support: NIH grants # NS15199, NS21011, & NS19920]

336.4 **MDA AND MDMA (ECSTASY) INTERACTIONS WITH BRAIN SEROTONIN RECEPTORS AND UPTAKE SITES: IN VITRO STUDIES** George Battaglia, Michael J. Kuhar and Errol B. De Souza. Neuroscience Branch, ARC, National Institute on Drug Abuse, Baltimore, MD 21224.

MDA and MDMA are methylenedioxy derivatives of amphetamine and methamphetamine, respectively. These compounds exhibit psychotomimetic and stimulant properties and have been increasingly encountered in illicit drug traffic. Recent data suggest that MDA and MDMA may be toxic to brain serotonin neurons. Although the serotonergic system has been implicated in some of the physiologic and behavioral responses to MDA and MDMA, the precise interactions of these compounds with brain serotonin recognition sites have yet to be elucidated. These studies were designed to delineate an in vitro pharmacological profile of MDA and MDMA on pre- and post-synaptic serotonin recognition sites using radioligand binding techniques.

$5HT_{1A}$, $5HT_2$ and 5HT uptake sites were selectively labelled using ^3H WB4101 (+30 nM prazosin), ^3H-Ketanserin and ^3H-Paroxetine, respectively, in appropriate brain regions. At 5HT uptake sites in frontoparietal cortex, the affinities (K_i's) of (+)MDA (2.1 uM) and (+)MDMA (1.1 uM) were comparable with the S(+) isomer of each being 4- and 8-fold more potent, respectively, than the R(-) isomer. These compounds were 11-13 fold more potent at this site than their respective parent compounds, (+)amphetamine (22.7 uM) and (+)methamphetamine (15 uM). Additionally, S(+)MDMA (0.64 uM) was only 5-fold less potent than serotonin (0.14 uM). With repect to hippocampal $5HT_{1A}$ sites, the affinities of MDA (12 uM) and MDMA (7 uM) were significantly less than those observed at the uptake site. Furthemore, the stereospecificity at $5HT_{1A}$ receptors was the opposite of that at the uptake sites with the R(-) isomer of both MDA and MDMA being more potent than the S(+) isomer. At cortical $5HT_2$ receptors (+)MDA (5.4 uM) and (+)MDMA (4.9 uM) were equipotent and exhibited a sterospecificity similar to that observed at $5HT_{1A}$ receptors.

Of interest, MDA and MDMA appear to have agonistic properties at $5HT_2$ receptors in that they can discriminate high and low affinity components of binding and exhibit GTP-sensitivity.

In conclusion, these data suggest that, in vivo, both MDA and MDMA may exert a potent and preferential effect on the 5HT uptake system associated with serotonin axons and presynaptic terminals. Also, the findings of different potencies and of reverse stereospecificity at uptake sites vs. receptors may help elucidate the primary site(s) responsible for the psychotomimetic effects in man. For example, the S(+) isomer of MDMA and the R(-) isomer of MDA are reported to be the more potent psychotomimetic in man, suggesting that the effects of MDMA may primarily involve 5HT uptake sites while those of MDA may be mediated by its interaction with $5HT_2$ receptors.

336.5 **EFFECTS OF MDA AND MDMA (ECSTASY) ON BRAIN MONOAMINERGIC SYSTEMS: IN VIVO STUDIES.** S.Y. Yeh*, G. Battaglia, E. O'Hearn*, M.E. Molliver, M.J. Kuhar and E.B. De Souza (SPON: A. Goldberg). Neuroscience Branch, Addiction Research Center, National Institute on Drug Abuse, and Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21224

(+)3,4 methylenedioxyamphetamine (MDA) and (+)3,4 methylenedioxymethamphetamine (MDMA) are compounds which possess psychotomimetic and stimulant properties. These so called "designer drugs" have found increased popularity among recreational drug users. Recent studies suggest that MDA may exert potent neurotoxic effects on brain serotonergic systems (Science 229:986, 1985). Our studies were designed to investigate the effects of in vivo administration of MDA and MDMA on the regional concentration of various brain monoamines and their metabolites as well as on the pre- and post-synaptic binding sites (i.e. uptake sites and receptors).

Male, Sprague Dawley rats were injected s.c. with either saline (1 ml/Kg), MDA or MDMA (20 mg/Kg) twice a day for four consecutive days. Two weeks following the last injection the animals were killed and various brain regions dissected. Brain concentrations of monoamines and their metabolites were measured using RP-HPLC. Radioligand binding techniques were employed to monitor changes in the affinity and/or density of uptake sites and receptors.

MDA caused dramatic decreases in 5HT (65, 26 and 33%) and 5HIAA (58, 64 and 40%) content in frontoparietal cortex (FPC), hippocampus (HIP), and hypothalamus (HYP), respectively. MDMA produced similar but less dramatic decreases in 5HT (42, 15, and 18%) and 5HIAA (46, 55 and 26%) in FPC, HIP and HYP, respectively. These compounds caused smaller changes in striatal 5HT content (10-20%), while the decreases in striatal 5HIAA levels elicited by both MDA (33%) and MDMA (36%) were comparable to that in other regions. In parallel with the observed decreases 5HT and 5HIAA content in FPC, significant and quantitatively similar decreases in the B_{max} of ^3H-Paroxetine-labeled 5HT uptake sites were elicited by both MDA(69%) and MDMA(62%). In contrast, neither MDA nor MDMA elicited any changes in the levels of NE, DA, DOPAC or HVA in any of the brain regions examined. Also, there was no difference in the number of ^3H-Mazindol-labeled NE uptake sites in FPC following either MDA or MDMA administration. Monoamine receptor adaptations to the indirect or direct effects of these compounds are presently being investigated.

In summary, our finding of selective and profound decreases in presynaptic serotonin markers following chronic administration of MDA and MDMA support our immunocytochemical studies which indicate that these changes represent the selective destruction of serotonin terminals.

336.6 NEURONAL RECEPTORS OF ALCOHOL-PREFERRING (P) AND NON-PREFERRING (NP) RATS. D. T. Wong, L. Lumeng*, P. G. Threlkeld*, L. R. Reid* and T. K. Li*. Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46285, and Indiana University School of Medicine, Indianapolis, IN 46223.

Neurochemical studies show that P rats have lower levels of serotonin (5HT) in cerebral cortex and hippocampus and higher levels of norepinephrine in cerebral cortex in comparison with NP rats (Murphy et al, Pharmacol. Biochem. Behav. 16:145, 1982). In the present studies, by means of radioreceptor assays, we found a higher binding of ^3H-5HT to $5HT_1$ receptors in membranes isolated from frontal cortex and hippocampus of 6-month-old P rats with significant increases in receptor numbers (Bmax) of 47% and 90%, respectively, but without change in affinity (Kd). No consistent difference was seen between P and NP rats in Kd and Bmax values for ^3H-ketanserin binding to $5HT_2$ receptors. Similarly, no differences in ^3H-WB4101, ^3H-clonidine and ^3H-dihydroalprenolol binding to α_1, α_2 and β-adrenergic receptors in cortical membranes was observed between these rats. These preliminary findings have been replicated at least once and are consistent with the involvement of the serotonergic system in alochol preference.