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5-Hydroxyindoleacetic acid in cerebrospinal fluid reflects serotonergi damage induced by 3,4-methylenedioxymethamphetamine in CNS of non-human primates

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This study examined whether 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid (CSF) could be used to detect seroto ergic damage induced by (±)-3,4-methylenedioxymethamphetamine (MDMA) in the central nervous system (CNS) of non-hum primates. Monkeys were administered toxic doses of MDMA; two weeks later, the animals were lightly anesthetized with ether ar CSF was obtained by means of cervical puncture. Later that same day, the animals were killed for direct determination of CNS ser tonin and 5-HIAA concentrations. Monkeys with 73–94% depletions of serotonin and 5-HIAA in brain and 42–45% depletions serotonin and 5-HIAA in the spinal cord had a 60 ± 7% reduction of 5-HIAA in CSF, without any change in homovanillic acid (HV or 3-methoxy-4-hydroxyphenethyleneglycol (MHPG). These findings indicate that CSF 5-HIAA can be employed to detect centr serotonergic damage produced by MDMA in non-human primates, and suggest that CSF 5-HIAA may be useful for detectir MDMA-induced neuronal damage in humans.

There is now substantial evidence that (±)-3,4-methylenedioxymethamphetamine (MDMA), a popular recreational drug[1], damages central serotonergic nerve fibers in a variety of experimental animals including rats[3,7,17,19,21,26,30], guinea pigs[7], mice[31] and monkeys[22,23,32]. Animals given MDMA show long-term reductions in the concentration of brain serotonin[3,7,23,26,30], the number of serotonin uptake sites[3,7], the activity of tryptophan hydroxylase[30], and the level of 5-hydroxyindoleacetic acid[7,17,26,30]. Anatomical studies indicate that MDMA produces these neurochemical deficits by damaging serotonergic nerve fibers[7,19,23,32]. Whether humans who use MDMA sustain serotonergic damage is not yet known. However, since the toxic dose of MDMA in the monkey (5 mg/kg) is only 2–3 times higher than that typically taken by humans (1.7–2.7 mg/kg)[24,27], this possibility needs to be carefully explored. Unfor tunately, there is currently no direct way of evaluat ing living humans for central serotonergic damage For this reason, the present study was undertaken t determine if 5-HIAA in CSF might be used to detec serotonergic damage induced by MDMA in the cen tral nervous system (CNS) of non-human primates.

Six 3-year-old female squirrel monkeys (*Saimir sciureus*) weighing 500–700 g were used. Animals were housed individually in standard primate steel cages (1 m^3) with free access to food (Purina monkey chow) and water. MDMA, dissolved in saline as the hydrochloride salt, was administered subcutaneously at a dose of 5 mg/kg twice a day (09.00 and 17.00 h) for 4 consecutive days. Three monkeys received MDMA; 3 were given saline. The aforementioned regimen of MDMA was employed because it is known

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to damage a large (80–90%) fraction of serotonergic nerve fibers in the monkey brain[23,24,32]. Two weeks after drug treatment, CSF was collected from each monkey between 11.00 and 12.00 h by means of cervical puncture as follows. Monkeys were lightly anesthetized with ether, the nape of the neck was shaved and cleansed with betadine. The animal was then held suspended by the shoulders, with the neck in full flexion. While holding the animal in this position, a 25-gauge needle 5/8" in length attached to a 1 ml syringe was slowly inserted along the midline into the subarachnoid space between the first and second cervical vertebrae, and 300 μl of CSF were slowly aspirated. CSF was then transferred to a 1 ml screw-cap polypropylene tube and immediately stored frozen in liquid nitrogen until assay. Shortly after CSF had been collected, monkeys were killed, brains were removed and concentrations of monoamines and their metabolites were measured by means of high-performance liquid chromatography coupled with electrochemical detection (HPLC-EC) according to the method of Kilpatrick and colleagues[13].

5-HIAA concentration in cervical CSF of monkeys given MDMA two weeks previously was reduced by 60% (Table IA). Serotonin in the caudate nucleus of these same animals was decreased by 90% (Table IB). Concentrations of HVA and MHPG in CSF of MDMA-treated monkeys were normal. Normal HVA and MHPG concentrations in CSF were associated with normal levels of dopamine and norepinephrine in the caudate nucleus and hippocampus, respectively (Table IB). MDMA-treated monkeys also showed a marked depletion of serotonin in the hippocampus (−73%), hypothalamus (−82%) and thalamus (−86%) (Table II). The most severely affected brain region was the somatosensory cortex where serotonin was reduced by 94%. MDMA also reduced the concentration of serotonin in the cervical spinal cord, but to a lesser extent (−44%) (Table II). Measurements of 5-HIAA showed that this serotonergic marker was also lowered by MDMA. Regional 5-HIAA depletions paralleled those of serotonin in all CNS regions (Table II).

These results demonstrate that prolonged depletions of serotonin and 5-HIAA induced by MDMA in the CNS of the squirrel monkey are associated with a substantial decrement of 5-HIAA in the CSF of this experimental animal. Since long-term depletions of serotonin and 5-HIAA are good indicators of serotonergic damage[7,19,23], the present findings suggest that CSF 5-HIAA can be used to detect serotonergic damage in living primates. The significance of this observation is twofold. First, it provides a way for probing for serotonergic damage in monkeys while they are alive, something that will be of particular value when expensive non-human primates need to be studied repeatedly over time. Second, and perhaps more importantly, the present findings provide a basis for evaluating MDMA-exposed humans for possible serotonergic damage. It should be emphasized, however, that in the monkey CSF was sampled from the cervical subarachnoid space, whereas in humans, CSF is generally obtained from the lumbar region. Hence, the question arises as to whether lumbar CSF 5-HIAA also reflects serotonergic damage induced by MDMA. To address this question, attempts were made to collect CSF from the lumbar subarachnoid space of the squirrel monkey. However, in keeping with the experience of others (Coe, personal communication) these efforts were unsuccessful. For such purposes, a larger primate species may be more suitable.

Since CSF 5-HIAA has been reported to be influenced by a variety of factors including age[6], diet[8,9], volume of CSF withdrawn[4,11,12], body height[28,33], mo-

TABLE I

Relationship between 5-HIAA in CSF and serotonin in brain of control and MDMA-treated monkeys

A. Selective reduction in 5-HIAA in the CSF of monkeys administered MDMA 2 weeks previously. Values represent the mean ± S.E.M. (expressed in μg/ml of CSF). $^*P < 0.05$, two-tailed Student's *t*-test.

Treatment	*n*	*5-HIAA*	*HVA*	*MHPG*
Saline	3	101 ± 7	255 ± 44	30 ± 4
MDMA	3	41 ± 7*	264 ± 20	40 ± 11

B. Selective reduction in serotonin in the caudate nucleus of monkeys administered MDMA 2 weeks previously (same animals as in Table IA). Values represent the mean ± S.E.M. (expressed in μg/ml of CSF). $^*P < 0.05$, two-tailed Student's *t*-test.

Treatment	*n*	*Serotonin*	*Dopamine*	*Norepinephrine*
Saline	3	0.218 ± 0.023	11.6 ± 0.9	2.59 ± 0.18
MDMA	3	0.021 ± 0.005*	9.7 ± 0.2	2.84 ± 0.91

TABLE II

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TABLE II

Regional brain concentrations of serotonin and 5-HIAA in monkeys given MDMA 2 weeks previously. Values represent the mean S.E.M. (expressed in μg/g of tissue)

	Hippocampus	*Hypothalamus*	*Cortex*	*Caudate*	*Thalamus*	*Cervical cord*
Serotonin						
Control	0.11 ± 0.01	0.77 ± 0.07	0.081 ± 0.013	0.22 ± 0.02	0.61 ± 0.17	0.30 ± 0.02
MDMA	0.03 ± 0.01*	0.14 ± 0.05*	0.005 ± 0.001*	0.02 ± 0.01*	0.09 ± 0.02*	0.17 ± 0.03*
5-HIAA						
Control	0.31 ± 0.02	0.80 ± 0.04	0.130 ± 0.020	0.42 ± 0.03	1.41 ± 0.05	0.24 ± 0.01
MDMA	0.08 ± 0.01*	0.20 ± 0.02*	0.014 ± 0.005*	0.07 ± 0.02*	0.35 ± 0.07*	0.14 ± 0.02*

*$P < 0.05$, two-tailed Student's *t*-test.

tor activity[8,20], and, in humans, certain neuropsychiatric syndromes[2,5,14,15], it is important to consider if any of these factors influenced the results of this study. Age, diet or amount of CSF withdrawn are unlikely to have altered the present results since control and experimental monkeys were of the same age, were fed the same diet and had an identical volume of CSF removed. Motor activity, while not formally measured in this study, is unlikely to have influenced the findings because all monkeys were active and exploring their cages for several hours prior to cervical puncture. Body height is not a critical variable in the present context because CSF was collected from the high cervical rather than the low lumbar region, thus minimizing the influence of body height on the ventriculo-spinal gradient of 5-HIAA in CSF[4,11]. Moreover, all monkeys were comparable in weight and size. Given these considerations, it seems reasonable to conclude that the lower concentration of 5-HIAA found in the CSF of the MDMA-lesioned monkey is not related to any of the aforementioned factors, but instead reflects serotonergic damage resulting from MDMA administration[7,23,32].

The relative contribution of brain and spinal cord to 5-HIAA in spinal CSF has been a matter of considerable controversy[10]. The results of this study throw some light on this question for they suggest that 5-HIAA in cervical CSF of the squirrel monkey originates from both brain and spinal cord. This can be surmised from the fact that a 60% reduction of 5-HIAA in cervical CSF was associated with only a 42% depletion of 5-HIAA in the cervical spinal cord, but an 80% depletion of 5-HIAA in brain (computed by averaging the depletion of 5-HIAA in the various brain regions shown in Table II). If 5-HIAA in cervical CSF were originating only from the spinal cord, the depletion of 5-HIAA in cervical CSF would n be expected to exceed that in the cord. This is b cause 5-HIAA concentrations in CSF generally p allel those in adjacent CNS areas[16,25,28]. The mag tude of 5-HIAA depletion in cervical CSF of t monkey is noteworthy in one other respect. Wh the depletion seems to overestimate the extent serotonergic damage (as indexed by serotonin and HIAA depletion) in the cervical cord, it substantia underestimates serotonergic damage in regions the brain such as the somatosensory cortex whe serotonin is depleted by 94% (Table II). This tende cy of CSF 5-HIAA to substantially underestima serotonergic damage in brain will be important bear in mind when attempts are made to detect ser tonergic neurotoxicity in MDMA-exposed huma by means of CSF 5-HIAA analysis.

To the best of our knowledge, the depletion serotonergic markers produced by MDMA in t cervical spinal cord of the monkey provides one the first indications that MDMA is toxic to seroto ergic fibers not arising from nerve cells in the dors raphe nucleus. Until now, evidence has suggest that the toxic effect of MDMA may be restricted this serotonergic cell group[19,23]. The present findi that serotonergic fibers in the spinal cord are dar aged by MDMA argues against this notion since ser tonergic projections to the spinal cord do not ori nate from the dorsal raphe nucleus[29]. A toxic effe on serotonergic fibers in the spinal cord has also bee recently noted by Nencini et al.[18] working wi MDMA in the rat.

In summary, the present results demonstrate th 5-HIAA in CSF can be used to detect serotoner damage induced by MDMA in the CNS of non-h man primates. Whether 5-HIAA in lumbar CSF

humans can be used for similar purposes is as yet unclear. However, given the large number of individuals who continue to self-administer MDMA for recreational purposes, this needs to be investigated. Such studies could be of value in defining the long-term effects of MDMA on serotonergic neurons in the human CNS.

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1 American Medical News, DEA to outlaw hallucinogenic 'Ecstasy' drug, June 14, 1986, p. 96.
2 Asberg, M., Thoren, P., Traksman, L., Bertilsson, L. and Ringberger, V., 'Serotonin depression' — a biochemical subgroup within affective disorders, *Science*, 191 (1976) 478–480.
3 Battaglia, G., Yeh, S.Y., O'Hearn, E., Molliver, M.E., Kuhar, M.J. and DeSouza, E.B., 3,4-Methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroy serotonin terminals in rat brain: quantification of neurodegeneration by measurement of [^{3}H]paroxetine-labeled serotonin uptake sites, *J. Pharmacol. Exp. Ther.*, 242 (1987) 911–916.
4 Bertilsson, L., Asberg, M., Lantto, O., Scalia-Tomba, G., Traksman-Bendz, L. and Tybring, G., Gradients of monoamine metabolites and cortisol in cerebrospinal fluid of psychiatric patients and healthy controls, *Psychiat. Res.*, 6 (1982) 77–83.
5 Bowers, M., Lumbar CSF 5-hydroxyindoleacetic acid and homovanillic acid in affective syndromes, *J. Nerv. Ment. Dis.*, 158 (1974) 325–330.
6 Bowers, M.B. and Gerbode, F.A., Relationship of monoamine metabolites in human cerebrospinal fluid to age, *Nature (Lond.)*, 219 (1968) 1256–1257.
7 Commins, D.L., Vosmer, G., Virus, R., Woolverton, W., Schuster, C. and Seiden, L., Biochemical and histological evidence that methylenedioxymethylamphetamine (MDMA) is toxic to neurons in the rat brain, *J. Pharmacol. Exp. Ther.*, 241 (1987) 338–345.
8 Curzon, G., Joseph, M.H. and Knott, P.J., Effects of immobilization and food deprivation on rat brain tryptophan metabolism, *J. Neurochem.*, 19 (1972) 1967–1974.
9 Eccleston, D., Ashcroft, G.W., Crawford, T.B., Stanton, J.B., Wood, D. and McTurk, P.H., Effect of tryptophan administration of 5-HIAA in cerebrospinal fluid in man, *J. Neurol. Neurosurg. Psychiat.*, 33 (1970) 269–272.
10 Garelis, E., Young, S.N., Lal, S. and Sourkes, T.L., Monoamine metabolites in lumbar CSF: the question of their origin in relation to clinical studies, *Brain Research*, 79 (1974) 1–8.
11 Jacupcevic, M., Lackovic, Z., Stefoski, D. and Bulat, M., Non-homogeneous distribution of 5-hydroxyindoleacetic acid and homovanillic acid in the lumbar cerebrospinal fluid of man, *Brain Research*, 31 (1977) 165–171.
12 Johansson, B. and Roos, B., Concentration of monoamine metabolites in human lumbar and cisternal cerebrospinal fluid, *Acta Neurol. Scand.*, 52 (1975) 137–144.
13 Kilpatrick, I., Jones, M. and Phillipson, O., A semiautomated analysis method for catecholamines, indoleamines, and some prominent metabolites in microdissected regions of the nervous system: an isocratic HPLC technique employing coulometric detection and minimal sample preparation, *J. Neurochem.*, 46 (1986) 1865–1876.
14 Koslow, S.H., Maas, J.W., Bowden, C.L., Davis, J.M., Hanin, H. and Javaid, J., CSF and urinary biogenic amine metabolites in depression and mania, *Arch. Gen. Psychiatr.*, 40 (1983) 999–1010.
15 Mendels, J., Frazer, A., Fitzgerald, R., Ransey, T. and Stokes, J., Biogenic amine metabolites in cerebrospinal fluid of depressed and manic patients, *Science*, 175 (1972) 1380–1381.
16 Moir, A.T., Ashcroft, G.W., Crawford, T.B., Eccleston, D. and Guldberg, H., Cerebral metabolites in cerebrospinal fluid as a biochemical approach to the brain, *Brain*, 93 (1970) 357–368.
17 Mokler, D.J., Robinson, S.E. and Rosencranz, J.A., 3,4-Methylenedioxyamphetamine produces long-term reductions in brain 5-hydroxytryptamine in rats, *Eur. J. Pharmacol.*, 138 (1987) 265–268.
18 Nencini, P., Woolverton, W. and Seiden, L., Enhancement of morphine induced-analgesia after repeated injections of MDMA, *Brain Research*, (1988) in press.
19 O'Hearn, E.G., Battaglia, G., De Souza, E.B., Kuhar, M.J. and Molliver, M.E., Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause ablation of serotonergic axon terminals in forebrain: immunocytochemical evidence, *J. Neurosci.*, in press.
20 Post, R.M., Kotin, J., Goodwin, F.K. and Gordon, E., Psychomotor activity and cerebrospinal fluid amine metabolites in affective illness, *Am. J. Psychiatr.*, 130 (1973) 67–73.
21 Ricaurte, G.A., Finnegan, K.T., Nichols, D.E., DeLanney, L.E., Irwin, I. and Langston, J.W., 3,4-Methylenedioxyethylamphetamine (MDE), a novel analog of MDMA, produces long-lasting depletion of serotonin in the rat brain, *Eur. J. Pharmacol.*, 137 (1987) 265–268.
22 Ricaurte, G.A., Forno, L.S., Wilson, M.A., DeLanney, L.E., Irwin, I., Molliver, M.E. and Langston, J.W., MDMA exerts selective toxic effects on central serotonergic neurons in primates, *Soc. Neurosci. Abstr.*, 13 (1987) 905.
23 Ricaute, G.A., Forno, L.S., Wilson, M.E., DeLanney, L.E., Irwin, I., Molliver, M.E. and Langston, J.W., MDMA selectively damages central serotonergic neurons in the primate, *J. Am. Med. Assoc.*, in press.
24 Ricaurte, G.A., DeLanney, L.E., Irwin, I. and Langston, J.W., Toxic effects of 3,4-methylenedioxymethamphetamine (MDMA) on central serotonergic neurons in the primate: importance of route and frequency of drug administration, *Brain Research*, in press.
25 Roos, B., On the occurrence and distribution of 5-hydroxyindoleacetic acid in brain, *Life Sci.*, 1 (1962) 25–27.
26 Schmidt, C.J., Neurotoxicity of the psychedelic amphetamine, methylenedioxymethamphetamine, *J. Pharmacol. Exp. Ther.*, 2-
27 Seymour, R. San Francisco
28 Stanley, M., Correlations ly obtained fr 1279–1286.
29 Steinbusch, H tivity in the c terminals, *Ne*
30 Stone, D.M., The effects (MDMA) an aminergic sys

Exp. Ther., 240 (1987) 1–7.
27 Seymour, R.B., *MDMA*, Haight Ashbury Publications, San Francisco, CA (1986).
28 Stanley, M., Traksman-Bendz, L. and Dorovini-Zis, K., Correlations between aminergic metabolites simultaneously obtained from human CSF and brain, *Life Sci.*, 37 (1985) 1279–1286.
29 Steinbusch, H.W., Distribution of serotonin immunoreactivity in the central nervous system of the rat cell bodies and terminals, *Neuroscience*, 6 (1981) 557–618.
30 Stone, D.M., Stahl, D.S., Hanson, G.L. and Gibb, J.W., The effects of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine on monoaminergic systems in the rat brain, *Eur. J. Pharmacol.*, 128 (1986) 41–48.
31 Stone, D.M., Hanson, G.R. and Gibb, J.W., Differences in the central serotonergic effects methylenedioxymethamphetamine (MDMA) in mice and rats, *Neuropharmacology*, (1987) 1657–1661.
32 Wilson, M.A., Ricaurte, G.A. and Molliver, M.E., Distinct morphologic classes of serotonergic axons in primates exhibit differential vulnerability to the psychotropic drug 3,4-methylenedioxymethamphetamine, *Neuroscience*, in press.
33 Wode-Helgodt, B. and Sedvall, G., Correlations between height of subject and concentrations of monoamine metabolites in cerebrospinal fluid from psychotic men and women, *Comm. Psychopharm.*, 2 (1978) 177–183.