The behavioural effects of EOS-induced changes in substantia nigra GABA levels

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GABA levels in the substantia nigra (SN) are raised after local injection of ethanolamine-*O*-sulphate (EOS), an inhibitor of GABA: glutamate transaminase (Dray, Oakley & Simmonds, 1975). Dray, Fowler, Oakley, Simmonds & Tanner (1975) have reported lowered striatal dopamine levels on the side of the EOS injection and rotational behaviour consistent with asymmetrical striatal functioning. These effects faded after 5 days. We have studied the changes in spontaneous and amphetamine induced motor behaviour after *bilateral* injection of EOS into the SN (zona reticulata).

Rats are implanted bilaterally with 23 gauge cannulae to within 1 mm of a site in the zona reticulata of the SN. The co-ordinates were A, 3.0; L, 2.0; V, 6.8 from the atlas of Pellegrino and Cushman. For the EOS injection the rat was held firmly and a 30 gauge injection cannula was lowered through the guide to a site 1 mm beyond the guide tip. EOS (200 μg/kg) was injected in 1.5 μl of saline over a 2 min period. Control rats received a 1.5 μl of vehicle solution under identical conditions. Thirty minutes elapsed between the injections on the two sides of the brain. Behaviour was observed immediately following the EOS injection. Twenty-four and 72 h after the injection spontaneous locomotor behaviour was measured for 30 min and stereotyped behaviour was rated. (+)-Amphetamine (1.5 mg/kg) was then administered and these behaviours recorded for 2 hours. The EOS injection was repeated at weekly intervals. The cannulae placements were verified histologically at the end of the experiment.

Immediately following the EOS injection contralateral turning occurred (i.e. away from the injection side). When the injection was given on the second side turning in the opposite direction occurred. Stereotyped behaviours including sniffing and biting were also seen soon after the injection and were still pronounced 24 h later. At this time (+)-amphetamine (1.5 mg/kg) intensified the on-going stereotyped behaviour both in its nature and duration. These effects became more pronounced after each weekly EOS injection.

The results suggest that raising GABA levels in the zona reticulata of the SN results in heightened functional activity of the dopamine-containing nigro-striatal tract. This activity results in spontaneous stereotypy and enhanced motor responses to amphetamine. The nigro-striatal system appears to become progressively sensitized to the EOS treatment.

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Platelet uptake of [^{14}C]-5-hydroxytryptamine in 'emotional' and 'non-emotional' rats

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A method has been developed in our laboratory for selecting rats characterized by a high level of emotionality (Boissier, Simon & Soubrie, 1975). We have undertaken studies in order to look for biochemical differences between 'emotional' (E) and 'non-emotional' (NE) rats. We report here data concerning 5-hydroxytryptamine (5-HT) uptake by blood platelets. Such a study is of interest because the uptake of amines by platelets is thought to be a model for some aspects of aminergic brain function.

The experiment was performed on 14 E rats and 14 NE rats. Two fractions of 1 ml of platelet-rich plasma were prepared for each rat. The uptake of [^{14}C]-5-HT (25 nmol, 1 μCi/ml) was measured at suitable time intervals. Statistical analysis (Student's t test) of the results (expressed in d.p.m. $\times 10^3$ per 10^7 platelets) indicates that 5-HT uptake by platelets is lower in E rats than in NE rats:

Time of incubation (min)	*5*	*15*	*30*
E rats	1.97 ± 0.13	3.60 ± 0.13	6.52 ± 0.35
NE rats	2.00 ± 0.07	4.68 ± 0.21	7.82 ± 0.40
	ns	$P < 0.001$	$P < 0.02$

If the mechanism of uptake by platelets is indeed a model for uptake by neurons (Trenchard & Turner, 1975), then our results are indicative of a reduced 5-HT uptake at the neuronal level in E rats. Using the method proposed by Carlsson, Corrodi, Fuxe & Hökfelt (1969), we tried to further evaluate uptake of 5-HT in the brain. According to these authors the rate of 5-HT depletion caused by H75/12 compound (4-methyl-α-ethyl-*m*-tyramine) gives an indication of the rate of uptake at the level of the neuronal cell membrane.

The experiment was performed on 12 E rats and 12 NE rats. We observed that, when E rats are treated with H75/12 (25 mg kg^{-1}) for 1 h, the brain 5-HT level is decreased 21%. Under the same conditions the brain 5-HT level of NE rats is decreased 43%. The difference between these two percentages is significant ($0.02 < P < 0.05$).

This result is in agreement with the hypothesis that 5-HT uptake at the neuronal level is reduced in E rats. But investigations of uptake by synaptosomal preparations are necessary to provide further support for this hypothesis.

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Effects of *p*-chlorophenylalanine and α-methyltryptophan on rat social behaviour

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Previously we have studied the effect on rat motor activity of an inhibitor of 5-hydroxytryptamine (5-HT) synthesis, *p*-chlorophenylalanine (PCPA), and its reversal by the 5-HT precursor tryptophan (Marsden & Curzon, 1976). In the present communication the effects of PCPA on social behaviour are compared with those of α-methyltryptophan (AMTP), a drug which induces tryptophan pyrrolase and thus decreases brain 5-HT synthesis by depleting the precursor tryptophan (Sourkes, Missala & Oravec, 1970).

Male Sprague-Dawley rats (110–130 g) were housed 3 to a cage under a 12 h light/dark cycle for ten days. Six cages of rats were then injected with PCPA (200 mg/kg i.p.) and six with (±)-AMTP (150 mg/kg i.p.). Controls were injected with the vehicle medium (0.5% Tween in 0.9% saline). Various components of social behaviour in the home cage were observed for 5 min under red light during the first 90 min of the dark period. Observations were made 24 and 72 h after injection. Locomotor activity was measured simultaneously using an Animex activity meter. The amount of food eaten/24 h was also measured. Either 24 or 72 h after drug treatment the rats were killed and brain 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) determined (Curzon & Green, 1970).

Both drugs reduced food intake during the first 24 h after injection but it was normal by 72 hours. PCPA markedly reduced 5-HT synthesis at 24 and 72 h with a significant decrease of 5-HT (24 h, −42%; 72 h, −53%) and 5-HIAA (24 h, −51%; 72 h, −67%). This was associated at 24 h (but not at 72 h) with increased locomotor activity ($P < 0.05$) and increased interactions between rats involving sniffing and biting ($P < 0.01$), fighting ($P < 0.01$) and mounting ($P < 0.01$). Self-grooming was decreased ($P < 0.05$). AMTP also decreased 5-HT turnover as brain 5-HIAA was reduced (24 h, −41%; 72 h, −43%) but 5-HT concentrations appeared to increase presumably due to the formation of α-methyl-5-HT (Roberge, Missala & Sourkes, 1972). In contrast to PCPA, AMTP had no significant effect on any of the above components of social behaviour or net locomotor activity. Self-grooming was increased at 24 h ($P < 0.01$) and at 72 h ($P < 0.05$) and burrowing was increased at 24 h ($P < 0.01$). α-Methyl-5-HT is formed by the action of tryptophan hydroxylase (Gal & Christiansen, 1975). To investigate whether α-methyl-5-HT was involved in the behavioural effects of AMTP tryptophan hydroxylase was inhibited by injecting PCPA (100 mg/kg) on three consecutive days to 24 rats and