coupling of olivary neurones (see Headley, Lodge & Duggan, 1976, and references therein). These authors suggested that harmaline acts by inhibiting synaptic 5-hydroxytryptamine (5-HT) release, but other evidence (Wiklung, Sjölund (Björklund, 1978) is more compatible with an *increased* 5-HT release by harmaline.

We here describe experiments in which harmaline was examined for effects on the uptake and release of 5-HT and other neurotransmitters. The inferior olive is in practice too small (2 mg wet weight/rat) for making such measurements so we used rat cortex which also contains 5-HT terminals.

Freshly dissected chilled cortices were cut on a McIlwain chopper into 0.1 × 0.1 × 2.0 mm 'minislices' which were then suspended in 37°C Krebs-Ringer solution which, in experiments on monoamines, contained ascorbic acid and a monoamine oxidase inhibitor. Incubation with 0.1–1.0 μCi tritium-labelled compounds was as follows: 5-HT and noradrenaline 10^{-7}M; γ-aminobutyric acid (GABA) 4×10^{-7}M; glycine 1×10^{-9}M; L-glutamate and L-aspartate 4×10^{-9}M; D-glutamate and D-aspartate 2×10^{-6}M. In uptake experiments the tissue was then filtered, washed and assayed for radio-activity. In release experiments the tissue was filtered, washed and transferred to a perfusion chamber; 2.7 min superfusate collections were assayed. Harmaline (⩾3 concentrations) was tested for antagonism of uptake or of spontaneous or 20–40 mM potassium – stimulated release.

Harmaline inhibitory concentration 50% values were above 5×10^{-4}M for uptake of all the amino acids ($n \geqslant 3$); for noradrenaline, $4.38 \pm 0.55 \times 10^{-5}$M (mean ± s.e. mean) and for 5-HT $2.43 \pm 0.14 \times 10^{-5}$M. Harmaline 10^{-5}M had no effect on 5-HT release and at 10^{-4}M did not affect spontaneous release; potassium-stimulated release was however reduced on some occasions, but in double-label experiments [^{14}C]-GABA release was inhibited concurrently.

Tremor is generated in rats when harmaline concentrations in whole brain exceed 1.5×10^{-5}M (Zetler, Back & Iven, 1974). In our experiments harmaline at this concentration did not affect 5-HT release but did reduce the uptake of noradrenaline and 5-HT (see also Buckholtz & Boggan, 1977). If our results on cortical tissue *in vitro* are relevant to olivary tissue *in vivo* then it is unlikely that harmaline acts by modifying 5-HT release.

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Biochemical and morphological aspects of kainic acid injection into rat cerebellum

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Kainic acid is a potent neurotoxic agent when injected *in vivo* and it has been shown to destroy intrinsic neurones whilst sparing axons which pass through the injected area (Coyle & Schwarcz, 1977; McGeer & McGeer, 1977). The mechanism of this action is as yet unknown, but there is evidence to suggest that it may, at least partially, involve postsynaptic glutamate receptors (Campochiaro & Coyle, 1978). In support of this, Herndon & Coyle (1977) have shown that kainic acid when injected into the rat cerebellum, results in a loss of all neurones except the granule cells. These cells are thought to be glutamatergic and are known to synapse onto all other cell types in the cerebellum. We have investigated further this kainic acid-resistance of the granule cells.

Female Wistar rats (200–300 g) were anaesthetized with sodium pentobarbitone, placed in a stereotaxic frame and injected with doses of kainic acid (in 2 μl Tris citrate buffer, pH 7.1) at a depth of 3 mm below the vermis, through a burr hole in the calvarium. Animals were killed at various times following injection and the cerebella removed for histological examination or biochemical studies.

The preservation of granule cells following kainic acid injection was found to be both dose and time dependent. Twenty four h after an injection of 2 μg, there was extensive degeneration of neurones over most of the vermis and part of the cerebellar hemispheres but which was not apparent in the granule cell layer. At higher doses however, or with time intervals of greater than 24 h following injection, the granule cells were also affected and the whole layered structure of the cerebellar cortex was disrupted. Injection of dihydrokainic acid (2 μg) which is neuropharmacologically inactive, caused no apparent degeneration of any cellular components under iden-

tical conditions.

The behavioural characteristics of the kainic acid injected animals were tonic extensions of the hind limbs which were pronounced 24 h after injection but tended to dissipate at longer survival times. At all survival times, a general lack of muscle tone and coordination was apparent – these symptoms being consistent with a cerebellar lesion. In no instance with the dihydrokainic acid injected rats, was any behavioural abnormality evident.

The neuronal cell types lesioned under these conditions are all inhibitory and, may utilize γ-aminobutyric acid (GABA) as a transmitter. Accordingly, we have examined the high affinity uptake of [^{3}H]-GABA into cerebellar synaptosomes and, have found that the V_{max} in kainate-lesioned cerebellum was reduced to 55% of control and dihydrokainate injected rats, whilst the K_m remained unaltered (Table I). This finding correlated well with the histological data which showed that approximately 50% of the cerebellum was affected by the lesion. In close correspondence with the preservation of the granule cells, was the finding that the high affinity uptake of [^{3}H]-glutamate was unaffected by the kainic acid lesion (Table I). These findings provide further evidence for the hypothesis that glutamate is the transmitter of the cerebellar granule cells.

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Table 1 Effect of kainic acid lesion on the uptake of [^{3}H]-GABA and [^{3}H]-glutamate into rat cerebellum

[^{3}H]-GABA	*K_m (μM)*	*V_{max} (nmole mg protein^{-1} 5 min^{-1})*
Control	2.35 ± 1.05	13.04 ± 1.80
kainic acid	2.63 ± 0.99	5.50 ± 0.67*
dihydrokainic acid	2.39 ± 0.47	12.20 ± 0.75
[^{3}H]-gluatamate	*K_m (μM)*	*V_{max} (nmole mg protein^{-1} 3 min^{-1})*
Control	11.84 ± 4.88	14.52 ± 2.11
kainic acid	8.68 ± 1.11	12.41 ± 0.52
dihydrokainic acid	6.90 ± 1.10	16.35 ± 0.69

Significance of difference from control by *t*-test; *$P<0.01$

Some observations on the behavioural and biochemical effects of L-tryptophan plus a monoamine oxidase inhibitor in immature rats

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Adult rats given L-tryptophan (L-TP) plus a monoamine oxidase inhibitor (MAOI) develop a characteristic behavioural syndrome including hyperactivity which results from increased brain 5-hydroxytryptamine (5-HT) synthesis and function (Grahame-Smith, 1971). This behavioural model has been used to study the mechanisms controlling central 5-HT function and the effects of drugs on this function (Green & Grahame-Smith, 1976). We have now investigated the behavioural and biochemical responses of immature rats to L-tryptophan plus a MAOI with a view to studying the effects of drugs on developing brain 5-hydroxytryptaminergic systems.

Adult male Sprague-Dawley rats (150–200 g) or 21-day old male pups (40–50 g) were injected with tranylcypromine (TCP 10 mg/kg i.p.). Thirty minutes later L-TP was administered (5–100 mg/kg i.p.) and activity recorded (movements/min) using LKB Animex activity meters (sensitivity and tuning: 30 μA). At 120 min brains were removed and tryptophan (Denckla & Dewey, 1967) and 5-HT (Curzon & Green, 1970) measured.

Basal brain 5-HT concentrations were 0.37 ± 0.02 μg/g wet weight (21 day; $n = 6$) and 0.43 ± 0.01 μg/g (adult; $n = 8$; $P < 0.05$). Tryptophan concentrations were 4.45 ± 0.4 (21-day; $n = 6$) and 2.86 ± 0.03 (adult; $n = 8$; $P < 0.01$).

Brain tryptophan concentration 90 min after L-TP injection was directly related to the L-TP dose in both adults and pups. However, accumulation of brain tryptophan over this 90 min period (corrected for basal levels) revealed that 21-day rat brain accumulated more tryptophan than adult brain at corresponding doses of L-TP (126% of adult brain tryptophan accumulation at 25 mg/kg and 188% at 50 mg/kg L-TP).

The accumulation of adult brain 5-HT over 90 min increased up to an L-TP dose of 100 mg/kg whereas 21-day brain 5-HT accumulation plateaued at around