

A non-invasive method for the preparation of split brain rats using ultrasonics

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Severance of the corpus callosum after physical separation of the cerebral hemispheres can be performed in the cat (Myers, 1956) and the monkey (Downer, 1959). This method is not ideally suited to the rat due to the damage caused to the sinuses and the very soft cerebral hemispheres. Using a shielded-knife, good results have been obtained by Goodman & Russell (1974). However, such methods cause unavoidable neural damage in the process of inserting the cutting device through tissues on approaching the corpus callosum.

An appropriate alternating voltage supplied to a concave piezoelectric crystal produces ultrasonic waves which pass through a fixed focal point dissipating sufficient energy at that point to cause a lesion (Johnston & Dunn, 1976). Thus, ultrasonics offer a non-invasive approach and cleavage of the corpus callosum can be achieved by a series of small overlapping lesions.

Male albino rats (190–220 g) under chloral hydrate anaesthesia (500 mg/kg, intraperitoneally) were placed in a head-holder. A midline incision was made in the scalp, the underlying membranes scraped away from the skull, and a 4 mm hole trephined at a skull landmark (lambda). A rectangle of bone approximately 10 mm long and 4 mm either side of the midline suture was then removed to expose the cortex. The ultrasonic transducer was held in a micro-

manipulator and coordinates of the corpus callosum determined from the intersection of the sagittal and transverse sinuses. Ultrasonic energy from the transducer is coupled into the cortex by saline (37°C) flowing through a hollow cone from the transducer and over the brain. A total acoustic power output of 2 W at 3 MHz at approximately 200 W/cm² is used. Approximately spherical lesions of 0.9 mm diameter are produced with a 14 s exposure. Lesions can be observed in 100 μ sections prepared using a vibratome from brains removed 48 h later.

This method has the advantage of reaching deep-lying structures without cutting surrounding tissues and is of special importance where any extra damage may be harmful or make interpretation of results difficult.

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Effects of long-term treatment with contraceptive steroids on plasma and brain tryptophan, brain 5-hydroxytryptamine, and locomotor activity in female mice

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Although contraceptive steroids may have marked effects on monoamine metabolism and behaviour, little work has been carried out on animals with normal

ovarian function. We have studied effects of prolonged daily injections of norethistrone acetate (200 μ g/kg) alone and in combination with ethinyl oestradiol (100 μ g/kg) compared with daily vehicle injection. Locomotor activity (Animex activity meter) was determined continuously for 2 oestrus cycles prior to injection, then every 7th day throughout 42 days of treatment. Free and total plasma tryptophan and brain tryptophan and 5-HT were determined on the 43rd day (Bender, Boulton & Coulson, 1975; Denckla & Dewey, 1967; Curzon & Green, 1970) and compared with dioestrus values.

Biochemical results are shown in Table 1. Locomotor activity declined after both treatments. On the final day of treatment the activity of the

Table 1 Plasma and brain tryptophan and brain 5-HT levels in dioestrus and hormone-treated female mice

	Dioestrus	Norethistrone	Norethistrone and oestradiol
Plasma total tryptophan ($\mu\text{g/ml}$)	24.2 ± 1.9	26.0 ± 0.5	$22.0 \pm 0.6^*$
Plasma free tryptophan ($\mu\text{g/ml}$)	12.2 ± 1.3	15.2 ± 0.3	$8.91 \pm 0.44^*$
Brain tryptophan ($\mu\text{g/g}$)	2.41 ± 0.20	2.60 ± 0.09	3.02 ± 0.47
Brain 5-HT (ng/g)	624 ± 21	632 ± 18	$867 \pm 12^{**}$

Mean \pm s.e. mean.

* Significantly different from norethistrone alone $P < 0.001$.

** Significantly different from both norethistrone alone and dioestrus values $P < 0.001$.

norethistrone group was 47% while that of the norethistrone plus ethinyl oestradiol group was 54% of initial dioestrus values. Vehicle controls resumed cyclic changes in locomotor activity within 8 days, while the hormone treatments abolished these cyclic changes.

The ineffectiveness of norethistrone in changing brain tryptophan is perhaps surprising in view of the acceleration of 5-HT turnover reported after progestogen treatment (Ladisch, 1974). The reduction in plasma total and free tryptophan on combined treatment with oestradiol may be due to acceleration of protein synthesis by the latter (Hamilton, Widnell & Tata, 1968). The rise in brain 5-HT could also be due to changes in storage following increased protein formation in nerve endings or perhaps to inhibition of monoamine oxidase by oestradiol (Morrison & Pritchard, 1971).

These findings do not support the hypothesis that brain tryptophan levels and hence 5-HT synthesis may be controlled by plasma free tryptophan (Knott & Curzon, 1972), since both brain tryptophan and 5-HT were raised by combined treatment, while plasma levels were lowered. The functional activity of these raised 5-HT levels is, however, in question since 5-HT has been suggested to be involved in mechanisms inhibitory to spontaneous locomotor activity (Neuburg & Thut, 1974; Grabowska & Michaluk, 1974), yet this activity declined to approximately the same extent in both treatment groups.

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