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DEMONSTRATIONS

A superfusion technique for isotope efflux studies and concurrent electrophysiological investigation in the rat cuneate nucleus

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The superfused rat cuneate nucleus has been developed in our laboratory for various studies of the pharmacology and physiology of evoked excitatory and inhibitory phenomena. Techniques have been described for superfusion, within a plastic ring, of the pial surface over a small region of primary afferent terminals and second-order neurones while recording their evoked activity, or measuring presynaptic inhibition, with a concentric tungsten micro-electrode (Davidson & Southwick, 1971; Davidson & Simpson, 1976).

A recent modification of this technique has permitted a study of the efflux characteristics of a variety of isotopically labelled putative amino acid neurotransmitters from a region of the cuneate which provides a maximal evoked response following ipsilateral forepaw stimulation in the chloralose-urethane anaesthetized rat (see also Roberts, 1974). A small Perspex ring, internal diameter 2.5 mm, internal volume approximately 30 μl ., is sealed in place over the predetermined area with petroleum jelly. Isotopic labelling of the cuneate region is achieved by closed-cycle perfusion at 62.5 $\mu\text{l. min}^{-1}$ for 1 hr through a peristaltic pump connected to fine-diameter polythene inlet/outlet tubes inserted through the Perspex ring. After the labelling period, perfusion with an artificial cerebrospinal fluid (c.s.f.) solution (Merlis, 1940) is commenced from a Braun constant-perfusion pump at 62.5 $\mu\text{l. min}^{-1}$. A second peristaltic pump is used to collect 5 min fractions of the superfusate in glass vials containing 0.2 ml. water and 7 ml. Multisol II (Intertechnique Ltd.). Radioactivity is estimated by liquid scintillation spectrometry. Quench curves for [$1\text{-}^{14}\text{C}$] γ -amino-*n*-butyric acid (GABA) in the c.s.f. solution are shown, indicating that quenching is not normally a serious source of counting error in this preparation.

Data are shown for efflux curves obtained over a 100 min collection period after perfusion with solutions containing $2.0 \mu\text{C ml.}^{-1}$ of $[1-^{14}\text{C}]$ -GABA. With this technique levels of radioactivity can be recovered in the samples ranging from more than 5000 counts min^{-1} at the start of perfusion to a minimum of 100 over the course of the experiment, following correction for background. At least two distinct components of the spontaneous efflux curve can be distinguished, an initial rapid efflux lasting up to 15 min, followed by a much slower component.

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A simple, inexpensive mechano transducer system using frequency modulation

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The increasing cost of force-displacement transducers using solid-state strain-gauges or tensioned-wire bridges, and the difficulty of repairing such devices, have made it worth while to re-examine other designs for mechano-electric transducers.

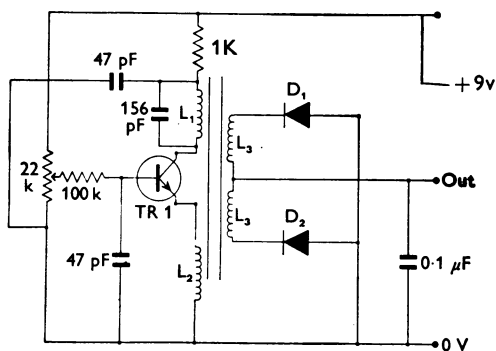


Fig. 1. Circuit diagram. L_1 , L_2 and L_3 wound on common 0.5 in form with 0.25 in ferrite core. L_1 (collector coil) 15 turns; L_2 (emitter) 10 turns; L_3 (secondary coil) 25 turns bifilar wound. Lever mounted above coil varies its inductance. TR 1: BF 180. D_1 , D_2 : IN 4148. $0.1 \mu\text{F}$ filters any RF signal left output.

The design demonstrated consists of a simple oscillator; the inductance of the coil is varied by moving a piece of iron closer to it or further away, and this produces modulation of the basic frequency of the oscillator. The frequency-modulated signal is detected by a Foster-Seeley discriminator which produces a d.c. output proportional to the shift in frequency.

Similar designs have been used in the past, but suffered from the severe draw-back of thermal drift. Modern silicon-type semiconductors have a much smaller drift, and tunnel diodes, integrated circuit operational amplifiers or U.H.F. transistors are all suitable for the oscillator circuit. The U.H.F. transistor has been selected because of low cost and high stability.

The complete circuit can be made very compact, occupying no more space than a conventional transducer element. By the use of suitable levers the device can be used for isotonic or isometric recording, or as a pressure transducer. By suitable selection of components the circuit can be made very linear, and is highly sensitive, producing an output of up to 0.5 V for a movement of the iron-sensing element of less than 0.5 mm. This high sensitivity eliminates the need for high-gain preamplifiers. The total cost of components is less than £5.00.

A simple and effective ruminal cannulation technique for sheep

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The cannula consists of a 60 ml. polythene bottle (85 × 38 mm of 3 mm wall thickness) with a screw cap. The bottom is sawn off and six saw cuts 40 mm long made through the wall. Over a flame, each of the six sawn sections is bent outwards perpendicular to its original position to form a lug, near the end of which a small hole is bored. A large rubber washer is needed which will fit firmly over the cannula.

The operative procedure involves a sheep kept without food overnight and anaesthetized with a halothane: oxygen mixture, during which time a cuffed endotracheal tube is inserted. Using conventional aseptic surgical procedures throughout, a skin incision made in the sublumbar triangle is lengthened until a piece of sheet Perspex can be inserted tightly into it. The Perspex has a length exactly equal to half the external circumference of the cannula. The underlying muscle layers and peritoneum are split grid-iron fashion parallel to the muscle fibres. Using two pairs of peritoneal tissue forceps the rumen wall is lifted to the level of the edges of the skin incision. Interrupted nylon sutures are used to fasten the skin edges to the rumen wall, leaving an oval area of rumen wall free of sutures. This oval is now incised lengthwise to form a fistula. Rumen gases and liquor escape at this point but the prior suturing of the skin to the rumen wall prevents

their leakage into the subcutaneous tissue. The cannula with each pair of lugs tied together is inserted. A scalpel inserted into the cannula cuts these ties, so that the lugs spring apart again. A large rubber washer is placed around the cannula over the skin. It is pushed down as far as possible and fastened with adhesive tape. The lid is screwed on. Anaesthesia is stopped and the endotracheal tube is removed before chewing movements commence. It is important that the sheep's head should be made to hang below the level of the operating table before removing the tube, so that accumulated saliva and possibly regurgitated rumen contents drain from the mouth without risk of being inhaled.

Hypoxia and the role of arterial chemoreceptors

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COMMUNICATIONS

On the acid-induced abolition of reticulo-ruminal motility in sheep

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Reticulo-ruminal motility in sheep and cattle may be abolished by acids either infused intraruminally (Ash, 1959; Svendsen, 1974) or accumulated after overfeeding (e.g. Mullen, 1976). The results of intraruminal infusion of a variety of acids in abolishing reticulo-ruminal motility in sheep kept on one controlled feeding regime led Upton, Ryan & Leek (1976) to conclude that abolition coincided with intraruminal concentrations of *undissociated* volatile fatty acids (VFA) in the range 20–100 mmol/l, which paralleled the threshold concentration for acid-sensitive reticulo-ruminal epithelial receptors described by Leek & Harding (1975).

In the present investigation, acids were infused into sheep (i) which were unfed for the previous 18 hr and (ii) which were force-fed with commercial ewe nuts at a level of 1 kg 18 hr and 0.5 kg 1 hr prior to starting the acid infusions. After taking into account the greater rumen volumes (based on a polyethylene glycol dilution technique), relatively greater amounts of all the infused acids (hydrochloric, acetic and lactic) were required in order to abolish motility in all cases for the force-fed sheep compared with the unfed sheep. This was so despite the presence of greater amounts of endogenous VFA and hence, because of the lower pH attained, of much greater concentrations of *undissociated* VFA (48–82 mmol/l) in the force-fed sheep than in the unfed sheep (4–10 mmol/l).