A simple and inexpensive method for the intracerebral administration of drug solutions to the conscious rat

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Many different cannula systems have been designed to allow the direct application of drugs to discrete brain areas of conscious animals (Myers, 1971; Harvey & Stevens, 1975). The major technical problem lies in securing the device to the skull and consequently many of the cannula systems are complex and hence expensive. The system detailed here is both inexpensive and simple to construct.

Cannula guides consist of 23 swg stainless steel tubing cut to the required length and electrochemically cleaned of burr. The length of each guide is obviously dictated by the topographical location of the brain area under investigation but certain minimum requirements are apparent. Thus, 4 mm is sufficient to allow positioning in the stereotaxic cannula-guide carrier and a further 4 mm for cementation. This portion carries a small ball of epoxy-resin (2–3 mm diameter) which acts as guide anchorage and prevents movement of the tube in the vertical plane. The remaining part of the cannula guide which lies below the cranium is of length such that the tip rests 2–3 mm above the area under investigation.

Stilettes, which remain within the cannula-guides at all times other than when injecting, are also simply constructed. Stilette shafts of 30 swg, non-ferrous wire are coated with a water repelling agent (dimethyldichlorosilane) and bent, at one end, over a 3–4 mm length of polythene tubing (I/D 0.86 mm) and secured in place with a 3 mm ball of epoxy resin. This facilitates removal of the stilette from the guide, and the cylinder of polythene, which fits closely over the guide tube provides anchorage and prevents access of foreign material into the underlying tissue. The tip of the shaft lies flush with that of the cannula guide.

The cannula guides are stereotaxically positioned and then secured to the surface of the cranium with polymethyl methacrylate. Sufficient cement is used such that both the guide anchorage and the whole of the incised area are covered. The cement is dried by a stream of warm air for 3–4 min before unclamping the guide from the stereotaxic apparatus. Stilettes are then fitted and the animals housed individually for 5 days before experimentation.

Drug solutions are injected through a 30 swg cannula at a rate of 0.47 µl/min and in a volume of 0.5 µl. Injection placements have been histologically verified following the infusion of 0.5 µl of 1% methylene blue and 4 days storage of tissues in 8% formyl saline at 4°C. Placements were found to be consistently reproducible.

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Central and peripheral inhibition of the milk-ejection reflex: studies with µ-adrenoceptor antagonists

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Most species of mammal fail to milk eject when they are anaesthetized and suckled by their young. This could be the result of high circulating levels of adrenaline, either blocking the release of oxytocin from the neurohypophysis or preventing the action of the hormone on the mammary gland (Cross, 1955). Rats are an exception and will milk eject when anaesthetized (Lincoln, Hill & Wakerley, 1973), though an appreciable number, too many for some forms of experimental study, still fail to respond to the suckling stimulus. The rat mammary gland is sensitive to the actions of adrenaline, but the effects can be eliminated by β-adrenoceptor antagonists (Bisset, Clark & Lewis, 1967). Thus we have questioned whether central or peripheral adrenoceptor stimulation might explain the failure, or partial failure, of some anaesthetized rats to milk eject when suckled.

Rats, at day 7–10 of lactation and separated from their young for 16 h, were anaesthetized with urethane (1.2 g/kg, i.p.), and two teat ducts were cannulated for the measurement of intramammary pressure. Three hours later, and while the animals were still deeply anaesthetized, 10 pups were applied to the uncannulated nipples. Each milk ejection in the next 3 h was recorded; each ejection was associated with

an abrupt rise in intramammary pressure and a behavioural response from the young.

Of 166 rats studied, 90 began to milk eject within 60 min of the young being applied to the nipples and thereafter gave milk ejections at regular intervals. Propranolol (1 mg/kg, i.v. or i.p.) was given to 49 of those which did not milk eject within 1 h, and 42 (86%) subsequently milk ejected. Of 27 rats that were left untreated only 3 (11%) began to milk eject. By contrast, practolol (1 mg/kg, i.v.) promoted milk ejection in only 1 of 10 animals—8 of the 9 failures milk ejected after being given propranolol. Intravenous infusions of adrenaline or isoprenaline (0.1–1.0 μg/min) abolished the milk-ejection reflex but simultaneously reduced the mammary response to exogenous oxytocin; both these actions were antagonized by propranolol. It is unlikely, however, that the failure of some rats to milk eject when anaesthetized and suckled is due to circulating adrenaline depressing the gland response. Such animals display a normal sensitivity to exogenous oxytocin, i.e. compared with animals which do milk eject. Thus, it would appear that the failure of rats to milk eject is the result of a central dysfunction, possible involving an adrenergic mechanism though not necessarily related to circulating amines.

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Effects of ethanol and chlordiazepoxide on social interaction in rats

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Although it is widely held that small doses of ethanol increase social behaviour in man, there is little experimental evidence to support this. In general ethanol has been found to decrease social behaviour in animals (e.g. Krsiak & Borgesova, 1973), but this may well be due to the large doses used. Ethanol (0.4 g/kg) was found to increase exploration in rats (File, unpublished observations) and it was thought that this dose might also increase social behaviour. In order to investigate whether any such increases could be related to anxiety reduction, social interaction between pairs of male rats was studied in conditions in which the level of fear was manipulated. This was done by altering the intensity of illumination and the rats' familiarity with the situation.

In the low fear condition ethanol (0.4 g/kg) did not change the duration of active social contact, but in conditions of moderate fear this dose increased active contact. In the high fear condition active contact was not significantly increased. A higher dose (1.2 g/kg) reduced active contact in all the test conditions, but this may have been secondary to motor impairments.

The effect of the anxiolytic drug, chlordiazepoxide, on social interaction was also studied in the same conditions. Chlordiazepoxide produced a dose related (2.5–7.5 mg/kg) decrease in active social contact and an increase in passive contact.

Both ethanol (0.4 g/kg) and chlordiazepoxide (5 mg/kg) increase exploration in rats, measured by head-dipping in a hole-board, and this effect might be due to anxiety reduction. However, the two drugs differ in their effects on social interaction. The effects of ethanol are consistent with anxiety reduction but the effect of an acute injection of chlordiazepoxide appears to resemble that of a sedative. However, rats pretreated with chlordiazepoxide (5 mg/kg) for 5 days and then tested for social interaction showed significantly increased active contact in conditions of moderate and high fear.

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Reference

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