Wistar Albino rats weighing 150 g and anaesthetized with urethane 1.5-2 g/kg i.p. Extracellular recordings were made from single neurones in the substantia nigra using parallel multibarrel glass microelectrodes (Crossman, Walker & Woodruff, 1974b). GABA, imidazole acetic acid, glycine, ACh, noradrenaline and 5-HT were ejected iontophoretically from 0.25 M solutions in distilled water, pH 3.5-6. Picrotoxin and strychnine were ejected from saturated solutions in distilled water, pH 6. Current balancing was employed during the ejection of all drugs.

Results were obtained from a total of 32 spontaneously active neurones. All of these neurones were inhibited by GABA (5-50 nA). Glycine (50 nA) and imidazole acetic acid (50 nA) also inhibited all the neurones to which they were applied (16 and 5 cells respectively). The effects of picrotoxin and strychnine upon GABA and glycine inhibitions were examined. Picrotoxin (50 nA, 4 min) reversibly blocked the GABA but not the glycine response. Strychnine (50 nA, 2.5 min) reversibly blocked both the GABA and glycine inhibitions.

ACh (50 nA) was applied to 15 neurones and excited all but one of them. Noradrenaline (50 nA) was applied to 12 neurones; three were inhibited, two were excited, one gave a biphasic response (inhibition followed by excitation) and six were unaffected. 5-HT (50 nA) was applied to 14 neurones; eight gave a biphasic response characterized by inhibition followed by excitation, two neurones gave excitation alone and three were unaffected.

In conclusion, therefore, the results of this study are consistent with a possible role as transmitter agents in the substantia nigra for GABA, ACh, noradrenaline and 5-HT.

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## Oestradiol binding in neonatal rat brain cytosol

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There is a critical period in brain development (0-5 days post-natally, in rats) when exposure to some steroids imprints 'masculinity' on the brain, expressed in adulthood in the gender-related patterns of copulatory behaviour, aggressiveness and hypothalamic control of gonadotrophin secretion. Both oestradiol and testosterone elicit these effects, and it has been suggested that the effect of the latter requires preliminary conversion to the former in the brain. Cytosols from oestrogen sensitive areas contain high affinity oestrogen specific receptors; the present experiments were designed to investigate binding of oestradiol in brain from neonatal (5-day old) rats and to compare reaction parameters, specificity and regional distribution with those of adult brain (Ginsburg, Greenstein, MacLusky, Morris & Thomas, 1973 and 1974).

Brain anterior to pons and cerebellum was removed from 5-day old rats of either sex, chilled immediately and divided into two blocks; one containing hypothalamus and amygdala, the remainder in the other. Cytosol fractions were prepared (see Ginsburg *et al.*, 1973) from pooled tissues from five animals. After incubation (30 min; 30° C) with a range of [$^3$H]-17 β-oestradiol concentrations, bound oestradiol in the cytosol incubates was separated on small Sephadex LH 20 columns, at 2° C. Control incubates contained, additionally, at least 100-fold

molar excess of diethylstilboestrol (DES); unlabelled steroids were also present in specificity experiments.

Considerable amounts of DES suppressible binding of 17β-oestradiol (DSB) were found in cytosol from both brain areas of both sexes; no DSB was found in heart cytosol or plasma. Saturation binding capacities for 17β-oestradiol in both brain regions of either sex were of the order $1.5 \times 10^8$ sites/mg wet weight and equilibrium dissociation constants were all of the order $0.5 \times 10^{-9}$ M; similar magnitudes and ranking orders of the affinities of other steroids for the oestradiol binding moiety were also found. Kds were of the order $10^{-6}$ for corticosterone, progesterone, testosterone and 5α-dihydrotestosterone, $4 \times 10^{-8}$ M for 5α-androstan 3β 17β diol and 5α-androstan 3α-17β-diol, $10^{-9}$ M for 17α-oestradiol, oestriol, 16-epioestriol and DES. Thus the DSB we have measured and characterized from brain of 5-day-old rats is distinct from the high affinity receptors in adult brain in distribution, Kd, and specificity (Ginsburg *et al.*, 1973 and 1974) nor can it be identical with the oestradiol binding in perinatal rat brain cytosol described by Plapinger, McEwen & Clemens (1973) which, like oestradiol binding in blood, is not suppressed by DES and is of much lower oestradiol affinity. These oestradiol binding reactions do not necessarily represent the hormone receptor interactions involved in sexual differentiation—a protective function of the reaction should also be considered.

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## Assessment of the agonist and antagonist activities of narcotic analgesic drugs by means of the mouse vas deferens

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It has been shown (Kosterlitz, Waterfield & Berthoud, 1974) that the correlation between the analgesic effect in man and the depression of the electrically induced contraction of the longitudinal muscle of the guinea-pig ileum is very high ($r = 0.930$), $n = 13$, potency spread $>3$ log units). Moreover, the correlation between the antagonist potencies determined in the morphine-dependent monkey and those measured in the guinea-pig ileum is also very high ($r = 0.974$, $n = 10$, potency spread $>2$ log units).

Recently it has been found that the responses of the longitudinal muscle of the mouse vas deferens to electrical stimulation (0.1 Hz, 1 ms, supramaximal voltage) are depressed by morphine-like drugs and that naloxone antagonizes this effect (Henderson, Hughes & Kosterlitz, 1972). For the assessment of the agonist and antagonist potencies of narcotic analgesic drugs by this preparation a method was used which is similar to that developed for the guinea-pig ileum (Kosterlitz & Watt, 1968). Normorphine, which in both preparations is equipotent with morphine, was the standard of reference because the onset of action and the recovery from it are very rapid. After two successive dose-response curves for normorphine had been constructed, the drug to be assayed was added to the organ bath (3 ml Mg-free Krebs solution) in a concentration to give a depression of the twitch by 20-40%. The degree of antagonist activity and the recovery from it were tested by suitable concentrations of normorphine at intervals of 7 minutes. The agonist and antagonist potencies were calculated as described for the guinea-pig ileum.

The values of the relative agonist activities obtained on the guinea-pig ileum and on the mouse vas deferens showed good agreement for compounds without antagonist component (codeine, pethidine, diamorphine and levorphanol) although the mean absolute sensitivity of the mouse vas deferens is only one-seventh of that of the guinea-pig ileum. For compounds with dual agonist and antagonist activities, the agreement between the relative agonist activities was not as