suitable doses desensitizes or competitively reduces the size of evoked excitatory junction potentials (EJP).

The walking legs of *Eupagurus bernhardus* were removed at the autotomizing joint. The leg, with opener muscle and nerve bundles exposed, was placed in crab Ringer at room temperature, 18° C. The Ringer composition (mM) used was: NaCl, 490; KCl, 12.2; $CaCl_2$, 14.8; $MgCl_2$, 5.75; $NaHCO_3$, 1.79. The pH was 7.3. A constant volume (0.1 ml) of drug was added to the surface of the exposed muscle fibres.

The membrane potential changes and EJP were recorded from the dactyl abductor muscle of the leg using 3 M-KCl-filled glass electrodes. The EJP were evoked by stimulation of the excitatory nerve bundle with trains of pulses from a Devices Digitimer stimulator, via a suction electrode. The membrane potential and EJP were displayed on a Tektronix 502A oscilloscope and digital voltmeter and recorded on a Watanabe WTR-2C pen-recorder.

In most cases, concentrations of 1×10^{-4} M-L-glutamate or 5×10^{-4} M-L-aspartate were sufficient to depolarize the membrane and diminish EJP amplitude. When a solution of L-glutamate of sufficient concentration to give a submaximal response was combined with an equipotent solution of L-aspartate in the ratio 1 : 2 or 1 : 3 (0.3×10^{-4} M-L-glutamate + 3×10^{-4} M-L-aspartate), the depolarization of the membrane was greater than that produced by the original L-glutamate (1×10^{-4} M) or L-aspartate (5×10^{-4} M) concentration. This synergistic effect was also reflected in a reduction in amplitude of the evoked EJP.

These observations and others (Colton & Freeman, 1973), coupled with evidence that the concentration of L-aspartate in peripheral nerve of crab is five times that of L-glutamate (Evans, 1972) suggest that L-glutamate and L-aspartate may both play a role in transmission at the excitatory neuromuscular junction in crustacea.

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Pharmacological studies on single neurones in the substantia nigra of the rat

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γ-Aminobutyric acid (GABA) is the only substance for which there is good evidence for a neurotransmitter role in the substantia nigra. The substantia nigra contains high levels of GABA (Fahn & Côté, 1968; Okada, Nitsch-Hassler, Kim, Bak & Hassler, 1971). The inhibition of nigral neurones by electrical stimulation of the descending striato-nigral pathway is blocked by intravenously administered picrotoxin (Precht & Yoshida, 1971) and these nigral neurones are also inhibited by GABA (Feltz, 1971). Crossman, Walker & Woodruff (1974a) demonstrated that iontophoretically applied picrotoxin blocked the inhibition of nigral neurones both by stimulation of the caudate nucleus and by iontophoretically applied GABA.

Feltz (1971) reported that nigral neurones were inhibited only by GABA and not by glycine, acetylcholine (ACh) or dopamine. Gulley & Smithberg (1971), however, described four different types of synaptic terminals onto neurones in the substantia nigra and tentatively suggested that these might release GABA, ACh, noradrenaline and 5-hydroxytryptamine (5-HT). In the present study, therefore, we have examined the effects on neurones in the substantia nigra of iontophoretically applied GABA, imidazole acetic acid, glycine, ACh, noradrenaline and 5-HT.

Experiments were performed on 21 female

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