lized in bands between 1·0 M and 1·4 M sucrose. However, GABA-T and MAO were predominantly localized in fractions characteristic of mitochondria.

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Application of the dansyl technique to an investigation into the identity of the primary sensory transmitter in the spinal cord

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The identity of the primary sensory transmitter in the spinal cord is still unknown, although glutamate has been suggested as a candidate (Johnson, 1972). We have now developed a fully quantitative detection technique, based on the method described by Osborne, Briel & Neuhoff (1971), which has allowed us to measure, with a sensitivity in the picomole range, amino acids, amines and other possible transmitters in the form of their fluorescent ^{3}H-dansyl (1-dimethylaminonapthalene-5-sulphonyl chloride) derivatives, in rat dorsal sensory neurones.

It is widely accepted that a substance is likely to be uniquely, or disproportionately concentrated in those presynaptic cell bodies and nerve terminals where it has a transmitter function. We have therefore made a quantitative comparison of dorsal and ventral root chemical components. Of the 32 major substances detected, three amino acids were found in significantly higher concentrations in the dorsal roots: glutamate (1·27 times ventral root) and, in much lower total amounts, threonine (1·40 times ventral root) and arginine (1·27 times ventral root). The marked disparity between dorsal and ventral root levels of glutamate confirms the findings of other workers (Duggan & Johnston, 1970; Johnson & Aprison, 1970). This evidence suggests that the higher dorsal root levels of glutamate might result from synthesis in the cell body of the neurone, and subsequent anterograde transport towards the nerve terminals in a manner analogous to that found for other transmitters (Evans & Saunders, 1967; Dahlström, 1971).

We have tested this possibility in two ways: after an 8 h ligation of dorsal roots *in vivo*, analysis of 0·5 cm sections of roots indicated that a number of dansyl-reactive substances accumulated on both sides of the ligature, suggesting that accumulation was partly due to inflammatory and other non-specific changes. However, when compared with the central section there appeared to have been a selective accumulation of glutamate, alanine and glycine peripheral to the ligature. The second method employed was to inject the dorsal root ganglia with ^{14}C-glucose (2·5 μCi) in a manner similar to that described by Lasek (1968), in the expectation that label might be rapidly incorporated into the neurotransmitter, and then transported centrally along the axon.

Although there was a slow exponential passage of radioactivity into the nerve over a 6 h period, no rapid transport of radioactivity along the axon was detected, and radioactivity was not found to accumulate on the cell body side of a crush made at the point of entry of the dorsal root into the cord. However, this latter finding is not conclusive because the rapid turnover of labelled material could have prevented the detection of a rapid transport process.

These experiments did not establish the unique existence of any substance in the dorsal root, or the rapid transport of a substance along the dorsal root, but they do provide additional evidence for the role of glutamate as the sensory transmitter.

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Responses of midbrain neurones to iontophoretically applied 5-hydroxytryptamine

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In a recent study (Aghajanian, Haigler & Bloom, 1972), it was reported that cells in the raphé system of the rat were always depressed by small amounts of 5-hydroxytryptamine (5-HT) applied iontophoretically, whereas other midbrain cells were unaffected. These findings were not in agreement with earlier findings from the same laboratory, in which it was reported that raphé neurones in cats were insensitive to iontophoretically applied 5-HT, but were generally excited by noradrenaline (Crayton & Bloom, 1969), or that a large proportion of raphé cells in rats were excited by 5-HT (Couch, 1970). In view of these conflicting reports it was decided to re-investigate the sensitivity of raphé and non-raphé cells to iontophoretically applied 5-HT.

Male albino rats were anaesthetized with chloral hydrate (350 mg/kg), and, using standard recording and microiontophoretic techniques, the responses of eighty midline midbrain cells to iontophoretically applied 5-HT (25–100 nA) were studied. The results are summarized in Table 1. The response to noradrenaline was studied and is also summarized in Table 1.

Five raphé neurones out of seven tested were depressed by lysergic acid diethylamide, in currents which did not affect twenty non-raphé neurones tested. On these latter neurones, however, excitatory responses to noradrenaline and glutamate as well as to 5-HT, were antagonized by lysergic acid diethylamide.

TABLE 1

		Responses				
		−	+	−+	0	
Raphé t recov. = 69 ± 12 s	Spont.	10	0	0	0	5-Hydroxytryptamine 25–100 nA
	Glutamate excited	3	1	0	1	
Non raphé t recov. = 18 ± 4 s	Spont.	8	7	2	6	
	Glutamate excited	25	9	0	8	
Raphé		3	0	0	1	Noradrenaline 50–100 nA
Non raphé		10	6	3	3	

t recov. (±S.E. of mean) represents the time in seconds recovery for depressions produced by 50 nA currents of 5-hydroxytryptamine.
Raphé neurones were identified by their position and characteristically slow firing rates.
Spont. = spontaneously active.
Glutamate excited with 0–40 nA currents.

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