cates that, on the average, 2000 RNA molecules bear triphosphate termini in an *E. coli* cell growing logarithmically at 37°C. The finding that the 5′-terminal nucleotide of RNA made in vivo and in vitro (*1, 2*) is limited to a purine gives support to the notion that there must exist in the genome specific sites for the attachment of RNA polymerase. The finding of two 5′-terminal nucleotides on RNA suggests there are at least two functional categories of genes, those initiated by cytosine and those by thymidine.

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3. Symbols and abbreviations: P, phosphate; X, a ribonucleotide; A, adenosine, G, guanosine; C, cytidine; U, uridine; ADP and ATP, adenosine 5′-di- and triphosphates; GTP, CTP, and UTP, guanosine, cytidine, and uridine 5′-triphosphates. The α, β, and γ positions refer to the first, second, and third (outer) phosphates of a nucleoside triphosphate. DEAE-cellulose, diethylaminoethyl-cellulose; TCA, trichloroacetic acid; tris, tris(hydroxymethyl)aminomethane.
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11. RNA polymerase was prepared by D. Kaplan according to the method of M. Chamberlin and P. Berg [*Proc. Nat. Acad. Sci. U.S.* **48**, 81 (1962).]
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18. [(2 $\times$ 10^{5} dpm in pppXp region) (3 $\times$ 10^{23} molecules/mole of phosphate)]/[(2.2 $\times$ 10^{6} dpm/μc) (10^{9} μc of ^{32}P per mole of phosphate) (1.5 $\times$ 10^{10} cells)] $\cong$ 2000 molecules pppXp per cell. We assume two of the four phosphates of pppXp are labeled rapidly (*7*).
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Complex Synaptic Configurations in Planarian Brain

Abstract. *Complex synaptic configurations which appear to have especial evolutionary and functional significance are shown in the neuropil of the brain of the planarian* Dugesia dorotocephala. *Some of the endings in these synaptic attachments contain dense core vesicles, suggesting that nonadrenaline or serotonin or both are neurotransmitters at a more primitive phyletic level than reported hitherto. The spatial proximity and connectivity of the synapses suggest modes of action permitting greater functional complexity to the planarian brain than previously supposed. Closely adjacent cellular processes which contain polysomal ribosomes, unusual in the neuropil, suggest synaptic transmission–protein synthesis coupling and a possible role in memory.*

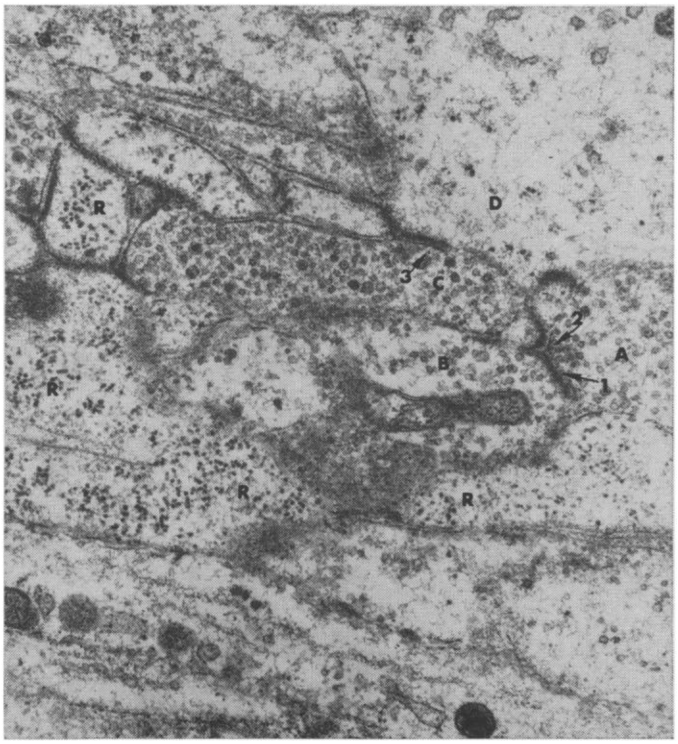
Previous studies have elaborated many aspects of the fine structure of the planarian brain (*1*) and shown that these primitive animals engage in complex and plastic patterns of behavior (*2*). Recent experiments have demonstrated that high population densities suppress fissioning in the planarian *Dugesia dorotocephala* and that this effect is mediated via the brain (*3*). It was shown in these earlier electron micrographic studies that all the synaptic junctions between the neurons of the brain occurred in the neuropil (*1*). These synapses were of two types. One involved a terminus of the presynaptic fiber onto the postsynaptic neuron. The other involved the juncture of the lateral surfaces of two more-or-less parallel neural processes. Both kinds of synapses possessed the usual characteristics of an aggregation of small, light core vesicles on the presynaptic side, a heavily staining subsynaptic web, and a sharply demarcated, lightly staining intrasynaptic space of about 150- to 200-Å thickness. These synapses seemed to involve the juncture of only two neurons and only light core synaptic vesicles (although clusters of larger electron dense neurosecretory granules were shown elsewhere in the neuropil).

In this report we describe another kind of synaptic configuration in the planarian brain that is significantly different from those described previously and in which, we believe, the particular ultrastructural morphology has important theoretical and functional implications.

The following methods were used. *Dugesia dorotocephala,* which had been maintained in the laboratory for more than a year in aged tap water, in white enameled dishpans, on a diet of fresh raw beef liver, were decapitated just behind the auricles. The heads were fixed immediately for 1 hour in a 1.0 percent solution of osmic acid buffered to *p*H 7.4 in phosphate buffer. The specimens were then embedded in Epon which was polymerized for 48 hours at 60°C. Sections were cut on an ultramicrotome with a diamond knife (Sorvall "Porter-Blum" M-2) and then stained with lead citrate and uranylacetate according to the method described by Reynolds (*4*). The sections were observed and photographed with a Philips model EM 200 electron microscope. Magnifications of 20,500 and 51,560 times were obtained in the original negatives and further enlargement was achieved photographically.

One feature worth noting is the presence of "dense core" vesicles along with the light core vesicles in the same ending of the presynaptic cell. The outside diameter of these dark core vesicles averages about 600 Å, compared to about 300 Å for the light core vesicles. There is now reasonably convincing evidence that such dense core vesicles are the vesicles of the biogenic amine neurotransmitters, such as serotonin and noradrenaline (*5*). To our knowledge no one has hitherto provided evidence which would suggest that noradrenaline or serotonin is employed as a neurotransmitter by any animal as phyletically primitive as the planarians. These observations would indicate an antiquity for such neurotransmitters in the evolution of the brain that is much greater than previously suspected.

Another curious feature is the com-



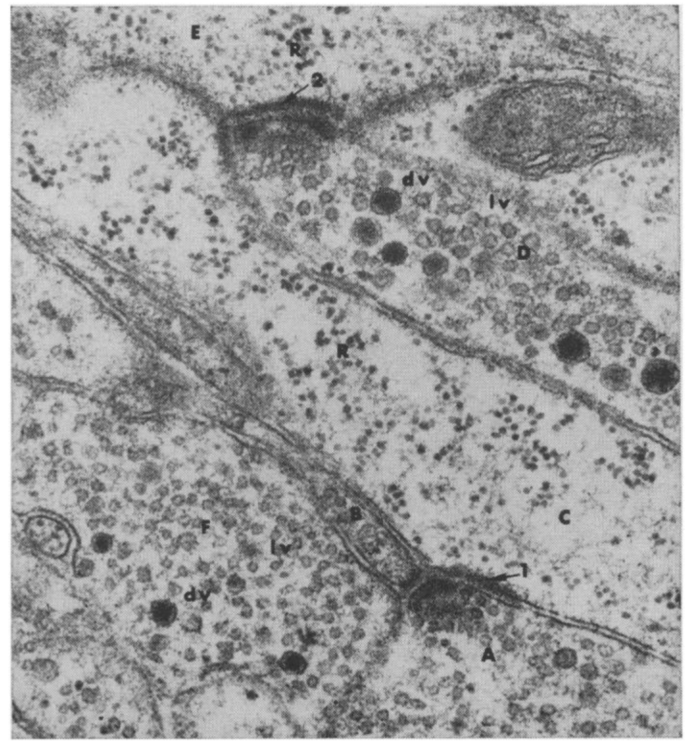


Fig. 1 (left). Electron micrograph showing complex synaptic configurations in the neuropil of the brain of the planarian *Dugesia dorotocephala*. Cell ending *A* is the presynaptic side and *B* the postsynaptic side for synapse *1; A* is also presynaptic and ending *C* postsynaptic for synapse *2,* but the synaptic clefts for *1* and *2* are joined. The same nerve fiber ending *C* is presynaptic and cell *D* postsynaptic in regard to synaptic junction *3*. Ribosomes (*R*) can be seen in some fibers. Uranyl acetate and lead citrate staining (× 25,300). Fig. 2 (right). Electron micrograph showing complex synaptic configurations and dark core vesicles in the neuropil of the brain of *Dugesia dorotocephala*. Neuronal process *A* is presynaptic for synaptic cleft *1* which is joined to cell processes *B* and *C;* neuronal process *D* is presynaptic to endings *E* and *C* across the single synaptic cleft *2*. Both light core (*lv*) and dense core (*dv*) synaptic vesicles can be observed in endings *F* and *D*. Both *C* and *E* contain ribosomes (*R*). Uranyl acetate and lead citrate staining (× 63,710).

plexity of the synaptic attachments. In Fig. 1, for example, it would appear that nerve cell ending *A* is presynaptic and *B* postsynaptic for synapse 1, *A* is presynaptic and *C* postsynaptic for synapse 2, but *C* is presynaptic and *D* postsynaptic for synapse 3. In Fig. 2 ending *A* appears to be presynaptic and *B* and *C* postsynaptic for synapse 1, with the same synaptic cleft joining all three cells. The analyses of Rall and Shepherd (*6*) in regard to similar, although not identical, synaptic configurations in higher animals show that these permit electrotonic interaction and the formation of complex functional contingencies without the necessity of excitation involving the entire neuron. By appropriate exploitation of these functional possibilities such synaptic configurations could enable an apparently limited brain, such as that of the planarian, to achieve much greater functional complexity than one might anticipate from more traditional conceptions of neural action. Thus some of the complex behaviors described previously for planarians (*2*) could be explicable on such grounds.

Yet another aspect of these synaptic configurations is the close proximity of cell processes containing ribosomes. Since these processes are in the neuropil of the brain, some distance from the cell bodies to which the Nissl substance is normally confined, it seems possible that there is a functional significance in this spatial proximity. One might conjecture that these ribosome-containing processes are concerned with protein synthesis that is intimately coupled with impulse transmission across the adjacent synapses. Bodian (*7*) has made similar conjectures in regard to endoplasmic reticulum-ribosomal systems which he has found associated with certain large boutons on primate spinal motoneurons. Since the theories currently favored to explain long-term memory envision such coupling between protein synthesis and synaptic transmission, such structural configurations are of interest as possible memory storage entities. The ribosomes in the processes displayed in the present report appear, however, to be of the polysomal type instead of membrane bound. Polysomal ribosomes are usually involved in systems in which there are shifts in the kinds of proteins synthesized, while endoplasmic reticulum-ribosomes are usually involved in the continued synthesis of a given kind of protein. It might be suggested therefore that the present system entails a synaptic modulation of the kinds of proteins synthesized, while the Bodian system would involve the turning on, or off, of the production of a specific protein.

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