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Histochemical evidence of the catecholamine-associated nervous system in certain schistosome cercariae

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Abstract. The localization of catecholamines was documented in the cercaria of *Schistosoma mansoni* and *S. japonicum* by a fluorescent histochemical method using glyoxylic acid (GA). Cell bodies and nerve fibres were spatially visualized in whole-mount preparations, and the fluorescent traces were investigated. The nervous system was bilaterally symmetrical, showing a similar formation in both species. A pair of cerebral ganglia with a transverse commissure showed a complex network of fluorophores, and each radiated two sets of anterior and posterior longitudinal nerve cords. A third pair of longitudinal nerve cords was observed in the most dorsal area. A posterior transverse commissure was seen to connect the posterior longitudinal cords, and the posterior terminals of the postero-ventral cords communicated with the tail cords. The glyoxylic acid-induced fluorescence (GAIF) method was demonstrated to be quite suitable for neuroanatomical and neurophysiological investigations of larval forms.

Certain trematodes have been demonstrated to contain acetylcholine (ACh) and biogenic amines as neurotransmitters. Bennett and Bueding (1971) reported that catecholamines (CAs) and 5-hydroxytryptamine (5-HT) were independently localized in the nervous system of adult schistosomes. The primary CAs are detected in the tissue extracts (Chou et al. 1972; Gianutsos and Bennett 1977). In the cercarial stage, however, only cholinergic esterase activity has been noted in consecutive studies (Bruckner and Voge 1974; Fripp 1967; Lewert and Hopkins 1965).

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The glyoxylic acid-induced fluorescence (GAIF) method of Lindvall and Björklund (1974) can quickly demonstrate the primary CAs through a simple procedure with high sensitivity and precision. In the present study, GAIF histochemical evidence showed that the cercariae of *Schistosoma mansoni* and *S. japonicum* developed a complex network of fluorophores, meaning that CAs localized in the nervous system.

Materials and methods

Cercariae of *S. mansoni* and *S. japonicum* were obtained from the respective snail hosts, *Biomphalaria glabrata* and *Oncomelania nosophora*, which were being maintained after experimental infection with miracidia. Based on the modified GAIF method of Sharpe and Atkinson (1980), the present histochemical procedure was executed as follows. The cercariae released naturally from the snails were immersed in 2% GA (0.1 *M* phosphate buffer, pH 7.0) at 4° C for 2 min, mounted on a glass slide and dried completely with a hair drier. The specimens were heated for 5 min in an oven at 100° C and then sealed in liquid paraffin or nonfluorescent emulsion oil. Fluorescent neuroanatomical tracings were examined with an Olympus BH-2 incident epifluorescence microscope equipped with a V (BP 405) excitatory filter.

Results

The results of the present histochemical studies are summarized in diagram form in Fig. 1. The revealed neural system is well developed and bilaterally symmetrical, with systematic communication in both the central and peripheral components. Cell bodies are large in size and distinguishable from varicosities that are formed throughout the nerve fibres.

The fluorescence is so strong and stable that identification is possible for a long time, over several minutes. The visualization that weakened after observation is completely recoverable with no spe-

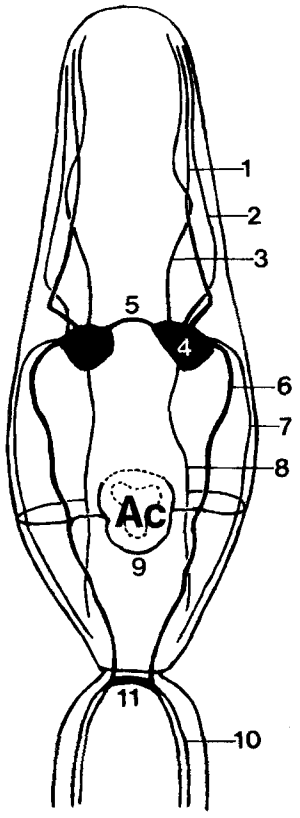


Fig. 1. Schematic pattern of the nervous system in schistosome cercariae revealed by the GAIF method. *Ac*, acetabulum; *1*, antero-ventral longitudinal nerve cord (AVC); *2*, antero-lateral longitudinal nerve cord (ALC); *3*, antero-dorsal longitudinal nerve cord (ADC); *4*, cerebral ganglion (CG); *5*, cerebral transverse commissure (CTC); *6*, postero-ventral longitudinal nerve cord (PVC); *7*, postero-lateral longitudinal nerve cord (PLC); *8*, postero-dorsal longitudinal nerve cord (PDC); *9*, posterior transverse commissure (PTC); *10*, tail lateral longitudinal nerve cord (TLC) (basal part); *11*, tail transverse commissure (TTC) (basal part)

cial treatments. As long as the specimens are preserved in a dark place, the fluorescence is condensed to yield the same localization for the following few weeks as on the initial day.

The central nervous system is clearly recognizable, located in the mid-field of the cercarial body. Cerebral ganglia (CGs) are very large, irregularly shaped and connected by a more dorsal cerebral transverse commissure (CTC) (Figs. 2, 5).

A set of two anterior and posterior longitudinal nerve cords is seen to emerge from each of the CGs. The anterior ones are identified as the antero-ventral nerve cord (AVC) (Figs. 2, 4) and the antero-lateral nerve cord (ALC) (Fig. 5) from their tracing locations. The posterior cords are demonstrated as the postero-ventral longitudinal nerve cord (PVC) (Figs. 2, 4) and the postero-lateral longitudinal nerve cord (PLC). The most prominent PVCs, evidently the thickest of all the nerve cords, are elongated via a few bipolar cell bodies. The PVCs extend along the ventro-lateral part of the cercarial body and continue posteriorly, coming in close proximity to each other. However, the PLCs are so faint and slender as to be indiscernible in the posterior part.

In the dorsal area parallel longitudinal nerve cords appear, based on cell bodies located behind the CTC. The anteriorly extending cord is designated as the antero-dorsal longitudinal nerve cord (ADC) (Fig. 6) and the posterior one, as the postero-dorsal longitudinal nerve cord (PDC). Occasionally the PDC is seen to emanate from the CG. Three pairs of anterior cords (AVC, ALC and ADC) are finally found to distribute around the oral sucker, as exactly illustrated in *S. japonicum*.

At the posterior part of the cercarial body, a transverse commissure is observed to connect the posterior longitudinal cords. This posterior transverse commissure (PTC) reveals very obvious communication between the PVCs of *S. japonicum* (Fig. 4). Lying under the ventral surface, the PTC embraces the acetabulum along its outer margin; moreover, it extends to reach the PLC and, occasionally, the PDC. However, the commissure between the two PDCs cannot be found. The dorsal area seems to be poorly innervated in comparison with the ventral area.

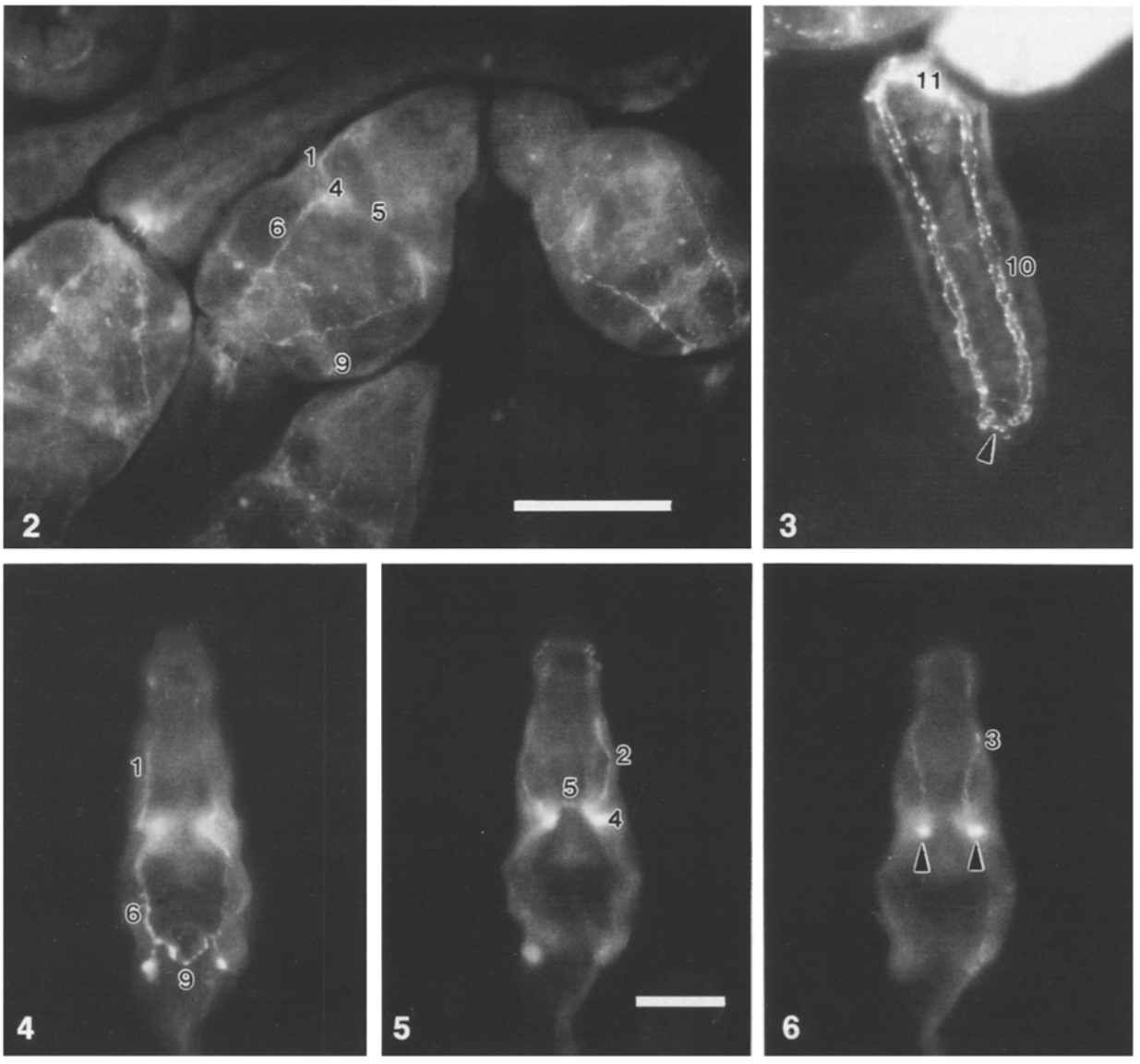
At the posterior extremity, the PVC communicates with a tail lateral longitudinal nerve cord (TLC) on each side. Each of the TLCs immediately separates, typically into two branches (Fig. 3). Some tail transverse commissures (TTCs) are found to join the TLCs, taking on a ladder-like form. Although the visualization of the TLCs and TTCs becomes faint toward the distal region, it is still recognizable as reaching the terminus of the tail (Fig. 3), and even entering the caudal forks.

The peripheral nerve tracts described above are seen to pass through subtegumental tissue, and because all of them cannot be identified in single worms, normal variations are likely to be present.

Discussion

The CA-associated nervous system was determined in the schistosome cercariae by using a modified method of the GAIF histochemistry of Lindvall and Björklund (1974). This method has been used for sections of various kinds of material and, occasionally, for small pieces, as is the case with adult helminths (Sharpe and Atkinson 1980; Tiekotter 1988). However, for small larval forms, the GA solution is sufficiently permeable for the whole body in a short time (Nakajima 1987). By this simple manner, the nervous system of schistosome cercariae could be readily studied. Because the very strong fluorescence was well preserved and completely recoverable, the localization could be investigated several times over the course of several days.

Using classic formaldehyde-induced fluorescence, the CA-associated nervous system has been studied in adult schistosomes (Bennett and Bue-



Figs. 2, 3. GAIF neural network of the *S. mansoni* cercaria. Bar = 50 μm. **Fig. 2** Cercarial body showing the (*1*) antero-ventral longitudinal nerve cord, (*4*) cerebral ganglion, (*5*) cerebral transverse commissure, (*6*) postero-ventral longitudinal nerve cord and (*9*) posterior transverse commissure. **Fig. 3** Cercarial tail showing the (*10*) tail lateral longitudinal nerve cord, comprising two branches, and (*11*) basal tail transverse commissure. The *arrowhead* denotes the terminal transverse commissure

Figs. 4–6. GAIF neural network of the *S. japonicum* cercaria in three phases of the identical specimen. Bar = 20 μm. **Fig. 4** Ventral phase showing the (*1*) antero-ventral longitudinal nerve cord, (*6*) postero-ventral longitudinal nerve cord and (*9*) posterior transverse commissure. **Fig. 5** Middle-dorsal phase showing the (*2*) antero-lateral longitudinal nerve cord, (*4*) cerebral ganglion and (*5*) cerebral transverse commissure. **Fig. 6** Most dorsal phase showing (*3*) the antero-dorsal longitudinal nerve cord. *Arrowheads* denote cell bodies

ding 1971; Machado et al. 1972). However, the GAIF method is more sensitive and yields better localization of monoamines than the former method (Björklund et al. 1972; Lindvall and Björklund 1974; Lindvall et al. 1974). The cercarial system was observed to be well developed, consisting of a complex network as shown in Fig. 1.

The major CGs, CTC and PVCs seem to trace nerve fibres reactive to acetylcholinesterase (Bruckner and Voge 1974). The general formations and patterns are similar to those of the well-developed cholinergic system recently reported in other species (Niewiadomska and Moczoń 1982). As was suggested in the adult worm (Bennett and Bueding

1971), the localization of CAs may also be parallel to that of ACh in the cercarial stage.

Transverse commissures are suspected to be poorly developed in the body part of the cercariae. The only identifiable PTC appears to be equivalent to a thick commissure that embraces the acetabulum in the adult worm (Bennett and Bueding 1971). Because no definitive difference in the GAIF localization was observed between *S. mansoni* and *S. japonicum*, the cercarial nervous system may, in fact, be identical in the two species.

Individual nerve cells and fibres predominantly contain a single transmitter, and neuropharmacological effects differ among various kinds of transmitters such as ACh, 5-HT and CAs (Mansour 1984; Smyth and Halton 1983). The activities and effects of CAs are complex in their mechanisms, resulting in the fact that different roles have been suggested (Bennett and Bueding 1971; Gianutsos and Bennett 1977; Pax et al. 1984; Tiekotter 1988; Tomosky et al. 1974).

In the cercarial stage, it is of interest that the well-developed neural network is constructed in the tail region, which assumes a major role in muscular movement and in which a poor sensory activity is involved. The tail musculature and its associated neurons have been ultrastructurally investigated (Reger 1976).

The GAIF method not only reveals the nervous system with high sensitivity and reliability but also possesses wide application (Chiba et al. 1976; König 1979; Korkala and Waris 1977). Therefore, this simple method appears to be convenient for various uses in neuroanatomical and neurophysiological investigations of larval forms.

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