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EFFECT OF DOPAMINE ON LARVAL CATECHOLAMINERGIC NEURONS AND METAMORPHOSIS OF THE SAND DOLLAR *ARACHNOIDES PLACENTA* (ECHINODERMATA: ECHINOIDEA)[1]

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Chang-Po Chen and Shu-Fen Huang (1990) Effect of dopamine on larval catecholaminergic neurons and metamorphosis of the sand dollar *Arachnoides placenta* (Echinodermata: Echinoidea). *Bull. Inst. Zool., Academia Sinica* **29**(2): 105-112. Larvae of the eight-arm, early-rudiment and advanced-rudiment stages were incubated with dopamine (0, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} M) for 24 hr. Advanced-rudiment larvae incubated with 10^{-3} M, 10^{-4} M and 10^{-5} M dopamine had an average metamorphosis rate of 3%, 53% and 66.7%, respectively. Early-rudiment larvae had a metamorphosis rate of 88% when incubated with 10^{-5} M dopamine, but none metamorphosis occurred at other concentrations. Nearly all of the eight-arm larvae died. During larval development, catecholaminergic neurons as indicated by the glyoxilic-acid-induced fluorescence increased in the rudiment and decreased in the apical neuropile, oral ganglion, epaulette and arm. Prior to settling, advanced-rudiment larvae displayed substratum-testing behaviors by contacting the substratum with the apical neuropile, while the dopamine level of the apical neuropile increased quickly and then decreased. This suggests that the apical neuropile may be the receptor for receiving environmental cues in induction of metamorphosis.

Key words: Dopamine, Larva, Metamorphosis, Neuron, Sanddollar.

Catecholaminergic neurons of echinoderm larvae can be visualized after glyoxilic acid staining, which show a blue-green fluorescence (Burke, 1983; Burke and Gibson, 1986; Chia *et al.*, 1986; Nakajima, 1987). Catecholamines include dopamine and its metabolic products, norepinephrine and epinephrine (Cooper *et al.*, 1986). The development of dopaminergic neurone can be enhanced by uptake of the external dopamine (Dietzel and Gottmann, 1988).

Larvae of the sand dollar, *Dendraster excentricus* contain catecholaminergic neurons and can be induced to metamorphose by dopamine (Burke, 1983). Metamorphosis of *D. excentricus* is controlled by the larval nervous system; apical neuropile being a stimulatory control and oral ganglion being an inhibitory control. However, the development of catecholaminergic neurons during larval development and at metamorphosis is known.

Larvae of the sand dollar, *Arachnoides*

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placenta developed quickly and became competent for metamorphosis at the seventh day (Chen and Run, 1989). In most echinoides, primary podia have been proposed containing sensory receptors to detect metamorphic factors from substratum (Burke, 1980). However, prior to settling, competent larvae of *A. placenta* use the region of the apical neuropile instead of primary podia to test the substratum. The process of metamorphosis of *A. placenta* takes several hours (unpublished observations) and is slower than that of *D. excentricus* (5 min. cf. Burke, 1983). The purposes of this study are to investigate the chronological development of catecholaminergic neurons in larvae and to demonstrate the relationship between catecholaminergic neurons and metamorphosis in *A. placenta*.

MATERIALS AND METHODS

Adult *Arachnoides placenta* were collected from Shun-sun beach, Hsin-chu, Taiwan. Spawning was induced by injection of 0.5 M KCl. The larvae were reared following the methods given by Chen and Run (1989).

(1) Effect of dopamine on larval metamorphosis

Since metamorphosis of *Arachnoides placenta* is probably induced by bacteria (Chen and Run, 1989), penicilline was used in this experiment.

Larvae of three developmental stages; eight-arm (n=20), early-rudiment (n=20) and advanced-rudiment (n=10) (Fig. 1) were incubated under a combination of 10^{-3} M penicilline and five concentrations of freshly prepared dopamine: 0 M, 10^{-3} M, 10^{-4} M, 10^{-5} M and 10^{-6} M. The number of larvae metamorphosed was recorded after 24 hr incubation. Three replicates were conducted.

(2) Development of catecholaminergic neurons

Ten larvae of each developmental stages as mentioned above were incubated with 10^{-5} M dopamine for 4 hr and then stained with glyoxilic acid. Larvae treated similarly but without dopamine were used as control. The fluorescence was observed under a fluorescent microscope (Olympus AH-Z, New Vanox, V Excitation: BP-405, Y-455). Specimen preparations are stable for repeated examination and can be stored for at least 3 weeks at room temperature (Burke and Gibson, 1986). The relative fluorescent intensity was recognized in three levele as 1 to 3, the stronger the fluorescence, the higher the number was given. In order to compare the fluorescence of neurons located at different parts of the larvae, the larval neurons

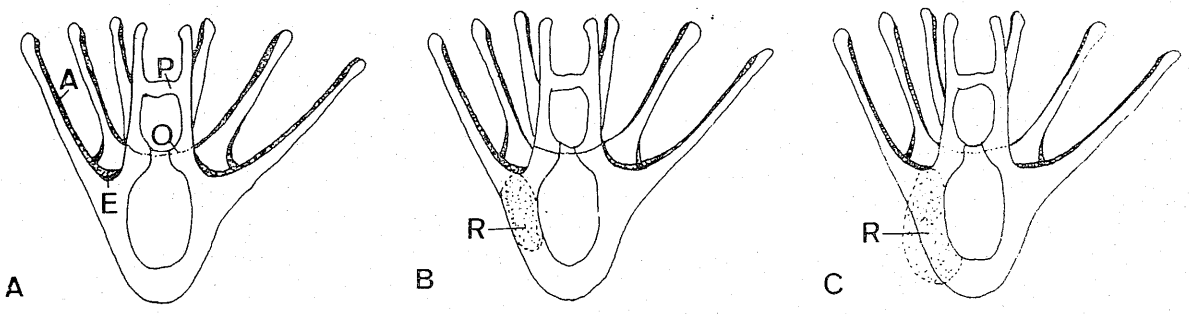

Fig. 1. Sketch drawings of *Arachnoides placenta* larvae showing dorsal view of three developmental stages. A, eight-arm: all larval arms with same length at 3.5 days. B, early rudiment: small adult rudiment at 5.5 days. C, advanced rudiment: large protrusive adult rudiment at 7 days. A: arm, E: epaulette, O: oral ganglion, P: apical neuropile, R: rudiment.

were divided into five parts: arm, apical neuropile, oral ganglion, epaulette and rudiment (Fig. 1). Chi-square test was used to detect the difference of relative fluorescent intensity between the addition of dopamine and the control in each parts of the larvae (Sokal and Rohlf, 1981).

(3) Temporal changes of catecholaminergic neurons in advanced-rudiment larvae when incubated with dopamine

About 9 advanced-rudiment larvae were incubated with 10^{-5} M dopamine for 0 hr, 0.5 hr, 1 hr, 2 hr or 4 hr, then stained with glyoxilic acid. The intensity of fluorescence of catecholaminergic neurons was recorded.

RESULTS

(1) Effect of dopamine on metamorphosis

The advanced-rudiment larvae incubated with 10^{-3} M, 10^{-4} M and 10^{-5} M dopamine showed an average rate of metamorphosis of 3%, 53% and 66.7%, respectively. Eighty-eight percent of the early-rudiment larvae incubated with 10^{-5} M dopamine metamorphosed but none of them metamorphosed when inbubated with 10^{-3} and 10^{-4} M dopamine; they were either died or severely damaged (Table 1). Nearly all of the eight-arm larvae incubated in dopamine died (but no observation with 10^{-6} M). The symptoms include: the blackening of the larval body, the retraction of epidermal tissues on the arms and the falling of the larval skeletons. Some advanced rudiment larvae incubated with 10^{-3} M dopamine had the same phenomenon as early rudiment larvae. Dopamine of 10^{-6} M concentration did not show any apparent damage to advanced-rudiment and early-rudiment larvae.

(2) Catecholaminergic neurons

Catecholaminergic neurons occurred in the apical neuropile, arm, epaulette, oral ganglion and rudiment of the larva (Fig. 2). Neurons appeared like a thread as in the larval arm (Fig. 2A) or a pile of bead net as in the apical neuropile (Fig. 2B) and oral ganglion (Fig. 2D). Some neurons are related to the body movement such as in the larval arms, epaulette (Fig. 2C), and rudimental spines (Fig. 2E).

The fluorescence of catecholaminergic neurons of the eight-arm larvae incubated with dopamine did not differ significantly from that of the control. After dopamine incubation, fluorescence of neurons increased significantly at the arm of both early and advanced rudiment larvae, decreased significantly at the oral ganglion of advanced rudiment larvae, and

Table 1
Average percentage (%) of larval metamorphosis of the sand dollar, *Arachnoides placenta* after dopamine incubation (n=3). Media contain 10^{-3} M penicilline to inhibit bacteria-induced metamorphosis

Concentration of dopamin (M)	Developmental stages		
	Eight-arm	Early-rudiment	Advanced-rudiment
0	0	0	0
10^{-3}	0	0	3.0± 4.7
10^{-4}	0	0	53.0±20.5
10^{-5}	0	88.0±10.3	66.7±12.5
10^{-6}	—	0	0

—: no data

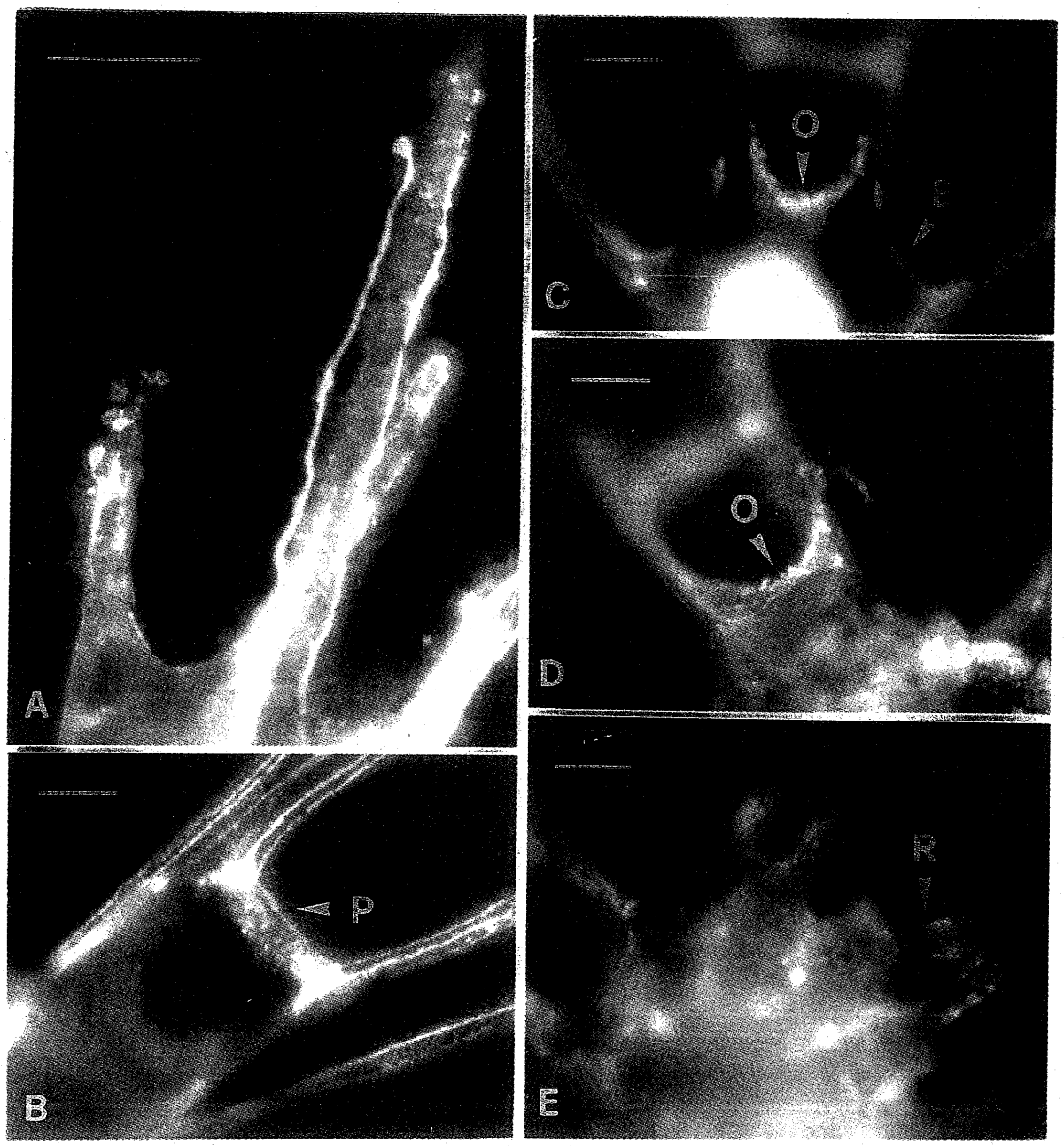

Fig. 2. Fluorescence of larval neurons. A: larval arms are histolyzed and the neurons are exposed, B: apical neuropile (P), C: oral ganglion (O) and neurons of epaulette (E), D: oral ganglion (O), E: neurons of rudiment juvenile (R), Bar=50 μm.

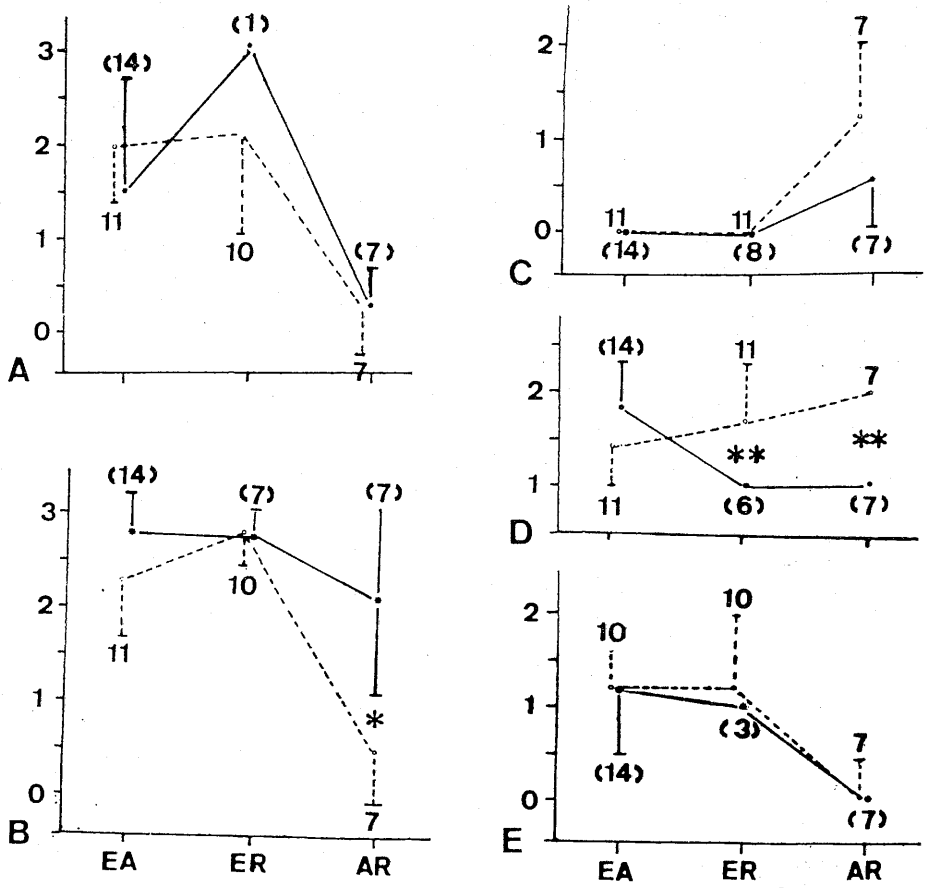

Fig. 3. Fluorescent intensity of neurons on three developmental stages of larvae incubated with (dotted line) and without (solid line) dopamine. EA: eight-arm, ER: early-rudiment, AR: advanced-rudiment, A: apical neuropile, B: oral ganglion, C: rudiment, D: arm, E: epaulette. Mean, S. D. and numbers of larvae examined are given. The mark of * and ** indicates significant difference at 0.5 and 0.01 level, respectively.

did not differ significantly at other parts of early and advanced larvae (Fig. 3). One advanced rudiment larva metamorphosed within 4 hr dopamine incubation.

The developmental tendency of the catecholaminergic neurons from eight-arm larvae toward advanced-rudiment larvae was a gradual increase at the rudiment and decrease at the apical neuropile, oral ganglion, epaulette and arm. Larvae incubated with dopamine, had similar developmental tendency, except of their arms of which the fluorescent intensity increased from eight-arm larvae toward advanced-rudiment larvae (Fig. 3).

(3) Temporal changes of catecholaminergic neurons in advanced-rudiment larvae when incubated with dopamine

Fluorescence of neurons at the apical neuropile of advanced-rudiment larvae increased to a peak at 0.25 hr, then attenuated and vanished at 4 hr (Fig. 4). Fluorescence of neurons of rudiment increased gradually, reached a peak at 1 hr, and then sustained on a stable level. The fluorescent intensity in oral ganglion and epaulette decreased gradually and vanished finally. The tissues of larval arms were histolyzed first (Fig. 2A),

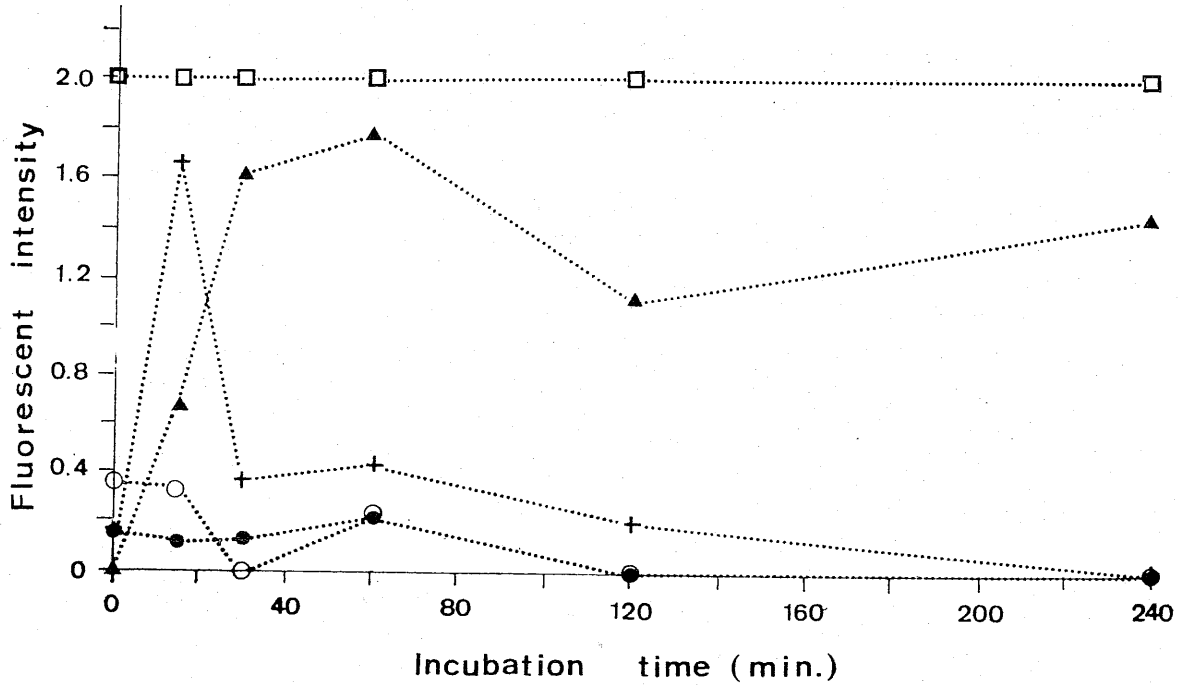

Fig. 4. Temporal changes of catecholaminergic neurons in advanced-rudiment larvae when incubated with dopamine. +: apical neuropile, □: arm, ○: epaulette, ●: oral ganglion, ▲: rudiment.

Table 2
Temporal changes of catecholaminergic neurons of advanced-rudiment larvae when incubated with dopamine. Mean and standard deviation are given

	Incubated times (min.)					
	0 (n=8)	15 (n=9)	30 (n=8)	60 (n=9)	120 (n=10)	240 (n=9)
Arm	2.0±0.0	2.0±0.0	2.0±0.0	2.0±0.0	2.0±0.0	2.0±0.0
Apical neuropile	0.1±0.4	1.7±0.5	0.4±0.5	0.4±0.5	0.2±0.4	0
Oral ganglion	0.2±0.5	0.1±0.3	0.1±0.4	0.2±0.4	0	0
Epaulette	0.4±0.5	0.3±0.5	0	0.2±0.4	0	0
Rudiment	0	0.7±0.5	1.6±0.7	1.8±0.4	1.1±0.9	1.4±0.7

exposed the neurons directly to external dopamine and increased the fluorescent intensity (Table 2 and Fig. 4).

DISCUSSION

Dopamine apparently can induce metamorphosis in the sand dollar, *Arachnoides placenta*, at the concentration of 10^{-5} M, but it is lethal at higher concentrations. Earlier larvae are more sensitive to the lethal effect than advanced ones. Histolysis and retreatment of larval epidermis and other larval tissues are critical responses during metamorphosis (Cameron and Hinegardner, 1974; Chia and Burke, 1978; Burke, 1983). External addition of dopamine can trigger histolysis of larval tissues (Burke, 1983; present study). Therefore, the

dopamine-induced histolysis may cause the death of larvae which are not competent for metamorphosis.

However, catecholamine and dopamine are subject to oxidation, and they polymerize in solution to form heterogenous polymeric melanin pigments (Lindner and Dooley, 1976). The blackening of larval tissues is caused by oxidation products, but not by dopamine *per se*. The oxidation products is toxic to larvae of the tube building polychaetes, *Phragmatopoma lapidosa californica* (Pawlik, 1988). Therefore, the explanation of the cause of death of larvae *Arachnoides plancenta* should be cautious.

Dopamine but not epinephrine, is able to induce larval metamorphosis of the sand dollar, *Dendraster excentricus* (Burke, 1983) when added to external medium. Dopamine induces larval metamorphosis of the sand dollar, *Arachnoides placenta* and enhances the development of some larval catecholaminergic neurons. These data suggest that the catecholaminergic neurons of sand dollars contain a significant amount of dopamine which is involved in metamorphosis.

The apical neuropile has been proposed as a stimulatory control in larval metamorphosis of the sand dollar, *Dendraster excentricus* (Burke, 1983). During incubation, dopamine level at the apical neuropile of advanced rudiment larvae of *Arachnoides placenta* increases quickly to a peak then decreases immediately, indicating that dopamine has been uptaken and released. After the release of dopamine from apical neuropile, catecholaminergic neurons of rudiment increase greatly, suggesting that apical neuropile is the key receptor in triggering larval metamorphosis of the sand dollars.

In most echinoides, primary podia have been proposed containing sensory receptors to detect metamorphic factors from substratum (Burke, 1980). However, prior to settling, competent larvae of the sand dollar *Arachnoides placenta* display specific substratum-testing behaviors: the area of larval apical neuropile was downward and pull out and back occasionally (personal observation). This suggests that the apical neuropile is the first place to detect the environmental cue in the substratum. This cue may relate to bacteria film (Chen and Run, 1989). Thus, the apical neuropile of the larvae of the sand dollar, *Arachnoides placenta* may have two important functions, i. e., detecting the metamorphosing signals and stimulating metamorphosis.

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Dopamine 對海錢 *Arachnoides placenta* 幼生 Catecholaminergic 神經元及變態之作用

陳 章 波　　黃 淑 芬

海錢幼生在八腕期、原基早期及原基晚期的階段經 10^{-2}～10^{-6} M dopamine 浸泡 24 小時後，原基晚期幼生在 10^{-3}、10^{-4}、10^{-5} M 之下變態率分別爲 3%、53% 及 66.7%；原基早期者在 10^{-5} M 下變態率爲88%，但其他濃度下沒有變態；八腕期幼生則幾乎全部死亡。以 Glyoxylic acid 螢光染色法分析 Catecholaminergic 神經元在幼生之發育，發現 Dopamine 量在原基體逐漸增加，而在頂端神經堆 (Apical neuropile)，口神經結 (Oral ganglion)，肩帶 (Epaulette) 及腕則減少。原基晚期幼生附著前先以頂端神經堆碰觸底層以測試之，又頂端神經堆的 Dopamine 量有快速增加再減少之現象，顯示頂端神經堆可能爲環境中變態誘因的接受者。