Readout Mechanisms for the Optically Activated Skin Camouflage Reactions of *Ambystoma* Larvae

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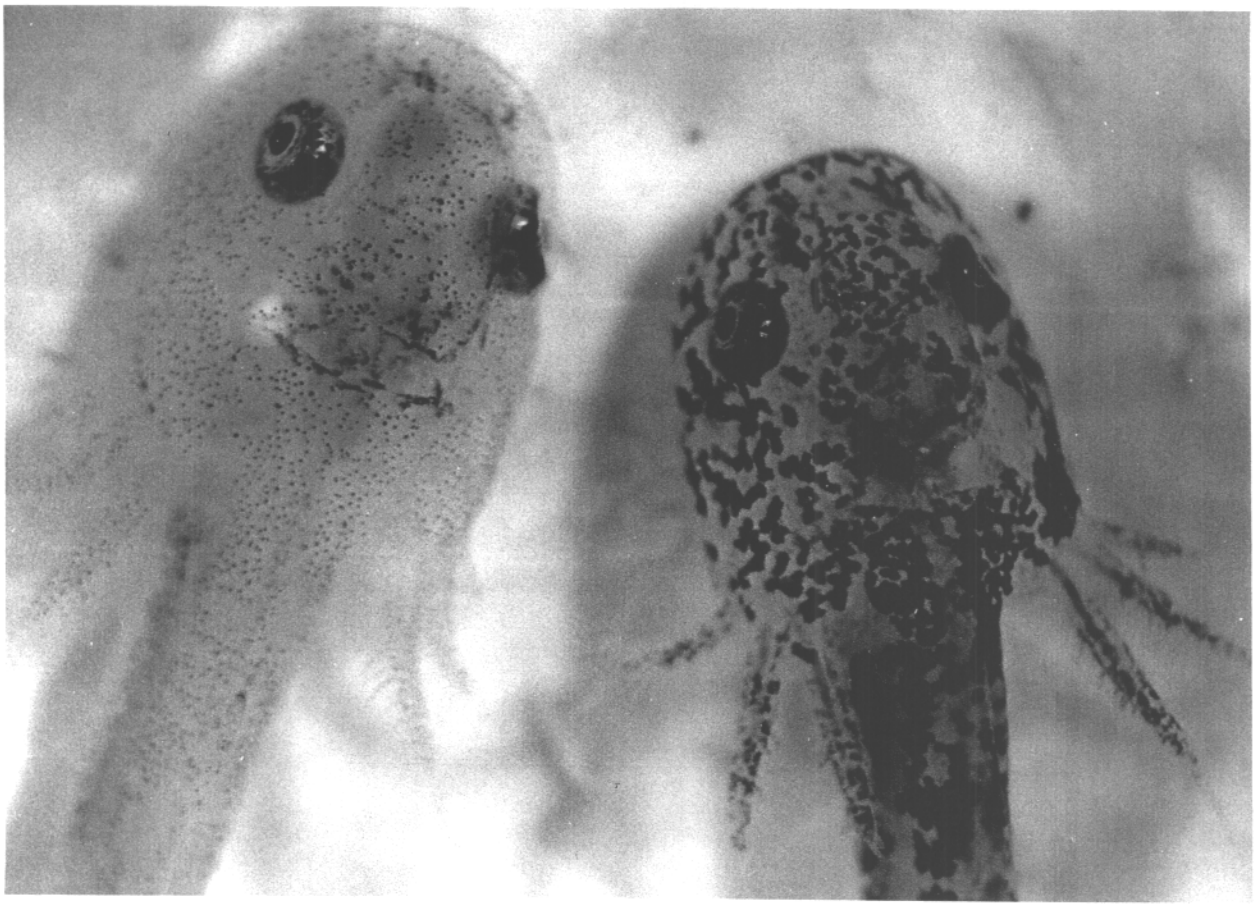
Introduction. Few wild species of *Ambystoma* would survive larvahood without the ability to adapt their skin's reflectance to changes in the immediate photic background. The adaptations afford camouflage from alert and agile predatory coinhabitants of their native environment. In the laboratory, the animals typically blanch in white cups but progressively darken when transferred to a black receptacle, eventually becoming all but visibly undetectable. At the beginning of the century Henry Laurens (1914, 1916, 1917) demonstrated that these camouflage reactions are evoked via the eyes. We have been exploiting his discovery for analysing the *Ambystoma* visual system and as a non-invasive, behavior-independent measure of the functional incorporation into the host site of grafted larval eyes (Pietsch and Schneider, 1985, 1988, 1990). Although our preoccupation has been primarily with the sensory and associations sides of the reaction, some of our experiments have dealt parenthetically with output. In the present communication, we turn our attention to the readout for the system, principally to experiments illustrating the generally but less than universally held belief (*ibid.* for references) that the hypophysis (presumably through release of MSH by the pars intermedia) is essential for the darkening phase of the reaction.

Definitions and Usage. The accompanying photomacrograph of two *A. tigrinum* larvae shows what we mean by blanching or darkening. The spots, which contract into puncta during blanching and progressively expand in darkening, are aggregations of melanosomes within dermal melanophores. (see Bagnara and Hadley for general treatise on pigmentation.) Epidermal melanophores, which can react to nonvisually absorbed radiation—with considerable individual and strain variation—to fine-tune an animal's apparent coloration, are not part of the reaction under consideration. Whether the melanosomes move into and out of permanently fixed ultramicroscopic extensions of the melanophore's cytoplasm or, alternatively, whether the cells themselves expand and contract is problematical for *Ambystoma*. Therefore, although our endpoints are the melanophores directly, we provisionally apply the term "spot" rather than "cell" to the effectors.

Blocking the Dark Phase of the Reaction. In one series, the principal subjects were 28 mm (and therefore very young) *A. tigrinum* larvae collected in Tennessee by Charles Sullivan and received at Harrison stage 24 in a jelly mass fortuitously large enough to permit the entire series to be conducted exclusively with siblings. All nociceptive procedures were carried out under MS 222 narcosis, and stock not used were returned to nature. As stock, animals were maintained on 12-hour cycles of light and dark but during the experiments (of short-term duration) were kept in continual fluorescent illumination of approximately 1400 lux, in a light chamber described in detail elsewhere (Pietsch and Schneider, 1985). Experimental subjects were kept in black plastic utility pans with luminances in the light chamber of approximately 5 nits; *i.e.*, stimulus conditions for maximal darkening *(ibid.)*. Controls for lighting included groups of larvae maintained throughout in either black or white (360 nits) pans. The test subjects were initially divided into three groups which, using the map shown in the accompanying drawing (from Pietsch and Schneider, 1985), were subjected to one of the following procedures: 1) extirpation of region B (B-less subjects); 2) hypophysectomy; 3) unoperated. Immediately after surgery animals were placed in black pans.

Within a few hours, during which the unoperated animals darkened, all B-less and hypophysectomized subjects had blanched. On the following day, using previously unoperated donors, a third of the B-less animals received a transplant of a freshly excised region B; another third received an amputated hypophysis, implanted into the still-patent mouth of the IVth ventricle; the status quo was maintained for the remaining third, now



constituting a B-less control group.

Four hours later, all recipients of either region B or the hypophysis alone had darkened. The B-less controls remained blanched.

The experiment under consideration was extended as follows. On the subsequent morning, region B was transferred back to the void in the cranium of its original donor, the latter animals having blanched in the interim. By evening these original donors were dark while the transitory hosts had blanched. The exchange was repeated among half the subjects. By morning the pigment patterns had again reversed while the unexchanged animals showed no changes. The animals in the accompanying photograph were subjects of the re-reversal experiment.

Forebrain Flipping. Another series was performed in which young (30 mm) *A. mexicanum* larvae were the subjects. Hypophysectomy (from inside the oral cavity) causes these animals to blanch. However, when the animals' detached hypophyses, instead of being discarded, were inserted into the brain ventricle, the animals darkened.

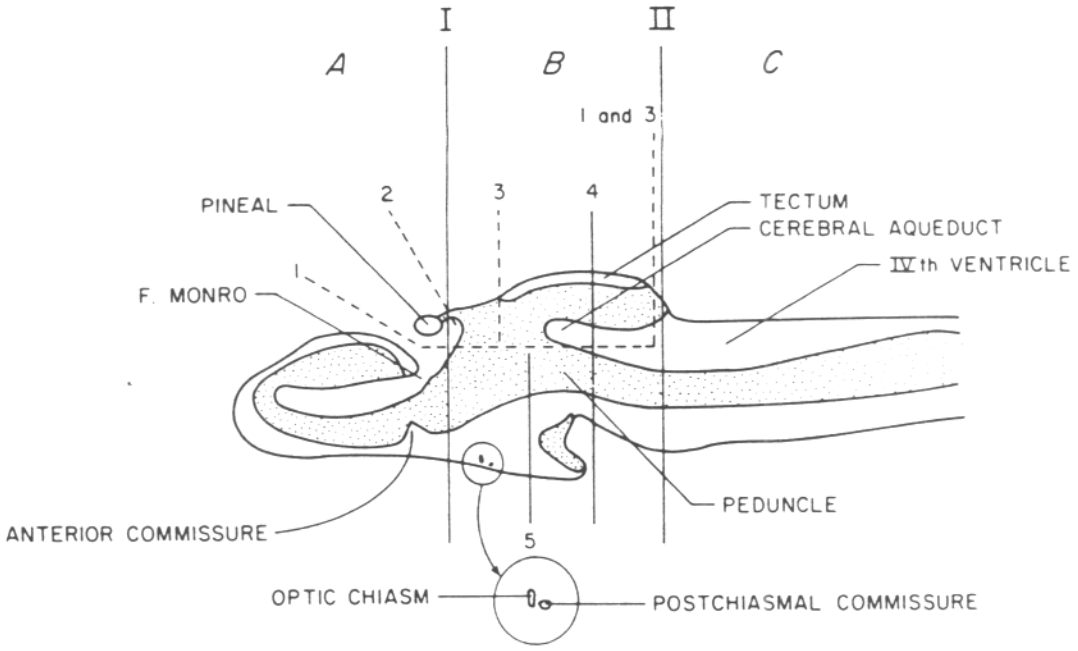
When we removed either region B or region AB as a unit (including the hypophysis), the subjects blanched. Returning B or AB reinstated darkening. After the return of A alone the animals remained blanched.

Most *Ambystoma* larvae, including bilaterally enucleated animals, blanch if kept in total darkness (<0.5 lux) for several days. Although the variables arising from the latter were well controlled in the experiments described, an exception to the rule permitted direct experimental elimination of any doubts: Genetically eyeless mutants (e/e) remain darkened even in total darkness (see Epp, 1972). Accordingly, experiments were conducted with young, genetically eyeless mutant *A. mexicanum* larvae as the subjects. With B ablated, the animals blanched; unoperated controls remained darkened. Darkening recovered when B was replaced, whether orthotopic or ventral surface facing up.

Extending the latter experiments, a lighting apparatus was constructed with all illumination directed from below rather than above. AB was the object of attention, (since it was easier to manipulate surgically than the hypo-

physis or B alone). AB was reimplanted right side up in some animals and upside down in others. The subjects darkened equally well whether AB faced up or down. When the subjects were then transferred to the standard light chamber (thus re-reversing the direction of the light source) no changes occurred in darkening.

Blanching of B-Less Subjects as Spastic Paralysis. The subtotally decapitated body can live for extended periods (Pietsch, 1981) and, as might be suspected from the absence of the hypophysis, generally does so in a permanently blanched state. However, when we denervated specific dermatomes in brainless *A. tigrinum* or *A. punctatum*, in some cases unilaterally, within 15 minutes the affected territory darkened, while the still-innervated neighboring or contralateral half dermatomes remained blanched. Localized cordectomy of debrained subjects failed to alleviate blanching, whereas unilateral sympathectomy was followed in 15 minutes by darkening. It would appear that the de facto removal of the hypophysis, thus inducing permanent blanching, establishes a condition comparable to spastic sympathetic paralysis, whereas either sympathectomy or denervation of a dermatome would seem to set up the equivalence of flaccid paralysis. In this connection, the camouflage reaction seems very similar to autonomic paresis in general, preganglionic lesions producing spasticity of the effector and postganglionic lesions causing flaccidity. The neurological strategy of the system would appear to be to use blood borne MSH to bring about a net inhibition of postganglionic sympathetics output to the dermatomes.



Semidiagrammatic map of the *Ambystoma* larva's brain in parasagittal section (after Pietsch and Schneider, 1985).

Acknowledgment

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Literature Cited

Bagnara, J. T., and M. E. Hadley. 1973. *Chromatophores and Color Changes*, Prentice-Hall, Englewood Cliffs.

Epp, L. G. 1972. Development of pigmentation in the eyeless mutant of the Mexican axolotl, *Ambystoma mexicanum*, Shaw. J. Exp. Zool. *181*:169-180

Laurens, H. 1914. The reactions of normal and eyeless amphibian larvae to light. J. Exp. Zool. *16*:195-210.

Laurens, H. 1916. The reactions of melanophores of *Amblystoma* larvae—the supposed influence of the pineal organ. J. Exp. Zool. *20*:237-261.

Laurens, H. 1917. The reactions of *Amblystoma tigrinum* larvae to light and darkness. J. Exp. Zool. *23*:195-205.

Pietsch, P., and C. W. Schneider. 1985. Vision and the skin camouflage reactions of *Ambystoma* larvae: the effects of eye transplants and brain lesions. Brain Res. *340*:37-60.

Pietsch, P., and C. W. Schneider. 1988. Transplanted eyes of foreign donors can reinstate the optically activated skin camouflage reactions in bilaterally enucleated salamanders (*Ambystoma*). Brain, Behav. and Evol. *32*:364-370.

Pietsch, P., and C. W. Schneider. 1990. Two-eyed versus one-eyed salamanders: does binocularity enhance the optically evoked skin blanching reactions of *Ambystoma* larvae? Physiol. Behav. *48*: 357-359.