# ROLE OF PREY-CAPTURE EXPERIENCE IN THE DEVELOPMENT OF THE ESCAPE RESPONSE IN THE SQUID *LOLIGO OPALESCENS*: A PHYSIOLOGICAL CORRELATE IN AN IDENTIFIED NEURON

THOMAS PREUSS*,‡ AND WILLIAM F. GILLY

*Hopkins Marine Station of Stanford University, Department of Biological Sciences, 120 Ocean View Boulevard, Pacific Grove, CA 93950, USA*

‡Present address: Department of Neuroscience, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA
*e-mail: tpreuss@aecom.yu.edu

## Summary

Although extensively used for biophysical studies, the squid giant axon system remains largely unexplored in regard to *in vivo* function and modulation in any biologically relevant context. Here we show that successful establishment of the recruitment pattern for the giant axon in the escape response elicited by a brief electrical stimulus depends on prey-capture experience early in life. Juvenile squid fed only slow-moving, easy-to-capture prey items (*Artemia salina*) develop deficits in coordinating activity in the giant axon system with that of a parallel set of non-giant motor axons during escape responses. These deficits are absent in cohorts fed fast-moving, challenging prey items (copepods). These results suggest that the acquisition of inhibitory control over the giant axon system is experience-dependent and that both prey-capture and escape behavior depend on this control.

Key words: giant axon, plasticity, ontogeny, behaviour, motor skill, learning, environmental complexity, motor pattern, squid, *Loligo opalescens*.

## Introduction

The control of complex types of behavior by the central nervous system involves recruiting an appropriate set of motoneurons with a firing pattern that depends on the underlying situational context. Although there has been much recent progress in understanding the mechanisms underlying motor control (Katz, 1996), considerably less is known about how motoneuron recruitment evolves in conjunction with the natural emergence of adaptive behavior patterns. Such emergent behavior patterns have been difficult to study because they are complex products of the interactions between an organism and those environmental features that ultimately define situational context (Chiel and Beer, 1997). In the present paper, we examine the maturation of the firing pattern of an identified giant motoneuron in conjunction with the ontogeny of two natural behavior patterns.

Squid giant axons innervate extensive fields of circular muscle fibers, and a single axonal impulse generates a powerful all-or-none muscle contraction that expels sea water from the mantle cavity (Young, 1938). Parallel non-giant motor axons can generate equally strong contractions, but only with repetitive firing (Prosser and Young, 1937; Wilson, 1960; Gilly et al., 1996). During escape responses, adult squid display two distinct recruitment patterns for the giant axons (Otis and Gilly, 1990). Sudden visual (flash) stimuli produce a stereotyped, short-latency startle response driven by a single giant axon spike. In contrast, superficial electrical stimuli produce more complex escape jets characterized by a concerted recruitment of non-giant and giant axons. In this case, the giant axon fires 50–100 ms after the onset of an intense burst of non-giant activity. Flexibility in the choice of recruitment pattern thus allows the giant axon to act either as the commanding element in the startle response or as an optional booster to an escape jet initiated by non-giant axons.

Although both giant and non-giant motor systems are functional at birth (Martin, 1965; Martin and Rungger, 1966; Marthy, 1987; Preuss et al., 1997), concerted recruitment of the two systems was not evident on the basis of recordings of stellar nerve activity during stimulated escape responses in embryos and hatchlings (Gilly et al., 1991). In the present study, we have employed improved recording techniques and describe how concerted recruitment emerges during late embryogenesis and becomes firmly established over the first few weeks post-hatching. We also demonstrate that the stable acquisition of concerted recruitment can be disrupted in animals whose environment does not demand skillful prey-capture behavior. Our results suggest that the development of inhibitory control over the giant axon system, essential for concerted recruitment in escape responses, is intimately linked with the ontogeny of prey-capture in an experience-dependent manner.

## Materials and methods
### Animals

Adult *Loligo opalescens* Berry were collected in Monterey Bay, California, USA, and allowed to spawn in circular tanks (2.5 m×1 m) supplied with flow-through natural sea water (13–16 °C). Five to ten cases of fertilized eggs were transferred into circular culture tanks (320 l) supplied with flow-through, filtered (10 µm) sea water. All eggs were removed 2–3 days after natural hatching commenced, and the day of most significant hatching activity during this period was defined as day 1 post-hatching. Squid were reared on two feeding schedules. One group received an *ad libitum* diet of live marine plankton, primarily copepods mixed with brine shrimp nauplii (*Artemia salina*) that were enriched with lipids, fatty acids and vitamins, prepared and provided by the Monterey Bay Aquarium (Preuss et al., 1997). The second group received only enriched *Artemia salina*. All experiments and procedures followed Universities Federation for Animal Welfare guidelines (Boyle, 1991) and Stanford University Institutional Animal Care guidelines.

### Electrophysiological recordings

Extracellular recordings of stellar nerve activity were carried out using modifications of methods described previously (Otis and Gilly, 1990). Animals were anesthetized in 0.4 % urethane in sea water, and the dorsal or ventral mantle surface was attached using cyanoacrylate cement to a 50 µm quartz capillary carried on a micromanipulator. A small hole was made in the mantle immediately over the stellate ganglion, and a polyethylene suction electrode was inserted for recording extracellular *en passant* activity from the main portion of the ganglion in embryonic and hatchling squid or from individual stellar nerves in larger juveniles. Recordings were amplified with an alternating-current-coupled preamplifier (DP-301, Warner Instruments, Hamden, CT, USA) and digitized on-line at 50 kHz using a laboratory computer and either a commercial (Digidata 1200, Axon Instruments, Foster City, CA, USA) or custom-built (D. R. Matteson, University of Maryland, Baltimore, MD, USA) interface. High- and low-pass filter frequencies ranged between 10 Hz and 3 kHz, respectively. Recordings were also sampled continuously through the audio input of a Hi-8 video recorder (Sony EVO-9700) and stored on Hi-8 tapes.

Flash stimuli were delivered using a camera strobe triggered by the computer used for data acquisition. Electrical stimuli were delivered from a stimulator (Grass Instruments, SD9, Quincy, MA, USA) through a coaxial, bipolar electrode (SNE-100, Rhodes Medical Instruments, Woodland Hills, CA, USA) and applied to the oral area between the arms. In the course of an experiment, a threshold stimulus for eliciting escape jetting in response to electrical stimulation was first identified. The stimulus strength was then increased and decreased arbitrarily around this value to elicit escape jets in which the giant axon was involved. Stimuli were delivered at intervals of at least 2 min. Experiments were conducted for a maximum of 2 h, after which the animals were anesthetized and decapitated.

### Mantle kinematics

A magnified lateral view of the animal was filmed continuously (Sony SSC-M374 camera; 30 frames s$^{-1}$), and these data were stored on the Hi-8 video tapes together with the electrical recordings (see above). Video sequences of escape responses were digitized at 60 Hz (LR-3 capture card, Scion Corporation, Frederick, MD, USA) and stored on a magneto-optical drive (Tahoe 230, Pinnacle Micro, Irvine, CA, USA). Mantle diameter was measured at a point one-third of the distance from the anterior end of the mantle in successive video images using NIH-Image 1.61 software. Percentage mantle contraction during an escape response was calculated from the fractional change in diameter as $[1-d(t)/d(0)]\times100$, where $d(t)$ is diameter at time $t$ and $d(0)$ is the mean diameter computed from the four time points immediately before the stimulus.

## Results
### Normal development of recruitment pattern

Neural recordings of the motor discharge from the stellate ganglion in developing squid reveal several ways in which the giant axons contribute to escape reactions. At all developmental stages, including the adult, flash stimuli produce a short-latency (40–50 ms) startle response characterized by a giant axon spike that precedes a short burst of summed non-giant axon activity (Fig. 1). This response is all-or-nothing, and the discharge pattern is invariant.

Electrical stimuli, however, reveal plasticity in the contribution of giant axons to the escape response. Typically, during the first 2 weeks post-hatching, brief electrical stimuli lead to complex, multi-cycle escape responses and variability in giant axon recruitment. This variability can be broken down into different temporal combinations of the two patterns discussed in the Introduction. The +/− pattern is defined by a short-latency firing of the giant axon in the first jet cycle (cycle 1) (similar to the startle response) followed by several cycles driven only by non-giant activity (Fig. 2A). The +/+ pattern also shows short-latency firing of giant axons in cycle 1, but displays concerted recruitment of non-giant and giant axons in at least one later cycle (Fig. 2B). Finally, the −/+ pattern shows no giant axon activity in cycle 1 and concerted recruitment of non-giant and giant axons in at least one later cycle (Fig. 2C). The −/+ pattern most closely resembles that in adults; however, adults show no short-latency behavioral responses to electrical stimuli, i.e. the first (−) cycle (Otis and Gilly, 1990).

Variability in recruitment of the giant axon system appears to be an inherent property of the response early in development and does not reflect stimulus quality. Thus, in an individual animal, a relatively constant electrical stimulus generally produces a variety of recruitment pattern types from trial to trial (Fig. 2), and the overall probability of any individual pattern tends to be highly variable (see below). Furthermore, no overall correlation appears to exist between stimulus strength and response type, nor does the response type change
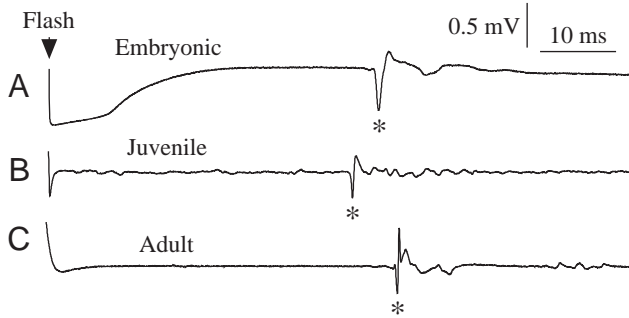
Fig. 1. Stellar nerve motor activity during a startle response caused by a sudden visual stimulus. A strobe flash elicits a stereotyped, short-latency recruitment of the giant axon (* indicates a giant axon spike). This startle response appears first in late-stage embryos (A) and remains essentially unchanged throughout development (B, 13-day-old juvenile; C, adult).
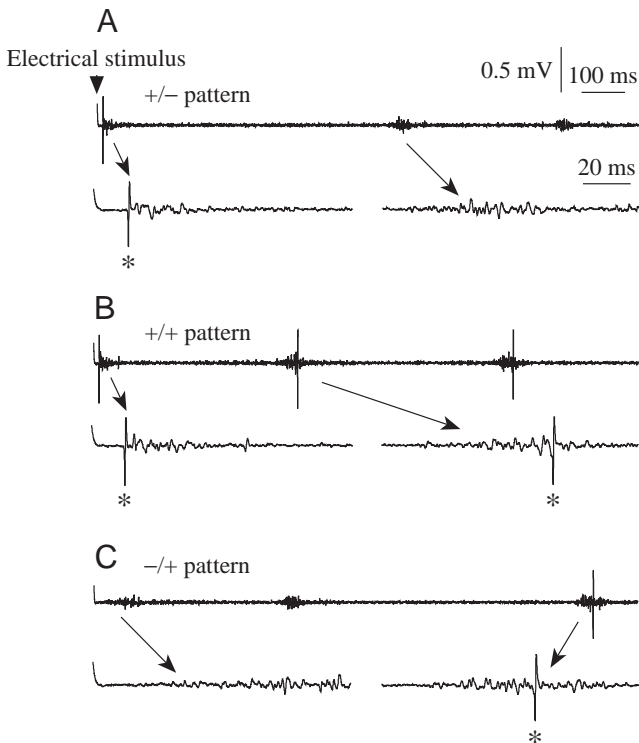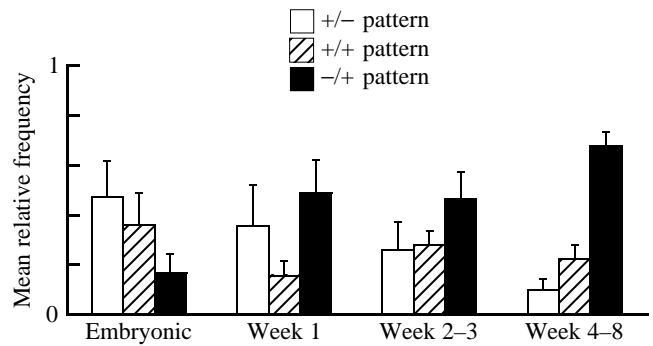


Fig. 3. Maturation of the giant axon recruitment pattern in copepod-fed squid. Data were collected and analyzed for embryonic squid (five squid; $N=42$) and for 1-week-old (nine squid; $N=138$), 2- to 3-week-old (nine squid; $N=130$) and 4- to 8-week-old (eight squid; $N=154$) juveniles. Variability of giant axon recruitment pattern exists in all groups, but the −/+ pattern (see Fig. 2), which is most similar to the pattern in adult squid, is rare in embryonic squid and predominant by 4–8 weeks. Values are means + S.E.M.; $N$ is the number of responses counted. Differences in +/− *versus* −/+ frequencies are significant ($P<0.05$ by *t*-test) only for embryonic and week 4–8 groupings.



Fig. 2. Complex multi-cycle escape responses to electrical stimuli in an 11-day-old squid. The recruitment pattern of the giant axon (* indicates a giant axon spike) in response to a constant stimulus (36 V; 0. 4 ms) shows a high degree of variability. Three prominent recruitment patterns emerge (A) +/−, (B) +/+ and (C) −/+, as described in detail in the text. In each set of traces, the upper trace is a continuous recording of three jet cycles following the stimulus. The lower trace shows selected regions (arrows) of this recording on an expanded time scale.

consistently during the course of an experiment (data not illustrated).

To assay this type of variability in giant axon recruitment and to characterize the maturation of the firing pattern, similar

experiments were carried out on developing animals from just before hatching to 8 weeks post-hatching. Electrical stimuli were used, and only responses in which giant axons participated were scored. Individual relative frequencies were computed for the three defined types of recruitment pattern observed in each animal, and mean values for four different age classes are plotted in Fig. 3. The predominant pattern in late-stage embryos is the +/− pattern. Although concerted recruitment of giant and non-giant activity is not uncommon (the +/+ pattern), the −/+ adult-like pattern occurs infrequently. In contrast, within 1 week of hatching, short-latency activation of giant axons (i.e. +/− and +/+ patterns) becomes considerably less frequent, and more complex escape jets, showing −/+ firing, become much more prominent. In juveniles (4–8 weeks old), the adult-like −/+ pattern predominates.

### Concerted recruitment and motor performance

In adult squid, concerted recruitment of giant and non-giant motor axons acts to boost the intra-mantle pressure transient that powers the jet escape (Otis and Gilly, 1990). To determine whether this boosting effect also occurs early in development when concerted recruitment is still maturing, we analyzed the mantle kinematics of hatchling and juvenile squid during escape jets of the type described above. The data in Fig. 4 indicate that concerted recruitment does boost motor performance in both hatchlings and juveniles. Mantle contraction due to short-latency firing of giant axons in the first cycle of an escape response was always weaker than that accompanying concerted recruitment in later cycles in a 46-day-old squid (cycle 1 *versus* cycle 3 in Fig. 4A). Repetitive firing of the giant axons during concerted activity produces an even larger boost (Fig. 4B). The data in Fig. 4C compare the peak contraction values in this animal (filled columns) and also

Fig. 4. Concerted recruitment of giant and non-giant axons boosts motor-performance. (A,B) Stellar nerve recordings (upper panels) and corresponding mantle contractions (lower panels) from a multi-cycle (+/+) escape response of a 46-day-old squid. (A) Short-latency recruitment of giant axons in cycle 1 produces smaller mantle contractions than concerted recruitment of giant and non-giant axons in cycle 3. (B) Repetitive giant axon firing during concerted recruitment (* indicates three giant axon spikes) enhances motor performance even further. (C) Comparison of peak mantle contractions during short-latency recruitment of the giant axon (cycle 1) and concerted recruitment in a 3-day-old ($N=6$) and 46-day-old ($N=6$) squid. Values are means + S.E.M.; $N$ is the number of measurements for each recruitment pattern. Differences are significant by $t$-test for both day 3 ($P<0.01$) and day 46 ($P<0.003$).
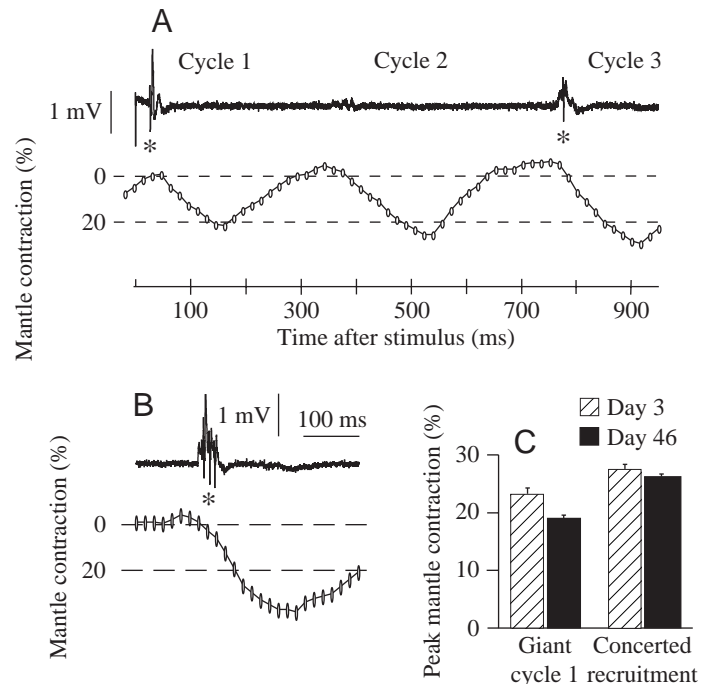
indicate that a similar, although less pronounced, boosting effect is present in a 3-day hatchling (hatched columns).

### Concerted recruitment is linked to prey-capture experience

All the results described thus far were obtained on squid reared on natural prey, primarily copepods. Because these crustaceans have an extremely fast escape response (Yen and Fields, 1992), juvenile squid must perfect complex, highly controlled behavior patterns to capture them. Surprisingly, this requires the elimination of high-speed jetting from the attack sequence (Chen et al., 1996). The ontogeny of prey capture occurs over the same period as does the maturation of concerted recruitment in escape responses (see Discussion). We therefore looked for an effect of prey-capture experience on the maturation of the giant axon system by using variability of its recruitment pattern in the escape response as an assay.

A series of experiments was performed on squid reared from birth only on easily captured *Artemia salina* nauplii, because such squid do not develop the prey-capture skills shown by their copepod-fed cohorts (Chen et al., 1996). During week 1, the most adult-like recruitment pattern (i.e. −/+) emerged in an apparently normal manner; however, this pattern becomes much less frequent during weeks 2–3 (Fig. 5). This trend is the opposite of that seen in copepod-fed animals (Fig. 3). Squid maintained on *Artemia salina* for up to 8 weeks showed a strong preference for the short-latency (+/−) pattern that is normally most common only in embryos (Figs 3, 5). Differences in mean relative frequencies at 4–8 weeks for the +/− and −/+ patterns for copepod-fed *versus Artemia*-fed squid are significant ($P<0.004$, $t$-test).

Another difference was noted between copepod-fed and *Artemia*-fed squid at 4–8 weeks post-hatching. Whereas every squid in the copepod-fed group showed a similar preference

for the −/+ pattern (Fig. 6A), *Artemia*-fed animals showed distinct individual differences. The majority of *Artemia*-fed animals studied showed no −/+ pattern at all (Fig. 6B), but three animals displayed the −/+ pattern at a frequency comparable with that of the copepod-fed group.

Copepods are a very challenging prey in comparison with *Artemia salina*, but copepods may also possess higher nutritional value. Although the *Artemia salina* nauplii used in our study were enriched with lipids, fatty acids and vitamins (Preuss et al., 1997), a check for possible nutritional differences between the two groups of squid was carried out by comparing mantle length as an index of growth. Seven samples of five animals each were taken between 15 and 40 days post-hatching, and a slightly larger mantle length was found in the *Artemia*-fed squid, the difference being significant
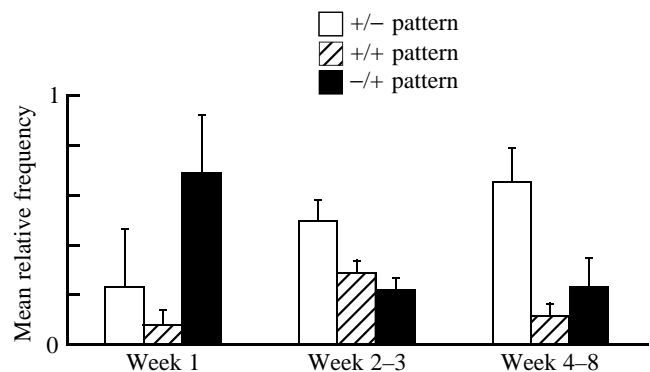


Fig. 5. Impaired development of giant axon recruitment in prey-deprived squid. Data for *Artemia*-fed squid were collected and analyzed for 1-week-old (four squid; $N=35$), 2- to 3-week-old (14 squid; $N=181$) and 4- to 8-week-old (nine squid; $N=117$) juveniles. Values are means + S.E.M.; $N$ is the number of responses counted.
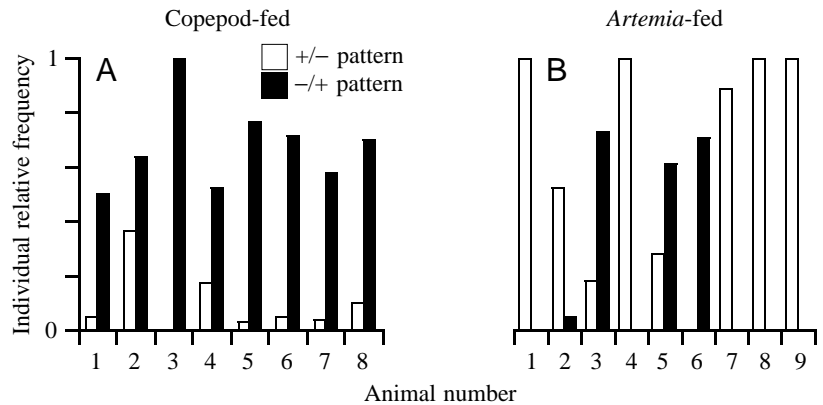
Fig. 6. Individuality in giant axon recruitment. Individual probabilities for the +/− and −/+ recruitment pattern (see Fig. 2) in 4- to 8-week-old animals (data from Figs 3, 6). (A) All copepod-fed squid show a preference for the −/+ pattern, whereas most *Artemia*-fed animals (B) demonstrate a distinct preference for the −/+ pattern, but three individuals do not.

at $P=0.001$ (Wilcoxon signed-rank test). In addition, we switched some squid that had been fed on copepods for 40 days to an *Artemia salina* diet for 1 week. Thereafter, we assayed the variability of giant axon recruitment in four of these animals and found a predominance of the −/+ pattern comparable with that seen in copepod-fed animals of the same age class (relative frequencies −/+=0.53±0.02; +/−=0.1±0.05; four squid, $N=48$; means ± S.E.M.).

## Discussion

Historically, the squid giant motor axon has been thought of as operating in a reflex mode with excitation provided by giant interneurons in the brain (Prosser and Young, 1937; Young, 1938). Recent studies of escape responses in adult squid, however, have revealed that the giant motor axons are typically recruited in concert with a parallel system of non-giant motor axons (Otis and Gilly, 1990). Concerted recruitment provides versatility in escape performance and may also permit participation of the giant axon system in other types of behavior such as prey capture (Packard, 1969).

The most important finding in the present study is that the stable acquisition of concerted recruitment of the two motor systems can be dramatically influenced by the acquisition of motor skills during prey-capture ontogeny. Although prey capture might appear to be a phenomenon unrelated to the maturation of the escape response, this view is undoubtedly simplistic. Because the giant axon system appears to play an important role in both behavior patterns in the adult animal (Packard, 1969), the existence of an ontogenetic link between the two might be expected. Such a link is suggested by two lines of evidence.

First, early maturation of concerted recruitment in the escape response is closely paralleled by distinct events in the ontogeny of prey capture. Over the first 1–3 weeks post-hatching, the attack sequence directed against copepods is modified through elimination of strong jetting by what appears to be a trial-and-error process (Chen et al., 1996). Powerful, 'forward'-directed attacks inevitably fail, because of the extremely fast escape response of the copepods, and inappropriate 'backward' retreats also thwart capture attempts. Thus, suppression of inappropriate jetting is materially rewarded by success in prey

capture, and failure to eliminate quick jetting within this time window leads to death by starvation. These are powerful selection pressures to mold emergent behavior.

It is precisely during this period that concerted recruitment of the giant axon system in escape responses becomes predominant, as indicated by the high frequency of the −/+ firing pattern during weeks 1–3. Between weeks 4 and 8, this recruitment pattern becomes even more firmly established, and during this time increasingly complex prey-capture behavior patterns evolve that allow the squid to approach the copepod stealthily and utilize capture strategies that rely much more on the arms and tentacles (Chen et al., 1996). Continued reward for not utilizing strong jetting in copepod attacks must remain a powerful influence during this period.

Second, rearing squid in an environment devoid of their natural, challenging prey items seriously disrupts the normal development of both prey capture and concerted recruitment. Hatchling squid can easily capture *Artemia salina* with rudimentary attacks that involve strong jetting and do not develop the normal suppression of jetting in prey-capture behavior, and this simple attack style does not change during development if *Artemia salina* is their only food source (Chen et al., 1996). Although *Artemia*-fed squid initially show concerted recruitment in escape responses, the −/+ pattern becomes increasingly rare after week 1, and by 8 weeks such squid are essentially embryonic-like in their escape response.

Presumably the appearance of the −/+ pattern is a programmed, innate feature of ontogeny, and it undoubtedly has adaptive value. Starting with its first appearance in hatchlings, concerted firing of giant and non-giant axons acts to boost motor performance during escape responses. This boost is likely to be involved in the hatching process itself, because hatching involves strong jetting as the embryo makes its final exit through the egg sheath. A boost in 'escape'-jetting performance at this time might therefore be advantageous.

Although the −/+ firing pattern may be an adaptive behavior released upon hatching, its successful long-term retention appears to be highly dependent on subsequent prey-capture experience. Whereas a strong link between prey-capture and escape response seems clear, it would be surprising if a behavior as critical as escape were absolutely dependent on one, and only one, set of experiences in an organism with a behavioral

repertoire as rich as that of a squid. Thus, experience in other types of skilled motor behavior might provide an alternative route to retention of the −/+ pattern in prey-deprived squid. For example, squid were frequently observed chasing and attacking a cohort that had just successfully captured a prey item, either an *Artemia salina* or a copepod. Such conspecific attacks certainly involve complex motor patterns and may only be performed by certain individuals within a group. This could explain why a minority of *Artemia*-fed animals, despite prey-capture deprivation, show essentially normal concerted firing during escape responses at 8 weeks of age. Other motor activities not controlled for in our studies could also conceivably influence development, and such alternative pathways would seem to be of considerable adaptive value.

Another set of behavioral observations showed that, given a choice between catching *Artemia salina* or copepods, squid show a clear preference to hunt for the quicker-moving copepods (T. Preuss and W. F. Gilly, unpublished results). This may explain why none of the tested animals from the copepod-fed group (4–8 weeks old) developed a preference for the +/− pattern, although these animals were in fact reared on a mixed diet of copepods and *Artemia salina* (see Materials and methods). Thus, the attraction towards copepods is probably another innate behavior in squid that, in turn, ensures appropriate experience for the development of the complex sensory–motor interactions that underlie skilled motor behavior.

At least two possible mechanisms can be postulated for the large decrease in short-latency firing of the giant axons (+/− and +/+ patterns) in copepod-fed squid. Sensory adaptation may take place and act to decrease the afferent activity that triggers short-latency escape responses. For example, the handling of copepods with its arms might produce painful sensations in the hatchling and lead to excitation of the giant axon system during prey capture (see also Chen et al., 1996). If these afferents were excited by the electrical stimuli employed in our experiments, adaptation in the sensory pathway could lead to a heightened threshold for the +/− response in copepod-fed animals. However, stimulus thresholds for the short-latency escape responses (+/− and +/+) were the same in *Artemia*-fed and copepod-fed squid, and these thresholds did not change during the developmental period studied. Moreover, no difference existed between the two groups in the frequency of electrically stimulated escape responses that involved no giant axon activity at all.

Although sensory adaptation of the type postulated may occur, the above observations suggest that it is not likely to be the primary mechanism leading to the elimination of short-latency responses to electrical stimuli during normal development. This mechanism would also not be expected to produce the elimination of the −/+ pattern in *Artemia*-fed squid. An alternative mechanism, which we favor, involves increasing inhibitory control over the giant axon system.

Suppression of jetting to enable copepod capture undoubtedly involves inhibitory control over both the giant and non-giant motor systems (Chen et al., 1996), and concerted recruitment during escape responses must involve similar control, especially over the giant axon system. It is very likely that development of the relevant inhibitory control mechanisms underlies the ontogenetic link between these behavior patterns and that developing inhibitory inputs are strengthened and stabilized by prey-capture experience through the powerful reinforcement provided by success. Increasingly fine-tuned inhibitory control would also gradually improve escape behavior through the incorporation of concerted recruitment. Ultimately, concerted recruitment may come to be employed in prey-capture behavior itself. As the squid grows and requires larger prey, it begins to feed on shrimp (Packard, 1969) and fish (T. Preuss and W. F. Gilly, unpublished observations) which, unlike copepods, can be captured by powerful jet-propelled attacks.

Successful development of inhibitory control in this experience-dependent manner would thus ensure survival for the critical first weeks after hatching and provide the juvenile squid with the basis for motor control that could affect a variety of behavior patterns during the rest of its life. Such a profound developmental influence is similar to that occurring in higher vertebrates such as birds (Margoliash, 1987; Bottjer and Arnold, 1997), rats (Walsh, 1981; Greenough, 1984) and humans (Black, 1998; Sackett et al., 1999). In these latter cases, the concept of a critical period is well established (Kandel, 1985). We have not yet identified a critical period for squid, although the first 2 weeks appear to be most important. It is clear, however, that rearing squid on *Artemia salina* alone for 4–8 weeks produces animals that show prey-capture behavior patterns, as well as escape responses, that are comparable with those of a recent hatchling. Furthermore, when switched to a copepod diet, these animals show no sign of developing the suppression of jetting necessary for successful captures (Chen et al., 1996; T. Preuss and W. F. Gilly, unpublished data). Thus, the plasticity evident at 1–2 weeks post-hatching is not apparent at these later times.

Inhibitory control of the squid giant axon system has not yet been described, although this has been suggested (Stanley, 1984). Anatomical data, however, suggest the existence of inhibitory inputs at the level of the first-order giant interneuron in the brain (Young, 1938; Martin, 1977; Mackie, 1990), making this cell a likely site for the hypothesized developmental modification of inhibitory control. This cell receives multimodal sensory input as well as descending inputs from the vertical lobe, a major area of higher integration (Young, 1979, 1991). The soma, dendrites and distal axon of the first-order giants are densely covered with synaptic boutons of several types (Gervasio et al., 1971; Froesch and Martin, 1972; Pozzo-Miller et al., 1998), some of which appear around the time of hatching (Marthy, 1987). Our physiological data suggest that inhibitory control over the giant axons in squid begins to function at about the time of hatching. This situation therefore appears to be analogous with the development of inhibitory control in the teleost Mauthner cell pathway (Eaton et al., 1977; Eaton and DiDomenico, 1986) and demonstrates once more the striking evolutionary convergence between cephalopods and fish (Packard, 1972).

Long-term modification of individual neurons resulting from biologically relevant experience may be a fundamental feature of all nervous systems (Wiesel, 1982). A clear difference between squid and higher vertebrates, such as mammals, is that one pathway being modified is composed of identified, giant neurons that show a relatively direct connection with specific behavioral events. Moreover, these giant cells are uniquely amenable to a variety of cellular-level experimental approaches. Mechanisms controlling long-term, or even permanent, modifications of neural properties remain to be elucidated, however, and further studies of giant neuron pathways may provide important insights (Bullock, 1993).

## References

**Black, J. E.** (1998). How a child builds its brain: some lessons from animal studies of neural plasticity. *Prev. Med.* **27**, 168–171.

**Boyle, P. R.** (1991). *The UFAW Handbook on the Care and Management of Cephalopods in the Laboratory.* Potters Bar, Herts: Universities Federation for Animal Welfare.

**Bottjer, S. W. and Arnold, A. P.** (1997). Developmental plasticity in neural circuits for a learned behavior. *Annu. Rev. Neurosci.* **20**, 459–481.

**Bullock, T. H.** (1993). How are more complex brains different? *Brain Behav. Evol.* **41**, 88–96.

**Chen, D. S. , Van Dykhuizen, G. , Hodge, J. and Gilly, W. F.** (1996). Ontogeny of copepod predation in juvenile squid (*Loligo opalescens*). *Biol. Bull.* **190**, 69–81.

**Chiel, H. J. and Beer, R. D.** (1997). The brain has a body: adaptive behavior emerges from interactions of nervous system, body and environment. *Trends Neurosci.* **20**, 553–557.

**Eaton, R. C. and DiDomenico, R.** (1986). Role of the teleost escape response during development. *Trans. Am. Fish. Soc.* **115**, 128–142.

**Eaton, R. C. , Farley, R. D. , Kimmel, C. B. and Schabtach, E.** (1977). Functional development in the Mauthner cell systems of embryos and larvae of the zebra fish. *J. Neurobiol.* **8**, 151–172.

**Froesch, D. and Martin, R.** (1972). Heterogeneity of synaptic vesicles in the squid giant fibre system. *Brain Res.* **43**, 573–579.

**Gervasio, A. , Martin, R. and Miralto, A.** (1971). Fine structure of synaptic contacts in the first order giant fibre system of the squid. *Z. Zellforsch.* **112**, 85–96.

**Gilly, W. F. , Hopkins, B. and Mackie, G. O.** (1991). Development of giant motor axons and neural control of escape responses in squid embryos and hatchlings. *Biol. Bull.* **180**, 209–220.

**Gilly, W. F. , Preuss, T. and McFarlane, M. B.** (1996). All-or-none contraction and sodium channels in a subset of circular muscle fibers of squid mantle. *Biol. Bull.* **191**, 337–340.

**Greenough, W. T.** (1984). Structural correlates of information storage in the mammalian brain: a review and hypothesis. *Trends Neurosci.* **7**, 229–233.

**Kandel, E. R.** (1985). Early experience, critical periods and developmental fine tuning of brain architecture. In *Principles of Neural Science* (ed. E. R. Kandel and J. H. Schwartz), pp. 757–770. New York: Elsevier.

**Katz, P. S.** (1996). Neurons, networks and motor behavior. *Neuron* **16**, 245–253.

**Mackie, G. O.** (1990). Giant axons and control of jetting in the squid *Loligo* and the jellyfish *Aglantha. Can. J. Zool.* **68**, 799–805.

**Margoliash, D.** (1987). Neural plasticity in birdsong learning. In *Imprinting and Cortical Plasticity* (ed. J. P. Rauschecker and P. Marler), pp. 23–54. New York: John Wiley & Sons, Inc.

**Marthy, H. J.** (1987). Ontogenesis of the nervous system in cephalopods. In *Nervous Systems in Invertebrates* (ed. M. A. Ali), pp. 443–459. New York: Plenum.

**Martin, R.** (1965). On the structure and embryonic development of the giant fibre system of the squid *Loligo vulgaris. Z. Zellforsch.* **67**, 77–85.

**Martin, R.** (1977). The giant nerve fibre system of cephalopods. Recent structural findings. *Symp. Zool. Soc., Lond.* **38**, 261–275.

**Martin, R. and Rungger, D.** (1966). Zur Struktur und Entwicklung des Riesenfasersystems erster Ordnung von *Sepia officinalis. Z. Zellforsch.* **74**, 454–463.

**Otis, T. S. and Gilly, W. F.** (1990). Jet-propelled escape in the squid *Loligo opalescens*: Concerted control by giant and non-giant motor axon pathways. *Proc. Natl. Acad. Sci. USA* **87**, 2911–2915.

**Packard, A.** (1969). Jet propulsion and the giant fibre response of *Loligo. Nature* **221**, 875–877.

**Packard, A.** (1972). Cephalopods and fish: the limits of convergence. *Biol. Rev.* **47**, 241–307.

**Pozzo-Miller, L. D. Moreira, J. E. and Llinás, R. R.** (1998). The first-order giant neurons of the giant fiber system in the squid: electrophysiological and ultrastructural observations. *J. Neurocytol.* **27**, 419–429.

**Preuss, T. , Lebaric, Z. N. and Gilly, W. F.** (1997). Post-hatching development of circular mantle muscles in the squid *Loligo opalescens. Biol. Bull.* **192**, 375–387.

**Prosser, C. L. and Young, J. Z.** (1937). Responses of muscles of the squid to repetitive stimulation of the giant nerve fibres. *Biol. Bull.* **73**, 237–241.

**Sackett, G. P. , Novak, M. F. S. X. and Kroeker, R.** (1999). Early experience effects on adaptive behavior: theory revisited. *Ment. Retard. Dev. Dissabil. Res. Rev.* **5**, 30–40.

**Stanley, E. F.** (1984). The action of cholinergic agonists on the squid stellate ganglion giant synapse. *J. Neurosci.* **4**, 1904–1911.

**Walsh, R. N.** (1981). Effects on environmental complexity and deprivation on brain anatomy and histology: a review. *Int. J. Neurosci.* **12**, 33–51.

**Wiesel, T. N.** (1982). Postnatal development of the visual cortex and the influence of environment. *Nature* **299**, 583–591.

**Wilson, D. M.** (1960). Nervous control of movement in cephalopods. *J. Exp. Biol.* **37**, 57–72.

**Yen, J. and Fields, D. M.** (1992). Escape responses of *Acartia hudsonica* (Copepoda) nauplii from the flow field of *Temora longicornis* (Copepoda). *Arch. Hydrobiol. Beih.* **36**, 123–134.

**Young, J. Z.** (1938). The functioning of the giant nerve fibres of the squid. *J. Exp. Biol.* **15**, 170–185.

**Young, J. Z.** (1979). The nervous system of *Loligo.* V. The vertical lobe complex. *Phil. Trans. R. Soc. Lond. B* **285**, 311–354.

**Young, J. Z.** (1991). Computation in the learning system of cephalopods. *Biol. Bull.* **180**, 200–208.