MICROSCOPY RESEARCH AND TECHNIQUE 49:511–520 (2000)

Structure and Function in the Cerebral Ganglion

RONALD CHASE*
Department of Biology, McGill University, Montreal, Quebec, H3A 1B1 Canada

KEY WORDS snails; *Helix*; olfaction; mating; mesocerebrum; procerebrum; metacerebrum

ABSTRACT Evidence is reviewed to evaluate whether the term "brain" is justified in referring to the snail's cerebral ganglion. The focus of the review is terrestrial species, with particular attention given to the genus *Helix.* In accordance with a standard definition of "brain," the cerebral ganglion is found to be differentiated both structurally and functionally. It receives convergent sensory inputs from a variety of anterior sensory organs plus the posterior body wall. Its outputs comprise motor commands directed towards anterior muscle systems, e.g., the tentacles and the penis, as well as premotor commands directed towards executory centers in other ganglia, e.g., the buccal, visceral, and pedal ganglia. Of the three major divisions in the ganglion, the procerebrum and the mesocerebrum are the most differentiated, whereas the metacerebrum is the least differentiated. The specializations of the procerebrum for olfactory functions, and the mesocerebrum for reproductive functions, reflect the importance of adaptations for feeding and mating in the evolution of the Gastropoda. *Microsc. Res. Tech. 49:511–520, 2000.* © 2000 Wiley-Liss, Inc.

INTRODUCTION

Historically speaking, early studies of the nervous system are mostly revealing of structure, whereas later studies are mostly revealing of function. So far as the nervous system of snails is concerned, one can point to Bullock and Horridge's monumental work on invertebrate nervous systems, published in 1965 (see Bullock, 1965), as marking the shift of attention from structure to function.

This paper concerns the cerebral ganglion of terrestrial snails and slugs belonging to the order Stylommatophora, subclass Pulmonata, class Gastropoda. Unless I say otherwise, all data reviewed here are garnered from experiments with a few closely related snail species, namely *Helix aspersa, Helix pomatia*, and *Helix lucorum*. It should be noted that many taxonomists no longer include the species *aspersa*, or *aspersus*, in the genus *Helix*. There is controversy, however, as to whether *aspersa* should be reassigned to *Cantareus, Cornu*, or *Cryptomphalus*. I retain the name *Helix aspersa* in this paper, to be consistent with the neurobiological literature, but readers are warned that the name may be on the way out. It is also useful to bear in mind that while the study of snails may contribute to a general understanding of how all nervous systems work, the details of structure and function in *Helix* do not necessarily generalize even to other land snails, of which there are approximately 30,000 species. When appropriate, I will use taxonomic comparisons to shed light on the relationship between structure and function.

Even early workers referred to the cerebral ganglion as the snail's "brain." The organization of the snail's central nervous system is derived from a primitive condition, called "orthogony," in which pairs of ganglia are arranged in a ventral series with connections made longitudinally by nerve cords and laterally by commissures. Indeed, the central nervous systems of most bilateral animals probably derived from an early orthogonal plan (Hanström, 1928). In extant invertebrates, the ganglia at the most anterior end of the chain are usually the largest, and one of them is exceptionally situated on top of, rather than underneath, the esophagous. It is this large dorsal ganglion that has traditionally been referred to as the brain. More recently, morphological and functional criteria have appeared (Delcomyn, 1998). Delcomyn states that brains process information, organize motor outputs, and make executive decisions. He further emphasizes (p. 56) the important criterion of regional specialization, which allows areas to be identified with different structures and different functions. This review focuses on the extent to which these brain-like features are present in the cerebral ganglion of snails.

HISTORY AND NOMENCLATURE

Already in 1883, Böhmig (cited in Kunze, 1921) recognized three divisions of the cerebral ganglion that correspond, more or less, to what we now call the procerebrum, the mesocerebrum, and the metacerebrum. The procerebrum is an obvious outcropping that causes little problem for identification (Fig. 1). In many familiar species, such as *Helix*, the mesocerebrum is also unmistakable, at least in its right anterior part, where numerous large neurons are evident. Even though the left mesocerebrum is smaller, as first recognized by Schmalz (1914), it too is obvious enough once comparison is made with the right side. However, anatomists have long argued two issues relating to the mesocerebrum. The first issue is whether there exists a neuropil that can be specifically associated with the mesocerebrum. All early workers, up to and including Kunze (1921), maintained that there is no distinctive mesocerebral neuropil. The question of the putative mesoce-

Grant sponsor: NSERC Canada.

*Correspondence to: Ronald Chase, Department of Biology, McGill University, 1205 Ave. Docteur Penfield, Montreal, Quebec, H3A 1B1 Canada.
E-mail: rchase@BIO1.LAN. mcgill.ca

Received 15 November 1999; accepted in revised form 12 December 1999

© 2000 WILEY-LISS, INC.

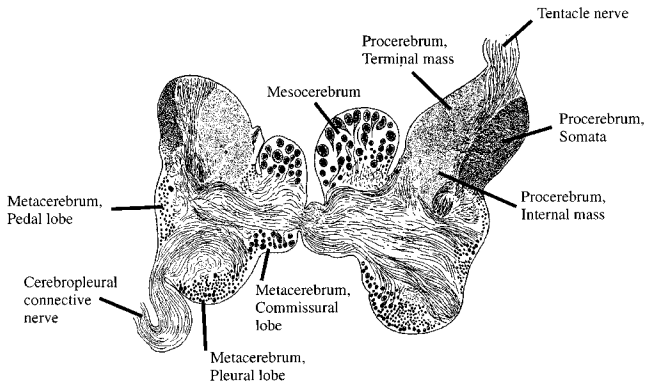

Fig. 1. A horizontal section of the cerebral ganglia (brain) of *Helix pomatia*. The procerebrum consists of a somatic region and two neuropilar regions. The mesocerebrum is larger on the right side than the left side. The metacerebrum, synonymous with postcerebrum, is divisible into three lobes. Actual width is about 2 mm. Modified from Kunze (1921).

rebral neuropil was important, in the eyes of early anatomists, because it bore on the second issue, which is the status of the cell population lying posteriorly to both the mesocerebral outcropping and the cerebral commissure (Fig. 1). De Nabias (1894) placed this region in the mesocerebrum, but Schmalz (1914) placed it in the metacerebrum (commissural lobe), evidently (according to Kunze) because Böhmig's histology had shown that the nearby neuropil was like that elsewhere in the metacerebrum. Kunze (1921) went along with Schmalz's conclusion, if not his logic, because she felt that to do otherwise would destroy the integrity of the metacerebrum. In my opinion, the so-called commissural lobe is nothing but a poorly developed part of the mesocerebrum, because in specimens that have an especially prominent right mesocerebrum, there is no break in the distribution of cell bodies from anterior to posterior across the ganglion. Ideally, the allegiance of this contentious region should be decided by studying the anatomical and functional properties of the resident neurons, but this has not yet been done. It is conceivable that the application of these criteria could assign the region in question to the metacerebrum on the left, and to the mesocerebrum on the right.

The metacerebrum is comprised of a flat central plane bounded by two or three lobes, depending upon whether one includes the commissural lobe. De Nabias (1894) introduced the terms visceral lobe and pedal lobe, which Kunze later changed to pleural lobe and pedal lobe (Fig. 1). As the names imply, these rather subtle lobes are, respectively, associated with the origins of the cerebropleural and cerebropedal connective nerves. Unfortunately, Hanström (1925) interchanged the labels for these two nerves in one of his drawings and his error was perpetuated by the reappearance of the drawing in Bullock's influential review (1965). Regardless, it is apparent that once the procerebrum and the mesocerebrum have been defined, the rest of the brain belongs to the metacerebrum. But there remains one more curiosity, which is the whereabouts of the postcerebrum. This term has been used by De Nabias (1894), Hanström (1925), and Bullock (1965) to refer to metacerebral structures. It is the Latin equivalent of the Greek metacerebrum. Since the prefixes pro- and meso- are used by all workers, and they are Greek, the corresponding term should be metacerebrum, not postcerebrum. Certainly it should not be imagined that there is both a postcerebrum *and* a metacerebrum.

PROCEREBRUM

Although circumstantial morphological evidence has long been available to link the procerebrum with olfactory function, physiological confirmation for such a function is recent; it will be reviewed later in this section. The tentacles were recognized in the nineteenth century as olfactory organs, and the origin of the posterior tentacle's main nerve (the olfactory nerve) at the apex of the procerebrum is obvious, as is the extensive course of the nerve within the procerebrum (Fig. 1). Also, although different in size and shape, the procerebrum and the tentacle ganglion (located in the tentacle at the peripheral end of the olfactory nerve) are similar in histological appearance, each possessing small neurons and a finely textured neuropil. Several nineteenth century studies (summarized by Van Mol, 1967) describe a special embryological origin for the procerebrum. It is said that the procerebrum and the remainder of the cerebral ganglion each derive from separate ectodermal invaginations, with the two only fusing at a relatively late stage in development.

Unlike the rest of the cerebral ganglion, and in contrast to the general pattern in invertebrate ganglia, the cell bodies and the neuropil lie laterally with respect to each other, rather than in the usual concentric arrangement. The procerebral neurons are very small and very numerous. Estimates of cell numbers range from 20,000 per lobe in *Helix aspersa* (Ratté and Chase, 2000) and *Achatina fulica* (Chase and Tolloczko, 1993) to approximately 50,000 in *Limax maximus* (Gelperin and Tank, 1990). The number of procerebral neurons is approximately equal to the number of all other central neurons. By using bromodeoxyuridine (BrdU) immunohistochemistry to label proliferating cells, Zakharov et al. (1998) discovered that cells continue to be born within the procerebrum for at least the first 4 weeks after hatching. Presumably these are mostly nerve cells, although the authors reported that at least some glial cells were also labelled. The origin of the newly generated cells is at the apical end of the procerebrum, i.e., near the entry point of the olfactory nerve; after birth the cells migrate toward the basal end, i.e., towards the metacerebrum. Whether neurogenesis continues at later ages, and if so, what consequences this has for function, are questions that require further investigation.

An influential argument for the role of the procerebrum in olfaction was put forward by Van Mol in 1967 based on a comparative morphological study of the cerebral ganglion in pulmonate gastropod molluscs. Van Mol brought to our attention the fact that the procerebrum is absent in aquatic species, or members of the order Basommatophora. It is present in all terrestrial forms, the Stylommatophora, but to different degrees. The species that are considered to be the most "highly evolved" have the largest procerebra. According to Van Mol, all of this demonstrates that the procerebrum evolved as an adaptation to the terrestrial life-

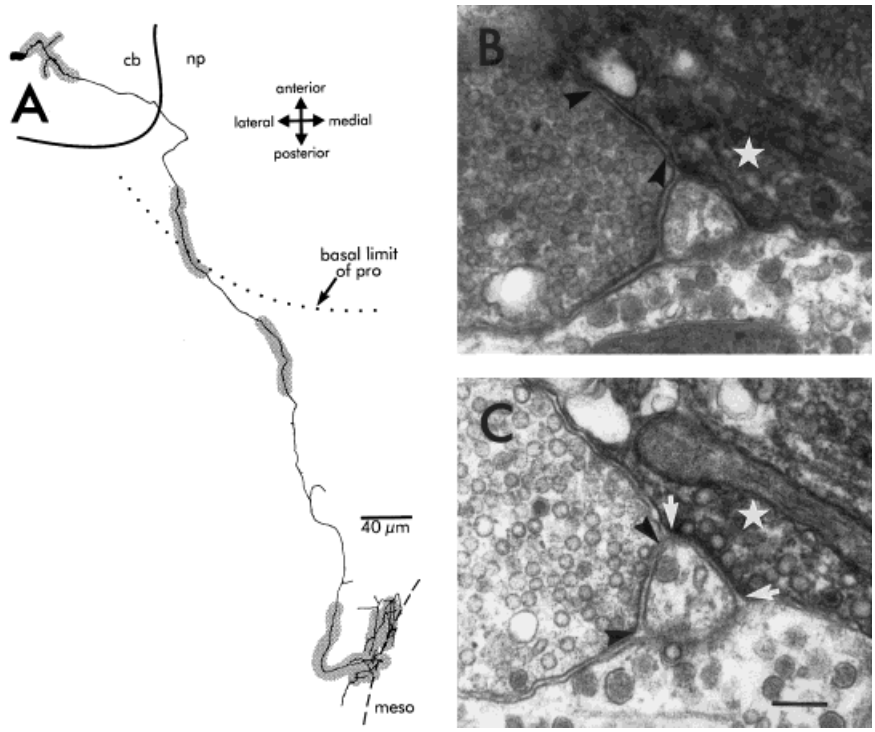

Fig. 2. A procerebral neuron that projects to the neuropil underlying the mesocerebrum. The cell was labelled by an intracellular injection of biocytin. **A:** Camera lucida drawing from thick sections. Shadings highlight areas that were serially sectioned and analyzed with electron microscopy. cb, cell body region; np, neuropil. **B,C:** Serial sections taken close to the mesocerebrum showing input and output synapses of the procerebral neuron. In B, an unidentified profile synapses on the labelled cell (asterisk). In C, the labelled neuron is itself presynaptic to a third, unidentified, profile. Note that the first two cells converge onto the third. Synapses are marked by arrows and arrowheads. Scale bar for B,C = 0.2 μm. Reproduced from Ratté and Chase (2000) with permission of the publisher.

style, one in which olfactory perception is of paramount importance. A shortcoming of this approach is that Van Mol fails to state his criteria for judging which taxa are the most evolved, and there is an implicit circularity in the argument because his judgments are influenced by the degree of brain differentiation. Also, it is not evident that the terrestrial environment is more demanding of olfaction than the aquatic environment.

Olfactory signals originate in sensory organs possessing specialized epithelial and sub-epithelial structures. Each tentacle bears one such olfactory organ at its tip (Chase, 1986a; Chase and Tolloczko, 1993). The olfactory nerve and the medial lip nerve carry the signals to the procerebrum from the superior and inferior pairs of tentacles, respectively. The output targets of the procerebrum have been difficult to determine owing to the small size of the procerebral neurons and to the fact that their processes do not form a noticeable tract in the neuropil, both features in striking contrast to the mesocerebrum (see below). Because silver impregnation methods are not able to reveal the cells' extremely fine neurites (as small as 0.1 μm), it was previously thought that the procerebral neurons have no axons and only local interactions (Hanström, 1925). This view was supported by anatomical findings showing that distant nerve cells send long processes of evident dendritic function into the procerebrum. Chase and Tolloczko (1989), working with *Achatina fulica*, reported that a group of about 25 cells on the ventral side of the pedal ganglion send processes through the cerebropedal connective to the procerebrum. Using hexamminecobalt to label this pathway, we confirmed, by electron microscopy, that the processes coming from the pedal ganglion are postsynaptic structures. The pedal ganglion-to-procerebrum pathway is also present in the slug *Limax maximus*, where a second pathway, again involving long receptive neurites, connects the buccal ganglion to the procerebrum. Both of these pathways in *Limax* were revealed by observing the transport of a lipophilic dye (DiI) into the procerebrum after crystals of the dye were placed in either the pedal ganglion or the buccal ganglion (Gelperin and Flores, 1997). Electrophysiological recordings revealed that activity in the pedal cells and the buccal cells is coupled to the field potential oscillation in the procerebrum (see below).

Despite the small size of the procerebral neurons, it is occasionally possible to impale them with a micropipette to introduce an intracellular label for morphological studies. We succeeded in labelling 43 neurons with biocytin for light microscopic examination (Ratté and Chase, 1997). Of these, 15 (35%) had neurite projections outside the procerebrum. Neurites were seen extending into several regions of the central neuropil and some approached the cerebral commissure before terminating or becoming invisible. The extensive projections of many procerebral neurons was confirmed by additional experiments that examined the retrograde transport of biocytin after focal injections into the neuropil, including contralateral placements. These findings raised the question whether the projecting neurites function as axons, i.e., as output pathways, or as dendrites. To find the answer, we (Ratté and Chase, 2000) performed correlated light and electron micrographic observations on intracellularly labelled procerebral neurons. We discovered that cells that project outside the procerebrum, for example to the mesocerebrum, do indeed have mostly output synapses on their distal processes, although the intermixing of some input synapses suggests synaptic integration or modulation at certain sites (Fig. 2). By contrast, the proximal

TABLE 1. Electrophysiological, optical, metabolic, and morphological correlates of olfactory function in the procerebrum[1]

Functional correlate	Spatial limit?	Stimulus odor	Stimulus quality	Species	Ref(s)
Uptake of dye	Yes	Cuke/carrot	Aversive (cond.)	*Limax marginatus*	a
Uptake of metabolic marker	No	Amyl acetate	Aversive	*Achatina fulica*	b
Power spectrum change	—	Several	Aversive	*Helix pomatia*	c
Wave frequency increase	—	Potato	Attractive	*Limax maximus*	d,e
	—	Garlic	Aversive	*Limax maximus*	d
	—	Several	Attractive (cond.)	*Limax marginatus*	f
Wave frequency decrease	—	Potato	Attractive	*Limax maximus*	g
	—	Amyl acetate	Aversive	*Limax maximus*	e
	Yes	Several	Aversive (cond.)	*Limax marginatus*	f,h
Wave amplitude/ form change	—	Potato	Attractive	*Limax maximus*	g
	Yes	Carrot	Aversive (cond.)	*Limax marginatus*	h
	—	Carrot	Attractive	*Helix aspersa*	I
Disruption of phase gradient	No	Potato	Attractive	*Limax maximus*	d,e
	No	Garlic	Aversive	*Limax maximus*	d
	No	Amyl acetate	Aversive	*Limax maximus*	e
	Yes	Potato	Attractive	*Limax maximus*	j

[1]*Spatial limit* refers to whether or not the correlate has a limited spatial distribution within the procerebrum. Stimuli marked as *cond.* are odors conditioned by associative pairing. *Cuke* is an abbreviation of cucumber. References: a, Kimura et al. (1998b); b, Chase (1985); c, Schütt et al. (1999); d, Delaney et al. (1994); e, Gervais et al. (1996); f, Kimura et al. (1998a); g, Gelperin and Tank (1990); h, Kimura et al. (1998c); I, Chase (unpublished data); j, Delaney and Kleinfeld (unpublished data; see Gervais et al., 1996).

neurites of the same cells, observed within the procerebrum, are typically postsynaptic at points of contact. Thus, the ultrastructural evidence supports the view that some procerebral neurons are true projection neurons. They receive functional inputs within the procerebrum and they transmit to locations outside the procerebrum, presumably in a manner that generates behaviors appropriate to the olfactory signal.

Other cells have neurites that are confined to the procerebrum, in some cases largely within the somatic region. These neurons are characterised by multiply branched arborizations that are adorned with varicosities. In the electron microscope we observed that input synapses are mostly localized to sites between the varicosities, whereas the varicosities themselves are foci for output synapses. Still other neurons have a special affinity for the internal mass within the procerebral neuropil (Fig. 1). They have a neurite that precedes with minimal branching and few synapses to the internal mass where it meanders extensively and forms mostly output synapses. Although we are a long way from understanding the functional microcircuitry of the procerebrum, a tentative model based on the foregoing results can be constructed (Ratté et al., in preparation).

In 1990, Gelperin and Tank discovered the fascinating electrical oscillations, or waves, in the procerebrum of the slug *Limax maximus*. These oscillations are easily recorded as extracellular field potentials, even in the absence of sensory stimulation, using either a blunt glass micropipette or a suction electrode. Depending on the species, the type of electrode used and its placement, the oscillations have various waveforms, but they are always continuous at a frequency of approximately 0.7 Hz. Similar oscillations have been reported in a number of olfactory structures from other animals, both invertebrate and invertebrate. In all cases, they are generated by the synchronized activity of a large neuronal population. The cellular basis of the oscillations in the procerebrum has been studied by Gelperin and his colleagues using whole cell recordings, optical measurements of membrane potential, and other techniques (Delaney et al., 1994; Gelperin, 1999). From their analysis, it seems that two classes of procerebral cells contribute to the oscillations. One class is endogenously active and fires in bursts. A second class is periodically hyperpolarized by cells of the first class. Electrical currents summating and conducting in the volume of the procerebrum generate the extracellular waveform.

One might expect that stimulation of the tentacles with odors would modify the spontaneous procerebral oscillation. Actually, several different effects have been described, as summarized in Table 1, including both increases and decreases in oscillation frequency. Gelperin's group has also reported (Gervais et al., 1996) that olfactory stimulation causes the phase lag between proximate and distal regions of the procerebrum to disappear. Ordinarily, in the absence of odors, the waves propagate from distal to proximal, but this movement stops when odors are presented. Most recently, Teyke and Gelperin (1999) have reported that disruption of the oscillations impairs odor discrimination but not odor recognition.

In an early experiment designed to demonstrate the olfactory function of the procerebrum, I examined the influence of olfactory stimulation on metabolic activity by using autoradiography to measure the cellular uptake of 2-deoxyglucose (Chase, 1985). I found that unilateral stimulation caused a unilateral increase in radiographic labelling within the procerebrum. Recently, Kimura et al. (1998b) described a much simpler method to visualize the procerebral neurons activated by olfactory stimulation. Their experiments begin with a conditioning procedure in which a food odor is presented to a slug paired with quinidine sulfate, an aversive stimulus. Then, 20 minutes after conditioning, they inject a small quantity of Lucifer Yellow into the animal's body cavity. When they later examine the brains in wholemount, they find clusters of dye-labelled neurons in the procerebrum. The dye uptake is thought to depend on endocytosis, presumably induced by electrical activity. If a similar experiment is conducted in which the food odor and the quinidine sulfate are pre-

sented *un*paired, much less labelling is seen. Curiously, the clusters appear in one, but not both, procerebra even though the odor is presented bilaterally. In many cases, the labelled cells are distributed as a band stretching across the procerebrum parallel to the dorsoventral axis. These bands of dye-labelled neurons bear a striking resemblance to the bands of BrdU labelled neurons reported by Zakharov et al. (1998) in their study of neurogenesis, but it is difficult to imagine how they could be related.

To summarize, it is evident from Table 1, as well as from other data not listed there, that the procerebrum has a function in olfaction. However, the inconsistent nature of the correlative measures shown in Table 1 suggests that we do not yet fully understand what the procerebrum does or how it does it. The procerebrum is responsible for, or at least involved in, the recognition and discrimination of odors, and given that experience is an important determinant of a snail's response to an odor (Croll and Chase, 1980), the procerebrum probably contains a learning mechanism. Still, judging from the results summarized in Table 1, no reliable correlate of procerebral learning has yet been identified. Much remains to be discovered about the neural circuitry of the procerebrum and how it works physiologically. Also, the role of the procerebral electrical oscillation, if any, remains a mystery. Is it part of the odor learning mechanism, or is it an epiphenomenon? Since similar questions remain to be answered in the olfactory structures of other taxa, both vertebrate and invertebrate, and since small neurons and electrical oscillations are common features of all these systems, the procerebrum is a valuable experimental model (Chase, 1986a; Gelperin, 1999).

MESOCEREBRUM

The asymmetry of the left vs. right lobes of the mesocerebrum (Fig. 1) was first noted by De Nabias (1894). Since the right lobe is always larger than the left lobe (in mature specimens), it occurred to me that this might be explained by function, given that the animal's reproductive organs are located on the right side (Chase, 1986b). Earlier authors may have been dissuaded from this interpretation because De Nabias himself had described a fiber tract running from the mesocerebrum to the pedal connective nerve, not the penial nerve (a cerebral nerve). However, our investigations showed that many mesocerebral axons exit this tract to enter the penial nerve (LaBerge and Chase, 1992; Li and Chase, 1995). Besides, it is now clear that the pedal ganglion is itself the source of fibers that innervate the penis via the cerebropedal connective and the penial nerve. The pedal ganglion also innervates several male reproductive organs via its own peripheral nerves, principally the nervus cutaneus primus dexter (NCPD). We determined the axon projections of 145 mesocerebral cells by intracellular injections of tracers (summarized in Chase and Li, 1994). All mesocerebral cells have an axon in the cerebropedal connective, and about half of them have additional axons in other nerves. About 25% of the cells have an axon in the penial nerve (approximated from the sum of projections into the penial nerve and the medial lip nerve, since the former often emerges from the latter).

Apart from two giant neurons in the metacerebrum, the mesocerebral neurons are the largest in the ganglion (Fig. 1). I measured the soma diameters of the 20 largest cells in 7 snails (Chase, 1986b). The mean diameter in the right lobe was 77 μm; in the left lobe it was only 62 μm. The right lobe also contains more neurons than the left lobe (Chase, 1986b; LaBerge and Chase, 1992), but the actual numbers depend on how the borders of the lobe are drawn. Roughly, the right mesocerebrum, defined to exclude the posterior, or commissural, lobe, contains about 140 neurons. Like the procerebrum, the mesocerebrum undergoes a considerable development after hatching. While the procerebrum adds new cells, the mesocerebrum adds branches, length, and bulk to its existing cells (LaBerge and Chase, 1992). The time course of the cells' growth parallels that of the penis, with the maximum cell size achieved at about 17 weeks of age (*Helix aspersa maxima*), or about 4 weeks before the penis attains its maximum weight.

The function of the mesocerebrum was first explored in vitro (Chase, 1986b). Synaptic inputs were found to enter the lobe from numerous peripheral nerves, principally the peritentacular nerves and the lip nerves. Cells in the right lobe are more likely to respond to nerve stimulation with an EPSP than are cells in the left lobe, and stimulation of nerves on the right side is much more effective than stimulation of nerves on the left side. These results suggested to me that the mesocerebrum has a sensory function biased towards the right side of the animal. Because the genital pore is located on the right side, most of the contacts between a mating pair occur on the right side, at least in the later stages of courtship and copulation. The right side bias is also evident in the motor output of the mesocerebrum. Stimulation of the entire right mesocerebrum, but not the left mesocerebrum, causes simultaneous movements of the penis and the dart sac in vitro (Chase, 1986b). The asymmetry of the mesocerebrum has led most workers to focus on the right side. The left mesocerebrum may serve to integrate sensory activity resulting from tactile and chemical stimulation early in courtship, but its properties have not been well investigated.

Confirmation of the role of the mesocerebrum in mating function has come from recent experiments in which the activity of right-side neurons was recorded in vivo using an implanted fine wire electrode (Koene et al., 2000). Stimulation of the skin of the animal evoked activity in the mesocerebrum when the probe contacted either one of the posterior (superior) tentacles, or the area between them. On the right side, the receptive field extended downward to encompass the genital pore, but not beyond; there was no comparable area of sensitivity on the left side of the animal. Results from in vitro experiments and in vivo experiments also converge to credit the mesocerebrum with a motor function. Stimulation of the lobe through an implanted fine wire causes genital eversion and, when combined with tactile stimulation, penial eversion and dart shooting (Koene et al., 2000). In vitro, intracellular stimulation of single neurons causes movements of the penis, the dart sac, or both (Chase, 1986b).

By recording with an implanted fine wire electrode, we were able to monitor mesocerebral electrical activ-

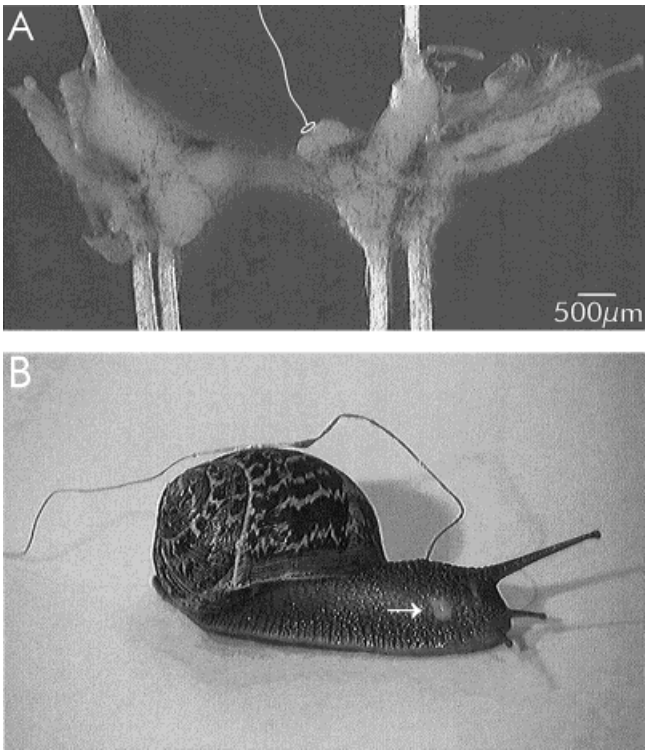

Fig. 3. In vivo recording of mesocerebral neuronal activity during natural mating behavior. **A:** Photograph of the cerebral ganglia with a drawing of a fine wire shown to indicate its size, shape, and site of implantation. **B:** A snail implanted with the fine wire for electrical recording and stimulation. The arrow points to the partially everted genital pore. Reproduced from Koene et al. (2000) with permission of the publisher.

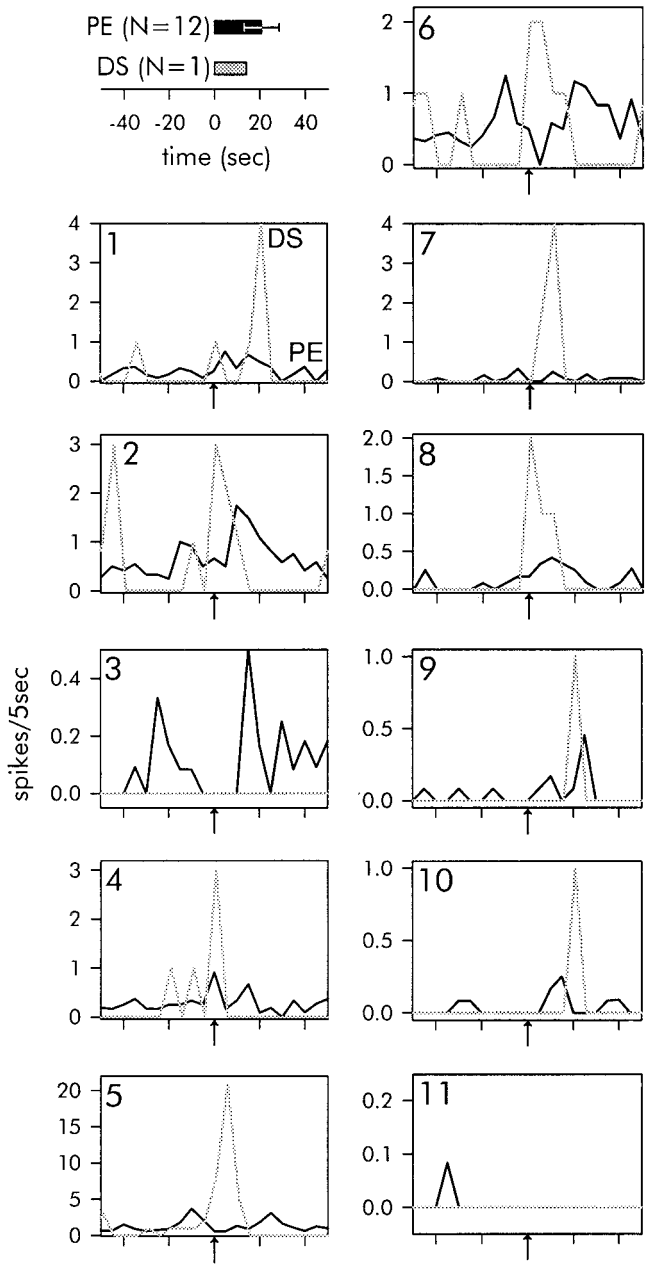

Fig. 4. The spiking activity of 11 units shown in relation to penial eversion (PE) and dart shooting (DS). The recording was obtained with a fine wire electrode as shown in Figure 3. Units were isolated from the multiunit record using a spike sorting software program. Activity during penial eversion was averaged over 12 occurrences, whereas dart shooting was a unique event. Both behaviors begin at time zero (arrows) and continue for durations as indicated at the top left, as determined from a video record. Most units have a peak of activity during, or just after dart shooting (grey lines), and most units show changes of activity around the penial eversion events (black lines). Reproduced from Koene et al. (2000) with permission of the publisher.

ity during natural mating (Fig. 3; Koene et al., 2000). A spike sorting program allowed us to analyze 11 units presumed to represent individual nerve cells located underneath the electrode. Most units were highly active during courtship, but their activity sharply decreased or entirely ceased soon after simultaneous intromission. All units but one were excited by physical contacts between the two mating snails. The activity of the units during the dart shooting event, as well as during 12 penial eversion events, is illustrated in Figure 4. It is evident that the majority of units fired brief bursts of spikes in association with dart shooting, and most units also showed a modulation of their activity in association with penial eversion. For penial eversion, a unit's activity typically increased just prior to the observed motor act. This was followed by a brief pause, and then another increase of activity above baseline levels.

In summary, there is ample evidence to conclude that the mesocerebrum plays a role in the control of mating behavior, at least for male functions. Structures homologous to the mesocerebrum of *Helix* exist in related taxa, and they probably have the same function. In particular, a strong case can be made for homology with the anterior lobe of *Lymnaea stagnalis*, representing the basommatophoran pulmonates, and with the H-cluster of *Aplysia californica*, representing the anaspidean opisthobranchs. The named structures in these taxa are located, like the mesocerebrum, at the anteromedial margin of the cerebral ganglion, and they have the same morphological asymmetry with a right-

side bias. Electrophysiological evidence similar to that summarized above for *Helix* indicates sensory and motor functions linked to the male reproductive organs. All the structures, including the mesocerebrum, contain significant amounts of APGWamide, a peptide that is capable of causing penial eversion when injected into an intact animal (*Lymnaea*: De Boer et al., 1997; *Helix*: Koene et al., 2000). While these findings suggest evolutionary conservation of the location for central neural control over mating behavior, it is noteworthy that the details of mating behavior differ considerably between species. *Helix* mates as a simultaneous reciprocal hermaphrodite; *Lymnaea* is a serial reciprocal hermaphrodite; and *Aplysia* is a simultaneous nonreciprocal hermaphrodite. Even among the stylommatophoran pulmonates (terrestrial taxa), there are large species differences. Courtships may last for only a few minutes or they may continue for as long as 34 hours, and only a few species shoot darts. Some species mate side by side, some mate standing upright with apposed soles, and still others mate hanging from mucus threads. Spermatophores are used to transfer sperm in some species but not in others. The two closely related species *Helix aspersa* and *Helix pomatia* differ significantly in the occurrence of biting during courtship, the posture during mating, and the duration of copulation (Adamo and Chase, 1988). This splendid diversity of behavior should challenge neurobiologists to look for structural and functional differences in the mesocerebrum and its homologues.

Some studies, including some of my own unpublished work, suggest a further role for the mesocerebrum in the control of spermatogenesis. Sokolove and Van Minnen (1983) injected slugs (*Limax maximus*) with homogenates of the mesocerebrum and found that injections increased the rate of DNA synthesis by spermatogonia in the ovotestis, presumably by mimicking an endogenous endocrine route. Later, Gomot (1993) performed the opposite experiment in *Helix aspersa*, by lesioning the mesocerebrum, but he also observed an increase in spermatogenic DNA synthesis! Clearly this phenomenon needs to be re-examined to explain the seemingly contradictory results, but it is possible that the mesocerebrum could both promote *and* inhibit spermatogenesis. A fine branch of the intestinal nerve innervates the ovotestis by branching profusely over its tubules. The mesocerebrum is implicated in the function of this nerve because backfills of the nerve (over a distance of 14 mm) consistently label two or three neurons in the mesocerebrum while leaving all other cerebral neurons unlabelled. Additional neurons are backfilled in the visceral and parietal ganglia. When I searched for the mesocerebral cells with a microelectrode, I failed to find any cell that would respond to stimulation of the ovotestis nerve with an antidromic spike, but I did find that about half the recorded mesocerebral cells (7 of 15) showed dramatic changes in spiking activity following nerve stimulation. Some cells are excited by the stimulus while others are inhibited. In all cases the effects far outlast the stimulus, continuing for several minutes in some cells. Taken together, the anatomical and physiological findings suggest that the mesocerebrum has both afferent and efferent connections with the ovotestis. Thus, whether the mesocerebrum exerts an excitatory or inhibitory influence on spermatogenesis might depend on the current state of the ovotestis as monitored by nervous feedback.

METACEREBRUM

Unlike the two regions already discussed, the metacerebrum has no distinctive features and it has no single, or easily characterized, function. Structurally, it is composed of neurons of various sizes, nearly all of which are larger than the procerebral cells but smaller than the mesocerebral cells. Their arrangement on three minor bulges (the pedal, pleural, and commissural lobes) and an interior flat plane is unremarkable and similar to that seen in other pulmonate ganglia. Generally speaking, metacerebral neurons collect sensory information from anterior sense organs and, when appropriate signals are received, they command motor actions. The presence of two giant neurons, first described by De Nabias in 1894, provides a good opportunity for structure/function investigations. As described below, these two giants have functions in tentacle withdrawal and feeding, respectively, and the function of the metacerebrum as a whole may not be significantly broader than that of these two cells. However, the particular functions of individual metacerebral cells have not been studied in a systematic fashion. Likewise, the available data are not sufficient to say whether different functions are represented in different areas of the metacerebrum, although the presence of lobes suggests that this might be the case.

Most of the cerebral nerves have mixed sensory and motor functions, but the small nerve that innervates the posterior tentacle's retractor muscle appears to be purely motor (Prescott et al., 1997). A cluster of presumed tentacle withdrawal motoneurons is labelled in the commissural lobe by backfilling this nerve (Prescott et al., 1997; Zakharov et al., 1982). The number of axon profiles that can be counted in histological sections of the nerve (22) is approximately equal to the number of backfilled somata (21) (Prescott et al, 1997). Another 29 metacerebral neurons are backfilled from the peritentacular nerves (internal and external); these cells probably control bending, length, and angle of the tentacle.

Foremost amongst the metacerebral neurons participating in tentacle withdrawal is the large identified neuron, C3, illustrated in Figure 5A. This cell was first recognized by De Nabias (1894) as the Upper Giant Cell. Its function in tentacle retraction has been clearly established (Cottrell et al., 1983; Prescott et al., 1997; Zakharov et al., 1982). The large size of the cell's soma ($\sim 110\ \mu m$) can be attributed to metabolic demands for support of its multiply branched axon and, to a lesser extent, its central, presumedly dendritic, arborization. The high rate of metabolism required by the cell is associated with large numbers of ribosomes, Golgi complexes, and other organelles, and multiple copies of genes (Chase and Tolloczko, 1987). The axon branches of C3 innervate both the retractor muscle of the posterior tentacle and the tegmental muscle at the base of the tentacle. C3 fires at a short delay and very rapidly when the tentacle is stimulated with a noxious stimulus, whether tactile or chemical (Chase and Hall, 1996). Interestingly, the sensitivity of the neuron to chemical stimulation of the tentacle is not affected by removal of the procerebral neuronal cell bodies. The stimulus-

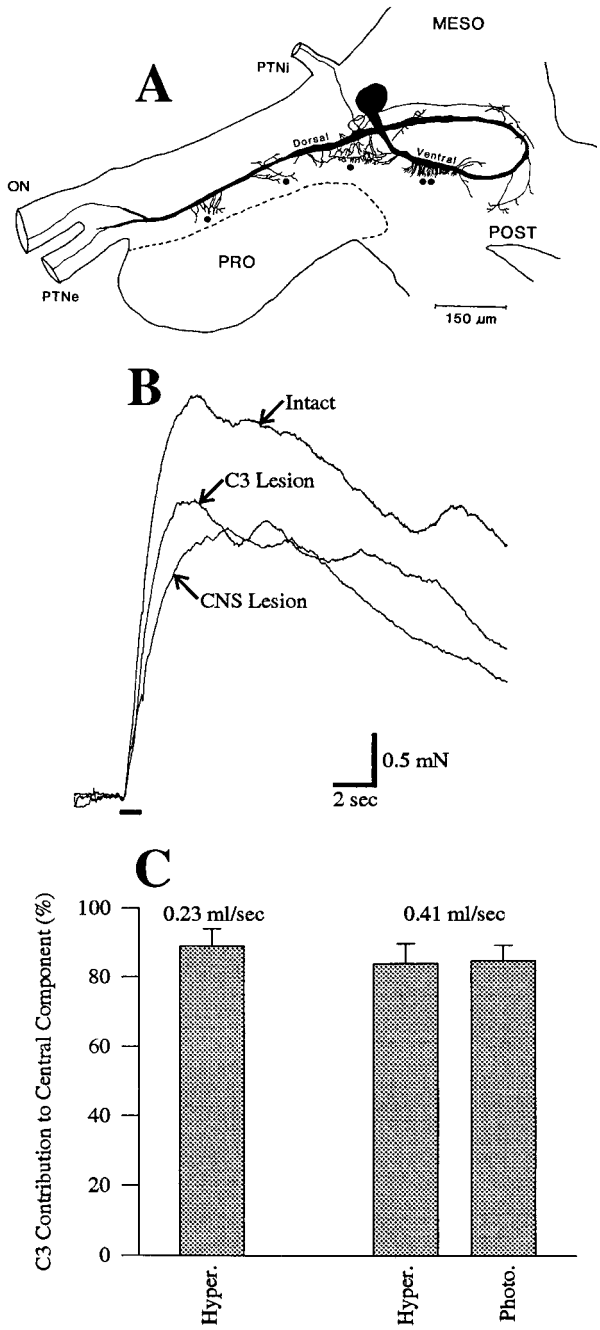

Fig. 5. The giant cell C3 is a motoneuron that mediates tentacle withdrawal. **A:** Camera lucida drawing of a cell filled by injection of hexamminecobalt chloride. Dorsal view of left cerebral ganglion. A portion of the neurite travels ventrally in the ganglion before looping dorsally. Dots mark the presumed sites of tentacular afferent inputs, physiologically distinguished as fast-conducting, low-threshold fibers (single dots) and slow-conducting, high-threshold fibers (double dots). **B:** Three traces of muscle tension showing the effects of lesions on the tentacle withdrawal reflex. The response that remains after the CNS lesion is mediated by peripheral pathways. Horizontal line indicates delivery of a saline jet to the tentacle tip (mechanical stimulus). **C:** The single neuron, C3, is responsible, on average, for 85.3 ± 2.8% (SE) of the central contribution to reflex tension, as determined by hyperpolarization and photoinactivation lesions. Stimuli were delivered at two strengths as indicated above the bars. **A** reproduced from Chase and Hall (1996), **B** and **C** from Prescott et al. (1997) with permission of the publishers.

evoked burst of activity in C3 accounts for 85% of the central contribution to reflexive tentacle retraction (Fig. 5B,C), which indicates the importance of this neuron for defense of the tentacles.

Feeding is another activity regulated by the metacerebrum. Some metacerebral neurons have a motor or premotor function while others are modulatory. The lips are a major source of sensory input and they are also a target for some of the motor outputs. Hernádi and co-workers found that about 240 neurons could be backfilled from the three lip nerves (anterior, medial, posterior or inner, medial, outer) (summarized in Kemenes, 1994). These are mostly in the metacerebrum, and mostly on the ventral surface of the ganglion. Interestingly, the neurons seem to mediate efferent discharges in the lip nerves but they are also sensitive to stimulation of the lips. In one study, 80% of neurons sampled on the ventral surface responded to either tactile stimulation or chemical stimulation, or both. The responses were diverse in sign (excitation, inhibition), stimulus specificity, duration, and receptive field location. Together, these findings reveal a population of neurons that is likely responsible for contacting foods and maintaining contact with them until they are ingested.

Other neurons, also on the ventral surface, are responsible for initiating and modulating the feeding motor program. Since the central pattern generator for the feeding motor program is located in the buccal ganglion, neurons of this type have axons travelling in the cerebrobuccal connective nerves, and they can be identified by backfilling those nerves. Delaney and Gelperin (1990a) found 14–18 such neurons in *Limax maximus*, all in the lateral lobe, which is probably equivalent to the pedal lobe of *Helix*. In contrast to the neurons described in the preceding paragraph, these neurons have no axons in any peripheral nerve. When stimulated through an intracellular electrode, some cells can initiate the feeding motor program, and all cells but one can enhance the bursting behavior of buccal ganglion neurons firing in phase with the motor program (Delaney and Gelperin, 1990b). When food stimuli are applied to the lips, the cerebral interneurons are excited, but when aversive chemical stimuli are applied, at least one class of interneurons is inhibited (Delaney and Gelperin, 1990c).

One of the cerebral feeding interneurons is also the largest neuron in the entire CNS. It has been given several different names, among which are the metacerebral cell (MCC), the serotonergic cerebral cell (SCC), and C1. I prefer to call it the giant cerebral neuron, or GCN. The cell body, which has a diameter of about 150 μm in *Helix*, is located in the flat part of the metacerebrum, on the ventral surface, somewhat more anteriorly to the interneurons described by Delaney and Gelperin (1990a). Its size, easy recognition, and homology across the pulmonate and opisthobranch species (Pentreath et al., 1982), have permitted an extensive investigation of its structure and function. Eric Kandel first made his mark in molluscan neurobiology by describing the synaptic inputs to the GCN (Kandel and Tauc, 1966). All the major sensory nerves of the cerebral ganglion, including the three lip nerves and the olfactory nerve, have terminal fields that overlap the large dendritic field of the GCN (Chase and Tolloc-

zko, 1992). We injected the cell with hexamminecobalt to label its synaptic contacts within the cerebral ganglion. In every one of 370 labelled contacts, the GCN was the postsynaptic cell (Chase and Tolloczko, 1992). While normally silent, chemostimulation with food substances excites the cell. The cell has somewhat different patterns of axonal branching in different species, but in all cases it has axons with profuse terminal arborizations in the ipsilateral buccal ganglion. The large size of the GCN soma, like that of C3, is due to the length of neurite that it must support.

The GCN has been frequently investigated as a model for understanding the modulatory actions of serotonin in gastropod molluscs (Pentreath et al., 1982). In some species, or at least in some experiments, the GCN can, by itself, initiate the feeding motor program, but this does not appear to be its main function. Rather, activity in the GCN increases both the rate and the effectiveness of the program. The former effect is achieved by modulating the central pattern generator (*Lymnaea*: Yeoman et al., 1996), while the latter effect is achieved at the level of the motor unit, from enhancement of transmission and from a facilitation of muscle excitation-contraction coupling (*Achatina*: Yoshida and Kobayashi, 1995). The overall function of the GCN is perhaps best demonstrated by lesion experiments, performed in *Aplysia* (Rosen et al., 1989), which show that animals bite more slowly and with reduced mouth movements when they have no GCN.

Whereas feeding and tentacle movements are certainly major functions of the metacerebrum, other functions are also likely, if not so well documented. A role in the control of mating behavior is indicated by the large numbers of neurons that can be backfilled from nerves that innervate the male reproductive structures. Thus, apart from filling cells in the mesocerebrum, backfills of the penial nerve label cells in the pedal lobe, and backfills of the nerve that innervates the dart sac (ncppd) label cells in the commissural lobe (Li and Chase, 1995). How these cells interact with mesocerebral neurons in the coordinated control of mating behavior is an interesting question for future study. A role for the metacerebrum in the initiation and modulation of locomotion is predicted from results in opisthobranchs, where cerebral neurons with these functions have been identified (Panchin et al., 1995). In stylommatophores, some metacerebral neurons project to the pedal ganglion, which contains the locomotor pattern generator (Ierusalimsky and Zakharov, 1994). Other metacerebral neurons pass through the pedal ganglion before terminating in the body wall. These latter neurons might be responsible for turning the snail during locomotion, by contracting the body wall on one side or the other.

Numerous peptides have been localized to the metacerebrum by immunohistochemical methods. Other papers in this issue (Croll; Elekes; Hernádi; Santama and Benjamin) treat peptidergic neurons in more detail. It is sufficient here to mention those peptides that are suspected to be in metacerebral neurons, and their possible functions. In *Helix*, positive immunohistochemical results have been reported for APGWamide, FMRFamide, insulin-related peptides, leucokinin I, substance P, tachykinin-related peptides, enkephalin, catch-relaxing peptide, vasoactive intestinal peptide, and neuropeptide F (for references, see papers in this issue). It needs to be said that few of the peptides indicated by immunohistochemistry have been confirmed by other methods, and in no case has a function for metacerebral peptidergic neurons been established. A peptide could be released into the circulation as a hormone or it could be used within the CNS as a transmitter or modulator. Some proposed regulatory functions involve structural growth, reproduction (gametogenesis, fertilization, oviposition), cardiac output, blood osmolarity, and activity cycles (hibernation, aestivation).

CONCLUSIONS

The evidence reviewed here is sufficient to acclaim the snail's cerebral ganglion as a true brain consistent with Delcomyn's criteria (1998). The ganglion receives convergent sensory input, not only from anterior sensory organs, but also from the statocyst, internal organs (e.g., the ovotestis), and the posterior body wall. After integration of the sensory information, it commands important motor behaviors including feeding, mating, defensive withdrawal, and (probably) locomotion. Most significantly, following Delcomyn, the cerebral ganglion is regionally differentiated. Two of the ganglion's main regions, namely the procerebrum and the mesocerebrum, are well differentiated both structurally and functionally. The metacerebrum does not appear to be differentiated; it seems to contain a heterogeneous mix of neuronal cells having multiple functions and organized in no apparent manner. The special functions of the procerebrum with respect to olfaction and feeding, together with those of the mesocerebrum in reproduction, are consistent with evolutionary patterns within the class Gastropoda in which traits associated with feeding and mating are highly diverse. Seen in this context, the specializations of the procerebrum and the mesocerebrum reflect the selective pressures that have acted to drive adaptive radiation.

REFERENCES

Adamo S, Chase R. 1988. Courtship and copulation in the terrestrial snail, *Helix aspersa* . Can J Zool 66:1446–1453.

Bullock TH. 1965. Mollusca:Gastropoda. In: Bullock TH, Horridge GA, editors. Structure and function in the nervous systems of invertebrates. San Francisco: WH Freeman, p1283–1386.

Chase R. 1985. Responses to odors mapped in snail tentacle and brain by ^{14}C-2-deoxyglucose autoradiography. J Neurosci 5:2930–2939.

Chase R. 1986a. Lessons from snail tentacles. Chem Senses 11:411–426.

Chase R. 1986b. Brain cells that command sexual behavior in the snail *Helix aspersa*. J Neurobiol 17:669–679.

Chase R, Hall, B. 1996. Nociceptive inputs to C3, a motoneuron of the tentacle withdrawal reflex in *Helix aspersa* . J Comp Physiol 179: 809–818.

Chase R, Li G. 1994. Mesocerebral neurons and their role in the control of mating behaviour. Netherlands J Zool 44:212–222.

Chase R, Tolloczko B. 1987. Evidence for differential DNA endoreplication during the development of a molluscan brain. J Neurobiol 18:395–406.

Chase R, Tolloczko B. 1989. Interganglionic dendrites constitute an output pathway from the procerebrum of the snail *Achatina fulica*. J Comp Neurol 2282:143–152.

Chase R, Tolloczko B. 1992. Synaptic innervation of the giant cerebral neuron in sated and hungry snails. J Comp Neurol 318:93–102.

Chase R, Tolloczko B. 1993. Tracing neural pathways in snail olfaction: From the tip of the tentacle to the brain and beyond. Microsc Res Tech 24:214–230.

Cottrell GA, Schot, LPC, Dockray GJ. 1983. Identification and probable role of a single neurone containing the neuropeptide *Helix* FMRF amide. Nature 304:638–640.
Croll R, Chase R. 1980. Plasticity of olfactory orientation to foods in the snail *Achatina fulica*. J Comp Physiol 136:267–277.
De Boer PACM, Ter Maat A, Pieneman AW, Croll RP, Kurokawa M, Jansen RF. 1997. Functional role of peptidergic anterior lobe neurons in male sexual behavior of the snail *Lymnaea stagnalis*. J Neurophysiol 78:2823–2833.
Delaney K, Gelperin A. 1990a. Cerebral interneurons controlling fictive feeding in *Limax maximus*. I. Anatomy and criteria for reidentification. J Comp Physiol 166:297–310.
Delaney K, Gelperin A. 1990b. Cerebral interneurons controlling fictive feeding in *Limax maximus*. II. Initiation and modulation of fictive feeding. J Comp Physiol 166:311–326.
Delaney K, Gelperin A. 1990c. Cerebral interneurons controlling fictive feeding in *Limax maximus*. III. Integration of sensory inputs. J Comp Physiol 166:327–343.
Delaney KR, Gelperin A, Fee MS, Flores JA, Gervais R, Tank DW, Kleinfeld D. 1994. Waves and stimulus-modulated dynamics in an oscillating olfactory network. Proc Natl Acad Sci USA. 91:669–673.
Delcomyn F. 1998. Foundations of neurobiology. New York: WH Freeman, 648 p.
Gelperin A. 1999. Oscillatory dynamics and information processing in olfactory systems. J Exp Biol 202:1855–1864.
De Nabias, B. 1894. Recherches histologiques et organologiques sur les centres nerveux des gastéropodes. Actes Soc Linn Bordeaux 47:11–202.
Gelperin A, Flores J. 1997. Vital staining from dye-coated microprobes identifies new olfactory interneurons for optical and electrical recording. J Neurosci Methods 72:97–108.
Gelperin A, Tank DW. 1990. Odour-modulated collective network oscilltions of olfactory interneurons in a terrestrial mollusc. Nature 345:437–440.
Gervais R, Kleinfeld D, Delaney KR, Gelperin A. 1996. Central and reflex neuronal responses elicited by odor in a terrestrial mollusc. J Neurophysiol 76:1327–1339.
Gomot P. 1993. Studies on the control of spermatogenic DNA synthesis by the mesocerebrum in the snail *Helix aspersa*. Cell Mol Neurobiol 13:517–527.
Hanström B. 1925. Über die sogenannten Intelligenzsphären des Molluskengehirms und die Innervation des Tentakels von *Helix*. Acta Zool Stockh 6:183–217.
Hanström B. 1928. Some points on the phylogeny of nerve cells and of the central nervous system of invertebrates. J Comp Neurol 46:475–491.
Ierusalimsky VN, Zakharov IS. 1994. Mapping of neurons participating in the innervation of the body wall of the snail. Neurosci Behav Physiol 24:33–46.
Kandel ER, Tauc L. 1966. Input organization of two symmetrical giant cells in the snail brain. J Physiol 183:269–286.
Kemenes G. 1994. Processing of mechano- and chemosensory information in the lip nerve and cerebral ganglia of the snail *Helix pomatia* L. Neurosci Behav Physiol 24:77–87.
Kimura T, Toda S, Sekiguchi T, Kirino Y. 1998a. Behavioral modulation induced by food odor aversive conditioning and its influence on the olfactory responses of an oscillatory brain network in the slug *Limax marginatus*. Learning Memory 4:365–375.
Kimura T, Suzuki H, Kono E, Sekiguchi T. 1998b. Mapping of interneurons that contribute to food aversion conditioning in the slug brain. Learning Memory 4:376–388.
Kimura T, Toda S, Sekiguchi T, Kawahara S, Kirino Y. 1998c. Optical recording analysis of olfactory response of the procerebral lobe in the slug brain. Learning Memory 4:389–400.
Koene JM, Jansen RF, ter Maat A, Chase R. 2000. A conserved location for the CNS control of mating behaviour in gastropod molluscs: evidence from a terrestrial snail. J Exp Biol 203:1071–1080.
Kunze H. 1921. Zur Topographie und Histologie des Centralnervensystems von *Helix pomatia* L. Z Wiss Zool 118:25–203.
LaBerge S, Chase, R. 1992. The development of mesocerebral neurons in the snail *Helix aspersa maxima*. Can J Zool 70:2034–2041.
Li G, Chase R. 1995. Correlation of axon projections and peptide immunoreactivity in mesocerebral neurons of the snail *Helix aspersa*. J Comp Neurol 353:9–17.
Panchin YV, Popova LB, Deliagina TG, Orlovsky GN, Arshavsky YI. 1995. Control of locomotion in marine mollusk *Clione limacina*. VIII. Cerebropedal neurons. J Neurophysiol 73:1912–1923.
Pentreath VW, Berry MS, Osborne NN. 1982. The serotonergic cerebral cells in gastropods. In: Osborne NN, editor. Biology of serotonergic transmission. New York: John Wiley, p 457–513.
Prescott SA, Gill N, Chase R. 1997. Neural circuit mediating tentacle withdrawal in *Helix aspersa*, with specific reference to the competence of the motor neuron C3. J Neurophysiol 78:2951–2965.
Ratte S, Chase R. 1997. Morphology of interneurons in the procererbrum of the snail *Helix aspersa*. J Comp Neurol 384:359–372.
Ratte S, Chase R. 2000. Synapse distribution of olfactory interneurons in the procerebrum of the snail *Helix aspersa*. J Comp Neurol 417:366–384.
Rosen SC, Weiss KR, Goldstein RS, Kupfermann I. 1989. The role of a modulatory neuron in feeding and satiation in *Aplysia*: Effects of lesioning of the serotonergic metacerebral cells. J Neurosci 9:1562–1578.
Schütt A, Basar E, Bullock TH. 1999. Power spectra of ongoing activity of the snail brain can discriminate odorants. Comp Biochem Physiol A 123:95–110.
Schmalz E. 1914. Zur Morphologie des Nervensystems von *Helix pomatia* L. Z Wiss Zoologie 111:506–568.
Sokolove PG, Van Minnen J. 1983. Control of reproductive development in the giant slug, *Limax maximus*. In: Lever J, Boer HH, editors. Molluscan neuroendocrinology. Amsterdam: North-Holland, p 101–105.
Teyke T, Gelperin A. 1999. Olfactory oscillations augment odor discrimination not odor identification by *Limax* CNS. Neuroreport 10:1061–1068.
Van Mol JJ. 1967. Étude morphologique et phylogénétique du ganglion cérébroïde des Gastéropodes Pulmonés (Mollusques). Mém Acad R Belg (Classe Sci) 37:1–168.
Yeoman MS, Brierley MJ, Benjamin PR. 1996. Central pattern generator interneurons are targets for the modulatory serotonergic cerebral giant cells in the feeding system of *Lymnaea*. J Neurophysiol 75:11–25.
Yoshida M, Kobayashi M. 1995. Modulation of the buccal muscle contraction by identified serotonergic and peptidergic neurons in the snail *Achatina fulica*. J Exp Biol 198:729–738.
Zakharov IS, Hayes NL, Ierusalimsky VN, Nowakowski RS, Balaban PM. 1998. Postembryonic neurogenesis in the procerebrum of the terrestrial snail, *Helix lucorum* L. J Neurobiol 35:271–276.
Zakharov I, Mats VN, Balaban PM. 1982. Role of the giant cerebral neuron in control of defensive behavior of *Helix lucorum* (in Russian). Neirofiziologiya 14:353–358. Transl Neurophysiol 14:262–266, 1983.