STRUCTURAL ORGANIZATION OF THE MORMYRID ELECTROSENSORY LATERAL LINE LOBE

J. MEEK[1],*, K. GRANT[2] AND C. BELL[3]

[1]*Department of Anatomy, University of Nijmegen, PO Box 9101, 6500 HB Nijmegen, The Netherlands*, [2]*Institute Alfred Fessard, CNRS, Gif sur Yvette, France* and [3]*Neurological Science Institute of Oregon Health Science University, Portland, OR 97209, USA*

*e-mail: J.Meek@Anat.KUN.nl

Summary

The electrosensory lateral line lobe (ELL) of mormyrid teleosts is the first central stage in electrosensory input processing. It is a well-developed structure with six main layers, located in the roof of the rhombencephalon. Its main layers are, from superficial to deep, the molecular, ganglionic, plexiform, granular, intermediate and deep fiber layers. An important input arises from electroreceptors, but corollary electromotor command signals and proprioceptive, mechanosensory lateral line and descending electrosensory feedback inputs reach the ELL as well. The ELL input is processed by at least 14 cell types, which frequently show plastic responses to different inputs. The large ganglionic and large fusiform cells are the ELL projection cells. They are glutamatergic and project to the isthmic preeminential nucleus and the midbrain lateral toral nucleus. Interneurons are located in all ELL layers and are mostly GABAergic. The most remarkable interneurons are large multipolar cells in the intermediate layer, which have myelinated dendrites making presynaptic terminals contacting granular cells. With respect to the synaptic organization and microcircuitry of the ELL, a number of qualitative and quantitative aspects have been elucidated using electron microscopical and intracellular labeling techniques. However, the pathways by which primary afferent input influences the ELL projection cells are still undetermined: primary afferents do not seem to contact large fusiform or large ganglionic cells directly, but seem to terminate exclusively on granular cells, the axonal properties of which are not known. Consequently, more information of the structural organization of the ELL is still necessary for a detailed understanding of the neural basis of the plastic electrosensory input processing in mormyrids.

Key words: electroreception, lateral line system, central nervous system, synaptology, microcircuitry, teleost, mormyrid, *Gnathonemus petersii*.

Introduction

The electrosensory lateral line lobe (ELL) is the first stage in the processing of electroreceptive input in the brain of mormyrids. Most of the electrosensory input of these fishes is generated by the animals themselves by way of the electric organ (EO) in their tail, which generates pulse-like electric organ discharges (EODs; Bass, 1986). These pulses are received by two types of tuberous electroreceptors in the skin: knollenorgans and mormyromasts (Zakon, 1986). Knollenorgans have a high sensitivity but do not encode stimulus intensity. They are activated by the EODs of the animal itself as well as by the EODs of other fishes and are involved in electrocommunication (Bell, 1986). Mormyromasts nicely encode the amplitude of reafferent EODs and are thus sensitive to changes induced by (conductive or insulating) objects in the nearby environment of the fish and are involved in active electrolocation (Bell, 1986). In addition, mormyrids have ampullary electroreceptors, specialized to detect the low-frequency electrical signals generated by various structures in the environment of the fish (e.g. plants and small prey animals), thus being involved in passive electrolocation (Bell, 1986).

The ELL receives ipsilateral input from all three types of electroreceptors by way of the lateral line nerves. Primary afferents innervating knollenorgans project to the nucleus of the ELL, primary afferents innervating ampullary organs project to the ventrolateral zone of the ELL, and primary afferents innervating mormyromasts project to the dorsolateral and medial zones of the ELL (see Fig. 1; Bell and Szabo, 1986; Bell et al., 1989). The nucleus of the ELL projects in turn to the exterolateral nucleus of the midbrain torus semicircularis. Interestingly, the nucleus of the ELL receives not only excitatory knollenorgan input but also an inhibitory corollary input from the electromotor command nucleus *via* a γ-aminobutyric acid (GABA)-ergic sublemniscal cell group (Bell, 1986; Mugnaini and Maler, 1987a; Denizot et al., 1987). The GABAergic terminals appear to be activated

simultaneously with the arrival of the knollenorgan activity that is evoked by the animal's own EOD and cancel this reafferent EOD input completely (Bell and Grant, 1989). Consequently, the exterolateral toral nucleus receives exclusively exafferent EOD input, the processing of which is not disturbed by reafferent input that is irrelevant for electrocommunication (Bell, 1986; Mugnaini and Maler, 1987b; Bell and Grant, 1989).

The ventrolateral ampullary zone of the ELL receives a corollary electromotor command input as well, to eliminate reafferent input effects that would impair the passive electrolocation of objects (Bell, 1986, 1989). The ventrolateral zone does not project to the exterolateral nucleus, but to the lateral nucleus of the midbrain torus semicircularis. In addition, the ventrolateral zone projects to the nucleus preeminentialis in the isthmus region (Bell and Szabo, 1986).

By far the largest part of the ELL, i.e. the dorsolateral and the medial zones on both sides, is involved in the processing of mormyromast input, and particularly complex and interesting signal processing features have been observed in this part of the ELL. These include different types of facilitating and inhibitory interactions between electrosensory and corollary electromotor signals (Bell, 1986, 1989, 1990a; Bell and Grant, 1992; Bell et al., 1992; Meek and Grant, 1994), integration of electrosensory and descending feedback signals from higher brain centers (Bell, 1986; von der Emde and Bell, 1996) and a marked plasticity in a number of input interactions (Bell et al., 1993, 1997b,c). A big advantage of the ELL over structures showing similar synaptic plasticity phenomena, such as the cerebellum, the hippocampus and the cerebral cortex, is that the plastic effects in the ELL can be interpreted and understood in terms of subtraction of expected sensory effects (as generated by, for example, tail movements, water movements or electric organ activity in the absence of nearby objects) from unexpected signals, thus allowing new and unexpected biologically relevant stimuli to stand out more clearly (Bell et al., 1997a,c).

Detailed anatomical information about the morphological and synaptic organization of afferents and cell types is necessary to understand the mechanisms that underlie the plastic interactions between different ELL inputs. Such information provides a basis for interpreting extra- or intracellularly recorded responses. Accordingly, this paper surveys our understanding of the structural organization of the mormyrid ELL, including both the extrinsic and intrinsic connectivity patterns. The paper focuses on the medial mormyromast zone of *Gnathonemus petersii*, since the most recent data have been obtained in this region of the mormyrid ELL. Apart from some quantitative differences, the dorsolateral mormyromast zone seems to have a similar organization (Grant et al., 1996). A recent review of the organization of the nucleus of the ELL has been presented by Meek and Nieuwenhuys (1998). No data on the ventrolateral ampullary zone have been published since the review by Bell and Szabo (1986).

Extrinsic connections of the ELL

The extrinsic connections of the (medial zone of the) ELL are summarized in Fig. 2. As mentioned above, an important input arises from primary afferents that innervate mormyromast electroreceptors. It has been demonstrated that the mormyromast projections to both the medial and dorsolateral zone are topographically organized and project a map of the body surface to each ELL zone (Maler et al., 1973a,b; Bell and Russell, 1978). These maps arise from different mormyromast receptor cells: the lateral receptor cells are connected with the medial zone, and the basal cells are connected with the dorsolateral zone (Bell et al., 1989; Bell, 1990a,b). It should be mentioned that the ELL is the only target of electrosensory primary afferents. This is in contrast to the mechanosensory lateral line primary afferents, which have more than one central target in mormyrids (Bell, 1981) and other teleosts (Meek and Nieuwenhuys, 1998).

A second input to the ELL arises from the preeminential nucleus, which is also a target of the ELL efferents. ELL efferents project by way of the lateral lemniscus almost exclusively to the preeminential nucleus in the isthmus region and to the lateral nucleus of the torus semicircularis in the midbrain (Bell et al., 1981; Finger et al., 1981; Grant et al., 1996). The lateral toral nucleus, which also receives input from the ampullary ELL region (Bell and Szabo, 1986), projects to the valvula cerebelli, either directly or *via* the dorsal preglomerular nucleus, and to the nucleus preeminentialis (Bell et al., 1981; Finger et al., 1981). The dorsal preglomerular nucleus, previously referred to as the dorsal anterior pretectal nucleus (see Meek and Nieuwenhuys, 1998), also projects to cerebellar lobe C_3 (Meek et al., 1986). The nucleus preeminentialis projects both directly and indirectly to the ELL. The direct projection terminates in the deep part of the molecular layer of the ELL. The indirect projection is *via* the cerebellar eminentia granularis posterior.

The eminentia granularis posterior (egp) is a mass of granule cells that covers a large part of the surface of the ELL (Fig. 1). The granule cells project with small unmyelinated parallel fibers to the molecular layer of the ELL (see below). The egp not only relays electrosensory feedback signals from the preeminential nucleus to the ELL, but also proprioceptive input as received *via* the first and second lateral funicular nuclei in the obex region (Bell et al., 1981; Szabo et al., 1990; Szabo, 1993), mechanosensory lateral line input and corollary electromotor command input (Bell et al., 1992).

Corollary electromotor command signals arise from the electromotor command nucleus in the brainstem, a small nucleus with a small number of small cells that have widely extending dendrites (Grant et al., 1986). These neurons generate the electromotor command signals under the influence of a variety of inputs on their dendrites (Elekes and Szabo, 1985) and of terminals from the precommand nucleus on their somata (Grant, 1993). The electromotor command nucleus projects primarily to the large and electrotonically coupled cells of the medullary relay nucleus, located just above the
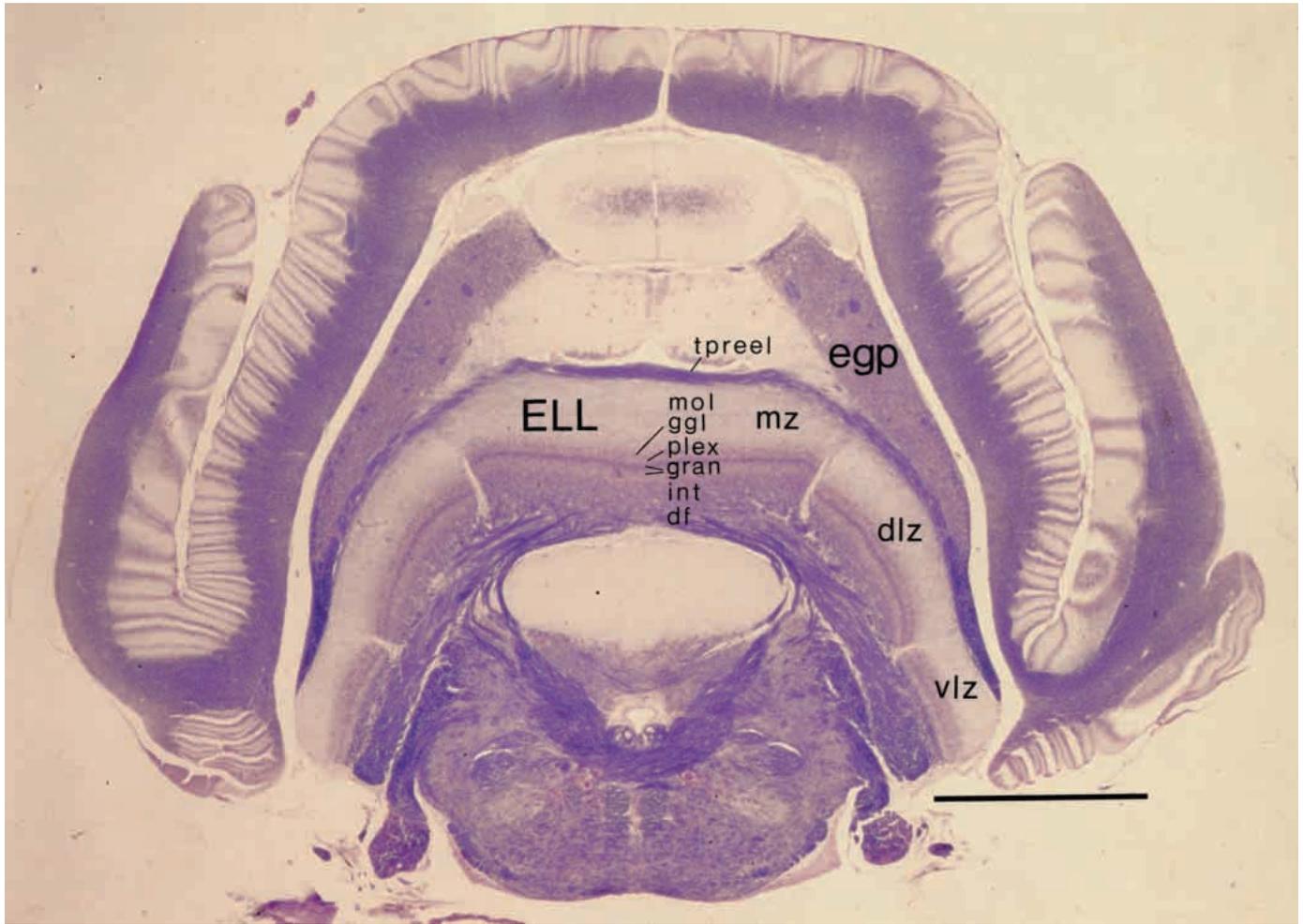
Fig. 1. Transverse Kluver–Barrera-stained section of the rhombencephalon of *Gnathonemus petersii* at the level of the electrosensory lateral line lobe (ELL). Myelinated fibers are dark purple and cell bodies light purple. df, deep fiber layer; dlz, dorsolateral ELL zone; egp, eminentia granularis posterior; ggl, ganglionic layer; gran, granular layer; int, intermediate (cell and fiber) layer; mol, molecular layer; mz, medial ELL zone; plex, plexiform layer; tpreel, preeminential–ELL tract; vlz, ventrolateral ELL zone. Scale bar, 1 μm.

command nucleus (Elekes et al., 1985), which in turn projects to the electromotor neurons in the spinal cord that innervate the electric organ (Fig. 2; Grant et al., 1986). However, collaterals of the short axons between the command and relay nucleus in the brainstem project to the bulbar command-associated nucleus, which in turn projects to the medullary relay nucleus and to the paratrigeminal and the mesencephalic command-associated nuclei (Bell et al., 1983). The paratrigeminal command-associated nucleus projects to the egp (Bell et al., 1983), which relays the paratrigeminal corollary electromotor command signals to the ELL (see above). The mesencephalic command-associated nucleus, recently analyzed by Bell et al. (1995), projects to juxtalemniscal cells located medial and ventral to the lateral lemniscus in the region of the preeminential nucleus. Juxtalemniscal cells project either directly to the ELL or *via* the juxtalobar nucleus, which is located just rostromedial to the ELL (Bell and von der Emde, 1995).

In addition to the inputs just enumerated and illustrated in

Fig. 2, the ELL receives serotoninergic input from raphe nuclei (Grant et al., 1989; Meek and Joosten, 1989) and noradrenergic input from the locus coeruleus and/or from a group of cells in the caudal rhombencephalon (Meek et al., 1993). Different ELL zones also receive afferents from other zones. Both intrazonal or commissural connections (connecting the left medial zone with the right medial zone and the left dorsolateral zone with the right dorsolateral zone) and interzonal connections (between the left medial and dorsolateral zones and between the right medial and dorsolateral zones) have been described, and all are topographically organized (Bell et al., 1981; Bell and Szabo, 1986).

Layers of the ELL

The ELL consists of six main layers, termed, from superficial to deep, the molecular, ganglionic, plexiform, granular, intermediate (cell and fiber) and deep fiber layers (Maler, 1973; Bell and Szabo, 1986). The boundary between

the granular and intermediate layers has recently been redefined (Grant et al., 1996), and we will follow their subdivision. Each main layer can be subdivided into a superficial and a deep sublayer with a different population of neural elements, which means that the mormyrid ELL in fact consists of 12 distinct (sub)layers with different information-processing properties (Fig. 3).

The molecular layer is composed of numerous thin, unmyelinated fibers which course parallel to each other and parallel to the ELL surface, and thus are termed parallel fibers. These fibers originate from the egp granule cells and terminate on the spiny dendrites of more deeply located cells (see below). All these features are similar to those of the cerebellar molecular layer and, consequently, the ELL is frequently described as a cerebellum-like structure. In fact, the ELL molecular layer is a derivative of the cerebellar crest. This is a layer of parallel fibers that originate from the cerebellar granular eminences, two superficially located masses of granule cells in the caudal cerebellar region, sometimes referred to in the past as cerebellar auricles. The cerebellar crest parallel fibers run from rostral to caudal over the

(mechanosensory) octavolateralis region in teleosts and several other fish groups, terminating *en passant* on more deeply located Purkinje-like cells, known as crest cells (see Meek and Nieuwenhuys, 1998). Interestingly, the parallel fibers of the ELL molecular layer are not oriented rostrocaudally but transversely, a remarkable evolutionary topological transformation. There is a clear distinction between the superficial and the deep parts of the molecular layer. The superficial part is more 'crystalline', more regularly built up by exclusively transversely oriented thin parallel fibers and their targets, whereas the deep molecular layer, where the preeminential fibers terminate, has a less regular organization with fibers that have various diameters and are oriented in a variety of directions.

The ganglionic layer consists predominantly of cell bodies, a substantial proportion of which are relatively large ($>10\,\mu$m in diameter). Consequently, this layer has been compared with the cerebellar Purkinje cell layer, which in teleosts is known as the ganglionic layer because it contains not only Purkinje cells but also the teleostean eurydendroid cerebellar projection cells (see Meek and Nieuwenhuys, 1998). Nevertheless, the
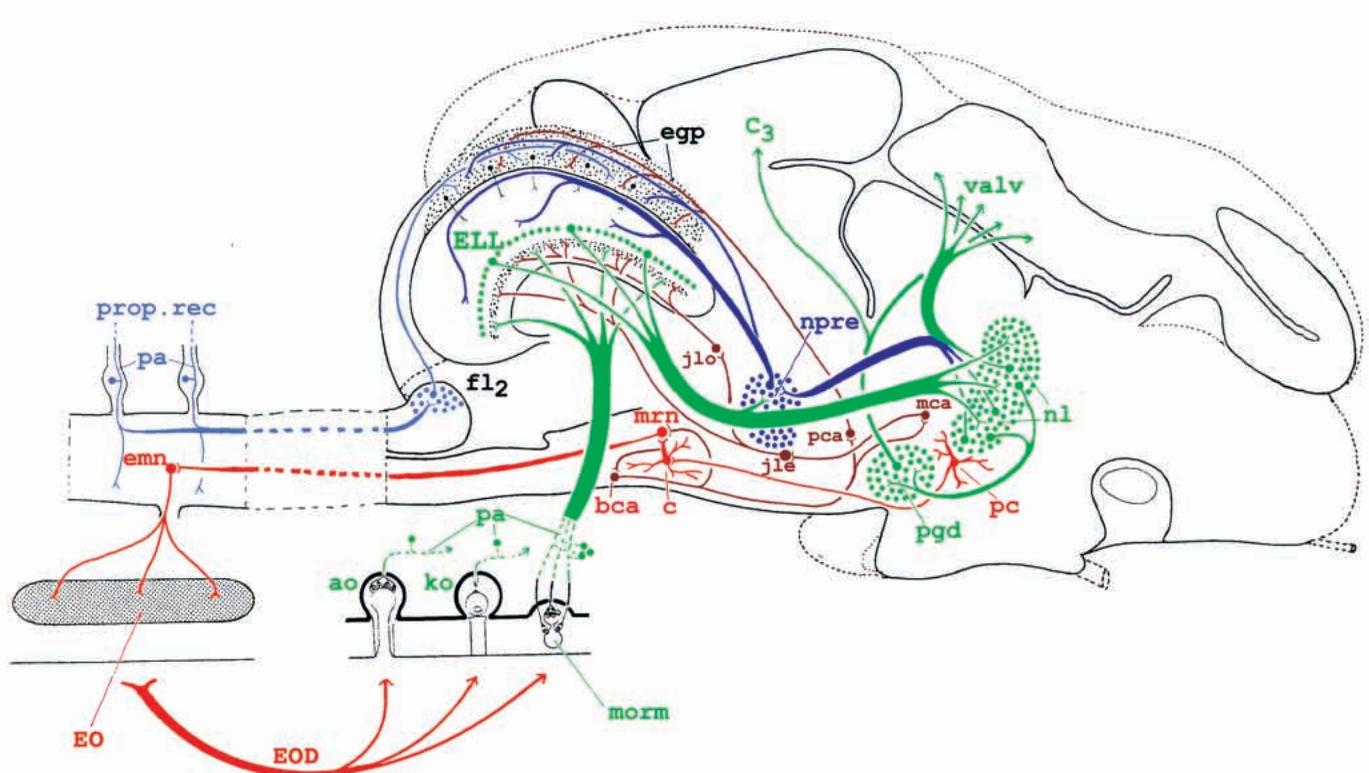

Fig. 2. The extrinsic connections of the medial (mormyromast) zone of the mormyrid electrosensory lateral line lobe (ELL) and some related circuits. Ascending electrosensory projections have been indicated in green, feedback electrosensory connections in blue, electromotor command pathways in red, corollary electromotor pathways in brown and ascending proprioceptive projections in purple. ao, ampullary (electroreceptive) organ; bca, bulbar command-associated nucleus; c, (electromotor) command nucleus; C_3, third cerebellar lobule; egp, eminentia granularis posterior; emn, electromotor neuron; EO, electric organ; EOD, electric organ discharge; fl_2, second funicular nucleus; jle, juxtalemniscal cells; jlo, juxtalobar nucleus; ko, knollenorgan; mca, mesencephalic command-associated nucleus; morm, mormyromast primary afferent; mrn, medullary relay nucleus; nl, nucleus lateralis (of the torus semicircularis); npre, nucleus preeminentialis; pa, primary afferent; pc, precommand nucleus; pca, paratrigeminal command-associated nucleus; pgd, dorsal preglomerular nucleus; prop. rec, proprioreceptors; valv, valvula cerebelli.
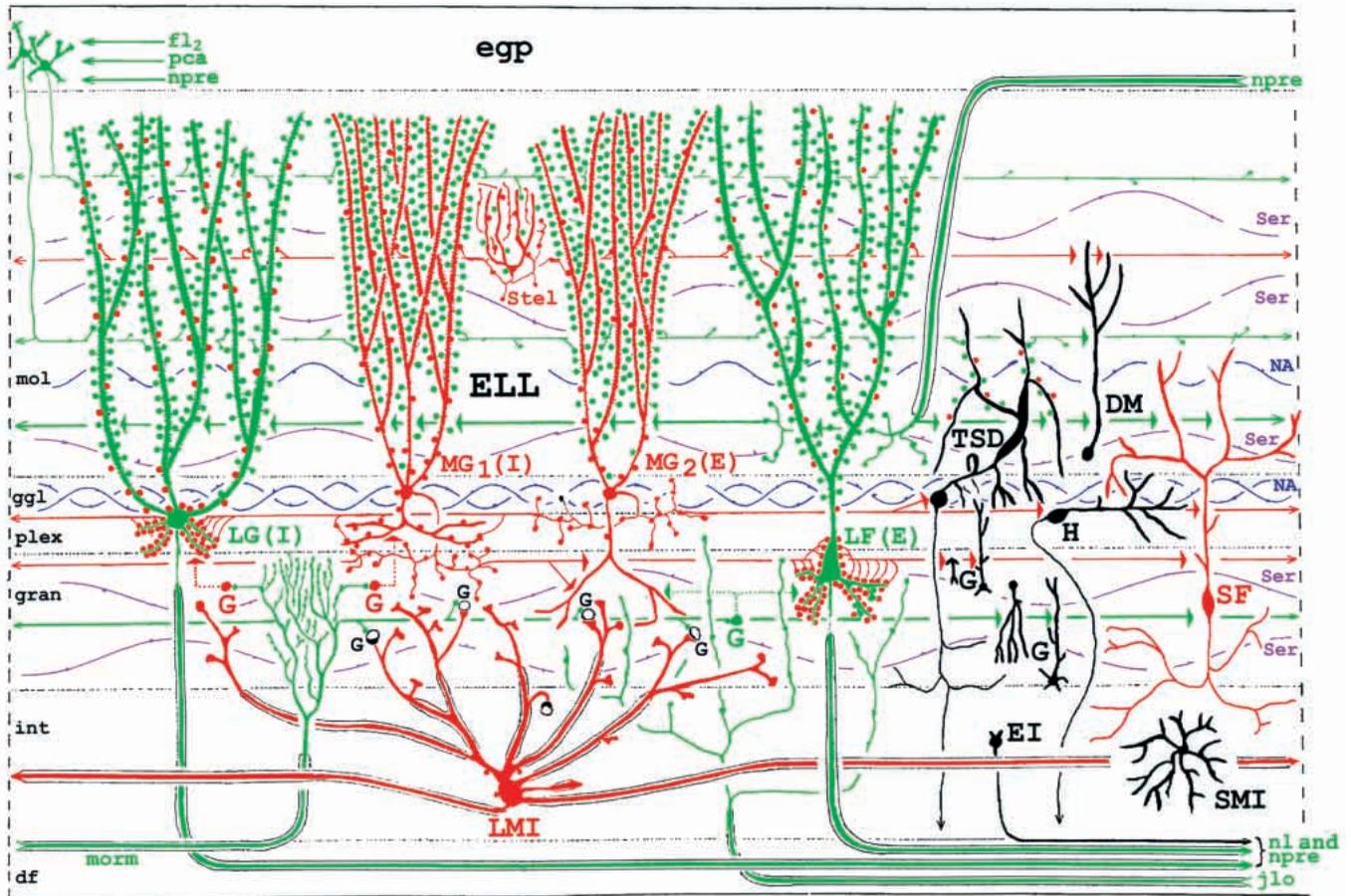
Fig. 3. The intrinsic circuitry of the medial (mormyromast) zone of the mormyrid electrosensory lateral line lobe (ELL). GABAergic (inhibitory) structures have been indicated in red, glutamatergic (excitatory) structures in green, noradrenergic fibers in blue and serotoninergic fibers in purple. Cells with unknown transmitters are indicated in black, while myelin sheaths are indicated by contours. Dendritic trees and axonal arborizations are drawn semi-schematically. Axonal arborizations are connected with horizontal lines to indicate their main layer of termination and their (possible) targets. Presynaptic terminals are indicated by small dots and unequivocally demonstrated synaptic connections are indicated by small dots contacting postsynaptic dendrites or cell bodies. Presumed but not (yet) definitely demonstrated synaptic contacts are indicated by blunt arrows, while sharp arrows indicate that structures continue beyond the frame of the drawing. df, deep fiber layer; DM, cell of the deep molecular layer; E, excited by primary afferent input; egp, eminentia granularis posterior; EI, efferent cell of the intermediate layer; fl_2, second funicular nucleus; G, granular cell; ggl, ganglionic layer; gran, granular layer; H, horizontal cell; I, inhibited by primary afferent input; int, intermediate (cell and fiber) layer; jlo, juxtalobar nucleus; LF, large fusiform cell; LG, large ganglionic cell; LMI, large multipolar intermediate layer cell; MG, medium-sized ganglionic cell; MG_1, first subtype of MG cell, with basal dendrites in the plexiform layer; MG_2, second subtype of MG cell, with basal dendrites in the granular layer; mol, molecular layer; morm, mormyromast primary afferent; NA, noradrenaline; nl, nucleus lateralis (of the torus semicircularis); npre, nucleus preeminentialis; pca, paratrigeminal command-associated nucleus; plex, plexiform layer; Ser, serotonin; SF, small fusiform cell; SMI, small multipolar intermediate layer cell; Stel, stellate cell; TSD, cell with a thick smooth dendrite.

term ganglionic layer is unfortunate, since it has led to the designation of its large cells as ganglion cells. This is misleading since they resemble neither the ganglion cells of afferent nerve ganglia nor the ganglion cells of the retina. In several respects, they resemble cerebellar Purkinje cells, and consequently the term 'Purkinje-like' cells is frequently encountered as an alternative. These cells are 'crest cells', but this is a term unfamiliar to most neuroscientists and would not distinguish them from the unspecialized mechanosensory lateral line crest cells. To make the confusion even greater, the comparable cells in gymnotids are known as pyramidal cells

(Carr and Maler, 1986). We now prefer the name ganglionic (thus not ganglion) cells or ganglionic layer cells for the large elements in the ELL ganglionic layer. Medium-sized, GABAergic ganglionic cells are concentrated in the superficial part of the ganglionic layer, and large glutamatergic ganglionic cells are concentrated in the deep part of the ganglionic layer (see below). It should be noted that the majority of cell bodies in the ganglionic layer are rather small ($<10\,\mu m$ in diameter; Meek et al., 1996), which is very different from the cerebellar ganglionic layer of mormyrids and other teleosts.

The plexiform layer is a relatively cell-poor zone between

the ganglionic and granular layers, and is visible with particular clarity at low magnifications (Fig. 1). At higher magnifications, it is obvious that this layer, in addition to a plexiform pattern of thin dendrites and axons, also contains a substantial number of small cells and that the boundaries with the deep ganglionic layer and with the superficial granular layer are not sharp. The origins of the small axons and dendrites in the superficial and deep parts of the plexiform layer are different (see below).

The granular layer consists predominantly of granular (i.e. small, round) cells, but it should be noted that these do not resemble cerebellar granule cells in any way: they are somewhat larger, do not receive (glomerular) mossy fiber input and do not send parallel fibers to the molecular layer (see below). Consequently, we term this layer the granular (and not granule cell) layer, and term the small cells granular (and not granule) cells. In addition to granular cells, the granular layer contains quite large and also medium-sized fusiform cells. The granular elements are packed quite densely in the superficial part of the granular layer. This (sub)layer alone was previously known as the granular layer (and now sometimes as the granular layer proper), whereas the deep part of the granular layer was previously included in the intermediate layer. However, the predominant neural elements in the deep granular layer are granular cells, in spite of the fact that they are somewhat larger and less densely packed than those in the superficial granular layer. Hence, and for several other reasons (Grant et al., 1996), we prefer the name deep granular layer to superficial intermediate layer for this ELL region.

The intermediate (cell and fiber) layer is characterized by a low density of small granular cells and by large and medium-sized multipolar neurons, interspersed in a meshwork of small and thicker myelinated fibers with various orientations. The cells are mostly in the superficial part of the intermediate layer, whereas the deep part of the intermediate layer consists almost exclusively of fibers.

The deep fiber layer contains almost exclusively myelinated axons, which are on average thicker than those in the intermediate layer and are concentrated in well-oriented bundles that run in the transverse plane or slightly obliquely. Juxtalobar afferents course through the deepest, periventricular part of the deep fiber layer (C. C. Bell, unpublished observations), whereas the primary afferents constitute the larger, more superficially located part of the deep fiber layer. Because all primary afferents enter the ELL laterally and terminate ipsilaterally, the deep fiber layer is rather thick ventrolaterally but virtually absent in the dorsomedial part of the ELL.

Laminar organization of afferents in the ELL

As mentioned above, electroreceptive primary afferents enter the ELL deep fiber layer laterally and course dorsomedially in this layer to reach their topographically determined termination region. Once they have arrived at this site, they bend more or less orthogonally to penetrate the intermediate layer, lose their myelin sheath (except for perhaps a few branches) and terminate in the (superficial as well as deep) granular layer, with a few terminals more deeply (Bell and Russell, 1978; Bell and Szabo, 1986; Bell et al., 1989; Bell, 1990a,b; Grant et al., 1996). They make mixed (i.e. combined gap-junctional and chemical) synaptic contacts with granular cells and small dendrites in the granular layer (Bell et al., 1989; J. Meek, C. C. Bell and K. Grant, unpublished observations) and have excitatory, glutamate-mediated effects on their targets (Bell, 1990a,b; Grant et al., 1998). No primary afferent terminals have been observed to contact the somata of the large or medium-sized fusiform cells in the granular layer or the large multipolar cells in the intermediate layer. However, some of the small dendrites in the granular layer that receive primary afferent input may belong to these cell types.

The juxtalobar fibers are another deeply arriving set of afferents to the ELL. These fibers relay excitatory corollary electromotor command signals to the ELL (Bell and von der Emde, 1995). Terminal arborizations of individual juxtalobar fibers are more widely distributed than those of primary afferents and make varicosities that strongly resemble terminals from the intermediate up to the deep molecular layer, with the largest density in the granular layer, however (Fig. 3; C. C. Bell and K. Grant, unpublished observations). Their target cells in the ELL are unknown.

Preeminential fibers enter the ELL superficially and form a distinct (preeminential–ELL) tract located between the ELL molecular layer and the egp (Fig. 1). From there, fibers descend through the molecular layer to lose their myelin and to terminate in the deep molecular layer in a topographically ordered manner (Bell et al., 1981; Bell and Szabo, 1986). In the deep molecular layer, they make type I (i.e. with large round vesicles and asymmetric synaptic specializations; thus excitatory) synaptic terminals with spiny and smooth dendrites (Meek, 1993; J. Meek and K. Grant, unpublished observations). It is not known to which ELL cell types these dendrites belong.

It has already been mentioned that the parallel fibers of the molecular layer arise from the egp and thus, in contrast to cerebellar parallel fibers, represent ELL afferents. Their origin from the egp has now been demonstrated by both anterograde and retrograde tracer experiments (J. Meek, K. Grant and C. C. Bell, unpublished observations) and by Golgi impregnation (J. Meek, unpublished observations). The precise distribution of parallel fibers originating at different sites in the egp over the ELL surface and at different depths in the molecular layer is unknown, but our experiments suggest that most parallel fibers extend over the entire surface of the ELL from left to right. The ELL parallel fibers make type I (thus excitatory) synaptic contacts with spines of the apical dendrites of the ganglionic cells and with the non-spiny dendrites of some other cell types, including stellate cells (Grant et al., 1996; Meek et al., 1996). It has been mentioned above that the egp parallel fibers relay proprioceptive, electroreceptive feedback, mechanosensory lateral line and corollary electromotor command signals to the ELL (Fig. 2), all of which can be

considered as predictive signals for the electrosensory input of the ELL. Our present attention is especially focused on the intrinsic ELL circuitry that connects the superficial molecular layer input of the ELL to its deep electrosensory input, since interactions between these two regions underlie the plastic interactions between electrosensory signals and their predictive inputs (Bell et al., 1993; Bell et al., 1997a–c).

Serotoninergic fibers course through almost all ELL layers (Grant et al., 1989; Meek and Joosten, 1989) whereas noradrenergic fibers are mostly restricted to the deep molecular and ganglionic layers (Meek et al., 1993). The layer(s) of termination and the targets of interzonal and intrazonal ELL connections are unknown. This also holds for the juxtalemniscal input to the ELL.

Cell types in the ELL

The ELL cells that project to the preeminential nucleus and the lateral toral nucleus *via* the lateral lemniscus, referred to as lemniscal projection neurons to distinguish them from interzonal and intrazonal or commissural projection cells, have been characterized in detail by Grant et al. (1996). They can be subdivided into large ganglionic (LG) and large fusiform (LF) cells and a population of small cells in the intermediate layer, which has not yet been characterized further (EI, efferent intermediate layer cells; Fig. 3). LG cells have a multipolar cell body (on average 13 μm×13 μm) in the deep ganglionic layer, whereas LF cells have a fusiform soma (on average 10 μm×20 μm) in the granular layer. Apart from the shape and location of their cell bodies, the properties of LG and LF cells are quite similar: They are both glutamatergic, have 6–12 long, spiny apical dendrites in the molecular layer and several short, smooth basal dendrites that arborize in the plexiform (LG cells) or in the granular (LF cells) layer. Their apical dendritic trees make on average approximately 10 000 spiny synaptic contacts per cell with egp parallel fibers, and in the deep molecular layer they are probably contacted by preeminential terminals. Their apical dendrites make, in addition, non-spiny synaptic contacts with GABA-positive terminals from stellate cells, approximately 3000 per neuron. The somata and proximal basal dendrites of both LG and LF cells are densely covered with GABAergic terminals that arise in part from medium-sized ganglionic cells (see below). Electrophysiological recordings with subsequent intracellular labeling have shown that LG cells are I-cells, which means that they are inhibited by electric stimulation of their receptive field center, whereas LF cells are E-cells and thus are excited by a similar stimulus (Bell et al., 1997a).

All layers of the ELL contain several types of interneurons, which makes the intrinsic organization and wiring pattern of the mormyrid ELL quite complex. Stellate cells are present throughout the molecular layer and have properties that are quite similar to those of cerebellar stellate cells. In the deep molecular layer, an additional type of deep molecular layer (DM) cell is encountered. Meek et al. (1996) have reported that ELL stellate cells are GABAergic and have mostly apically

oriented, smooth dendrites that receive both excitatory parallel fiber input and inhibitory stellate cell input. Their axons terminate on the shafts of the spiny dendrites of large ganglionic cells, large fusiform cells and medium-sized ganglionic cells, as well as on the non-spiny dendrites of stellate cells and TSD cells (see below). Terminals on other cells with dendrites in the molecular layer may also exist, but have not (yet) been demonstrated. Deep molecular layer cells have smooth apical dendrites that are restricted to the lower half of the molecular layer. Their axonal properties and transmitter are presently unknown.

The most numerous interneurons of the ganglionic layer are GABAergic medium-sized ganglionic (MG) cells, which are particularly concentrated in the superficial part of the ganglionic layer. Their properties have been described in detail by Meek et al. (1996). Like LG cells, they have spiny apical dendrites in the molecular layer contacted by parallel fibers (on their spines) and stellate cell axons (on their shafts). However, in the medial ELL zone, the number of MG apical dendrites is on average larger and their spine density greater than those of LG cells, resulting in approximately twice as many spiny synapses per cell with parallel fibers (approximately 20 000). In contrast, the number of inhibitory stellate axon synapses on MG cell dendritic shafts is considerably lower (approximately 1000–2000 per cell). MG cells have one smooth thin basal dendrite that branches and terminates either in the plexiform layer (MG$_1$ subtype) or in the granular layer (MG$_2$ subtype). The dendrites of the MG$_1$ subtype and the proximal basal dendrite of the MG$_2$ subtype receive a low density of GABAergic synaptic inputs in the plexiform layer, in part arising from MG cell axons. The contacts of the basal dendrites of MG$_2$ cells in the granular layer are unknown. Meek et al. (1996) showed that the axons of MG cells terminate in the deep ganglionic and the superficial plexiform layers on basal dendrites of other MG cells and on the somata and proximal dendrites of LG cells. Intracellular labeling (Han et al., 1999) revealed that the axonal properties of MG$_1$ and MG$_2$ cells are different: the MG$_1$ cells, with relatively superficially located dendrites in the plexiform layer, have more deeply located axons, whereas the MG$_2$ cells, with their dendrites in the granular layer, have more superficially located axons. They also showed that MG$_2$ axons contact the cell bodies of LG cells and that MG$_1$ axons contact the cell bodies of LF cells (Fig. 3). Han et al. (1999) suggest that MG$_1$ cells are I-cells and that MG$_2$ cells are E-cells on the basis of the locations of their basal dendrites, which resemble those of LG neurons (I-units) and LF neurons (E-units) respectively. They also suggest that MG$_1$ axons synapse predominantly upon LF and MG$_2$ neurons, whereas MG$_2$ axons mostly terminate on LG and MG$_1$ cells. This would mean that I-interneurons predominantly inhibit E-cells and that E-interneurons predominantly inhibit I-cells (Fig. 3).

Apart from MG interneurons, the ganglionic layer contains numerous small cells with unknown dendritic and axonal properties, and the cell bodies of so-called TSD cells. The latter have a thick, smooth dendrite (TSD) that courses obliquely in

the deep molecular layer. This dendrite has thin branches that ascend to approximately the middle of the molecular layer and recurrent branches that descend into the ganglionic and plexiform layers. In the molecular layer, these branches receive both excitatory and inhibitory inputs (Meek et al., 1996). Their axons have collaterals in the deep granular layer and then course to the intermediate layer (Han et al., 1999; Fig. 3). They probably establish interzonal connections, since TSD cells were never labeled after tracer injections into lemniscal targets. The same probably holds for a type of horizontal cell encountered in the plexiform layer. The axon of this cell type also courses to the intermediate layer, but without terminals in the granular layer (Han et al., 1998; Fig. 3).

Granular cells are the most mysterious population of ELL neurons. Some are quite small ($5\,\mu m$ in diameter), but others are larger (up to approximately $10\,\mu m$ in diameter); some have only apical dendrites, others only basal dendrites and yet others have both; some are GABA-positive, others glutamate-positive, and a subpopulation is calbindin-positive, but the correlations between these different morphological and biochemical features are uncertain. Granular cells are the major, and perhaps the only, target of primary afferents, but it is not known whether all (subtypes of) granular cells receive mormyromast input. The manner in which they relay the electrosensory input further is also unknown, since the axonal properties of granular cells have not been unravelled. Consequently, several attempts are being made in our laboratories to analyze the functional morphology of (distinct types of) granular cells. Since LG (and possibly also MG_1) cells give I-responses on electrical stimulation, and LF (and possibly also MG_2) cells give E-type responses, we have to assume that the granular cell input is relayed to these neurons. The simplest way to explain the E- and I-responses of LG, MG and LF cells is to assume that inhibition arises from an inhibitory granular cell subtype, whereas excitation arises from an excitatory type of granular cell (Han et al., 1999; Fig. 3). However, this has not been demonstrated anatomically and is not in line with the observation that the surface of LF cells is almost completely covered with GABAergic synapses (Grant et al., 1996). For LG and MG_1 cells, the suggested circuitry might well be possible, since their somata and basal dendrites indeed receive predominantly inhibitory synapses (see above; Fig. 3).

Apart from granular cells and LF cells, discussed above, the granular layer also contains small fusiform (SF) neurons. These are GABAergic and have an apical, non-spiny dendritic tree that branches in the ganglionic and the deep molecular layers (Meek, 1993). The axon of these cells terminates in the deep granular and superficial intermediate layers (Han et al., 1998). The sources of synaptic input to SF neurons and the targets of their axons are not known.

The most curious type of ELL interneuron has a large multipolar cell body in the intermediate layer and is consequently referred to as LMI (large multipolar intermediate layer) neuron (Fig. 3). The unusual aspect of these cells is that they have dendrites that are all myelinated and make presynaptic, not postsynaptic, terminals in the granular layer (Meek et al., 1994). Their dendrites have a widespread distribution and may even cross the midline. They have a clearly distinguishable axon with a probably commissural intrazonal projection. The LMI cells are GABAergic, and thus their dendritic presynaptic terminals, which make huge synaptic contacts with granular cells, may inhibit the latter strongly. This is consistent with electrophysiological observations (Bell, 1990a). However, it is not known how LMI cells may be excited by primary afferent (or other) input since they do not receive synaptic input on their dendrites and have only a few inhibitory synapses on their soma. Consequently, we assume that their dendritic terminals are ephaptically excited by the granular cells on which they synapse, but this has to be analyzed further. Other cell types that occur in the intermediate layer are small efferent (EI) and small multipolar (SMI) neurons, the properties of which are not known.

Intrinsic ELL circuitry

Combined light and electron microscopical analyses of the ELL using a variety of tracer, impregnation and immunohistochemical techniques have revealed the following microcircuits in the mormyrid ELL (Fig. 3; for references, see above). Primary afferents terminate with both gap junctions and chemical synapses on granular cells. It is not known whether they also terminate on the basal dendrites of MG_2 cells or on SF cells, and it is quite unlikely that they terminate directly on LF cells, in spite of the fact that these are surrounded by terminal fields of primary afferents. The granular cells might ephaptically excite presynaptic dendritic LMI terminals, which in turn might inhibit the granular cells that excite them. Simultaneous excitation of many LMI terminals might well result in activation of the entire dendritic arborization of LMI cells and thus in strong lateral inhibition. Granular cells must also relay primary afferent signals to the LF and LG ELL projection neurons and to the MG interneurons, since these respond to primary afferent input but do not seem to be contacted directly by primary afferents, but it is unclear how granular cells do this. We assume that inhibitory granular cells contact LG and MG_1 cells, whereas excitatory granular cells contact LF and MG_2 cells. We also hypothesize, as discussed above, that MG_1 cells inhibit LF and MG_2 cells, whereas MG_2 cells inhibit FG and MG_1 cells. Under this hypothesis, E-cells would nicely inhibit I-cells and *vice versa*. The role of juxtalobar fibers, TSD axons and SF axons, which all terminate in the granular layer, is unknown.

In the molecular layer, preeminential fibers make excitatory synaptic contacts with spines and non-spiny dendrites. They probably terminate on all postsynaptic structures present in the deep molecular layer, including the dendrites of LG, MG, LF, TSD, DM and SF cells, but this is not yet certain. Eminentia granularis posterior (egp) parallel fibers terminate massively on the spines of LG, MG and LF cells and on the smooth dendrites of TSD and stellate cells. The latter, in turn, make GABAergic inhibitory contacts with LG, MG and LF cells. It

is conceivable that parallel fibers and stellate axons also contact DM and SF cells, but this has not yet been demonstrated. The effects and targets of serotoninergic and noradrenergic fibers in the ELL have not been analyzed, but they probably have a general, possibly nonsynaptic, modulatory effect on ELL activity.

In conclusion, the present picture of the ELL circuitry presents a complex substrate for the plastic interactions between electrosensory signals that enter the deep ELL layers and the predictive signals that are relayed to the molecular layer. The predictive inputs are predominantly relayed to spines, which are considered to be the main locus of synaptic and neuronal plasticity. Remarkably, none of the cells that receive spiny input in the molecular layer receives direct electrosensory input. The latter seems to be exclusively relayed into the ELL circuitry by granular cells, which have complex relationships with inhibitory LMI cells and excitatory juxtalobar terminals and whose axonal properties are still unknown. The mechanisms involved in ELL signal processing and plasticity will be more fully understood when we know more about the granular cells and their roles in electrosensory input processing.

References

Bass, A. H. (1986). Electric organs revisited: Evolution of a vertebrate communication and orientation organ. In *Electroreception* (ed. T. H. Bullock and W. Heiligenberg), pp. 13–70. New York: Wiley & Sons.

Bell, C. C. (1981). Central distribution of octavolateral afferents and efferents in a teleost (Mormyridae). *J. Comp. Neurol.* **195**, 391–414.

Bell, C. C. (1986). Electroreception in mormyrid fish: Central physiology. In *Electroreception* (ed. T. H. Bullock and W. Heiligenberg), pp. 423–452. New York: Wiley & Sons.

Bell, C. C. (1989). Sensory coding and corollary discharge effects in mormyrid electric fish. *J. Exp. Biol.* **146**, 229–253.

Bell, C. C. (1990a). Mormyromast electroreceptor organs and their afferent fibers in mormyrid fish. II. Intra-axonal recordings show initial stages of central processing. *J. Neurophysiol.* **63**, 303–318.

Bell, C. C. (1990b). Mormyromast electroreceptor organs and their afferent fibers in mormyrid fish. III. Physiological differences between two morphological types of fibres. *J. Neurophysiol.* **63**, 319–332.

Bell, C. C., Bodznick, D., Montgomery, J. and Bastian, J. (1997a). The generation and subtraction of sensory expectations within cerebellum-like structures. *Brain Behav. Evol.* **50**, 17–31.

Bell, C. C., Caputi, A. and Grant, K. (1997b). Physiology and plasticity of morphologically identified cells in the electrosensory lobe. *J. Neurosci.* **17**, 6409–6422.

Bell, C. C., Caputi, A., Grant, K. and Serrier, J. (1993). Storage of a sensory pattern by anti-Hebbian synaptic plasticity in an electric fish. *Proc. Natl. Acad. Sci. USA* **90**, 4650–4654.

Bell, C., Dunn, K., Hall, C. and Caputi, A. (1995). Electric organ corollary discharge pathways in mormyrid fish. I. The mesencephalic command associated nucleus. *J. Comp. Physiol.* A **177**, 449–462.

Bell, C. C., Finger, T. E. and Russell, C. J. (1981). Central connections of the posterior lateral line lobe in mormyrid fish. *Exp. Brain Res.* **42**, 9–22.

Bell, C. C. and Grant, K. (1989). Corollary discharge inhibition and preservation of temporal information in a sensory nucleus of mormyrid electric fish. *J. Neurosci.* **9**, 1029–1044.

Bell, C. C. and Grant, K. (1992). Sensory processing and corollary discharge effects in mormyromast regions of mormyrid electrosensory lobe. II. Cell types and corollary discharge plasticity. *J. Neurophysiol.* **68**, 859–875.

Bell, C. C., Grant, K. and Serrier, J. (1992). Sensory processing and corollary discharge effects in the mormyromast response of the mormyrid electrosensory lobe. I. Field potentials, cellular activity in associated structures. *J. Neurophysiol.* **68**, 843–858.

Bell, C. C., Han, V. Z., Sugawara, S. and Grant, K. (1997c). Synaptic plasticity in a cerebellum-like structure depends on temporal order. *Nature* **387**, 278–281.

Bell, C. C., Libouban, S. and Szabo, T. (1983). Pathways of the electric organ discharge command and its corollary discharges in mormyrid fish. *J. Comp. Neurol.* **216**, 327–338.

Bell, C. C. and Russell, C. J. (1978). Termination of electroreceptor and mechanical lateral line afferents in the mormyrid acousticolateral area. *J. Comp. Neurol.* **182**, 367–382.

Bell, C. C. and Szabo, T. (1986). Central structures and pathways of the mormyrid electrosensory system. In *Electroreception* (ed. T. H. Bullock and W. Heiligenberg), pp. 375–421. New York: Wiley & Sons.

Bell, C. C. and von der Emde, G. (1995). Electric organ corollary discharge pathways in mormyrid fish. II. The medial juxtalobar nucleus. *J. Comp. Physiol.* A **177**, 463–479.

Bell, C. C., Zakon, H. and Finger, T. E. (1989). Mormyromast electroreceptor organs and their afferent fibers in mormyrid fish. I. Morphology. *J. Comp. Neurol.* **286**, 391–407.

Carr, C. E. and Maler, L. (1986). Electroreception in gymnotiform fish: Central anatomy and physiology. In *Electroreception* (ed. T. H. Bullock and W. Heiligenberg), pp. 319–374. New York: Wiley & Sons.

Denizot, J. P., Clausse, S., Elekes, K., Geffard, M., Grant, K., Libouban, S., Ravaille-Veron, M. and Szabo, T. (1987). Convergence of electrotonic club endings, GABA- and serotoninergic terminals on second order neurons of the electrosensory pathway in mormyrid fish, *Gnathonemus petersii* and *Brienomyrus niger* (Teleostei). *Cell Tissue Res.* **249**, 301–309.

Elekes, K., Ravaille, M., Bell, C. C., Libouban, S. and Szabo, T. (1985). The mormyrid brainstem. II. The medullary electromotor relay nucleus: an ultrastructural horseradish peroxidase study. *Neurosci.* **15**, 417–429.

Elekes, K. and Szabo, T. (1985). The mormyrid brainstem. III. Ultrastructure and synaptic organization of the medullary pacemaker nucleus. *Neurosci.* **15**, 431–443.

Finger, T. E., Bell, C. C. and Russell, C. J. (1981). Electrosensory pathways to the valvula cerebelli in mormyrid fish. *Exp. Brain Res.* **42**, 23–33.

Grant, K. (1993). Motor control of signal generation compared in mormyrid and gymnotiform fish. In *Contributions of Electrosensory Systems to Neurobiology and Neuroethology* (ed. C. C. Bell, C. D. Hopkins and K. Grant). *J. Comp. Physiol.* A **173**, 729–731.

Grant, K., Bell, C. C., Clausse, S. and Ravaille, M. (1986). Morphology and physiology of the brainstem nuclei controlling the electric organ discharge in mormyrid fish. *J. Comp. Neurol.* **245**, 514–530.

Grant, K., Clausse, S., Libouban, S. and Szabo, T. (1989). Serotoninergic neurons in the mormyrid brain and their projection to the preelectromotor and primary electrosensory centers: immunohistochemical study. *J. Comp. Neurol.* **281**, 114–128.

Grant, K., Meek, J., Sugawara, Y., Veron, M., Denizot, J., Hafmans, T., Serrier, J. and Szabo, T. (1996). Projection neurons of the mormyrid electrosensory lateral line lobe: Morphology, immunohistochemistry and synaptology. *J. Comp. Neurol.* **375**, 18–43.

Grant, K., Sugawara, Y., Gomez, L., Han, V. and Bell, C. C. (1998). The mormyrid electrosensory lobe *in vitro*. II. Physiology and pharmacology of cells and circuits. *J. Neurosci.* **18**, 6009–6025.

Han, V., Bell, C. C., Grant, K. and Sugawara, Y. (1999). The mormyrid electrosensory lobe *in vitro*. I. Morphology of cells and circuits. *J. Comp. Neurol.* **404**, 359–374.

Maler, L. (1973). The posterior lateral line lobe of a mormyrid fish. A Golgi study. *J. Comp. Neurol.* **152**, 281–298.

Maler, L., Karten, H. J. and Bennett, M. V. L. (1973a). The central connections of the posterior lateral line nerve of *Gnathonemus petersii*. *J. Comp. Neurol.* **151**, 57–66.

Maler, L., Karten, H. J. and Bennett, M. V. L. (1973b). The central connections of the anterior lateral line nerve of *Gnathonemus petersii*. *J. Comp. Neurol.* **151**, 67–84.

Meek, J. (1993). Structural organization of the mormyrid electrosensory lateral line lobe. *J. Comp. Physiol.* A **173**, 675–677.

Meek, J. and Grant, K. (1994). The role of motor command feedback in electrosensory processing. *Eur. J. Morph.* **32**, 225–234.

Meek, J., Grant, K. and Hafmans, T. G. M. (1994). Multipolar intrazonal axons in the mormyrid electrosensory lateral line lobe: Myelinated dendrites and reciprocal synaptic connections involved in center surround inhibition. *Neurosci. Abstr.* **20**, 579.

Meek, J., Grant, K., Sugawara, Y., Hafmans, T., Veron, M. and Denizot, J. (1996). Interneurons of the ganglionic layer in the mormyrid electrosensory lateral line lobe: Morphology, immunohistochemistry and synaptology. *J. Comp. Neurol.* **375**, 43–65.

Meek, J. and Joosten, H. W. J. (1989). The distribution of serotonin in the brain of the mormyrid teleost *Gnathonemus petersii*. *J. Comp. Neurol.* **281**, 206–224.

Meek, J., Joosten, H. W. J. and Hafmans, T. G. M. (1993). Distribution of noradrenaline-immunoreactivity in the brain of the mormyrid teleost *Gnathonemus petersii*. *J. Comp. Neurol.* **328**, 145–160.

Meek, J. and Nieuwenhuys, R. (1998). (The brains of) holosteans and teleosts. In *The Central Nervous System of Vertebrates* (ed. R. Nieuwenhuys, H. J. ten Donkelaar and C. Nicholson), pp. 759–937. New York, Heidelberg: Springer.

Meek, J., Nieuwenhuys, R. and Elsevier, D. (1986). Afferent and efferent connections of cerebellar lobe C3 of the mormyrid fish *Gnathonemus petersii*. An HRP study. *J. Comp. Neurol.* **245**, 342–358.

Mugnaini, E. and Maler, L. (1987a). Cytology and immunocytochemistry of the nucleus of the lateral line lobe in the electric fish *Gnathonemus petersii* (Mormyridae): Evidence suggesting that GABAergic synapses mediate an inhibitory corollary discharge. *Synapse* **1**, 32–56.

Mugnaini, E. and Maler, L. (1987b). Cytology and immunocytochemistry of the nucleus extrolateralis anterior of the mormyrid brain: possible role of GABAergic synapses in temporal analysis. *Anat. Embryol.* **176**, 313–336.

Szabo, T. (1993). Common sense afferent pathways to the electric lateral line lobe in mormyrid fish. In *Contributions of Electrosensory Systems to Neurobiology and Neuroethology* (ed. C. C. Bell, C. D. Hopkins and K. Grant). *J. Comp. Physiol.* A **173**, 673–675.

Szabo, T., Libouban, S. and Denizot, J.-P. (1990). A well defined spinocerebellar system in the weakly electric teleost fish *Gnathonemus petersii*. A tracing and immunohistochemical study. *Arch. Ital. Biol.* **128**, 229–247.

von der Emde, G. and Bell, C. C. (1996). Nucleus preeminentialis of mormyrid fish, a center for recurrent electrosensory feedback. I. Electrosensory and corollary discharge responses. *J. Neurophysiol.* **76**, 1581–1596.

Zakon, H. (1986). The electroreceptive periphery. In *Electroreception* (ed. T. H. Bullock and W. Heiligenberg), pp. 103–156. New York: Wiley & Sons.