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Short Term Synaptic Plasticity Across Multiple Electrosensory Maps in the Weakly Electric Fish *Apteronotus leptorhynchus*

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Short Term Synaptic Plasticity Across Multiple Electrosensory Maps in the Weakly Electric Fish *Apteronotus leptorhynchus*

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List of abbreviations:

ACSF	Artificial cerebrospinal fluid
AMPA	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AM	Amplitude modulation
AP	Action potential
Ca^{2+}	Calcium
CMS	Centromedial segment
CLS	Centrolateral segment
DML	Dorsal molecular layer
EGp	Eminentia granularis Posterior
ELL	Electrosensory lateral line lobe
EOD	Electric organ discharge
EPSP	Excitatory post synaptic potential
GABA	Gamma-aminobutyric acid
GC	Granule cell
Hz	Hertz, unit of frequency (cycles per second)
JAR	Jamming avoidance response
K^+	Potassium
LL	Lateral lemniscus
LTE	Long term synaptic enhancement
LTD	Long term depression
LTP	Long term potentiation
LS	Lateral segment
MS-222	Tricane methanosulfate
NMDA	N-methyl-D-aspartic acid

nP	Nucleus praeminentialis
PECB	Praemenintial- cerebellar tract
PC	Pyramidal cell
PF	Parallel fiber
PSP	Post synaptic potential
PTP	Post tetanic potentiation
RAM	Random amplitude modulation
RF	Receptive field
SK	Calcium dependent slow conductance potassium channels
StF	Tractus stratum vibrosum
ST-I	Stellate cell Inhibited by increases in EOD amplitude
ST-E	Stellate cell excited by increases in EOD amplitude
STP	Short term plasticity
VML	Ventral molecular layer

Abstract:

The electrosensory lateral line lobe (ELL) is the first order electrosensory processing station in the brain of the electric fish *Apteronotus leptorhynchus*, and is the only nucleus to receive input from electroreceptors on the skin. The ELL is subdivided into three maps: the centromedial segment (CMS), centrolateral segment (CLS), and lateral segment (LS) which receive input from tuberous electroreceptors processing high frequency signals. Two feedback pathways from higher order nuclei, the nucleus praemenintialis (nP) and the eminentia posterior granularis (EGp) are integral to processing in the ELL and show synaptic plasticity on various time scales. This thesis focuses on characterizing short term plasticity (STP) in nP-ELL synapses between the CMS, CLS and LS, and the development of an in vitro slice containing the entire direct feedback pathway. We find that LS pyramidal cells show greater facilitation in response to high frequency stimulation of direct feedback fibres as compared to CMS.

Résumé :

Le lobe électrosensoriel de la ligne latérale (ELL) est la première étape du traitement électrosensoriel du cerveau du poisson électrique, *Apteronotus leptorhynchus*, et c'est le seul noyau qui reçoit les informations provenant des électrorécepteurs de la peau. Le ELL est subdivisé en quatre cartes électrosensorielles, dont trois (segment centromédial : CMS ; centrolatéral : CLS ; et latéral : LS) qui reçoivent les informations des électrorécepteurs traitant les signaux de hautes fréquences causées par les perturbations du champ électrique du poisson, tandis que le segment médial (MS) reçoit les informations électrorécepteurs sélectifs aux signaux biogéniques de basses fréquences. Il y a deux voies de rétroaction en provenance de noyaux supérieurs (le noyau pré-émenintialis (nP) et le noyau eminentia posterior granularis (EGp) nécessaire à cette étape initiale du traitement électrosensoriel. Ces projections de rétroaction font synapse sur les dendrites apicaux des cellules pyramidales du ELL. Ces synapses présentent diverses formes de plasticité synaptique à différentes échelles de temps. Ce mémoire cherche spécifiquement à caractériser la plasticité à courte terme (STP) de la voie de rétroaction directe traversant les segments CMS, CLS et LS et le développement d'une préparation in vitro contenant l'ensemble de la voie de rétroaction directe. Dans le second chapitre, je montre que les cellules pyramidales du LS présentent une facilitation plus importante (mesurée par le changement de l'amplitude des potentiels post-synaptiques excitateurs, PPSE) en réponse aux stimulations à hautes fréquences des fibres de la voie directe de rétroaction que les cellules du CMS. Dans le troisième chapitre, nous développons une nouvelle approche *in vitro* pour étudier la rétroaction en préservant la voie directe de rétroaction en tranche, permettant un meilleur contrôle de l'expérimentateur et une caractérisation des réponses des cellules pyramidales et étoilées (stellate) lorsque les noyaux et les projections demeurent intacts.

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Statement of contributions:

Portions of this thesis have been previously published in Mileva et al. 2008. All authors contributed to the writing of this paper but I was the primary author on the sections appearing in the Introduction to this thesis. I developed the nP-ELL slice, with technical assistance from Sally Groothuis and performed recordings from neurons in the nP.

CHAPTER ONE:
General Introduction

(Parts excerpted from Mileva et al. 2008)

1.1 Natural history of Apteronotus leptorhynchus

Extracting relevant information about the immediate environment is of critical importance to the survival, growth, and reproduction of most organisms. Many animals have developed a wide variety of sensory mechanisms to examine their environment through visual, somatosensory, and auditory modalities. Organisms living in areas where these more conventional sensory modalities are not useful require other methods to observe their environment. One such novel sensory modality, the electric sense, has arisen independently at least twice in two separate orders of fish; the mormyriformes in Africa and the gymnotiformes in South America (Rose, 2004). The convergent evolution occurring in fish on different continents underscores the importance of these less common modalities (i.e. the electric sense) for obtaining maximal information about the environment.

Whereas many aquatic organisms, and even some terrestrial animals (e.g. platypus), can sense biogenic, low frequency, electric signals in their surroundings, the electrosensory system in specialized fish allows for the active production of a high frequency electric field and is a novel way for these fish to sense their environment (for review see von der Emde, 1999; Rose, 2004). This electric field expands outward from their body and any perturbations of the field are sensed by electroreceptors on the skin, which are transmitted for further processing to the brain. The electric field is generated by discharge of current from electrocytes in the electric organ located in the tail of the fish (called electric organ discharge: [EOD]). In most mormyriformes and gymnotiformes , the electric organ is

myogenic (derived from muscle) but in the *Apteronotidae*, a family of gymnotiformes, the organ is derived directly from nerve tissue (Waxman et al., 1972).

Another interesting distinction between mormyrid and gymnotid fish, is that all mormyrids (except one species) are pulse-type fish, meaning they emit an EOD at irregular intervals. While some gymnotid fish are also pulse-type, many are wave-type, emitting a quasi sinusoidal EOD at a constant, and often high, frequency (Moller, 1995). These inherent differences in EOD firing pattern create tradeoffs: wave type fish can gather information continually at very high rates, whereas pulse type fish can do so only sporadically. With regards to communication, pulse type fish signals are less likely to interfere, and in fact, these fish communicate by phase locking with another fish's EOD and in essence 'echo' each other (For review see Kramer, 1999). In contrast, wave-type fish readily elicit the jamming avoidance response (JAR) when in the vicinity of conspecifics with similar frequency; the JAR is a type of EOD modulation allowing the fish to systematically change its frequency and thus avoid jamming one another (for review see Heiligenberg, 1991; Viete and Heiligenberg, 1991). The reason behind this divergence of EOD discharge in pulse and wave type fish has not been clearly identified, since both types of fish occupy a similar niche. In this thesis, only the wave-type brown ghost knifefish (*Apteronotus leptorhynchus*) will be discussed in detail.

The brown ghost knifefish is a nocturnal hunter residing in murky Amazonian waters, and therefore has to rely on its electrosensory system as its primary sensory modality (Hagedorn, 1986; Crampton, 1998). This specialized system allows for both active electrolocation (i.e. through its self generated EOD) and electrocommunication between

conspecifics. Brown ghost knifefish are known as weakly electric fish since their EOD is in the millivolt range emitted at astonishing rates of up to 1200Hz (Moller, 1995). As a product of this high frequency, electroreceptors are almost constantly receiving information about the environment. The EOD in wave type fish is one of the most stable oscillations found in nature (Moortgat et al., 1998) and its modulations have been implicated in electrolocation, prey-capture, and communication with conspecifics (Nelson and Maciver, 1999; Hupe et al., 2008; for review see Moller, 1995). During electrolocation, objects in the environment create perturbations in the electric field which are translated into differential input to electroreceptors on the skin. These electroreceptors in turn project to the primary processing station in the brain, the electrosensory lateral line lobe (ELL). The processing in the ELL is crucial in determining the information sent to higher order brain centers (Bell and Maler, 2005).

1.2 The ELL of A. leptorhynchus*: connectivity and electrosensory maps*

The ELL is the first order processing station in the brain of *A. leptorhynchus*, and is the only nucleus to receive inputs from the primary afferents of electroreceptors. These afferents synapse onto the basal dendrites of basilar pyramidal cells in a topographic manner across three electrosensory maps (Fig 1.1A). Pyramidal cells are the main output neuron of the ELL and exist in two varieties: basilar pyramidal cells, or E-cells, and nonbasilar pyramidal cells, or I-cells, named for the presence or absence of a basilar dendrite and whether they are excited (E-cell) or inhibited (I-cell) by increases in EOD amplitude modulations (AMs), respectively. Glutamatergic electroreceptor afferents

synapse onto the basal dendrite of E-cells, as well as several types of interneuron such as ovoid cells, granule cell type 1 (GC1) and granule cell type 2 (GC2)(Fig 1.1B, interneurons not shown; Maler et al., 1981). Due to the lack of a basal dendrite in I-cells they receive primarily inhibitory (γ-amino butyric acid A [$GABA_A$]) synapses from GC2 cells (Berman and Maler, 1998). These characteristics of E and I type pyramidal cells are analogous to ON and OFF type relay cells in the visual system (Maler et al., 1981; Krahe and Gabbiani, 2004).

Both E and I pyramidal cells are spread across the pyramidal cell layer (PCL) of the four electrosensory maps, or segments, in the ELL; the medial segment (MS), centromedial segment (CMS), centrolateral segment (CLS) and the lateral segment (LS) (Berman and Maler, 1999). Each segment receives input from primary afferents; the MS receives the majority of its input from ampullary receptors (associated with very low frequency/ biogenic electric signals) while the CMS, CLS and LS receive input from tuberous electroreceptors (associated with higher frequency/active electrolocation and electrocommunication signals) (Fig. 1.1A; Zakon, 1984). In the mormyrid ELL, there are two distinct subdivisions, each containing a population of pyramidal cells responsible for the processing of either high frequency (electrocommunication) or low frequency (electrolocation) stimuli (Bell, 1989). In gymnotids, however, each tuberous electroreceptor projection trifurcates and synapses onto all three tuberous segments of the ELL with no cross-connectivity between ELL segments (Fig 1.1A; Carr et al., 1982; Mehaffey et al., 2008).

In *A. leptorhynchus* and *Eigenmannia virescens*, another gymnotid, the receptive fields (RFs) of pyramidal cells across segments differ, with increasing size from CMS to LS (Shumway, 1989a; Shumway, 1989b; Maler, 2009). The E and I cells between segments also

differ *in vivo* in their response to high and low frequency AMs of the EOD, with I-cells across all maps responding preferentially to low frequencies (0-20Hz, i.e. low pass) while E-cells tend to shift their preference to higher frequencies (30-50Hz, i.e. high pass) from CMS to LS (Shumway, 1989a; Mehaffeyet al., 2008; Krahe et al., 2008). The different properties of each map can be tied to specific behaviourally relevant stimuli since it has been shown that the CMS is necessary and sufficient for low-frequency related processing such as eliciting the JAR, while the LS is necessary and sufficient in high-frequency processing implicated in communication (Metzner and Juranek, 1997; Mehaffey et al., 2008).

Another interesting aspect of trifurcation by the electroreceptor afferents to each of the tuberous segments in the ELL, is that each segment can be thought of as a parallel processor and is analogous to other sensory systems employing parallel processing such as the visual (Roska et al., 2006), auditory (Rauschecker, 1998), and somatosensory (Pons et al., 1992) systems. Parallel processing allows for each 'processor' (i.e map) to extract a different aspect of a stimulus. Thus, in the gymnotid ELL, pyramidal cells of the different segments may send distinct but complimentary information to higher brain centers.

1.3 Pyramidal cell projections out of the ELL: two distinct feedback pathways

E and I type pyramidal cells of each segment in the ELL project topographically to a higher order structure, the nucleus praeminentialis (nP). Projections back to the ELL from excitatory stellate and inhibitory bipolar cells in the nP constitute a direct feedback pathway while excitatory projections from the granule cells in the eminentia granularis posterior (EGp) to apical dendrites of pyramidal cells in the dorsal molecular layer (DML) form an

indirect feedback pathway (Fig 1.1B,C; Sas and Maler, 1983; Sas and Maler, 1987). Stellate cells send glutamatergic projections which synapse onto both pyramidal cell apical dendrites in the ventral molecular layer (VML) of the ELL and inhibitory (GABAergic) interneurons in the ELL (disynaptic inhibition). Bipolar cells, however, send GABAergic ($GABA_B$) projections to the ELL pyramidal cell apical dendrites in the VML (monosynaptic inhibition). The axons from stellate and bipolar cells travel to the ELL in a myelinated fiber bundle known as the tractus stratum fibrosum (StF). Within the StF, fibers from the stellate and bipolar cells remain relatively segregated, with stellate projections in the dorsal aspect, and those of bipolar cells in the ventral aspect. From the ELL, projections travel to the torus semicircularis, and from there, on to the nuclei responsible for generating and modulating the EOD (i.e. the pre-pacemaker nuclei, and the pacemaker nucleus whose projections travel to the electric organ).

Feedback pathways are a central feature of brain organization. An example of this can be found in the hierarchical pathways of the primate cerebral cortex as almost all nuclei send reciprocal connections (i.e. have both feedforward and feedback pathways) (for review see Felleman and Van Essen, 1991). However, the functional roles of neuronal feedback are difficult to study because of the complexities involved with the dynamics of closed-loop systems (i.e. *in vivo* work on whole brain). These dynamics result from the inherent delays in feedback pathways in addition to the synaptic plasticity expressed by feedback inputs. Although difficult to study functional roles, it has been postulated that neuronal feedback plays a role in the shaping of receptive fields (Maler, 2009).

In vivo studies that involve intact feedback networks under realistic conditions are required to develop a full understanding of the roles of feedback in neural processing, but generally the results of these studies can be difficult to interpret because of the decreased experimental control and higher degrees of freedom. An alternative is to use *in vitro* approaches, but of course, there are inherent difficulties in preserving the pathways between sub-networks, due to the complex three-dimensional architecture. In cases where it has been possible to develop brain slice preparations that preserve feedback pathways, they have provided an effective complement to more realistic *in vivo* studies (e.g. Agmon and Connors, 1992).

Like sensory systems in general, electrosensory systems show extensive feedback, and may be easier to study due to their more stereotyped organization within the brain. Much of the work on electrosensory feedback has focused on the ELL and its associated feedback pathways (Berman and Maler, 1999; Bell and Maler, 2005; Sawtell et al., 2005). Any processing occurring at the level of the ELL pyramidal cells will have a major influence on electrosensory-mediated behaviours. Several *in vivo* studies have shown that these feedback inputs are critical for effective electrosensory processing (Bastian, 1995; Bodznick et al., 1999; Bastian et al., 2004). In particular, these feedback inputs mediate the responses of pyramidal cells in dynamic ways, through changes in synaptic strength (i.e. synaptic plasticity).

1.3.1: Mechanisms underlying synaptic plasticity

Synaptic plasticity is defined generally as a change in the strength of synaptic connections between neurons as a function of previous activity. It is well accepted that this plasticity is due to changes in the probability of neurotransmitter release (including quantity) from the pre-synaptic cell, and/or changes in the post-synaptic cell (i.e. desensitization of receptors) (Citri and Malenka, 2008). Countless forms of synaptic plasticity occur ubiquitously in the brain differing widely in mechanism and time course. One type of synaptic plasticity, and the focus of this thesis, is short term plasticity (STP) (Kandel and Tauc, 1965; Abbott and Regehr, 2004; Citri and Malenka, 2008). STP occurs on very short time scales (in the 100s of millisecond range) and is usually associated with transient stimuli (Zucker and Regehr, 2002). STP is classified commonly into facilitation and depression processes (reviewed by Abbott and Regehr (2004). Generally, facilitation tends to increase the size of a post-synaptic potential (PSP), whereas depression tends to decrease PSP size. Both processes are associated with time constants that define the duration over which the effect on the PSP is evident, and both processes are often associated with changes in the probability of neurotransmitter release. For facilitation to occur, depolarization in the presynaptic cell causes Ca^{2+} to enter the cell, via voltage gated Ca^{2+} channels, and in turn causes neurotransmitter release. If a following pulse comes quickly after the first residual Ca^{2+} left in the presynaptic bouton sums with new Ca^{2+} influx, triggering a greater amount of neurotransmitter release into the synapse. This is known as the residual calcium hypothesis (Katz and Miledi, 1968; Zucker, 1989; Zucker and Regehr,

2002). Whether depression or facilitation occurs depends on both the initial amount of neurotransmitter released and on the recovery rate of this released neurotransmitter from the synapse back into the presynaptic terminal (Zucker and Regehr, 2002). Low initial probabilities favour facilitation (i.e. slower depletion) while high probabilities favour depression. Following this, if neurotransmitter recovery from the synapse back into the presynaptic terminal is fast, facilitation will most likely occur as it is not depleted, while the opposite is true when synaptic depression is elicited. In the following section, depression and facilitation processes found in feedback synapses associated with the ELL will be discussed in more detail.

1.4 Synaptic plasticity of ELL feedback pathways

While some functional insight into ELL feedback pathways has been generated by *in vivo* studies, the development of the ELL brain slice (Mathieson and Maler, 1988) has led to a large number of studies describing the plasticity, and dynamics, of ELL pyramidal neurons and their feedback inputs under open-loop conditions (e.g. in which a feedback pathway is isolated from its feed forward inputs). The intrinsic cellular dynamics of pyramidal neurons have also been characterized in terms of how different ionic currents/channels influence the cells likelihood to fire a burst of action potentials rather than single action potentials (e.g. Turner et al., 2002; Mehaffey et al., 2006; Mehaffey et al., 2008). Some of the synaptic plasticity found at the synapses of the direct and indirect feedback pathways has also been characterized (Wang and Maler, 1997; Berman and Maler, 1999; Lewis and Maler, 2002; Oswald et al., 2002; Lewis and Maler, 2004). Each pathway exhibits synaptic plasticity on a

wide-range of time scales (from tens of milliseconds to tens of minutes) and involves both excitatory and inhibitory components.

Mathematical modelling of plasticity observed experimentally at these synapses can be used to quantify the processes of facilitation and/or depression (Lewis and Maler, 2002; Oswald et al., 2002; Lewis and Maler, 2004). Simple models of facilitation and depression have been used successfully in previous studies of the rat cerebellum, in which the complex interplay of processes occurring in the presynaptic terminal were quantified (Dittman et al., 2000; Abbott and Regehr, 2004). The ability of simple models to accurately fit data obtained experimentally at these synapses implies that despite the complex and dynamically interacting processes involved, it is possible to separately quantify facilitation and depression. To illustrate the plasticity and synaptic dynamics found at each of the two feedback pathways in the ELL, and their proposed functional roles, previous experimental and modelling work will be briefly discussed below.

1.4.1 Indirect feedback pathway

Electrical stimulation of the parallel fibers of the indirect pathway (Fig 1.1B) evokes PSPs whose short-term frequency dependence can be described by a combination of just two processes, one facilitation and one depression, both with time constants of about 80 ms (Lewis and Maler, 2002). In this case, the contribution of disynaptic inhibition via inhibitory interneurons of the DML was addressed explicitly and modeled using a depression-like term with a one second time constant. In a subsequent study, longer-term dynamics were found to directly influence short-term facilitation and illustrate the

Figure 1.1. Circuitry of indirect and direct feedback pathways of the electrosensory lateral line lobe (ELL). (A) The trifurcation of tuberous electroreceptor input to the pyramidal cell layer (PCL) of the three electrosensory maps of the ELL; centromedial segment (CMS), centrolateral segment (CLS) and lateral segment (LS). Figure modified from Mehaffey et al 2008. (B) Connectivity of an E-cell in the ELL. The nucleus praeminentialis (nP) receives excitatory input from pyramidal cells (PC) of the ELL. PCs project to the torus semicircularis and nP. Stellate and bipolar cells of the nP project direct excitatory and inhibitory input respectively, via the tractus stratum fibrosum (StF), onto pyramidal cells of the ELL. Stellate cells in nP also activate inhibitory interneurons in ELL which provide disynaptic inhibition onto ELL PCs. Multipolar cells of the nP project to the eminentia granularis posterior (EGp) through the praeminential-cerebellar tract (PECB). The granule cells of EGp complete the indirect feedback pathway by projecting parallel fibers (PF) to ELL that mediate excitation and disynaptic inhibition onto ELL pyramidal neurons (inputs onto DP neurons are omitted for clarity). (C) Schematics of the sub-networks involving the direct and indirect pathways, emphasizing the closed-loop feedback in each, PC-nP–PC and PC–nP– EGp–PC, respectively (figure modified from Mileva et al 2008).

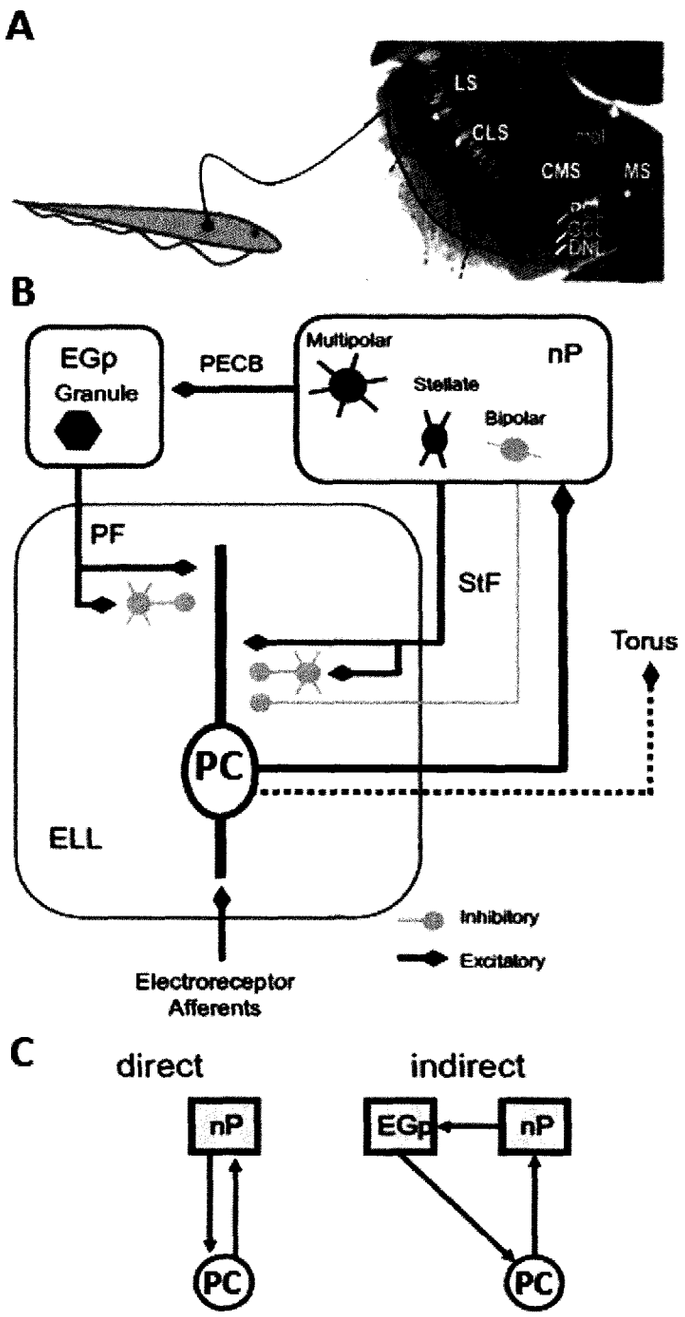

A
LS
CLS
CMS
MS
B
EGp
Granule
PECB
Multipolar
nP
Stellate
Bipolar
PF
StF
Torus
PC
ELL
Inhibitory
Excitatory
Electroreceptor
Afferents
C
direct
indirect
nP
EGp
nP
PC
PC

Figure 1.1

importance of observing the interaction between short and long term plasticity occurring in these pathways (Lewis and Maler, 2004). Postsynaptic long-term plasticity has also been well-characterized at these synapses. Bastian (1998) revealed an anti-Hebbian depression lasting several minutes, produced by concomitant parallel fiber stimulation and pyramidal neuron hyperpolarization *in vivo*. Briefly, Hebbian plasticity was first characterized by Donald Hebb in 1949, and outlines the theory that synaptic strength between two neurons will increase with their repeated interaction; however, as the name implies a decrease in synaptic strength with continual stimulation occurs in anti-Hebbian plasticity (Hebb, 1949; Caporale and Dan, 2008). In addition, recent slice studies have also found a type of anti-Hebbian plasticity in this pathway that requires the interaction of parallel fiber stimulation and pyramidal neuron bursts (Harvey-Girard and Maler, 2007).

1.4.2 Direct feedback pathway:

Synapses of the direct feedback pathway also exhibit both short and long-term plasticity. In a slice preparation, Oswald et al. (2002) showed that the dynamics of these synapses could be described by 5 different processes: two facilitation (time constants of about 20 ms and 900 ms) and two depression (time constants of about 1 sec and 8 sec) processes, and a longer-term post-tetanic potentiation (PTP) having a time constant of about 2 min. The influence of inhibition was not explicitly considered in this study but it was suggested that the depression terms may have been at least partially mediated by inhibitory synaptic inputs. In addition, Wang and Maler (1997) have shown that with an associative stimulation paradigm, (hyperpolarization of the pyramidal cell coupled with

stimulation of the direct feedback pathway) synapses show an anti-Hebbian depression, similar to that described *in vivo*, lasting about 10 min (Bastian, 1996a; Bastian, 1998). It remains unclear whether these synapses are capable of longer term plasticity such as long term potentiation or long term depression (LTP/LTD)(Wang and Maler, 1997). Current evidence suggests that overall, on slightly shorter time scales (many minutes), PTP will overwhelm any depression.

1.5 Functional roles of ELL feedback dynamics

As seen above, both direct and indirect feedback pathways exhibit synaptic plasticity on many time-scales. How these feedback dynamics might interact to influence natural electrosensory processing is still unknown. Several working hypotheses, described below, have been proposed and shown to be consistent with experimental data. That said, these hypotheses prescribe differing functions to the direct and indirect pathways.

1.5.1 Reafference suppression

One of the first demonstrations of a functional role for electrosensory feedback was to actively cancel predictable sensory inputs. Under conditions such as tail-bending, the electroreceptors on one side of the fish will be stimulated at a level (due to the position of the tail alone) that is much greater than that of any prey-related signal (Assad et al., 1999). It is important to note that the benefits of exploratory behaviours such as tail-bending are likely due to an increased contrast in the electric field perturbation from an external object

(Assad et al., 1999; Caputi and Budelli, 2006). Thus, effective electrosensory processing requires that any predictable components of the signal, such as those generated by swimming or the tail-bending itself, be suppressed. Specifically, this reafference suppression is achieved via the production of a negative image of the predictable inputs. Bastian and coworkers (1995, 1996, 2004) have demonstrated that the negative image is mediated by the long-term anti-Hebbian plasticity found at both direct and indirect feedback pathways. Similar mechanisms involving anti-Hebbian plasticity in cerebellar-like structures have evolved independently in other electrosensory systems (Bodznick et al., 1999; Roberts and Bell, 2000; Bell, 2001; Sawtell and Williams, 2008).

1.5.2 Feedback gain control and sensory processing

The indirect feedback pathway is also involved in gain control (Bastian, 1986a). A recent *in vitro* study has suggested that such gain control involves the activation of synaptic inhibition in combination with an intrinsic cellular mechanism resulting from bursting dynamics (Mehaffey et al., 2005). Although excitatory synapses were not considered explicitly in this study, it was assumed that the inhibition was mediated through disynaptic inhibition via inhibitory interneurons in the parallel fiber feedback pathway. A series of studies (Chacron et al., 2003; Chacron et al., 2005) showed that the frequency-dependence of information processing by ELL pyramidal neurons depends on the spatiotemporal nature of electrosensory stimuli and the recruitment of feedback inputs. In addition, a series of *in vivo* studies has characterized the sensory processing by pyramidal neurons under various conditions. Doiron et al. (2003) showed that the recruitment of bipolar neuron mediated

inhibitory feedback from nP can mediate a switch in ELL network dynamics. They showed that when a spatially global electrosensory stimulus was applied, diffuse inhibition (bipolar inhibition) caused the ELL network to switch to an oscillatory mode, whereas when applying this stimulus locally, no such change occurred. These examples highlight the complex interaction between primary sensory and feedback inputs. It is difficult, however, to determine the mechanisms underlying this interaction under the closed-loop feedback conditions *in vivo*. Traditional slice preparations typically lack intact feedback pathways, and can be used to describe the individual components of feedback networks in isolation, but the influences of feedback dynamics on pyramidal neuron responses under closed-loop conditions remain unclear.

1.5.3 Sensory filtering

Synaptic dynamics are commonly thought to provide a mechanism for filtering sensory inputs (Abbott and Regehr, 2004; Citri and Malenka, 2008). One way to look at this is to use the computational models of each of the feedback pathways, with the time constants of facilitation and depression as described above, (Lewis and Maler, 2002b; Oswald et al., 2002), to assess the nature of feedback inputs to pyramidal neurons under different conditions (modelling by J. Lewis). Fig. 1.2 shows the predicted PSP amplitude resulting from fixed-frequency stimulation of both direct and indirect feedback pathways (8Hz and 64Hz). The most noticeable difference between the two pathways is the magnitude of change, with the direct pathway resulting in up to three times more facilitation than the indirect pathway. In addition, as indicated by the differences in

response at the 3rd and 10th PSPs of the stimulus train, depression and inhibition are influencing the indirect pathway at much lower input frequencies (Fig 1.2B). In other words, the direct pathway is able to amplify transient inputs more rapidly, consistent with the hypothesis that this pathway may function as an attentional mechanism in the electrosensory system, amplifying fleeting changes on the surface of the skin (Berman and Maler, 1999; Oswald et al., 2002).

In Fig 1.2, it is important to note that both the facilitation and depression time constants were based on previous studies (Lewis and Maler, 2002; Oswald et al., 2002) where the data were obtained from intracellular and extracellular studies in ELL CMS only. To our knowledge, no work has been done on the synaptic plasticity between electrosensory maps in the ELL.

1.6 Thesis objectives

The direct and indirect feedback pathways are perfect candidates to study synaptic plasticity due to the knowledge of the many forms of plasticity and dynamics occurring at these synapses, coupled with the broadening understanding of their functional roles. More specifically, to help understand the functional role of STP at the direct feedback synapses (StF-Pyramidal synapses), this thesis aims to:

1) Characterize the difference in STP between E and I pyramidal cells in the LS of the ELL, using an *in vitro* ELL slice preparation,

2) Characterize differences in STP between the tuberous ELL electrosensory maps (CMS, CLS, and LS) using an *in vitro* ELL slice preparation,

3) Develop a novel *in vitro* brain slice preparation containing the entire direct feedback loop as a method of studying the direct feedback pathway with greater experimenter control than that found *in vivo*.

Figure 1.2. Short-term synaptic plasticity and filtering in the direct and indirect feedback pathways. (A) Amplitude of simulated post-synaptic potentials (PSPs) in ELL pyramidal neurons produced by periodic stimulus trains (8 Hz and 64 Hz) to the direct (black) and indirect (gray) feedback pathways. The underlying model description of these PSPs has been described previously (Lewis and Maler, 2002; Oswald et al., 2002). Arrows indicate the responses to the 3rd and 10th stimuli of the train. (B) PSP amplitude for the 3rd and 10th stimuli of the train as a function of stimulus frequency within the train. Figure modified from Mileva et al 2008.

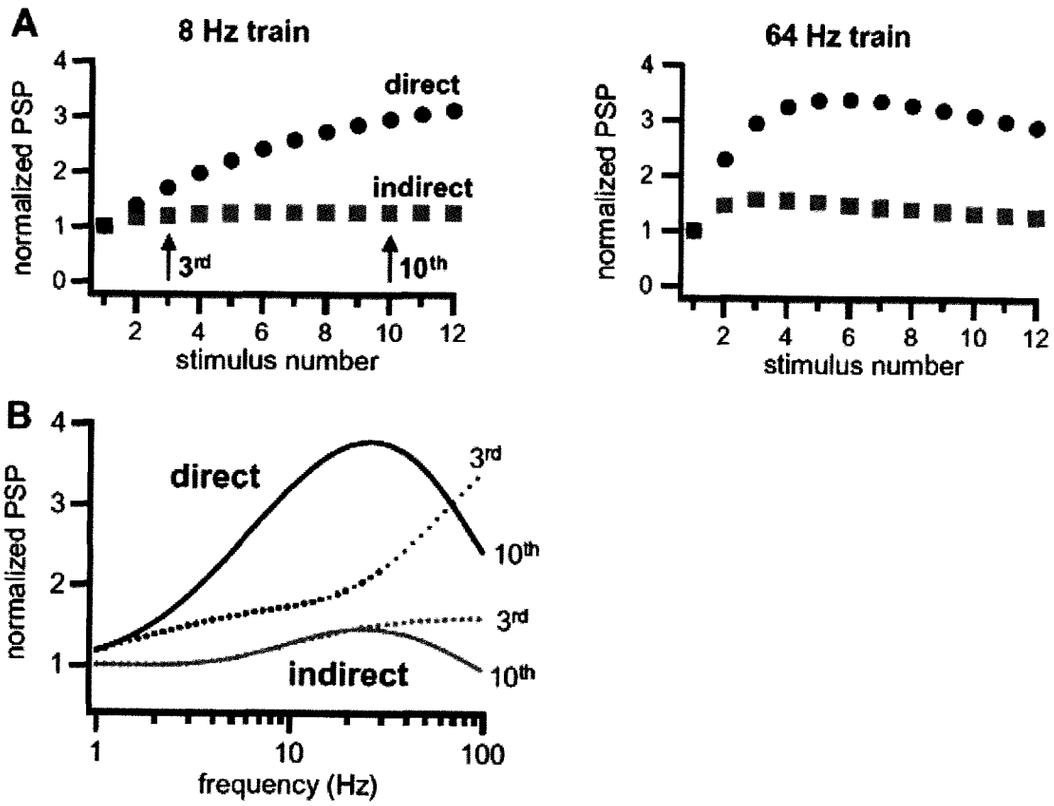

A
8 Hz train
direct
indirect
3rd
10th
normalized PSP
stimulus number
64 Hz train
B
direct
indirect
3rd
10th
frequency (Hz)

Figure 1.2

CHAPTER TWO:

Short-Term Synaptic Plasticity Across Multiple Electrosensory Maps in the Weakly Electric Fish *Apteronotus leptorhynchus*

2.1 Introduction:

Many studies *in vivo* (Bastian and Bratton, 1990; Bratton and Bastian, 1990) and *in vitro* (Mathieson and Maler, 1988; Wang and Maler, 1997; Lewis and Maler, 2002; Lewis and Maler, 2002b; Oswald et al., 2002) have investigated the roles played by the direct and indirect feedback pathways of the electric fish brain. These feedback pathways and the synaptic plasticity associated with them have been implicated in affecting the sensitivity (or gain) of pyramidal cells to sensory inputs (Berman et al., 1997), sensory filtering (Bastian, 1996a; Bastian, 1996b; Oswald et al., 2002) and suppressing redundant electrosensory information via the longer term anti-Hebbian plasticity (Bastian, 1995).

It appears that both direct and indirect pathways are necessary for proper electrosensory function. Ablation of the nucleus praeminentialis (nP; the key nucleus in the direct feedback pathway) directly affects electrodetection and electrolocation performance, and further implicates the direct feedback pathway as the attentional mechanism of the electrosensory system (Green, 1996; Berman et al., 1997). Ablating the praeminential-cerebellar tract (PECB) which connects the nP to the eminentia granularis posterior (EGp), and thus removes indirect feedback, changes the gain of ELL pyramidal cell responses (Bastian, 1986a; Bastian, 1986b). Revealing the importance of each feedback pathway to the animal through ablation studies is the first step; next, determining the underlying mechanisms of these functions is required. To do this, a thorough understanding of the synaptic plasticity underlying these feedback synapses is crucial.

In *A. leptorhynchus*, synaptic plasticity has been characterized in both feedback pathways. In a study of the direct feedback pathway StF-pyramidal cell synapses by Oswald et al. (2002), 5 distinct processes were described occurring on varying time-scales. Two types of facilitation (time constant of 20ms and 900ms) and depression (1sec and 8 sec) were found, as well as a longer term post-tetanic potentiation (around 2 minutes). That numerous processes occur in this pathway, at many time scales, underlines its importance in processing, with each process presumably making a nuanced contribution to the overall pyramidal cell response. In this chapter, the focus will be on the shortest timescales found by Oswald et al. (2002), and how this plasticity varies across the three tuberous maps in ELL.

To date, plasticity has only been studied in the centromedial segment (CMS) of the ELL. However, in both *A. leptorhynchus* (for review see Maler, 2009a; Maler, 2009b) and a related gymnotid, *Eigenmannia* (Shumway, 1989a; Shumway, 1989b) there are distinct physiological and anatomical differences in pyramidal cells between maps. Briefly, pyramidal cells of the lateral segment (LS) tend to have receptive fields (RFs) approximately two to three times larger than those of the CMS due, in part, to the convergence of electroreceptor afferents. The number of electroreceptor afferents going to each map is identical (Carr et al., 1982; Heiligenberg and Dye, 1982), but the number of pyramidal cells decreases from the CMS to LS, indicating that each pyramidal cell in the LS will receive input from a larger number of afferents. Shumway (1989) also found that the sensitivity of LS pyramidal cells to amplitude modulations (AMs) is three times higher than in those of other segments, and that this sensitivity is potentially governed by descending input from the nP.

Combined sensitivity and increased RF size in LS could confer the ability of pyramidal cells in this map to sense the weaker signals of more distant objects first (Nelson and Maciver, 1999).

The differences in both the anatomy and physiology of pyramidal cells across maps imply that each map is specialized to specific behavioural stimuli. One of the only studies of its kind carried out by Metzner and Juranek (1997) directly links the function of each map to well characterized electrosensory behaviours. Using selective ablations, the CMS was found to be necessary and sufficient to elicit the jamming avoidance response (JAR), a behaviour elicited when two fish have similar EOD frequencies, causing the fish to modulate their own EOD in response (for review see Viete and Heiligenberg, 1991). Other work has shown that the CMS is most sensitive to global low frequency stimuli (Shumway, 1989a; Krahe and Gabbiani, 2004). The LS on the other hand is necessary and sufficient for governing high frequency electrocommunication, such as 'chirps' (for review see Zakon et al., 2002; Metzner and Juranek., 1997). Chirping is defined as a global high frequency AM, and is consistent with recent work showing that E-type pyramidal cells in the LS tend to prefer broadband and high frequency signals while the E-type pyramidal cells of the CMS are tuned to lower frequencies (Mehaffey et al., 2008).

The divergence in proposed functions between maps could be a direct result of anatomical differences, as well as a product of the descending feedback pathways and their plasticity. This would imply that pyramidal cell response to feedback changes between maps, with each map differing in either synaptic plasticity at these synapses or processing

due to intrinsic cellular properties. Following this line of reasoning, pyramidal cells between the CMS, CLS and LS should show differences in STP at the direct feedback synapses.

In the direct feedback pathways of the ELL, STP has never specifically been characterized as most previous work has concentrated on the post-tetanic potentiation (Wang and Maler, 1997; Oswald et al., 2002). In this study, we aimed to characterize STP in the direct feedback synapses across the CMS, CLS, and LS. It is necessary to study STP *in vitro* (rather than *in vivo*) to both allow for greater experimenter control, and to isolate the specific synapse in question without involvement of other inputs. In this study, we will be focusing on the shortest time scales (less than one second) which could potentially involve both fast and slow facilitation and the 'fast' depression seen in the study by Oswald and colleagues (2002).

2.2 Materials and Methods:

2.2.1 Slice preparation:

Wild caught *Apteronotus leptorhynchus* from the Amazon Basin were obtained from local suppliers (Below Water, Montreal, Canada: DAP, Toronto, Canada), and kept at 26-28°C in freshwater holding tanks. All protocols were in accordance with the University of Ottawa Animal Care Committee protocol B-229.The basic procedure was similar to previous literature (Mathieson and Maler, 1988). Briefly, fish of 10-15g were anaesthetized using 0.2% Tricaine methanesulfonate (TMS, Syndel International Inc). Once the fish was anaesthetized, the brain cavity was opened, and a section of the brain containing the ELL was removed. Slices of approximately 350μm thickness were obtained using an OTS-5000 Vibratome (FHC Inc., Bowdoinham, ME). The slices were then transferred to a brain chamber perfused at 2ml/min with oxygenated artificial cerebrospinal fluid (ACSF). ACSF contained (in mM): 124 NaCl, 24 $NaHCO_3$, 10 D-glucose, 1.25 $KH2PO_4$, 2 KCl, 2 $CaCl_2$, 2 $MgSO_4$ (Fisher Laboratories, Ottawa, ON) and was oxygenated by bubbling a mixture of 95:5% O_2:CO_2. Slices were allowed a 1.5-2hr recovery period before intracellular recordings began.

2.2.2 Intracellular recording procedure:

In this *in vitro* preparation, the StF is a readily visible landmark because of its opaque appearance due to myelination. The dorsal StF was stimulated using bipolar or monopolar tungsten microelectrodes at 300-500k impedance (FHC Inc, Bowdoinham, ME) so as to elicit

excitatory post synaptic potentials (EPSPs). The dorsal aspect of the StF contains the excitatory stellate projections (glutamatergic) which synapse with pyramidal cell apical dendrites in the ventral molecular layer (VML) (Mathieson and Maler, 1988). By stimulating the dorsal StF, EPSPs were evoked with 100 μS pulses generated by a single channel stimulator (Digitimer, Hertz, UK) with 1-30V amplitude; amplitude was adjusted so that EPSPs did not trigger an action potential (AP).

Four basic synaptic stimulus protocols were used to observe STP in ELL pyramidal cells; 10 pulses were delivered at 10, 20 and 50Hz; and a longer 70 pulse train with multiple frequencies (1-50Hz; average of 7Hz). This 70 pulse train will be referred to as the 'random interval' train for the remainder of this paper, and is used to sample many different frequencies in a single trial. These trials provide a more comprehensive data set for future modelling studies (Lewis and Maler, 2002). A rest period of at least 30 seconds between trials was given, so that the response would not be biased from the previous stimulation. At least 3 trials were performed at each frequency. All cells were tested under each condition and cells in the LS were additionally subjected to random amplitude modulations (RAMs) in intracellular current to characterize E and I cells (Ellis et al., 2007; Mehaffey et al., 2008; Mehaffey et al., 2008). LS pyramidal cells are preferentially sensitive to either low frequency AMs (I-cells) or high frequency AMs (E-Cells) of the EOD. It has recently been established that mimicking these types of input with a RAM intracellular current injection is also an effective way to differentiate between the cells (Ellis et al., 2007; Mehaffey et al., 2008; Mehaffey et al., 2008). The RAM protocol consisted of a 30 second broadband

stimulus with frequency components of 0-60Hz. Typically, two 30 second recordings were taken when the cell was firing at a mean frequency of 10-30Hz (usually requiring ≤ 1nA current injection).

Sharp electrodes were made using boroscillicate glass in a Sutter P-2000 laser puller (Sutter Instruments; Novato, CA) with resistances of 80-130MΩ. Electrodes were filled with 3M KAc. Pyramidal cells were held at -80mV during all stimulus trials, with the exception of RAMs used to characterize E and I cells in LS, in which case the cell was forced to fire at 10-30Hz regardless of membrane potential as long as it stayed within physiological range (Mehaffey et al., 2008). All recordings were amplified using an Axoclamp-2B amplifier (Molecular Devices, Sunnyvale, CA, USA), digitized using a NIDAQ PCI-6036E board (National Instruments, Austin, TX) and recorded using Igor Pro (Wavemetrics) and NeuroMatic (Jason Rothman: ThinkRandom, University College London, UK) at a 5kHz sampling rate. Recordings were rejected if any of the trials were unsuccessful, or if no PSPs were generated during synaptic stimulation of the StF. A total of 33 recordings were obtained from pyramidal cells in the three segments of the ELL with maps being clearly identifiable *in vitro* (CMS:8, CLS:8, LS: 17).

2.2.3 Data analysis:

Electrophysiological data from periodic and random interval trains were assessed using custom made Igor Pro software. PSP amplitudes were measured and normalized to the first PSP.

Estimates of coherence (C) in E and I cells of LS were made using action potential trains and RAM stimulus protocols:

$$C(f) = \frac{P_{sr}(f)^2}{P_{ss}(f)P_{rr}(f)}$$

where P_{ss} and P_{rr} indicate the power spectra of the stimulus and the response, while P_{sr} is the cross-spectrum between stimulus and response (Mehaffey et al., 2008). Stimulus and response traces were analysed using custom made MatLab 7.0.4 software. Coherence ratios were obtained by taking the area under the curve between 30-50Hz (high) and 0-20Hz (low). Broadband cells were considered cells with coherence ratios of between 0.86 to 1.19, while anything below 0.86 was considered low-pass, and anything above 1.19 was considered high-pass (Based on Mehaffey et al., 2008, Ellis et al., 2007).

All statistical analyses were done using SPSS (Paws 7.0.2). Repeated Measures ANOVAs were performed on all periodic stimulation data. Variance was tested using Fmax ratios for all repeated measures ANOVAs and t-tests. These showed homogeneity of variance between all map types. Two-tailed t-tests assuming equal variance were done for pairwise post hoc comparisons of the 10^{th} PSP across all frequencies and segments of the ELL. Results were considered statistically significant if $p<0.05$.

2.3 Results:

The direct feedback pathway has previously been studied in detail in the CMS and exhibits synaptic plasticity on many time scales (Wang and Maler, 1997; Oswald et al., 2002). The synaptic plasticity in CLS and LS has not yet been characterized; this is a necessary step towards understanding differential processing across maps. When looking at the LS, it is important to differentiate between E and I cells since they show the greatest divergence in tuning to AMs among pyramidal cells of all segments in ELL (Mehaffey et al., 2008; Krahe et al., 2008). Therefore, characterization via intracellular RAMs was performed, and once E and I cells were clearly identified, both periodic and random interval synaptic stimulation protocols were carried out (see methods for details) to characterize the STP occurring at StF-PC synapses.

LS E and I cells show no difference in STP

In the LS, E-cells are tuned to high frequencies while I-cells are tuned to low frequencies (Shumway, 1989a; Mehaffey et al., 2008; Krahe et al., 2008). E and I cells were therefore identified based on their frequency preference, tested using stimulus-response coherence to an intracellular broadband stimulus 0-60Hz, (see methods for details) (Fig. 2.1A,B; Ellis et al., 2007; Mehaffey et al., 2008). As in previous studies, a cell was characterized as an I-cell when it showed greatest coherence to frequencies of 0-20Hz, or an E-cell when its coherence to frequencies of 30-50Hz was greatest. More specifically, when a cell's high to low frequency coherence ratio was >1.19, it was considered high pass (i.e. preferring higher frequencies), and low-pass when <0.89 (i.e. preferring lower

frequencies)(Mehaffey et al 2008). Any value between these ratios is considered to be tuned to broadband frequencies. E-cells of the LS can be classified as either high-pass or broadband, and were therefore pooled. Examples for each frequency ratio are shown in figure 2.1C. Using these criteria, a total of 11 E-cells (pooling high-pass and broadband cells), and 6 I-cells were identified in the LS.

Synaptic plasticity of LS E and I cells was assessed by eliciting EPSP via stimulation of the dorsal StF (Fig 2.2A,B). Stimulating the dorsal StF (yellow arrow Fig 2.2A) is known to preferentially recruit excitatory stellate cell fibers descending from the nP (Berman et al., 1997; Oswald et al., 2002). EPSP amplitude was measured for a periodic train of 10 pulses at 10, 20 and 50Hz and a 70 pulse random interval train (Fig 2.2B,C). The StF-PC synapses have been found to readily potentiate with high frequency stimulation both *in vivo* (Bastian, 1996b) and *in vitro* (approximately 50Hz) (Wang and Maler, 1997; Oswald et al., 2002).

EPSP amplitudes between E and I cells during periodic stimulation of the StF (10 pulses at 10, 20 and 50 Hz) are shown in Fig 2.3 (normalized to the first EPSP). Consistent with previous studies, EPSPs increase in amplitude significantly from the 1^{st} to 10^{th} pulse (Repeated Measures ANOVA, $p<0.05$). These data illustrate the frequency dependent properties of pyramidal cells between 10, 20 and 50Hz stimulation, where the amplitude of EPSPs at 50Hz is approximately double that at 10 and 20Hz. Further, EPSP amplitudes in LS E and I cells were very similar for each frequency of stimulation (Fig. 2.3; Repeated measures ANOVA marginal mean, $p>0.05$). This finding is interesting since it suggests that although E and I cells differ in their frequency tuning (Mehaffey et al., 2008) they do not necessarily

Figure 2.1. Identification of I and E cells in LS based on coherence ratios. (A) To identify between I and E pyramidal cells of the LS, coherence values were obtained by sending a broadband noise current with a range of 0-60Hz. (B) Close-up of broadband stimulus train and cell spike train. (C) Top and middle panels show the typical preference of LS E-cells to either high or broadband stimuli, with high/low coherence ratios of 1.41 and 1.14 respectively, while the lowest panel shows the preference of I-cells to low frequencies with a coherence ratio of 0.60.

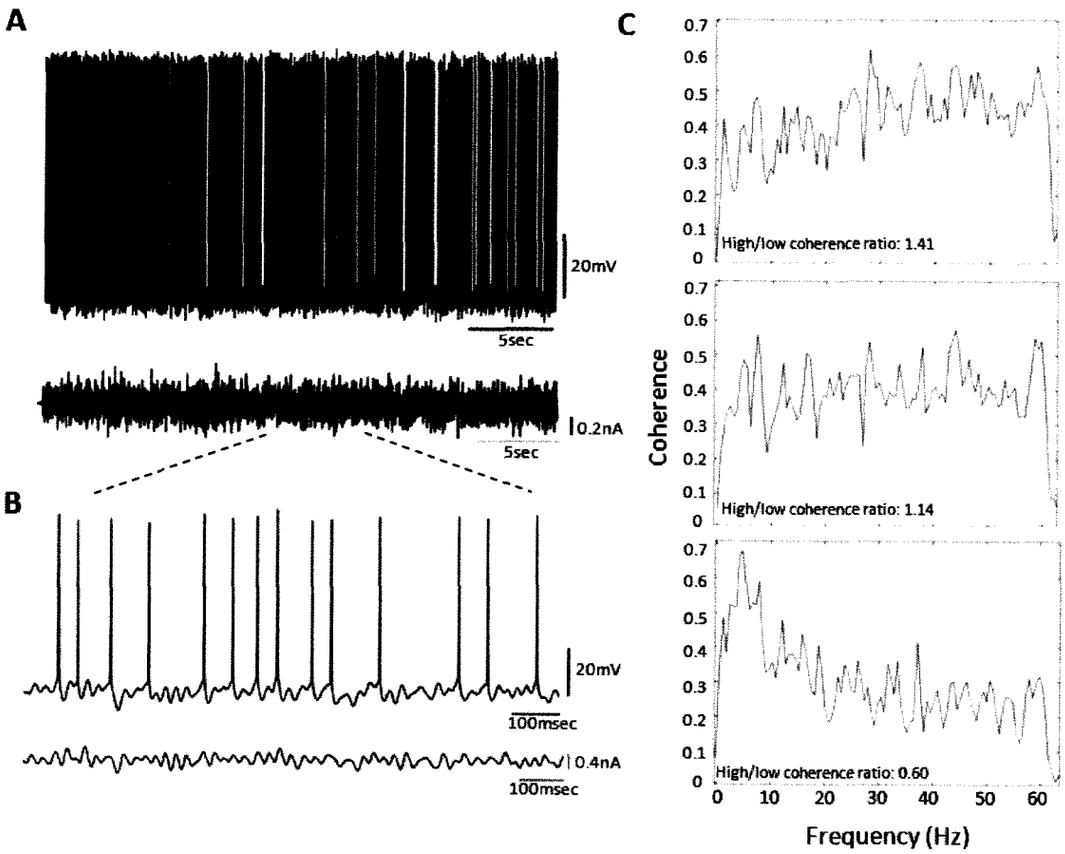

A
20mV
5sec
0.2nA
5sec
B
20mV
100msec
0.4nA
100msec
C
Coherence
High/low coherence ratio: 1.41
High/low coherence ratio: 1.14
High/low coherence ratio: 0.60
0 10 20 30 40 50 60
Frequency (Hz)

Fig 2.1

Figure 2.2: Synaptic stimulation of dorsal StF evokes EPSPs across all segments. (A) True transverse slice of ELL, grey line represents the StF and white line denotes the pyramidal cell layer from which recordings were made in all three segments. Black arrow denotes area of synaptic stimulation. (B) Trace showing example of synaptic stimulation of ELL pyramidal cell at 50 Hz for 10 pulses. (C) Part of the random interval stimulus train, where intervals between consecutive stimulations change (based on Lewis and Maler 2002). Stimulus artefacts omitted for clarity. Dots under trace represent timing of stimulus pulses.

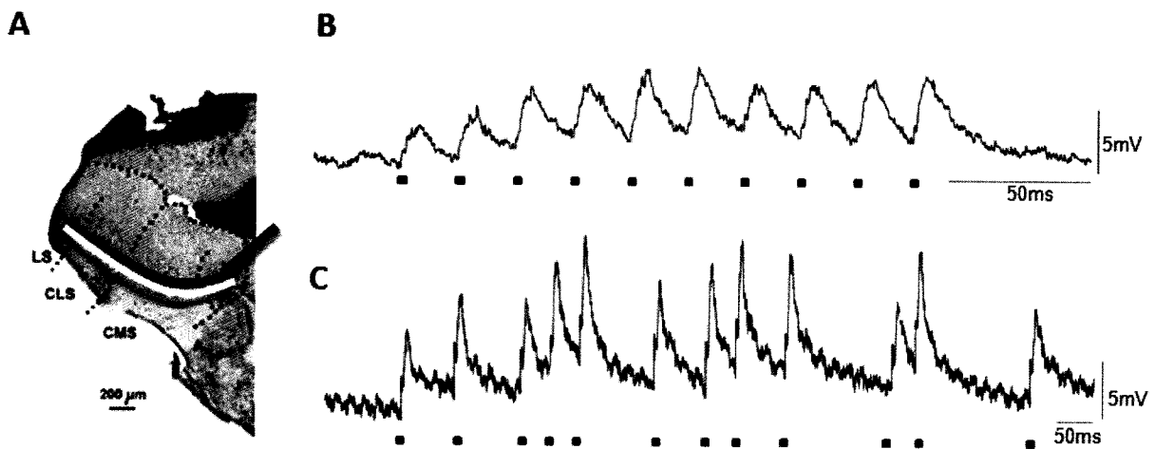


Fig 2.2

Figure 2.3. Normalized EPSP amplitude of E and I cells in the LS in response to a 10 pulse synaptic stimulation of the StF at 10, 20 and 50hz. (A) LS E-cell EPSP amplitude with each pulse number across 10 (n=10) , 20 (n=10) and 50hz (n=11) stimulation. (B) LS I-cell EPSP amplitude with respect to pulse number during a periodic synaptic stimulation at 10 (n=6), 20 (n=6), and 50hz (n=6) stimulation. Bars represent standard error.

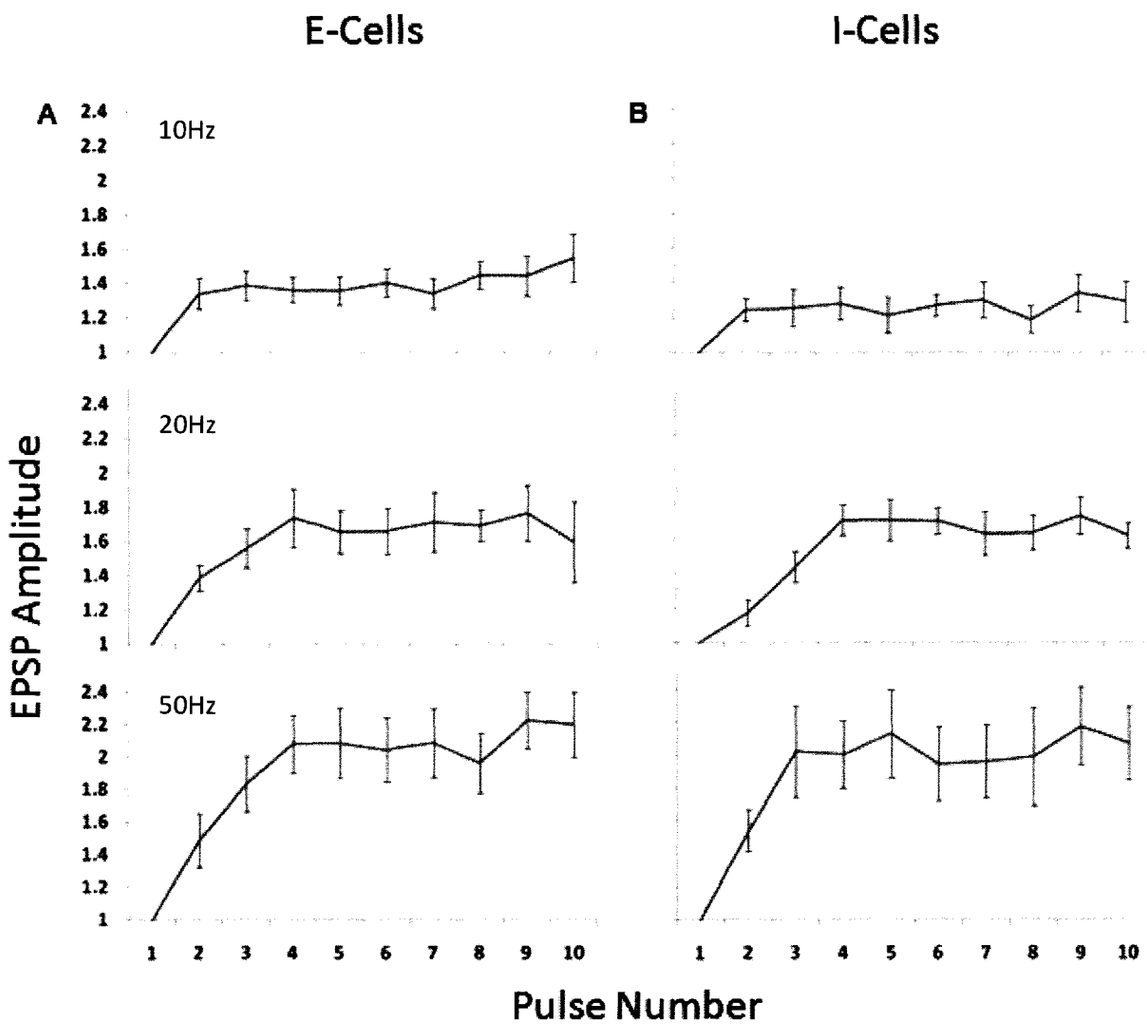

E-Cells
I-Cells
A
B
10Hz
20Hz
50Hz
EPSP Amplitude
Pulse Number
2.4
2.2
2
1.8
1.6
1.4
1.2
1
1 2 3 4 5 6 7 8 9 10

Fig 2.3

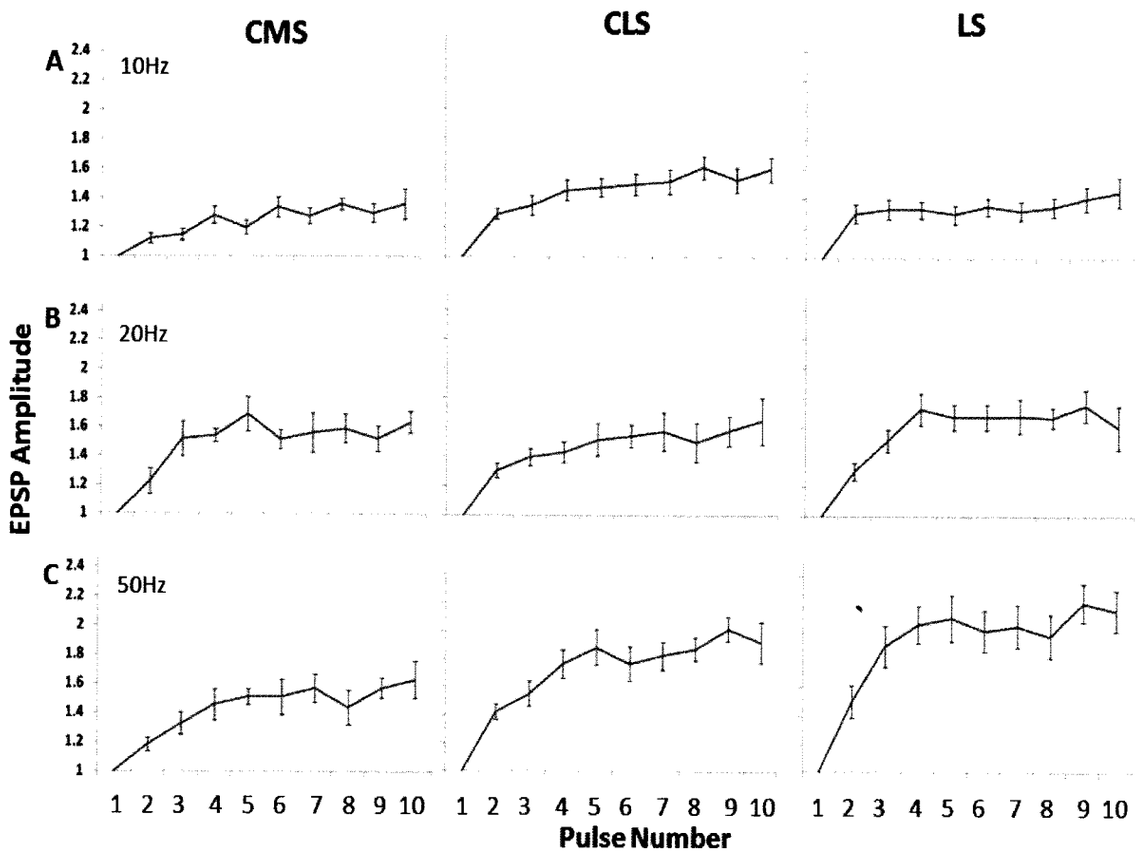
differ in their response to direct feedback. This may indicate that the difference in frequency tuning is solely dependent on intrinsic cellular factors such as the distribution of ion channels between maps (Ellis et al., 2007). Since no difference was observed between E and I cell facilitation to periodic synaptic stimulation, they were pooled for subsequent comparisons of STP between maps.

Synaptic plasticity and frequency dependence in ELL maps with periodic stimulation:

Following these observations, the synaptic responses of LS pyramidal cells (E/I pooled) were investigated and compared to those of CLS and CMS under similar stimulation frequencies of 10, 20 and 50Hz (Fig 2.4). Facilitation measured across the maps do not appear to vary significantly at 10 or 20Hz stimulation (Repeated measures ANOVA, $p>0.05$; Fig 2.4 A; B). However, at 50Hz synaptic stimulation there is a significant difference between CMS and LS (Repeated measures ANOVA marginal mean difference between CMS and LS 0.474 ± 0.154, $p=0.004$; Fig 2.4 C). These results show that STP varies across maps, and support the hypothesis that each map is optimized for a certain type of information processing (Krahe et al., 2008). The underlying mechanism may be related to Ca^{2+} dynamics, transmitter recovery and/or receptor desensitization (see discussion).

A post hoc pair wise comparison of EPSPs at the 10^{th} pulse (at 10, 20, 50Hz) further illustrates these differences (Fig 2.5). At 10 and 20Hz stimulation there is no significant difference between any of the segments (Pairwise two-tailed t-tests with equal variance assumed between all maps at 10 and 20 Hz= $p>0.05$) while at 50Hz stimulation, there is a significant difference in EPSP amplitude (CMS vs LS, $p<0.05$) with an increasing trend from CMS to LS.

Figure 2. 4. Normalized EPSP amplitude across the different segments of the ELL and frequency of stimulation. (A) EPSP amplitude of the CMS (n=8), CLS (n=8) and LS (n=16) pyramidal cells during a 10Hz, 10pulse synaptic stimulation. (B) EPSP amplitude of CMS (n=8), CLS (n=8) and LS (n=16) during 20Hz 10 pulse synaptic stimulation. (C) EPSP amplitude of CMS (n=8), CLS (n=8) and LS (n=17) during 50Hz 10 pulse synaptic stimulation. Error bars represent standard error.


CMS
CLS
LS
A
10Hz
B
20Hz
C
50Hz
EPSP Amplitude
2.4
2.2
2
1.8
1.6
1.4
1.2
1
1 2 3 4 5 6 7 8 9 10
Pulse Number

Random interval trains of stimuli characterize the time course of STP:

As seen during periodic stimulation of the StF, there is a frequency dependent response when increasing from 10 to 50Hz, with greater EPSP amplitudes occurring with increased frequency. Characterizing the response of pyramidal cells to the random interval stimulus train (e.g. 70 pulse train encompassing 1-50Hz frequencies with a mean at 7Hz) also shows frequency dependence (Fig 2.6). This type of protocol has also been used in previous studies to examine responses over a wide range of frequencies concurrently (Lewis and Maler, 2002). A common way to describe the resulting data is to plot EPSP amplitude versus the duration of the previous inter-stimulus interval (Lewis and Maler, 2002; Zucker and Regehr, 2002). Fig 2.6 shows plots (often referred to as recovery curves) for the results in each ELL map. With increasing intervals between stimuli, the amplitude of consecutive EPSPs will decrease until, if the interval is long enough, the amplitude will return to that of the 1^{st} EPSP (control level). These data show that in the CMS, EPSP amplitudes return to those of the first EPSP much sooner (τ=168ms) than in the CLS (τ=247ms) and LS (τ=237ms). In the LS the recovery may in fact involve two time constants, suggesting 'fast' and 'slow' facilitation processes (data not shown). This indicates that the timescale of STP is shorter in CMS than in CLS/LS by approximately 100ms. Thus, in CLS and LS, where we see a greater facilitation than CMS, there is also a much longer delay in recovery. This could suggest that the increased facilitation in EPSPs of the LS is due to slower recovery, perhaps directly related to the residual Ca^{2+} hypothesis (see discussion).

Figure 2.5. Relative EPSP amplitude of 10^{th} PSP between ELL segments and frequency of stimulation. CMS (blue), CLS (red) and LS (green) across. Error bars represent standard error. Asterisk implies significant difference. Star represents statistical significance at the p=0.05 level between the two bars.

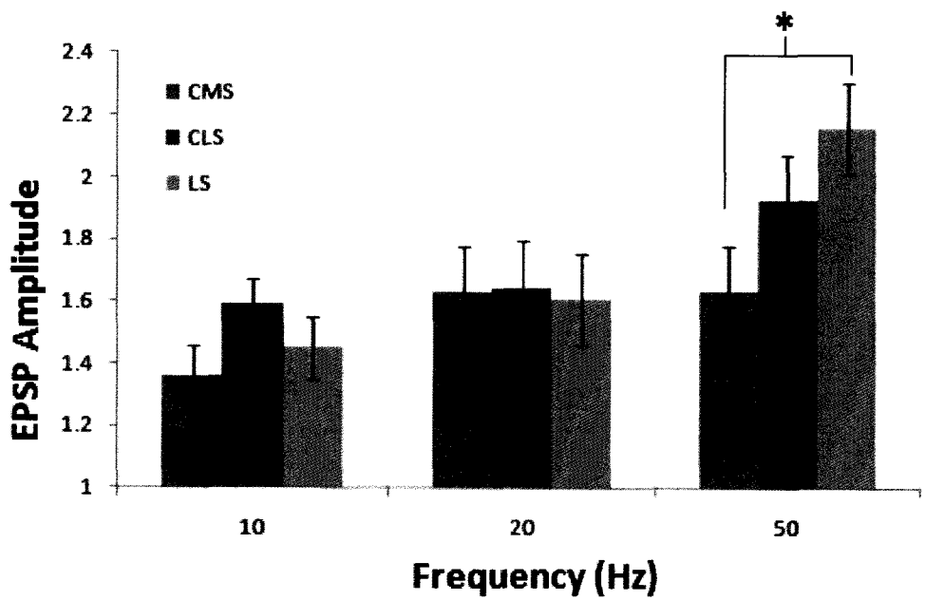

*
2.4
2.2
2
1.8
1.6
1.4
1.2
1
CMS
CLS
LS
EPSP Amplitude
10
20
50
Frequency (Hz)

Figure 2.5

Figure 2.6: EPSP amplitude with respect to stimulus interval between ELL segments. Mean EPSP amplitude of cells in CMS, CLS and LS shown at different intervals between stimulation. Stimulus intervals represent the distance between consecutive stimuli, for instance an interval of 0.02 would indicate that the stimulus is occurring at 50Hz. Lines of fit represent single exponential decay with tau indicated.

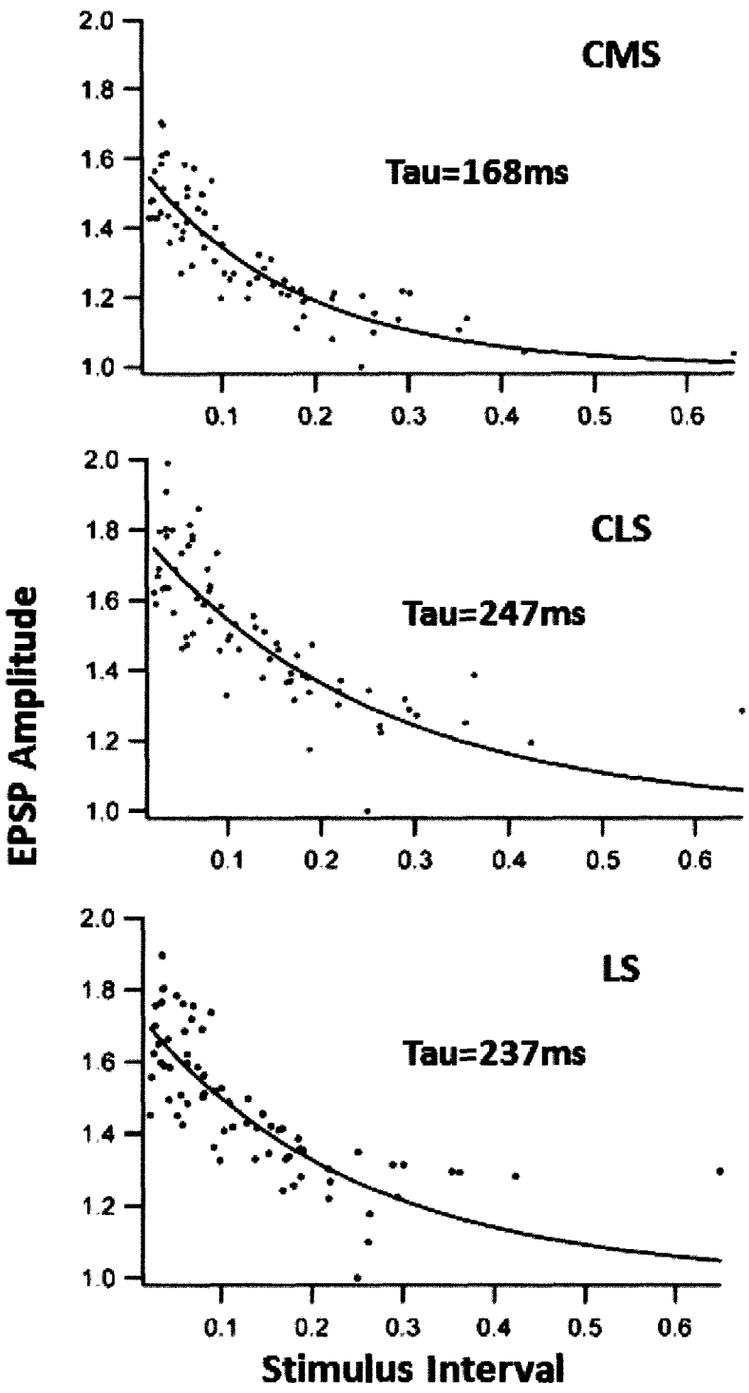

CMS
Tau=168ms
CLS
Tau=247ms
LS
Tau=237ms
EPSP Amplitude
Stimulus Interval
2.0
1.8
1.6
1.4
1.2
1.0
0.1
0.2
0.3
0.4
0.5
0.6

Figure 2.6

2.4 Discussion:

The replication of sensory maps is hypothesized to be the way critical processing nuclei can specialize without affecting the survival of the animal (Kaas, 1989). In other words, the original map is left to process the incoming information, while the newly duplicated map has time to develop specializations to further enhance feature extraction. That there are three separate electrosensory maps in *A. leptorhynchus* can also indicate that each acts like a spatial and temporal filter for some parts of the stimulus, with all maps processing the stimuli in both unique and redundant ways potentially controlled by the descending input from higher brain centers (Shumway, 1989a). In the brain of *A. leptorhynchus*, it has been hypothesized that the three tuberous electrosensory maps have separate functions in electrosensory processing, with the CMS implicated in low frequency processing, and the LS in high frequency processing (Metzner and Juranek, 1997; Mehaffey et al., 2008; Maler, 2009b). A recent study by Krahe and colleagues (2008) hypothesized that the CLS may be the most primitive map since the E-cells of this map are tuned to both high and low frequency AMs, while the LS and CMS are more specialized to either high or low frequency AMs respectively. Thus, characterizing pyramidal cell responses to direct feedback input (via nP stellate cells) between maps may reveal any specializations in electrosensory processing. Further, showing differences in STP between maps could potentially apply to feedback and its effect on neuronal processing in higher order organisms with multiple sensory maps.

Differences in frequency tuning of LS E and I cells may not be due to differences in contributions of the direct feedback pathway:

Within the three tuberous segments of the ELL, LS I and E-cells cells have the largest divergence in frequency tuning characteristics; E cells are tuned to high frequencies, and I cells are tuned to low frequencies (Mehaffey et al., 2008). Because of this difference between E and I-cells of the LS, it is interesting to find no significant difference in facilitation of EPSPs between cell type under stimulation of the StF (Fig.2.3). This implies that feedback from nP stellate cell input may not differentially affect E and I type pyramidal cells of the LS, and that their divergence in frequency response may be exclusively through electroreceptor afferent input as well as the distribution of intrinsic ion channels such as the Ca^{2+} activated slow conductance K^{+} (SK) channels (Ellis et al., 2007).

STP differs between maps

Stellate cells of the nP are known to respond preferentially to EOD AMs lower than 16Hz, but in return can respond with firing rates as high as 140Hz (Bastian and Bratton, 1990). However, in studies of the direct feedback inputs to ELL, it was found that stimulation of the StF at 50Hz caused the greatest increase in amplitude of EPSPs at the StF-PC synapse (Oswald et al., 2002). This is the main factor behind using 50Hz as the maximum stimulation frequency in this study. Through studying STP at StF-pyramidal cell synapses under a number of stimulation paradigms (10, 20, 50Hz and random intervals), we have been able to characterize the input of the direct feedback pathway to pyramidal cells across three electrosensory maps in the ELL. Differences in EPSP amplitude between maps of the

ELL will regulate the ability of cells in that map to reach the threshold for eliciting APs. The most profound difference between maps is seen at the highest frequency, 50Hz, where LS and CMS pyramidal cells diverge greatly in the relative amplitude of their EPSPs, with mean CMS EPSPs across all 10 pulses at 50Hz approximately 60% smaller than those in the LS (Fig 2.4).

It is important to note that any change in EPSP amplitude seen in Fig 2.4 and 2.5 are in terms of a relative increase from the first EPSP. From Fig 2.5 we see an increase in relative EPSP amplitude of approximately 50% at the 10^{th} PSP in LS (mean 2.15) and CMS (1.63) at 50Hz. In comparison with previous studies where EPSPs elicited at 50Hz had a maximal relative amplitude of 3 after normalization (Oswald et al., 2002), the amplitudes in our study were much smaller (maximal amplitude of 10^{th} PSP at 50Hz 2.13 in LS). The difference in EPSP amplitude facilitation between our study and Oswald 2002, may be due to the difference in experimental design: Oswald et al 2002 used extracellular recording techniques (and thus may have been measuring pooled responses from multiple pyramidal cells), whereas we used intracellular recordings (from single cells). In addition, in our study cells were held at -80mV using voltage-clamp to avoid eliciting APs. Overall, regardless of discrepancies in EPSP maximal amplitude between studies, our goal was to specifically observe STP across maps with an identical protocol between them.

Thus, as seen in Fig 2.4 and 2.5, the CMS and LS show the greatest divergence in facilitation where the LS has EPSP amplitudes almost double those of the CMS. This is congruent with other studies showing that the greatest difference in maps is usually

between the LS and CMS under various conditions (Shumway, 1989a; Shumway, 1989b; Maler, 2009a; Maler, 2009b). One possibility to account for this divergence between the LS and CMS, could be due to a frequency dependent increase in recruitment of disynaptic inhibition (via inhibitory interneurons) between the CMS and the LS (Fig 1.1B). In other words, higher frequencies of stellate cell firing may recruit disynaptic inhibition in the CMS to a greater extent than in the LS. This type of frequency dependent increase in disynaptic inhibition has been shown in a recent study of the synaptic plasticity of the indirect feedback pathway in the CMS, and was implicated in the cancellation of redundant input (Lewis and Maler, 2002; Lewis et al., 2007). One way to test this could be by blocking inhibition using a $GABA_A$ inhibitor (since disynaptic inhibition from the nP to ELL pyramidal cells is via $GABA_A$ receptors). We would expect that if disynaptic inhibition plays a role in the divergence between the LS and CMS, blocking with a $GABA_A$ specific antagonist (i.e. Bicuculline) would result in EPSPs of equal amplitude between all electrosensory maps. In fact, we might expect an overall increase in amplitude of EPSPs among all electrosensory maps since any contribution of disynaptic inhibition (weak or strong) would be eliminated (Wang and Maler, 1997). If the application of $GABA_A$ does not result in increased EPSP amplitude in the CMS we could presume there is either increased facilitation in the LS, or that synaptic depression plays a role in this difference.

Some basic mechanisms underlying STP, specifically short term depression and facilitation,could account for this difference, both at the presynaptic and postsynaptic terminals. There are several reasons synaptic depression may occur at the CMS StF-PC

synapse but not at the LS synapse. First, based on the residual calcium hypothesis, the amount of Ca^{2+} left in the presynaptic terminal following the first AP will determine the amount of neurotransmitter released when the next AP invades the terminal. This mechanism is based on Ca^{2+} dynamics and Fig 2.6 shows that the time constant of recovery in the CMS (τ=168ms) is much faster than in CLS (τ=247ms) and LS (τ=237ms), suggesting presynaptic Ca^{2+} stays in the CLS and LS for roughly 100ms longer than that in the CMS (leading to greater potentiation in CLS and LS maps of the ELL). We see that CLS and LS have similar recovery curves (Fig 2.6), while the only significant difference using periodic stimulation is between the LS and the CMS at 50Hz stimulation. This indicates that some other mechanisms, potentially involving Ca^{2+} dynamics are combined for the greater facilitation seen in LS.

A second possibility for the discrepancy in CMS and LS facilitation is vesicle turn-over rate. Vesicle recovery rate is crucial in determining the amount of neurotransmitter present in the presynaptic terminal (Zucker and Regehr, 2002). If turn-over rate is slow, there will not be enough neurotransmitter present to see facilitation when the next AP invades the terminal. An interesting follow up experiment would be to measure the whole cell capacitance via patch clamping the presynaptic terminal, but may prove inherently difficult. A significant change in capacitance could indicate increased neurotransmitter release, since the vesicle fuses with the cell membrane, and thus increases the overall capacitance (for review see (Matthews, 1996).

The mechanisms proposed above occur at the presynaptic terminal. There is also an important post-synaptic mechanism to consider: the desensitization of AMPA receptors. In other organisms, the desensitization of AMPA receptors can occur with high frequencies (Zhang and Trussell, 1994), and this may be what we are seeing in the CMS. This can be tested using cyclothiazide, an AMPA desensitization blocker, which would predictably result in an increase in the observed EPSP amplitude in the CMS (Zhang and Trussell, 1994).

Discrepancies across maps are likely due to a combination of all the mechanisms mentioned above. In our study, however, it is clear that facilitation overwhelms any possible overlapping depression (not specifically studied in this paper) described above in all maps of the ELL as no decreases in EPSP amplitude were observed (Fig 2.4, 2.5). This is confirmed by using simple computational models where the requirement for a fast depression term (as well as two facilitation terms) were found to fit the periodic and random interval data between maps presented in this Chapter (J. Lewis, unpublished observations).

A functional explanation for potentiation of EPSPs between maps: electrodetection and adaptive filtering

The difference in facilitation of EPSPs seen between the CMS and LS pyramidal cells is difficult to explain in terms of its function in electrosensory processing, although it may be implicated in the ability of the LS (over and above that of the CMS) to amplify transient inputs. A gain of 50% (seen in Fig 2.4 and 2.5) during transient input may have a significant effect on the ability of cells in the LS to help with electrodetection.

The direct feedback pathway may act as a sensory searchlight (Bastian and Bratton, 1990; Berman et al., 1997; Berman and Maler, 1999), the ability to 'focus' the electrosensory system on a specific target as it passes across the body surface (Berman et al., 1997). This was first proposed by Crick in 1984, for the visual system, but may exist in many sensory systems with associated feedback pathways. The three main constituents comprising Crick's (1984) sensory searchlight hypothesis are found in the ELL-nP feedback pathway: 1) Reciprocal excitatory connectivity, 2) an inhibitory surround (from inhibitory interneurons in the VML and the granule cell layer) and 3) a nonlinearity in the response of pyramidal cells to input (Crick, 1984; Berman et al., 1997). Based on the results of this study, it is possible that the second and third elements of this theory could differ between maps, through differences in disynaptic inhibition ('inhibitory surround'), as well as differences in facilitation of EPSPs between CMS and LS ('nonlinearity'). We can even extrapolate that due to the larger facilitation in the LS, it may serve as a 'better' map for electrodetection because of its larger RFs and greater sensitivity (Shumway, 1989a; Shumway, 1989b; Maler, 2009a; Maler, 2009b).

A recent study by Maler (2009) found a discrepancy (underestimation) between the anatomical and physiological calculations of RF sizes, and hypothesized that the direct feedback input from nP (due to its preserved topography) could actually contribute part of this missing area to the RF. Up to this point, the reasoning behind the function of the LS as the first and main component in sensing weak, distant prey, and low frequency signals holds true. The LS might further be implicated in the detection of high frequency

communication signals due to the LS E-cells' preferential sensitivity to high frequency AMs (Ellis et al., 2007; Krahe et al., 2008; Mehaffey et al., 2008) making it the map most sensitive to both high and low frequency external input. Recently, computational models have found that LS E-cells may in fact switch dynamically between high and low AM tuning based on the frequency of AMs presented to them (Maler, 2009a; Maler, 2009b). The ability of LS E-cells to switch their frequency tuning has also been found *in vivo* where both indirect and direct feedback pathways are intact and can contribute to this processing (Krahe et al., 2008). Neural network mechanisms are implicated in this switching ability, and we hypothesize that the difference in STP and specifically the facilitation of EPSPs between maps, can solve a small part of this puzzle, although further testing will be necessary to determine the exact mechanisms.

Mammalian STP has been implicated in a variety of functions, one of which is the synaptic filtering of inputs (Abbott and Regehr, 2004). The same can be said of the plasticity found in the direct feedback pathway, where Bastian and colleagues have characterized one of its functions as that of an adaptive filter for redundant input, albeit with much longer time scales due to the anti-Hebbian plasticity discussed in Chapter 1 (Bastian, 1995; Bastian, 1996a; Bastian, 1996b).

In terms of synaptic filtering, it has recently been shown that pyramidal cells respond to low frequency AMs (electrolocation) with spike bursts, while to high frequency AMs (electrocommunication) with single spikes (Oswald et al., 2004). This is consistent with work by Mehaffey and colleagues (2008) who found that LS E-cells had the lowest fraction

of bursting, since they are generally tuned to broadband and high frequency AMs. However, all I-cells and E-cells of the CMS are tuned to low frequencies so the burst fraction of these cells was significantly higher than those of LS-E cells. The presence of somatic SK channels on pyramidal cells in *A. leptorhynchus* increases in density from the CMS to the LS (Ellis et al., 2007). Ellis et al (2007) suggest that cells tuned to high frequency AMs require these SK channels in order to prevent the bursting dynamics in the cells, due to the role of SK channels in eliciting a medium afterhyperpolarization (mAHP) which overlaps with the back propagated depolarizing after potential (DAP) from the pyramidal cell apical dendrites. In other words, the presence of SK channels causes hyperpolarization of the cell, so that the DAP cannot elicit a burst response. These differences between maps in terms of fraction of burst firing, frequency tuning, and the presence of SK channels, along with the differential response to input from the direct feedback pathway can help shape many aspects of electrosensory processing in the weakly electric fish brain.

We propose that STP in the direct feedback pathway may modulate the RFs of pyramidal cells, specifically enlarging those of the LS (via increased facilitation). This can also be tied to the proposed 'attentional' mechanism of the direct feedback pathway, since increased RFs will increase the electrodetection ability of pyramidal cells. However, open-loop slices do not contain the entire feedback pathway and there has been little examination of nP stellate cell responses to ELL pyramidal cell input *in vitro*. More *in vitro* studies of the nP are necessary to assess the dynamics at these synapses and the presence of STP or long term plasticity in this part of the pathway. In Chapter 3 of this thesis,

development of an *in vitro* preparation preserving the entire direct feedback pathway is discussed, with specific focus on how it may increase our understanding of synaptic plasticity found at both nP-ELL and ELL-nP synapses.

CHAPTER THREE:

In vitro Preparation Preserving the Entire Direct Feedback Pathway

(Excerpted with modifications from Mileva et al. 2008)

3.1 Closed-loop feedback in vitro: an nP–ELL slice preparation

Typical brain slice preparations are 'open-loop', in that the brain region of interest is isolated from other regions that normally provide feed forward and feedback inputs (Yamamoto and McIlwain, 1966; Oertel, 1985). Open loop preparations are necessary to characterize the dynamics of feedback synapses independent of other network influences, but it is difficult in these cases to mimic natural activity patterns. Another approach is to develop an *in vitro* slice which contains an entire intact feedback loop. This often poses a significant challenge due to the difficulty of preserving three-dimensional architecture in a thin brain slice. Preservation of the indirect feedback pathway in a thin slice of the *A. leptorhynchus* brain is nearly impossible, due to the spatial topography of the three nuclei which comprise it (ELL, nP and EGp). However, the anatomy of the direct feedback pathway (i.e. the ELL and nP and their reciprocal connections via the StF and LL (Fig 1.1)), is favourable for a slice preparation. In the following sections, we outline an approach, focusing first on the anatomy of the nP, and second on the methodology behind the nP-ELL slice.

3.1.1. The nucleus praeminentialis, nP

The anatomy of nP has been characterized in detail (Sas and Maler, 1983). The nP is comprised of three distinct regions: the pars medialis, pars principalis, and the pars lateralis, each of which has differing cell types. The pars medialis, to which bipolar cells (direct inhibition to ELL) are unique, receives input mainly from the lateral LL while the pars

lateralis, to which boundary cells are the most common, from the descending fibers of the torus semi-circularis. The pars principalis has 12 different cell types, and makes up the bulk of the nP. Stellate cells (direct excitation to ELL) are the most common cell type in the pars principalis and *in vivo* research by Bratton and Bastian (1990) has elucidated their physiological characteristics. Stellate cells are typically silent and have two subtypes (ST-I and ST-E), depending whether they are inhibited (ST-I) or excited (ST-E) by increases in EOD amplitude (analogous to ELL E and I type pyramidal cells).

Studies have also been done on the nP in mormyriform fish (von der Emde and Bell, 1996). Although both fish evolved electroreception separately, the nP and its function seem to be conserved between mormyrids and gymnotids (Rose, 2004). In mormyrids, input from electroreceptors is somatotopically relayed to the nP from the different zones of the ELL (Bell and Maler, 2005). Similar to the gymnotid nP, the mormyrid nP also projects directly and indirectly onto ELL pyramidal cells and contains cells whose *in vivo* dynamics are comparable to the ST-I and ST-E cells (von der Emde and Bell, 1996). The large number of cell-types in the nP gives some idea of its importance in the electrosensory system of weakly electric fish, but makes their study more difficult. Developing an *in vitro* slice preparation can help characterize the dynamics and physiological function of cells in the nP, in addition to providing insight into the role of the direct feedback pathway in shaping ELL pyramidal neuron activity.

3.1.2 nP–ELL slice preparation

The basic experimental procedures were identical to those described previously (Mathieson and Maler, 1988; Berman and Maler, 1999; Lewis and Maler, 2002) and are in accordance with University of Ottawa Animal Care Committee protocol BL-229. Fish (10–15 g) were anesthetized using 0.2% Tricaine methanesulfonate (TMS, Syndel International Inc.) and surgery performed as previously described. The only difference in procedure was the angle and orientation of the slice. Here, the brain was cut transversely half-way along the optic tectum, at approximately section T19- T20 of the *A. leptorhynchus* brain atlas (Maler et al., 1991). The caudal portion of the blocked brain was glued to a chilled plastic chuck, dorsal-side down (schematically shown in Fig. 3.1A). The brain and chuck were then secured onto an OTS-5000 Vibratome (FHC Inc., Bowdoinham ME) and immersed in chilled ACSF. With the chuck positioned horizontally, ~600–700 µm of brain, from the tip of the brain stem, was removed (Fig. 3.1A, top). Thin sections were cut until only a small section of the brain stem remained and the myelinated fibers of the LL and the StF were barely visible. The chuck was then angled slightly (approximately 5 degrees), oriented with the caudal end up, and a 700 µm section of the brain was sliced (Fig. 3.1A, bottom).

Due to the thickness of this slice preparation (~700 µm), neuron viability was a concern. Although *in vitro* preparations involving other areas of the electric fish brain, such as the pacemaker nucleus, have shown that cells are viable even in thicker slices (Dye, 1988; Oestreich et al., 2006), studies with thicker whole brain preparations (4 mm) have suggested that viability in the deeper areas is questionable (Spiro, 1997). Our preliminary

recordings from nP stellate cells and ELL pyramidal cells in this slice show that they are indeed viable for several hours in the nP–ELL slice.

We illustrate our preliminary results in Fig. 3.1. First, we show that the feedback pathway to ELL is intact by electrically stimulating the StF/LL tracts at a location near the medial aspect of nP (Fig. 3.1B) and inducing one-for-one responses in an ELL pyramidal neuron (Fig. 3.1C(i)). Next, we show PSPs can be elicited in nP stellate neurons by stimulating the StF/LL tracts near the ELL (Fig. 3.1B and C(ii) and (iii)). As found *in vivo* (Bratton and Bastian, 1990), this stellate neuron had no spontaneous activity. The evoked PSPs show a slight depression at 50 Hz stimulation and the after-hyperpolarization following a spike is quite long, consistent with the high level of calcium-activated slow conductance potassium (SK) channels found in these neurons (Ellis et al., 2007). It will be important to fully characterize these dynamics in future studies.

Last, we show response curves (frequency versus injected current) for ELL pyramidal neurons (Fig. 3.1D) measured using a current ramp protocol. The maximum firing frequency is lower (30–55 Hz range) than that typically seen in the standard ELL slice (~100–250 Hz; e.g.Fernandez et al., 2005; Mehaffey et al., 2005). Given that the input resistance of these neurons at rest is similar to that in previous studies, we can speculate the discrepancy is due, at least in part, to the preserved feedback pathway in the nP–ELL slice. The possibility remains that the observed differences in maximum firing frequency could be due to differences in protocol as well. That said, intact feedback would also produce synaptic shunting or direct inhibition (Mehaffey et al., 2005), and thus limit firing frequency. The fact that pyramidal neurons usually fire at frequencies much lower than 100 Hz *in vivo* (Bastian

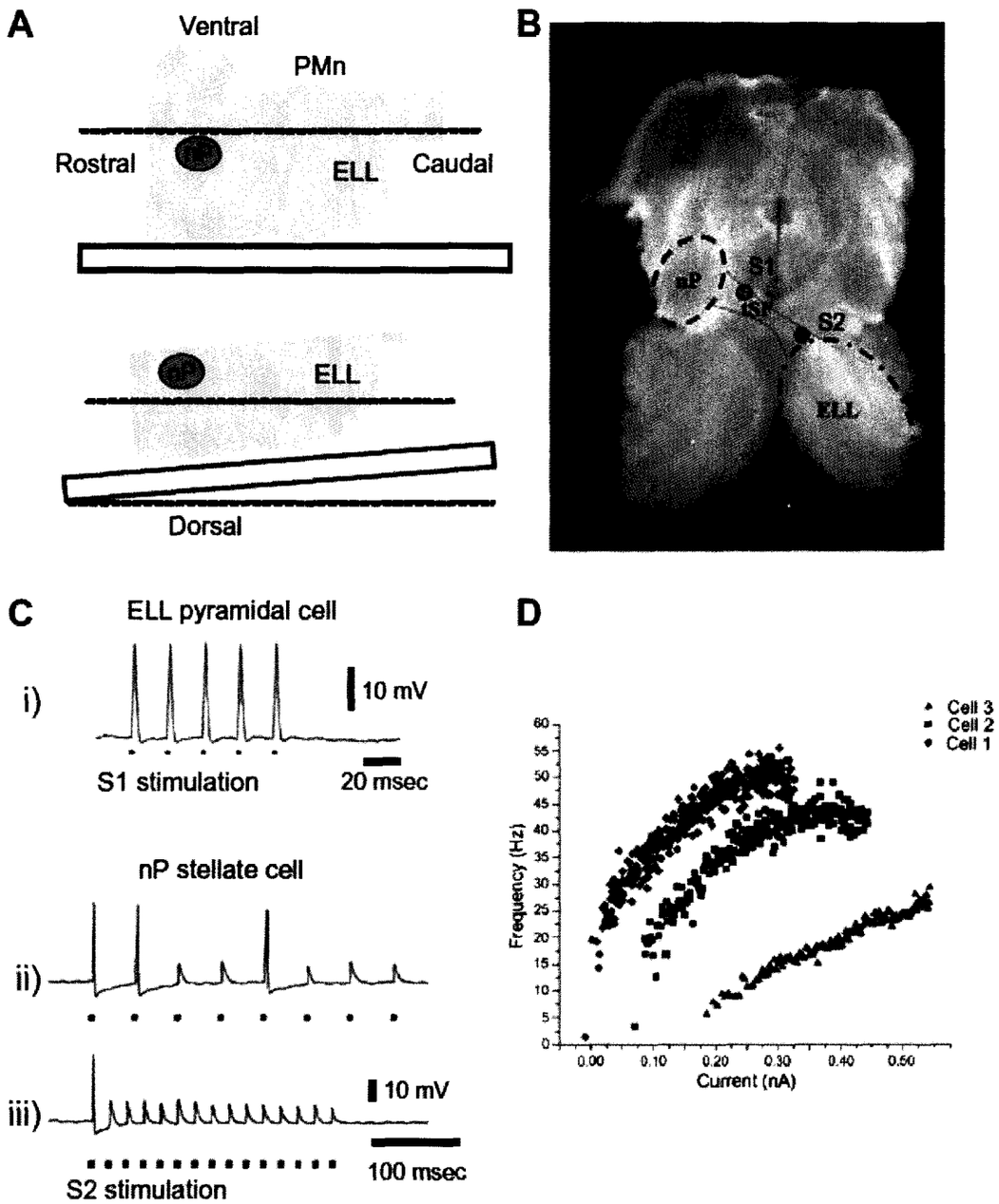
et al., 2002) provides more evidence that this is a viable hypothesis, but further experiments are required to test its validity. Overall, these observations show that the connections between nP and ELL are preserved in this slice. This suggests that it will be possible to record simultaneously from a neuron in each nucleus and identify reciprocal connections.

3.2 Discussion

Approaches similar to those just described have also proven useful for understanding cortical-thalamic dynamics. In mouse, a slice preserving connectivity between the ventrobasal nucleus of the thalamus and the sensorimotor "barrel" cortex (Agmon and Connors, 1991) has been widely used (e.g. Agmon and Connors, 1992; Feldman et al., 1999; Landisman and Connors, 2007). Recently, another mouse preparation containing feedback pathways linking the medial thalamus and the anterior cingulated cortex has been developed (Lee et al., 2007). In ferrets, a slice that preserves the retino-thalamic pathways and feedback connections inside the thalamus has been described (von Krosigk et al., 1993). This slice has been extensively and very effectively used (Kim et al., 1995; Destexhe et al., 1996; Kim and McCormick, 1998; Bal et al., 2000). Furthermore, a study using this slice showed a role for inhibitory feedback in information transfer from the thalamus to the cortex, possibly associated with the physiological mechanism responsible for sleep-arousal transitions (Le Masson et al., 2002). Interestingly, electrosensory feedback pathways resemble the structure and organization of cortical-thalamic loops (Berman and Maler, 1999). Studies at the cortical-thalamic level have shown that feedback connections

in between different circuit stages are critical for effective sensory processing (Sillito et al., 1994). In addition, studies in both systems have shown that delayed feedback inhibition plays a central role in determining network state (Contreras et al., 1996; Doiron et al., 2003). In weakly electric fish, we know that neuronal feedback is crucial. Extensive *in vivo* studies have shown that direct and indirect feedback pathways to the ELL play a role in gain control, reafference suppression as well as other aspects of sensory processing (Bastian, 1986a; Bell, 2001; Bastian et al., 2004). Studying synaptic dynamics in feedback pathways *in vitro* has also contributed to this understanding. By gradually building up the complexity of *in vitro* approaches, using methods such as those presented here we can gain new functional insights. Using the nP-ELL slice preparation, there was limited success in consistently recording from nP stellate cells of the pars principalis. Following this, we tried both intracellular and patch clamp electrophysiology on another novel slice preparation (not shown) containing only the nP and the LL (open-loop). Here as well there was limited success, and due to time constraints further attempts were not pursued.

Figure. 3.1. The nP–ELL slice preparation. (A) Schematic side view of brain showing nP, ELL, and pacemaker nucleus (PMn) for reference; dotted lines indicate the slicing orientation (see text). (B) Bright field microscopy of nP–ELL slice (cut deeper into the two nuclei for illustration purposes); nP and ELL outlined and connected via tSF/LL. S1 and S2 represent approximate stimulus sites for recordings in panel C. (C) Synaptically evoked activity recorded intracellularly from ELL pyramidal cell for a 50 Hz stimulus train (i). Synaptically evoked activity recorded intracellularly from nP stellate cell at 20 Hz (ii) and 50 Hz (iii). Dots under traces indicate stimulation times. (D) Instantaneous frequency-current (FI) curves of ELL pyramidal cells (n = 3, input resistances, R_{in} = 20–50 M X) determined using ramp current protocols of one second duration. (Image reproduced from Mileva et al., 2008)


A
Ventral
PMn
Rostral
ELL
Caudal
ELL
Dorsal
B
nP
S1
S2
ELL
C
ELL pyramidal cell
i)
10 mV
S1 stimulation
20 msec
nP stellate cell
ii)
iii)
10 mV
100 msec
S2 stimulation
D
Cell 3
Cell 2
Cell 1
Frequency (Hz)
Current (nA)

CHAPTER FOUR:

Brief Summary of Results and Discussion

The processing occurring at the level of the electrosensory lateral line lobe (ELL) is crucial since it is the sole nucleus receiving input from primary electrosensory receptors on the skin. Once information is processed it is sent, via projections, to higher brain centers, and in turn to the nuclei involved in generating the electric organ discharge (EOD). In other words, any processing at the ELL will therefore undoubtedly affect the whole organism. Two main feedback pathways affect the processing in the ELL: the indirect and direct pathways. These influence how information is processed and have been ascribed different possible functions (Chapter 1). The importance of these feedback pathways to sensory processing in the ELL motivates the study of synaptic plasticity occurring at their synapses. Specifically in this thesis, synaptic plasticity occurring at synapses of the direct feedback pathway (stemming from the higher order nucleus, the nucleus praeminentialis (nP)) were of interest. The goal of this thesis was to characterize the synaptic plasticity of descending inputs to the ELL. More specifically, we examined the short term plasticity (STP) occurring at the StF-PC synapses (Fig 1.1) between electrosensory maps, to better understand the role of the direct feedback pathway in shaping electrosensory processing during transient input.

The difference in STP between ELL maps found in Chapter 2 could suggest specializations for each of the three tuberous segments of the ELL. Replication of sensory maps is common in many sensory systems and is thought to be a way for unique processing across each map (Kaas, 1986). The two fold increase in amplitude of excitatory post synaptic potentials (EPSPs) in the lateral segment (LS) of the ELL compared with that of the centromedial segment (CMS) at high frequency synaptic stimulation could indicate its

heightened sensitivity to weak, distant objects, via larger receptive fields produced by increased facilitation in LS. This is also in line with the proposed function of the direct feedback pathway as a sensory searchlight, which has many properties similar to those of the mammalian thalamo-cortical feedback loops (Crick, 1984; Berman et al., 1997). The mechanism behind this increase in facilitation of EPSPs in the LS could be indicative of differential Ca^{2+} dynamics between the segments. This is shown in Fig 2.6 where the recovery curve decays at a slower rate in the LS, implying that a larger amount of residual Ca^{2+} is left in the presynaptic terminals in the LS than in the CMS. In turn, if a greater concentration of Ca^{2+} is found in the presynaptic terminal, greater facilitation will occur.

From these results future studies could focus on the potential contribution of frequency dependent disynaptic inhibition (via inhibitory interneurons), calcium dynamics occurring at the StF-ELL synapses, neurotransmitter recovery rates, and potential changes in the post synaptic membrane composition of receptors (AMPA). These characterizations will help clarify the mechanism by which plasticity in feedback synapses affects sensory processing, and in turn help further our knowledge of the function of these feedback pathways. With specific reference to multiple electrosensory maps, it would also be interesting to find if additional specializations exist between maps in terms of their response to other descending feedback (i.e. the indirect feedback pathway).

Finally, developing an *in vitro* slice containing the entire direct feedback loop is a necessary step in studying the functional roles of feedback. One of the main advantages of using *in vitro* slice preparations is the simplified connectivity which allows for experimental

manipulation of each aspect, without the whole network connectivity seen *in vivo*. Fig 3.1 shows that synaptic plasticity occurs in the lateral lemniscus/nP stellate cell synapse, although the underlying dynamics remain unknown. Understanding how this plasticity affects firing rate of stellate cells will undoubtedly be valuable in studying the feedback between pyramidal cells of the ELL and stellate cells of the nP.

Although a clear understanding of the functional roles of feedback (and its inherent synaptic plasticity) in sensory processing is still far off, simplified brain networks, such as those found in the weakly electric fish, can help clarify the mechanisms involved. In turn, this may lead to new functional insights and potentially applied to the brains of higher order organisms and the feedback pathways and plasticity associated with them.

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