

Memory-guided sensory sampling during self-guided exploration in pulse-type electric fish

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Dedicated to my mother who cultivated curiosity and love for knowledge in me,
and my father who instilled confidence in me when I needed it the most.

본 논문은 호기심을 길러주신 사랑많으신 어머니와 나는 할수있다라는 강한 믿음을
심어주신 아버지께 헌사합니다.

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List of Acronyms

EOD	Electric Organ Discharge
WEF	Weakly Electric Fish
EODR	EOD Rate
EODA	EOD Acceleration
CV	Coefficients of variation
RMS	Root-mean-squared
SNR	Signal-to-Noise Ratio
ISI	Inter-spike interval
IPI	Inter-pulse interval
PMn	Pacemaker neuron

Abstract

Animals must sense their surroundings to update their internal representations of the external environment, and exploratory behaviours such as sensory sampling are influenced by past experiences. This thesis investigates how voluntary sensory sampling activities undergo learning-dependent changes. Studies of freely behaving animals impose two major challenges: 1) the accuracy of biological measurements is compromised by movement-induced artifacts, and 2) large degrees of freedom in unrestrained behaviours confound well-controlled studies. Pulse-type weakly electric fish (WEF) are an ideal choice to study adaptive sensory sampling from unrestrained animals, since they generate readily observable and quantifiable sensory capture events expressed by discrete pulses of electric organ discharges (EODs). To study the voluntarily movements and sensory sampling while animals navigated in darkness, we developed three novel experimental techniques to track movements and detect sensory sampling from a freely behaving WEF: 1) an EOD detector to remotely and accurately measure the sensory sampling rate, 2) an electrical tracking method to track multiple WEF using their own EODs, and 3) visual tracking algorithm for robust body tracking through water under infrared illumination. These techniques were successfully applied to reveal novel sensory sampling behaviours in freely exploring *Gymnotus* sp. Cortical activity precedes self-initiated movements by several seconds in mammals; this observation has led into inquiries on the nature of volition. Here we demonstrate the sensory sampling enhancement also precedes self-initiated movement by a few seconds in *Gymnotus* sp. Next, we tested whether these animals can be trained to learn a location of food using electrically detectable landmarks and, if so, whether they can use their past experiences to optimize their sensory sampling. We found that animals revisited the missing food location with high spatial accuracy, and they intensified their sensory sampling near the expected food location by increasing the number of EOD pulses per unit distance travelled.

Résumé

Les animaux doivent ressentir leur environnement pour mettre à jour leurs représentations internes de l'environnement externe, et les comportements exploratoires telles que l'échantillonnage sensoriel sont influencés par les expériences antérieures. Cette thèse porte sur la façon dont les activités volontaires d'échantillonnage sensoriel subissent des changements qui dépendent de l'apprentissage. Les études sur les animaux se comportant librement imposent deux défis majeurs: 1) la précision des mesures biologiques est compromise par des artefacts induits par le mouvement, et 2) le grand nombre de degrés de liberté dans les comportements libres confondent les études bien contrôlées. Les poissons faiblement électriques (PFE) de type pulse sont un choix idéal pour étudier l'échantillonnage sensoriel adaptatif des animaux libres, car ils génèrent des événements de capture sensorielle facilement observables et quantifiables se manifestant par des impulsions discrètes de décharge de leur organe électrique (DOE). Pour observer les mouvements volontaires et l'échantillonnage sensoriel quand les animaux naviguent dans la noirceur, nous avons développé trois techniques expérimentales innovatrices pour suivre les mouvements et détecter l'échantillonnage sensoriel des PFEs se comportant librement : 1) un détecteur de DOE pour mesurer précisément et à distance le taux d'échantillonnage sensoriel, 2) une méthode de pistage électrique pour suivre plusieurs PFE en utilisant leur propre DOE, et 3) un algorithme de pistage visuel pour un suivi robuste du corps à travers l'eau sous illumination infrarouge. Ces techniques ont été appliquées avec succès pour dévoiler des comportements d'échantillonnage sensoriel nouveaux chez *Gymnotus* sp. en exploration libre. L'activité corticale précède de plusieurs secondes les mouvements auto-générés chez les mammifères; cette observation a mené à des questionnements sur la nature de la volonté. Ici, nous démontrons que l'échantillonnage sensoriel précède également les mouvements auto-générés de quelques secondes chez *Gymnotus* sp. Ensuite, nous avons vérifié si ces animaux pouvaient être entraînés à apprendre l'emplacement de nourritures en utilisant des balises électriquement détectables, et, le cas échéant, s'ils pouvaient utiliser leurs expériences antérieures pour optimiser leur échantillonnage sensoriel. Nous avons découvert que les animaux retournaient à l'emplacement de la nourriture manquante avec une grande précision spatiale, et qu'ils intensifiaient leur échantillonnage sensoriel près de l'endroit où la nourriture était attendue en augmentant le nombre de pulse de DOE par unité de distance parcourue.

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Chapter 1

Introduction

1.1 Motivation of study and conceptual framework

1.1.1 Motivation of studying animal behaviours

Animals exhibit seemingly complex behaviours that give them competitive advantages to survive in their environments. By using well-adapted bodies and specialized organs, animals behave in evolutionarily optimized manners under their unique environmental constraints such as available energy and information. In this regard, engineering problems in human activities share a common fundamental aspect with biological evolution, namely of striving for optimal solutions under given constraints. In fact, natural organisms inspired numerous technical solutions pertaining to novel structural designs, choice of materials, and coordination of behaviours. For example, the design of airplane wings was adapted from the airfoil shape of bird wings.

Similarly, the brain inspired the development of modern computers, and it continues to be the ultimate goal for computers to process information like biological brains. The brain plays the central role for synthesizing optimal and adaptive behaviours by coordinating sensors, actuators and information from past experiences (see Fig. 1.1). Thus, knowledge of brain functions that are optimized over evolutionary timescales could be applied to solve some of the big technological problems our humanity faces today. The brain in action can be best understood by observing the behaviours it generates, and the ecological context of behaviours aid our interpretation of neural activities. The complexity of animal behaviours is fascinating, such as intricate spider webbing, mating rituals of manikins, food caching in squirrels, long-distance migration in birds, echolocation of bats in total darkness to list a few. It is even more fascinating to know that these behaviours are controlled by tiny brains requiring minimal energies compared to man-made electronic controllers.

In understanding the brain functions at a physical level, quantitative approaches to naturally occurring behaviours have been successful. Certain behaviours are more accessible to quantitative observations in a particular animal model. Likewise, certain brains are structurally simpler and thus it is more practical to understand their detailed working mechanisms (see Fig. 1.2). Therefore, it is important to carefully choose an appropriate animal model that best represents the phenomena of interest, and also experimentally and theoretically

accessible. The brain collects information from animal's surroundings in an active manner by directing its sensing organs to the source of information within its detection range. One main example of this is the self-guided spatial exploration which can be quantified by the position of an animal and the direction of its sense organs. Experimenters are usually faced with competing goals between higher quality measurements and unperturbed observations of natural phenomena. At one extreme, experimenters may choose to restrain or even sacrifice an animal to obtain high-quality neural recordings under a well-controlled condition; or at the opposite extreme, experimenters may choose to passively observe spontaneous animal behaviours in their habitats to obtain ecological insights. Despite of technological advances, it is still difficult to obtain high quality physiological recordings from freely moving animals without influencing their naturalistic behaviours. Therefore, we chose to study pulse-type weakly electric fish as an animal model to uncover the fundamental neural principles underlying active sensing during exploratory behaviours due to many of its attractive properties for unperturbed experimental observations.

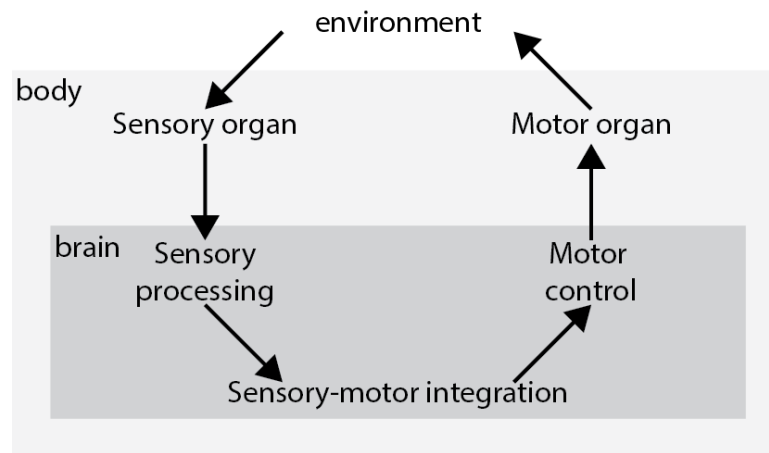


Figure 1.1. Conceptual framework of behavioural neuroscience. While behaviours are observed from how motor organs interacting with the environment, the ultimate goal is to understand the neural mechanisms of behaviour production.

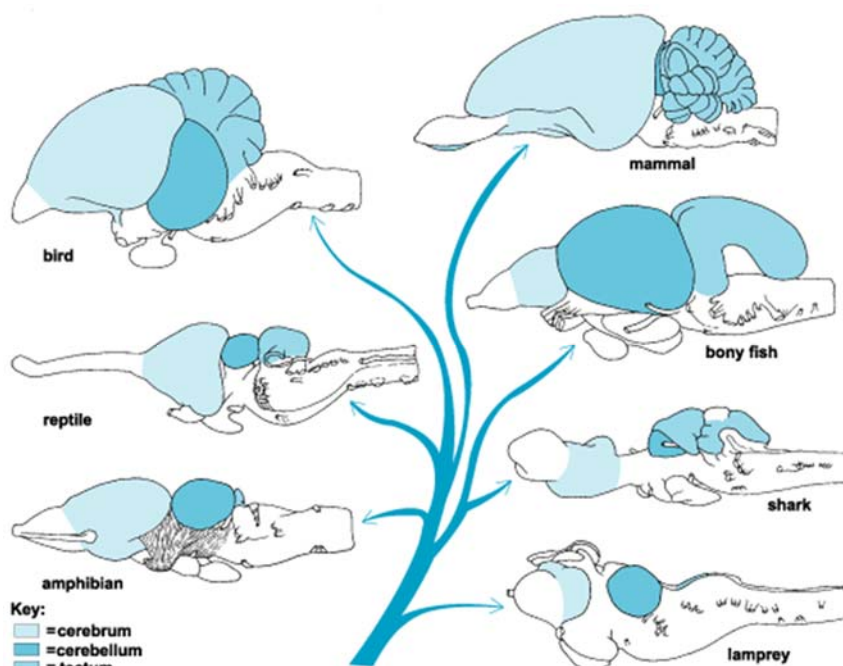


Figure 1.2. Comparison between different animal brains. The teleost (bony fish) brains are anatomically simpler and more compact than the mammalian brains. Figure is adapted from Northcutt et al. [2012].

1.1.2 Selection of a suitable animal model

In biology, some animal models such as rodents, zebrafish and fruit flies are much more commonly studied than other species. Although the use of standardized animal models enforces focused research efforts, no single animal model can appropriately address all research questions in behavioural neuroscience. For example, mice heavily rely on whisking and olfactory senses for navigation; but since they possess relatively poor visual acuity, it wouldn't make an appropriate model to study visually guided behaviours. Therefore, researchers can benefit from selecting the most suitable animal models to address their specific research questions by evaluating whether they display relevant behaviours, are physiologically accessible, and other logistic considerations. Some animal species display readily observable behaviours that lend themselves to objective quantifications and unambiguous interpretations. In retrospect, studies in previously unfamiliar animal preparations such as giant axon of squids [Hodgkin and Huxley, 1952] and sea slugs [Castellucci et al., 1970; Kandel and Schwartz,

1982] led to fundamental discoveries about the biophysics of neural activity owing to their anatomical simplicity and accessibility for physiological observations.

In particular, electric fish generate electrical signals that can be non-invasively measured from remotely positioned electrodes in water [Lissmann 1958; Bullock et al., 1972; Heiligenberg, 1985; Jun et al., 2012a; Jun et al., 2012b], and their one-dimensional signals in the temporal domain offer simpler interpretation and analysis compared to other behaviours involving many muscle groups and therefore large degrees of freedom. Owing to their electrical behaviours that do not require activation of muscles, the weakly electric fish (WEF) system has been successfully investigated to uncover the neural mechanism at each stage of processing from the sensory to motor areas by measuring neural activities and electrical behaviours from a restrained animal. Mice and rats have been popular models for studying spatial navigation and exploration because they can be trained to perform spatial tasks such as running through a maze. Yet the neural mechanisms of memory-guided spatial behaviours in mammals remain elusive due to their anatomically complex hippocampus, a brain structure responsible for the formation of spatial memory. Here, we make use of pulse-type WEF for studying memory-guided spatial exploration and sensory sampling because they possess anatomically simpler homologues of the hippocampus [Mueller et al., 2011; Harvey-Girard, 2012; Harvey-Girard, 2013], while still being trainable to perform spatial tasks such as food searching and retrieval.

1.1.3 Physical environment and behaviours

Animals' surroundings closely influence their behaviours because animals are physically coupled to their immediate surroundings (see Fig. 1.1). For example, signals generated by animals travel different distances depending on the type of surrounding media such as air, water or soil; and due to different attenuation characteristics, the physical media also affect the animals' sensing ranges. In electric fish, the detection range of objects is inversely proportional to the water conductivity, since the electrical energy dissipates over a longer distance in lower conductivity water [MacIver et al., 2001]. The laws of physics are highly relevant to understand certain quantitative aspects of these behaviours since they apply to all biological scales including the ecological, behavioural, brain system, and cellular levels.

The environment imposes physical limitations on the behavioural performance, and the accuracy of sensory representations in the brain, which in turn influences behavioural decisions. For example, the signal-to-noise ratio for the sensory stimuli and the thermal fluctuations in the sensory organs set an upper bound for the perceptual accuracy, and the behavioural performances closely follow the physically achievable performance bounds [Rieke et al., 1999]. Physical parameters such as the nerve diameter and the neuron's membrane resistance also influence the information processing capability of the brain by limiting the neural conduction speed. The evolutionary process achieved faster reaction times in the nervous system by tuning these and other parameters. For example, the diameters of squid giant axon became enlarged to lower the surface to volume ratio, and the axons of vertebrates became insulated with myelin to minimize the membrane leak currents and speed up conduction.

Field studies are necessary to measure the ranges of physical parameters found in an animal's natural habitat, and they can offer valuable insights for the ecological contexts of animal behaviours. Laboratory settings facilitate well-controlled experimental parameters and accurate measurements, but it is easy to overlook the ecological contexts of behaviours observed in artificial settings. Thus, in order to study animal behaviours that are relevant to their ecological niches, stimulus parameters and experimental conditions in the laboratory should be closely matched to the ranges found in the animal's natural environment. For example, South American WEF are naturally found in tropical freshwater. There the conductivity of water fluctuates over the annual cycle such as rainy or dry season, which affects the reproductive cycle of fish [Kirschbaum, 1979; Kirschbaum, 1987]. Thus, it is important to match the water conductivity in the lab to the values found in their natural habitats during reproductive seasons to study the electrocommunication behaviours between male and female WEF.

On a historical note, early ethologists such as Konrad Lorenz, Niko Tinbergen, and Karl von Frisch pioneered the study of animal behaviours in their natural environments between the 1930s and 1950s, when the mainstream biologists at that time primarily focused on behaviours occurring in the artificial settings far removed from animals' unique environmental conditions. Ethologists are concerned with naturalistic behaviours and stimuli

that are relevant to animals' ecological niches. The WEF system is particularly well-suited for the neuroethologists who study the neural mechanisms of naturalistic behaviours, since it is easy to electronically reproduce naturalistic stimuli and to control environmental parameters such as the resistivity and capacitance.

1.1.4 Spontaneous animal behaviours

Animals generate behaviours either spontaneously or in response to external stimuli (see Table 1.1). Between these two behavioural categories, neuroscientists primarily study stimulus-triggered behaviours because they can control when the behaviour occurs, and it is easier to establish constant baseline conditions. On the other hand, spontaneous behaviours such as self-guided spatial explorations can reveal the autonomous function of the nervous system. Self-initiated locomotion and foraging behaviours are the unifying phenomena across the animal kingdom, and their study permits general comparisons across species for the evolutionary understanding of spontaneous behaviours. However, it is more difficult to study spontaneous behaviours because they occur randomly and exhibit greater variability across trials. Experimenters can access the internal cognitive states of animals by passively observing their voluntary behavioural choices when presented with multiple possible outcomes.

Yet in order to accurately measure and objectively quantify spontaneous behaviours, experimenters must overcome several technical challenges. Due to their random nature, long-term observations are often necessary to capture infrequent spontaneous behaviours, and an automated detection of behavioural events from a large dataset may also be required. Next, to ensure the spontaneity of behaviours, the laboratory environment must be well-isolated from the external sources of physical stimuli that may elicit uncontrolled responses, and the background noise should be recorded for future validation. Unstrained animal movements generally involve large degrees of freedom in multiple joints, which are difficult to track reliably and accurately in the three-dimensional space. Further, measurement methods should be as non-invasive as possible and minimally interfere with spontaneous generation of behaviours, while achieving high accuracy and reliability. The pulse-type WEF is well-suited for studying spontaneous behaviours, because they possess a simpler body plan for easier motion tracking in shallow water; and it is possible to record their physiological signals non-invasively for extended periods. The high trial-to-trial variability of spontaneous behaviours

can itself be the subject of investigation to explain its evolutionary significance such as predator avoidance [Catania, 2009], and its relation to neural variability [Churchland et al., 2010; Churchland et al., 2011].

Table 1.1. Comparison between self-initiated and sensory-triggered actions.

	Self-initiated action	Sensory-triggered action
Source of action	Internal	External
Stochasticity	Random	Predictable
Adaptability	Flexible	Rigid
Timescale	Delayed	Immediate

1.1.5 Multidisciplinary approach in neuroscience

Neuroscience is one of the most interdisciplinary fields, and neuroscientists apply experimental and theoretical tools that originated in diverse disciplines. It takes many different perspectives to understand the brain and its behaviours, since these exhibit a vast array of dynamics and levels of organizations, from the molecular to the planetary scale. In particular, quantitative methods such as mathematical modelling and statistical analysis are widely applied in modern neuroscience research to process the large data sets. Recently in neuroscience, significant contributions have in fact been made by researchers with previous trainings in technical fields such as physics, engineering, and computer science. In relative terms, neuroscience is rich in data but still poor in theory. Physical models offer scalable means to synthesize unifying theories for different experimental studies. Physics is a useful theoretical platform to describe and predict interactions between multiple components across spatiotemporal scales. Thus, theoretical neuroscientists construct physically realistic models of neural circuits by defining how different types of neurons behave and interact with others, and validate their models by reproducing experimental data. Information theoretical quantities can further establish common grounds between the experimental observations and make theoretical predictions that lend themselves to experimental verification. For example, mutual information defines the information commonly present in the stimulus and response, thus providing a compact description of how a neural circuit encodes stimulus.

Likewise, experimentalists routinely apply techniques that require a working knowledge of computer programming, electronics and genetics. Proficiency in computer programming is highly needed in contemporary neuroscience because automated data analyses allow scalable means to process large datasets. For example, video-based behavioural tracking is widely used in neuroethological studies, and it is often not practical to manually process large quantities of image data. Machine vision algorithms can be implemented to automatically quantify massive image data for reliable, accurate and objective behavioural measurements. Although commercial solutions exist for popular behavioural experiments in standard animal models, such as Morris water maze tests with rodents, they often cannot meet specific demands of neuroethologists who study novel behaviours in exotic animals. Therefore, it is valuable to have hands-on technical skills to develop specialized hardware and software for establishing a new experimental paradigm involving novel organisms. This thesis develops such tools in the context of spatial memory in the WEF. .

1.1.6 Broader implications of neuroscientific discoveries to other fields

Fundamental discoveries in neuroscience have broad implications in other academic fields, clinical applications and societal values (see Fig. 1.3). The translational aspect of brain research is hugely relevant in today's changing economic and social conditions. Clinical applications of neuroscience such as neural prosthetics and psychiatric drugs have brought tangible improvements in everyday human conditions. Further, non-invasive technologies for monitoring the brain activities such as functional magnetic resonance imaging (fMRI), electroencephalography (EEG) and magnetoencephalography (MEG) are well-established diagnostic techniques. In addition, neural stimulation by means of deep brain stimulators and transcranial magnetic stimulation (TMS) demonstrate therapeutic potentials for the patients who have developed drug resistance. Next, robotics research offer promising solutions for the problems of today's aging societies face, and it is an active area of research to achieve flexible and autonomous behaviours in robots. Neuroethological studies on how various organisms generate adaptive behaviours inspired developments of various robots modelled after animals to mimic their behaviours such as walking, swimming and flying (see Fig. 1.4) [Sefati et al., 2013]. Long-standing philosophical notions such as the nature of free will and volition are even beginning to be challenged by recent studies in neuroscience. The discovery of preparatory brain activity before voluntary action (readiness potential) [Deecke et al., 1969;

Libet et al., 1983; Soon et al., 2008; Haggard, 2008] in humans and other animals has changed how philosophers view the voluntary cognitive process. The philosophical interpretation of human volition naturally extends to the legal interpretation, evident by recent court cases which involved the use of MRI data to prove the innocence of the accused by arguing the neurologically inevitable circumstances behind their actions. It is an active area of research in the neuroethics community to question whether such neurological data constitute valid evidences for making judicial decisions. Thus, understanding the fundamental mechanisms governing brain functions can impact both technology, and eventually, which in turn can impact social issues.

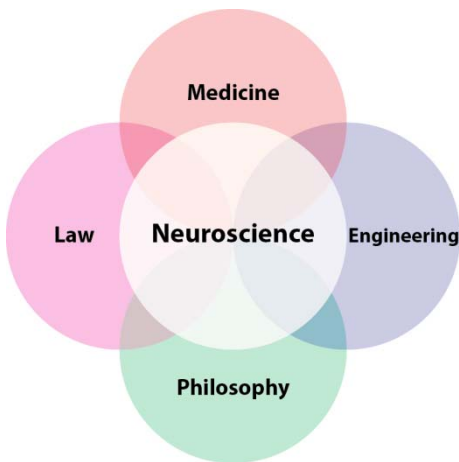


Figure 1.3. Translational aspects of neuroscience. Neuroscientific findings have transformed many important areas in today's society.

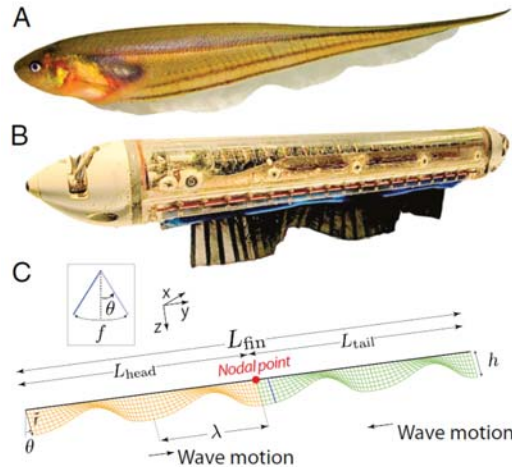


Figure 1.4. Design of an aquatic vehicle inspired by the ventral ribbon fin of certain weakly electric fish. Adapted from Sefati et al. [2013].

1.2 Sensory attention as a research problem

1.2.1 Sensory sampling

All animals acquire information about their surroundings, and how the sensory acquisition occurs in an efficient manner is one of the fundamental problems in neuroscience. Since the sensory acquisition costs energy and time, animals compromise between the cost of acquisition (motion etc...) and accuracy of sensory information by selectively attending to important sensory features. Animals prefer to orient towards novelties in their surroundings and ignore familiar features, and this involves tasks such as memory recall and decision making. Therefore, sensory sampling behaviours can reveal the integrative function of the brain at multiple levels beyond the primary sensory perception. Outwardly expressed sensing behaviours can reveal covert cognitive processes, and sensory acquisitions usually accompany physical movements of sensory organs such as saccadic eye movements, whisking in rodents, and movable pinnae (the external part of the ear) of rabbits and other animals. Certain types of sensory behaviours known as active sensing involve utilization of energy by animals for sensing purposes. For example, ultrasonic calls emitted by bats or electric fields generated by electric fish become modified by their surroundings, and animals analyse these perturbations

to detect obstacles for navigation and prey during hunting. These active sensing modalities can be readily quantified by their externally detectable movements (e.g. whisking, saccades) or signals (e.g. ultrasonic calls or electric pulses), which are absent in passive sensing modalities such as thermal or pain perceptions. In particular, the rate of energy use in active sensing modalities is linked to the bandwidth of the acquired sensory information [Salazar et al., 2013; Lewis et al., 2014], and is self-regulated [Caputi et al., 2003]. In addition, the target of an animals' selective attention can be measured by tracking the location and direction of its sensory fovea [Aytekin et al., 2004; Towal and Mitra, 2006; Engelmann et al., 2009], which contains the highest density of photoreceptors and is therefore well-suited for directed searches. In this thesis, we investigate voluntary active sensing behaviours in pulse-type electric fish, since we can infer the animal's autonomous attentional states from its electric organ discharge rate (EODR) and, from the location of its head which acts as a sensory fovea (because it has the highest density of electroreceptors), the target(s) of its active search.

1.2.2 Experience-guided sensory sampling

The environment offers a wealth of information accessible through the detection of various physical stimuli, and animals manage to efficiently extract relevant information that is important for survival, such as the locations of prey, predators and mates. How do animals learn to direct their attention to zero in on specific sensory features, and what kind of roles do their experiences play in guiding their targets of attention? Animals' sensory perceptions change due to extrinsic factors or to their own movements. Since each sensory sample is valid within limited temporal and spatial ranges, animals are required to frequently sample to maintain real-time internal representations of their surroundings. The frequency of sensory sampling needs to be at an optimal level, since suboptimal frequency may lead to a slower response time and excessively high frequency may saturate the limited information processing capacity of the brain.

For a given sensory feature, the rate of change of the feature and the desired accuracy of its detection and/or estimation are relevant parameters for determining the optimal sampling rate. For example, high sensory sampling rates are appropriate for tracking fast escaping prey, since the rate of change is high and prey capture requires a greater sensory accuracy. The primary targets of attention generally demand a high perceptual accuracy and thus an enhanced

sampling rate, and the target selection will presumably be based on an animal's past history of rewards or punishments associated with a target.

Animals can also adjust their sampling rates based on the rate of changes expected from previous experiences. When first exposed to a new environment, animals actively explore every feature within the environment; but after repeated exposures, they learn to ignore stable or slowly changing parts of the environment [Barchi et al., 2013]. Conversely, the detection of unexpected changes might trigger sensory sampling increases. Thus, the sensory sampling rates might express the internal states of surprise or familiarity based on the animal's recognition memory. Animals naturally orient toward sources of novel stimuli, known as the orienting responses [Sokolov, 1990], and the detection of novelty immediately triggers a shift of attention towards novel stimuli. Novel features in the sensory surroundings can be either explicit, such as the unpredictable onsets of loud stimuli, or implicit such as the displacement of an object requiring active comparisons between immediate perception and the memory of the object's prior location. *In this thesis, we investigate the implicit novelty detection abilities in the pulse-type WEF during self-guided spatial explorations. We hypothesize that the sensory sampling rates and the spatial target selections are efficiently determined based on the animal's expectations shaped by previous experiences.*

1.2.3 Animal models for active sensing

Active sensing occurs in various forms of sensory modalities in diverse species that are uniquely suited to the animal. In an open-field environment, the visual system is dominant in animals active during the daytime, and some combination of olfaction, audition (including echolocation) and somatosensation are dominant in nocturnal animals. In contrast, contact-based modalities, such as the whisker system in rodents, offer advantages in a dark and cluttered environment. To better locate the context of our research into active sensing, Table 1.2 introduces two other well-known examples of active sensing, and compares them with the active electrosensory system in terms of strengths and weaknesses for studying experience-guided sensory sampling. For example, the sensory sampling rates are simpler to quantify in the discrete ultrasonic calls in bats than the continuous rhythmic movements of whiskers in rodents.

Table 1.2. Comparison of active sensing modalities in four animals.

	Type of energy for sensing	Energy emitting organs	Medium of transmission	Energy sensing organs
Bats	Ultrasound	Mouth or nose	Air	Ears
Dolphins	Ultrasound	Mouth	Water	Ears
Rodents	Contact force	Facial muscles	Whisker	Hair follicles
Electric fish	Electricity	Electric organ	Water	Electroreceptors

Whisker system in rodents. Mice and rats primarily rely on their whisker system for navigating in darkness. The whisker system is an active sensing modality because animals themselves generate active movement of whiskers, and rely on the feedback of whisker movements for sensing [Hartmann, 2001; Towal, 2010; Grant et al., 2012]. Whiskers are rhythmically moved back and forth to detect nearby objects that cause physical deformation of whiskers, and forces are transmitted to the tactile nerve endings. The contact force is transmitted via stiff vibrissae (macrovibrissae) to a hair follicle that is innervated by 100-200 so-called primary sensory afferents nerve fibers. The physical arrangement of whiskers facilitates contacts with physical surfaces surrounding the animal. Macrovibrissae are arranged in rectangular grids consisting of 5 rows and 5-9 vibrissae per row, and the vibrissae lengths (up to 50 mm in rats and 30 mm in mice) become successively shorter toward the nose for efficient contacts with the surface in front of the animal. Macrovibrissae can be independently moved by muscles controlling the hair follicles, and the entire vibrissae rhythmically moves back and forth at 3-25 Hz. Thus, the movement and bending of whiskers allow direct measurements of tactile sensory information received by the animal. The grid-like arrangement of whiskers is topologically preserved and somatotopically (i.e. where spatial coordinates on the body are mapped onto coordinates in the brain) represented on the parts of the somatosensory cortex known as the barrel cortex.

Rodents exhibit highly adaptive whisking behaviours during self-guided spatial exploration. The whisking movement is adjusted when the animal is turning its head, such that the contact angle with the surface becomes optimal after the head turning [Towal and Mitra,

2006]. Whiskers from the left and right sides exhibit asymmetric movements when the animal is following a wall on one side. Animals modify the angle of whisker protrusion based on the anticipated surface location, and the head orientation shows similar experience-guided behaviours to achieve an optimal contact angle [Hartmann, 2001]. Thus, the whisker sensing system in rodents is a useful experimental model to study experience-guided sensory sampling. Other advantages include well-understood neuroanatomy, large availability of genetic tools, and trainable behaviours to solve experimental tasks. Automated whisker tracking systems for freely behaving rodents have been developed in several labs [Knudsen et al., 2005; Voigts et al., 2008; Perkon et al., 2011], but it is still technically challenging to track the three-dimensional movements of intact whiskers. Each vibrissa requires a large number of parameters to fully describe its shape and dynamics, and automated image tracking is further complicated by overlapping multiple vibrissae from different focal planes. Correct interpretation of the whisker-based sensory sampling activity may be complicated by the high degrees of freedom in whisking movements and difficulties in accurate measurements.

Echolocation in bats. Echolocating behaviours are observed in diverse species such as frogs, whales and bats, and echolocation requires self-generated acoustic energy production in the ultrasound range (15-150 KHz) for sensory acquisition. Most bat species generate ultrasonic calls to actively locate obstacles and prey in darkness [Lawrence and Simmons, 1982; Fuzessery et al., 1993; Surlykke and Moss, 2000; Aytekin et al., 2004; Jakobsen et al., 2013]. Echolocating bats measure the distance to the target by using the time delay between the call production and the arrival of echo reflected from the target. For example, the time delay becomes shorter as the bat approaches targets such as prey or landing sites. Due to the distance-dependent time delays to their echoes, bats vary the intervals between calls to avoid overlaps between the echoes. When a bat is far from a target, the inter-pulse intervals (IPI) are longer to allow the sound to travel a greater distance to the target and back; and as it closes in, the IPIs shortens for accurate target localization. In addition to modulating the call intervals (IPI), a bat also controls the ultrasonic beam width by changing the size of its mouth opening, which acts as the aperture of an ultrasonic emitter. The beam width is broader for locating distant targets during a search phase, and it becomes narrower as it flies towards the target during an approach phase. The ultrasonic beam spreads over distance due to the diffraction of the sound wave through an aperture having a diameter on the order of the wavelength. The angle of

spread is proportional to the wavelength and inversely proportional to the aperture diameter; thus a bat increases the aperture size by opening its mouth to narrow the beam directed at an approaching target. The size of the mouth opening (aperture diameter) is proportional to the size of the animal, but the beam width (angle of spread) at a fixed target distance is nearly constant in most bat species having different body sizes [Jakobsen et al., 2013]. The constancy of the beam width is achieved by variations of the call wavelength that is also proportional to the body size; *i.e.*, the decreasing effect of a larger aperture diameter on the beam width in a larger bat is equally cancelled by the increasing effect of a longer wavelength. The wavelength of ultrasound is proportional to the size of a target that can be detected, and shorter wavelength sound attenuates more readily in the atmosphere [Lawrence and Simmons, 1982]. Consistent with this, echolocating bats hunting small insects in densely vegetated areas typically use shorter wavelengths (higher frequency). The relationship between the wavelength of calls and the unique ecology of bat species is complicated, since some bats produce higher frequency sounds above the detection limit of ultrasound-sensing insects, while this severely limits their detection range in an open environment.

As in other mammals, bats are capable of forming spatial memories after repeated exposures to a stable environment, and their sensory sampling behaviours reflect the state of learning. Bats make decreasing number of echolocating calls as they are repeatedly exposed to a familiar environment, and the flight trajectories also converge to a stereotyped pattern over time [Barchi et al., 2013]. Neural recordings from freely behaving bats revealed three-dimensional place cells in their hippocampus [Yartsev et al., 2011], and comparisons with rodent hippocampal place cells show interesting parallels. Theta rhythm is a 3-8 Hz oscillation of extracellular field potential reflecting partial synchronization of localized synaptic activity [O'Keefe, 2007]. In rodents, the hippocampal theta rhythm occurs continuously, and was once thought to be a critical component for the formation of place cells. However, the hippocampal theta rhythm in bats occurs intermittently; and their theta powers are correlated with the frequency of sensory sampling or the echolocation call rate [Ulanovsky and Moss, 2007]. Together with the increasing theta power during running in rodents, these findings in bats suggest that the hippocampal theta rhythms are correlated with the sensory input rates. Thus, studies in echolocating bats reveal a critical link between the memory and the sensory sampling rate.

Although bats can offer important insights into the fundamental principles of active sensing and spatial memory, accurate measurements of their locomotive and sensory sampling strategies are technically challenging and resource-intensive in freely flying bats. The setup requires multiple high-speed infrared cameras installed at multiple viewing angles to reconstruct the three-dimensional flight paths, and also requires ultrasonic microphone arrays to capture the beam direction and angle. A flight room needs to be sufficiently large to allow unobstructed flights, and the walls need to be lined with sound-absorbing foam to suppress the echoes that they generate. In order to describe the complex movements and sensory sampling behaviours of freely behaving bats, large numbers of dynamic variables need to be tracked; these make the experimental studies both interesting and difficult to interpret at the same time. In summary, studies in the echolocating bats have revealed adaptive sensory sampling behaviours shaped by experiences; but the complexity of active sensing in bats and technical difficulties associated with tracking flight behaviours make the experimental preparation much too complicated to address a host of outstanding questions, such as how the sensory sampling rate is modulated by repeated exposures to a stable spatial layout and the neural mechanisms underlying the sampling adaptation.

1.3 Experience-guided sensory sampling in weakly electric fish

1.3.1 Weakly electric fish

Electric fish species generate electric current flow through the surrounding water by using electric organs composed of a cascade of electrocytes in series. Strongly electric fish discharge brief high voltage pulses (50-500 V) to stun their prey, whereas weakly electric fish (WEF) constantly generate low voltage discharges (<1 V) for sensing and communication purposes [Møller, 1995; Chacron, 2007]. Strongly electric fish species such as the Mediterranean electric torpedo rays (*Torpedo torpedo*) and African electric catfish (*Malapterurus electricus*) have been known since ancient times, and Roman historical records describe the numbing power of torpedo rays on humans [Zupanc and Bullock, 2005]. In comparison, the electrogeneration capabilities in WEF were more recently discovered (1950s) after amplifiers and oscilloscopes were invented to detect weak electric signals [Lissmann, 1958; Lissmann and Machin, 1958]. Most WEF species are found in freshwater since the signal detection range decreases as the water conductivity increases, thus the sea water will effectively short-circuit

the weak electric currents generated by the animal. WEF are nocturnal and many species have poor vision and live in turbid water. Thus active electroreception is optimally suited for such environments where the light is either absent or rapidly scattered. The electric currents generated by WEF can be approximated as a dipole current source:

$$V(\vec{r}) = \frac{1}{4\pi\sigma} \frac{\vec{r} \cdot \vec{p}}{|\vec{r}|^2} \quad (1.1)$$

where $\vec{r}$ is the field position vector; $\vec{p}$ the current dipole vector; σ is the conductivity of medium [Knudsen, 1975]. Thus the orientation of fish is an important parameter for determining the electric field geometry. Due to the rapid decay of the dipole field strength ($\sim d^{-3}$), the sensing range of WEF is approximately within one body length. The electrical properties of a nearby object interacts with the animal-generated electric field, and its presence modifies the electric potentials at different positions on the animal's body surface (see Fig. 1.6). The skin of WEF contains voltage sensors known as electroreceptors across the body, but they are particularly concentrated near the mouth and head region and sparser nearer the tail [Castello et al., 2000; Caputi et al., 2008; Push et al., 2008; Hollman et al., 2008]. WEF species from Africa (mormyriforms) and South America (gymnotiforms) arose and evolved independently after the continental separation (see Fig. 1.5), and the parallel evolution of the active electroreception from two continents resulted in different neural mechanisms to achieve similar sensory behaviours. For example, mormyriforms receive efferent copies of the self-generated pulse-type electric organ discharges or EODs in their sensory processing brain area (nucleus of the electrosensory lobe); such copies are called corollary discharges and inform the sensors of when pulses are being sent out to sample the environment. In contrast, gymnotiforms lack such corollary input to their sensory area (electrosensory lateral line lobe), and must thus process actively sensed objects in a continuous online mode. In this thesis, our experimental investigation is limited to the gymnotiform species due to their simpler brain structure and the fact that their EOD rates exhibit less variability.

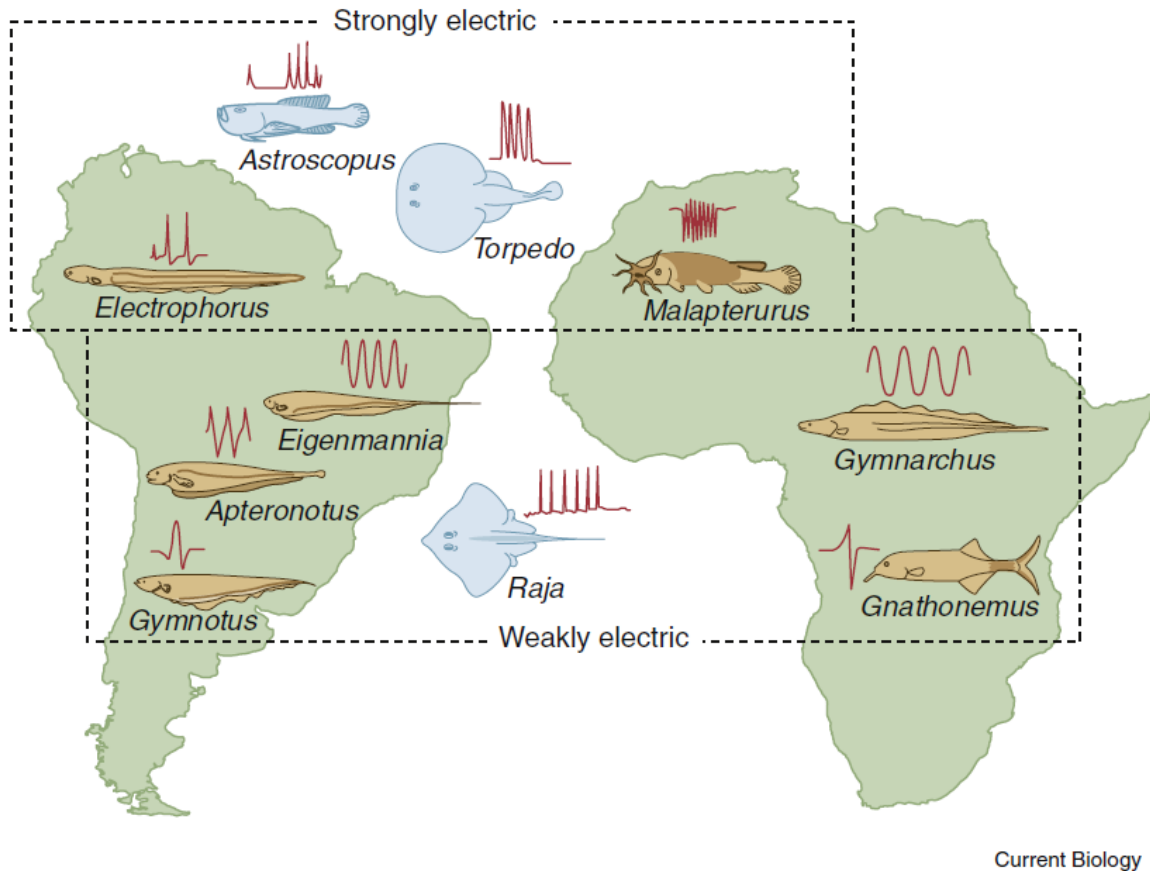


Figure 1.5. Evolution of electric fish. Figure is adapted from Nelson [2011], which was originally adapted from Moller [1995]. Blue colouring represents salt water species and brown colouring represents fresh water species. Typical EOD waveform of each species is shown above.

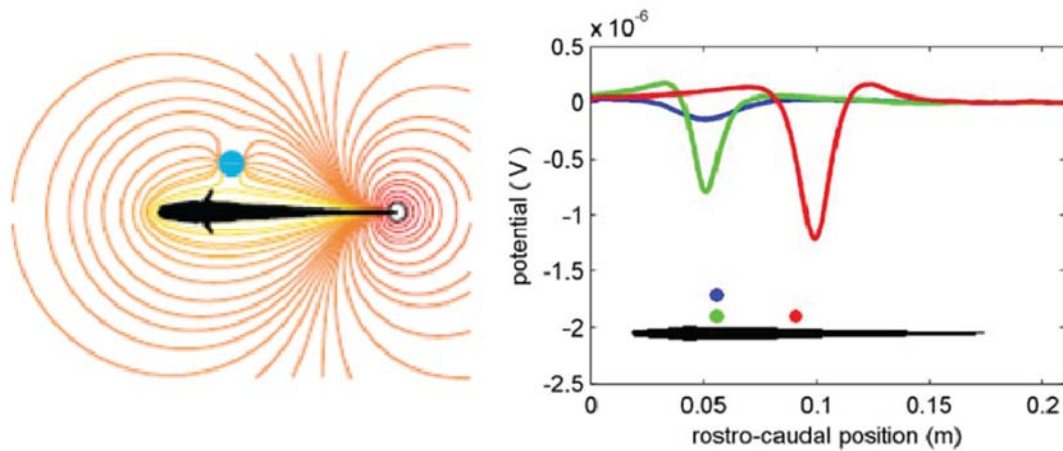


Figure 1.6. Electroréception of weakly electric fish. An object nearby fish distorts the electric field (left), and casts an electric image on fish's skin that appears as a perturbation from the usual transdermal potential in the absence of objects (other than water). Figures adapted from Krahe and Gabbani [2004] (left) and Babineau et al. [2007] (right).

Many animal species possess the ability to sense weak electric fields without the capability to self-generate the electric fields (passive electroreception). Passive electroreception is typically used for detecting low-frequency signals originated by the muscle activities in aquatic prey, and is found in sharks, rays, duck-billed platypus, and other species. In particular catfish are known to utilize passive electroreception for prey capture [Moller, 1995]. Passive electrolocation is made possible by the ampullary electroreceptors, a class of receptors tuned to the low-frequency range below ~ 50 Hz [Carlson, 2011]. The immediate common ancestor of gymnotiform and siluriform fish (catfish) developed ampullary receptors that were inherited by both orders [Baker et al., 2013]. WEF species possess two classes of electroreceptors, ampullary and tuberous types, and the tuberous electroreceptors are specifically tuned at the animal's own electric organ discharge (EOD) frequency range to detect self-generated signals carrying information about their surroundings [Zakon and Meyer, 1983; Meyer et al., 1986]. The high-frequency tuning (< 10 KHz) of tuberous receptors allows the detection of signals from other conspecifics for communication purposes. Tuberous receptors are further specialized into two sub-types of sensory neurons which either code for the EOD signal amplitude or the phase shifts, both of which can be modified by objects. The amplitude coding

neurons have spike firing rates that are proportional to the signal amplitudes, thus they ideally encode the resistivity of the surroundings. In contrast, the phase coding neurons fire once per EOD cycle at a fixed phase from the EOD waveform, and thus they convey the object's capacitive information (note that amplitude and phase are also altered by the presence of other WEF).

Electrogenesis. WEF can be classified as either wave- or pulse-type depending on their EOD waveforms. As their names suggest, wave-type species generate continuous quasi-sinusoidal EOD waveforms at higher rates (20-2200 Hz), and pulse-type species produce discrete pulsatile waveforms (~1 ms pulse width) at much lower rates (1-120 Hz) [Crampton and Albert, 2005]. Most mormyriforms belong to the pulse-type except in one extant species (*Gymnarchus*), whereas both types are prevalent in South America. Wave-type species exhibit nearly constant EOD rate with an animal, whereas pulse-type species exhibit far more variable EOD rates. In fact, the EOD pacemakers of the wave-type species are known as one of the most precise biological oscillators exhibiting sub-microsecond inter-spike interval (ISI) fluctuations [Moorgat et al., 2000].

Pulse-type species modulate their EOD rates under various behavioural contexts, and mormyriforms exhibit more variable inter-pulse intervals (IPIs) than gymnotiforms. When confronted with unexpected stimuli, most pulse-type species rapidly shorten their IPIs within one pulse cycle. These phenomena are known as the novelty responses, and will be discussed further in the next section. The electrocytes, cells responsible for generating the EODs, are embryonically developed from myocytes (myogenic, meaning that they are derived from muscle cells) in most WEF species except in the apteronotids, whose electrocytes developmentally originate from nerve cells (neurogenic). Thus, neurogenic species continue to generate EODs under the influence of curare, a commonly-used neuromuscular blocker injected during neural recordings to immobilize animals. In contrast, EOD production is shut down in myogenic species after curare treatment; but their spinal command signals can be measured and used to trigger artificial EODs that are generated by a pair of electrodes placed near the mouth and tail. Table 1.3 summarizes classifications of WEF according to different criteria. WEF actively avoid signal interferences between conspecifics during close physical interactions by modifying their EOD production [Heiligenberg, 1977; Heiligenberg, 1980].

In wave-type species, the EOD frequency is shifted away from each other's to prevent jamming (jamming avoidance response); and pulse-type species exhibit similar shift in their EOD pulse timing when the pulses from two individuals closely overlap in time. The basal EOD rates are influenced by the surrounding water temperature, and are individually specific and sexually dimorphic. For example, males have higher EOD frequencies in apteronotids, and the frequencies are correlated with the levels of testosterone [Maler and Ellis, 1987].

Table 1.3. Classification of weakly electric fish according to three categories.

By geography	Mormyriforms from Africa	Gymnotiforms from South America
By waveforms	Wave-type (e.g. Apterontidae, Gymnarchidae)	Pulse-type (e.g. Gymnotidae, Mormyridae)
Electric organ types	Neurogenic (e.g. Apterontidae)	Myogenic (e.g. Gymnotidae)

1.3.2 Novelty responses in pulse-type WEF

Animals naturally attend to outliers in their surroundings by orienting their heads or primary sense organs toward these novel sources of stimuli, and this phenomenon is commonly referred as the orienting response [Sokolov, 1990]. Pulse-type WEF exhibit an explicit form of the orienting response upon encountering unexpected changes such as a sudden appearance or disappearance of stimuli by rapidly and transiently increasing their EOD pulse rate (EODR) (see Fig. 1.7). *Such overt novelty responses to novel stimuli make the pulse-type WEF an ideal model for studying experience-guided sensory sampling behaviours.* The purpose of novelty responses is thought to be enhanced sensory sampling in an attempt to rapidly close the gap between the animal's internal representation and the current perception of the environment upon encountering mismatches between the two. Novelty responses can be elicited by various stimulus modalities such as electricity, light, sound, and vibration in the pulse-type WEF species from South America [Post and Emde, 2000; Schuster, 2000; Caputi et al., 2003] and

also from Africa [Engelmann et al., 2008; Pluta and Kawasaki, 2008]. Active electrical stimuli such as artificially generated electric fields [Post and Emde, 2000], or passive stimuli such as changes in the resistance or capacitance of an object [Caputi et al., 2003] are both effective at eliciting the novelty responses. The amplitude of the novelty response as measured by the change of IPI ratio follows the log ratio of the change in stimulus [Caputi et al., 2003]:

$$\frac{\Delta I}{I_0} = k \log\left(\frac{\Delta P}{\Delta P_0}\right) \quad (1.2)$$

where I_0 is the baseline IPI, ΔI is the maximum IPI change from the I_0 immediately after the stimulus, ΔP is the electric potential change on the fish's skin due to a step stimulus and ΔP_0 is the reference change in the electric potential. Strong active electrical stimuli can also interrupt the EOD production for up to a few minutes, because pulse-type WEF can enter a “stealth mode” upon detecting strong electrical discharges of their natural predators such as electric eels to hide from them [Schuster, 2000]. The EODR decreases back to the baseline level after the presentation of a prolonged stimulus, and the amplitude of the novelty response decreases over repeated stimuli presentations (this is caused by habituation, i.e. decay of novelty) [Grau, 1983; Post and Emde, 1999; Caputi et al., 2003]. The habituating responses to persisting or repeated stimuli may serve an important role in conserving the metabolic energy expenditure associated with active sensing [Salazar et al., 2013] by reducing the sensory sampling rate when the environment is stable or changes in a predictable manner. Conversely, when multiple novel stimuli of different sensory modalities are simultaneously presented, the novelty response amplitude exceeds the linear summation of amplitudes (supralinear enhancement) associated with each sensory modality in isolation [Pluta and Kawasaki, 2008]. Thus, the novelty responses exhibited by pulse-type WEF directly reveal the internal states of “sensory expectation” in intact animals, but the related studies were mostly conducted in restrained animals using sensory stimuli that were artificially delivered by experimenters [Heiligenberg 1977; Grau, 1984; Caputi et al., 2003; Post and Emde, 2000]. Unlike the previous paradigm of eliciting externally-driven novelty responses, this thesis investigates how the environmental and spatial novelties influence the sensory sampling behaviours that are self-driven by observing exploratory behaviours in freely-swimming pulse-type WEF.

1.3.3 Neural control of the EOD rate

In WEF, each EOD cycle is directly triggered by the spinal command signal that originates in the medullary EOD pacemaker, and the EOD pulses are precisely phase-locked to the action potentials of the pacemaker neurons. Thus, each EOD pulse essentially broadcasts the neural activity in the hindbrain (medulla) at the level of individual spikes. Relay cells in the medullary pacemaker have a large soma ($\sim 65\ \mu\text{m}$), and they send out axons to the spinal cord to activate the electric organs. Pacemaker neurons (PMn) are electrically coupled to each other and they synchronously drive the relay cells via electrical coupling [Kawasaki et al., 1988; Caputi et al., 2005]. In the gymnotiform pulse-type species, the EOD rate (EODR) changes relatively smoothly over time.

The instantaneous slopes of the EODR reveal the sum of inputs from multiple pre-pacemaker cells, which temporally modulate the pacemaker firing rate (see Fig. 1.8). The PMn and relay cells in the medullary pacemaker receive excitatory (glutamatergic) or inhibitory (GABAergic) inputs from the pre-pacemakers located in the diencephalon and medulla, and they control the EOD accelerations or interruptions [Wong, 1997; Comas and Borde, 2010]. The medullary pre-pacemaker contributes to the early rapid phase of the novelty responses, whereas the diencephalic pre-pacemaker contributes to the late phase by providing slowly decaying inputs to the pacemaker, according to pharmacological and lesioning studies [Comas and Borde, 2010]. The diencephalic pre-pacemaker receives inputs from the telencephalon, and controls electrocommunication behaviours in many WEF species.

The telencephalon of teleost (which includes all bony fish) is functionally and anatomically homologous to the mammalian forebrain, which plays a central role in advanced cognitive functions such as learning and memory. However, the role of the telencephalon in controlling the novelty responses is not clear according to the studies conducted in restrained animals utilizing stimuli delivered by experimenters. For instance, telencephalic lesions did not abolish the novelty responses triggered by sensory stimuli [Grau, 1984], nor affected the habituating responses to repeated stimuli [Corrêa and Hoffmann, 1998]. The telencephalon of a pulse-type WEF likely has extensive synaptic plasticity that underlies learning when it displays novelty responses during self-guided explorations.

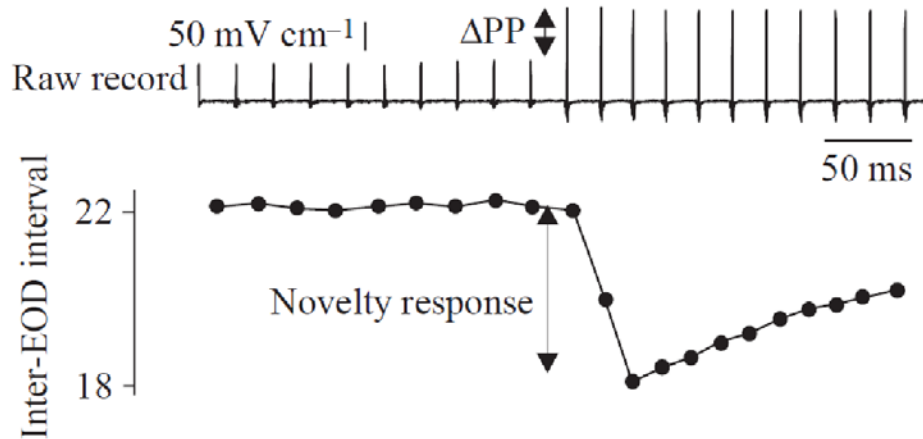


Figure 1.7. Novelty response in *Gymnotus* species. A novelty response is triggered by an abrupt change in the local conductivity. Note that the novelty response plot shows an opposite polarity when plotted as a function of instantaneous rate. Figure is adapted from Caputi et al. [2003].

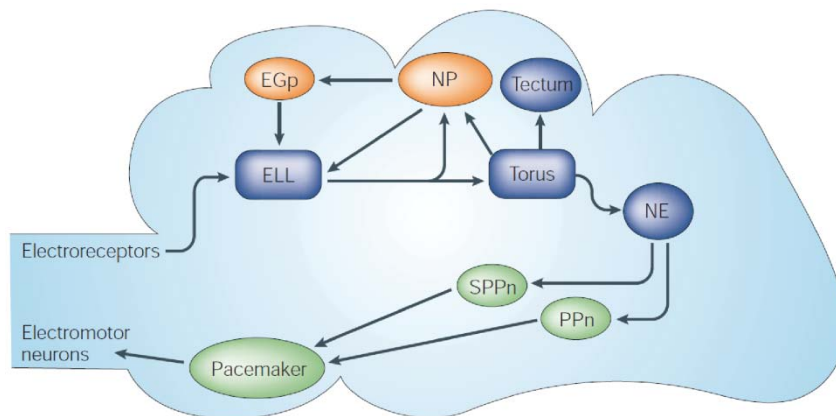


Figure 1.8. The neural pathway of governing the sensory-motor integration in weakly electric fish. The EOD pacemaker is controlled by prepacemakers (SPPn, PPn) that are located in the diencephalon. Figure is adapted from Rose [2004].

1.3.4 Spatial learning and memory

Animals demonstrate various forms of learning under different behavioural contexts, and multiple types of memory require specific neural structures. When animals experience repeatedly occurring stimuli that do not cause pain or pleasure (neutral stimuli), they learn to ignore the stimuli by decreasing the responses (habituation learning). Conversely, sensitization learning occurs when animals show increased responses to repeated stimuli that are noxious or painful. Habituation and sensitization are the simplest forms of learning that do not require association between multiple stimuli, and they are even observed in animals possessing a primitive nervous system such as round worms (*Caenorhabditis elegans*) [Rankin et al., 1990] and molluscs (aplysia) [Castellucci et al., 1970]. Associative learning refers to the formation of mental association between two or more stimuli, usually a neutral stimulus followed by another stimulus that is either rewarding or punishing. This type of learning was initially demonstrated in Pavlov's experiments, in which he measured how much dogs salivate in anticipation of food after hearing a bell sound that predicts an imminent food delivery.

Not all types of learning require exposures to repeated stimuli; for example, fear learning can develop after a single presentation of a stimulus when the animal experiences a second painful or otherwise aversive stimulus paired with this single stimulus. Humans can recall an event that they personally experienced at a specific time and place, and declarative memory refers to such memories that could be told as stories. Non-human animals also demonstrate a form of declarative memory known as the episodic memory, an ability to remember episodic events. Spatial learning requires the formation of episodic memories, because animals need to remember specific sequences of locations that they visited to efficiently search and navigate their environments.

Spatial behaviours are highly suitable for studying unifying neural mechanisms of memory-guided behaviours, since animals generally perform spatial tasks in one form or another, and the physical location of an animal is a clearly measurable quantity that lends itself to theoretical predictions. Animals rely on multiple strategies to navigate and orient themselves in space. For example, directional information could be obtained from global cues such as skylight polarization, magnetic fields, and constellations of stars, whereas the distance to a target location could be determined from local cues or spatial landmarks. It is also possible to

orient in space without any external cue by keeping track of self-generated movements, a process called path integration or dead reckoning [Mittelstaedt and Mittelstaedt, 1982; McNaughton et al., 1996; Whishaw and Gorny, 1999]. Path integration could be understood as a process of vector summation from a starting position to determine a current location, but error accumulates as an animal travels further from the start. Thus, animals must combine multiple strategies to accurately locate themselves based on the available spatial information. Below we will hypothesize that WEF possess an advanced form of spatial memory due to the severely limited spatial range of their electrical sense, and that they heavily rely on an internal cognitive map of their environment.

1.3.5 Neural basis of spatial learning

The formation of episodic and spatial memories requires a forebrain structure known as the hippocampus. One of the earliest evidences on the hippocampal function was discovered from a patient named H.M. who underwent a bilateral removal of the hippocampus to cure his epilepsy. The surgery impaired his ability to form new declarative memories (anterograde amnesia) while sparing his old memories and motor learning skills (non-declarative memories) [Scoville, 1954; Scoville and Milner, 1957; Penfield and Milner, 1958; Milner, 1962]. Evidences from comparative studies also support the role of the hippocampus in memory since the hippocampal volume (which reflects the number of cells dedicated to the task) relative to the rest of the brain is correlated with the geographic distances regularly visited and remembered by each species [Sherry, 1989; Healy and Krebs, 1993]. For example, food-caching species such as squirrels and jays have significantly greater hippocampal volume ratios compared to the species that are closely related but not engaging in such extensive spatial behaviours; and certain prairie vole species exhibit sexual differences in hippocampal volumes, in which males travel wider ranges than the females [Jacobs et al., 1990]. Similar findings were reported by the MRI studies in humans that taxi drivers have greater hippocampal volumes than control groups who engage less often in complex spatial tasks such as driving in a large metropolis [Maguire et al., 2000]. Hippocampal lesions in animals result in impaired spatial performances such as food searching and maze learning in rodents, birds, and teleost fish [Rodríguez et al., 2002]. Spatial learning based on sensory cues have been demonstrated in teleost fish (bony fish), which includes goldfish and WEF [Toerring and Moller, 1984; Cain et al., 1994; Cain, 1995; Walton and Moller 2010]. Y-maze experiments

have shown that WEF can learn to use the electric field patterns for selecting the correct compartment containing food [Graff et al., 2004]; and they can also use landmarks in an open environment to visit food locations or escape mazes [Walton and Moller, 2010].

Cellular-network basis of memory. The hippocampal pyramidal cells exhibit one of the highest levels of synaptic plasticity in the brain, and the synaptic strengths between two cells undergo long-term changes after paired electrical stimuli [Bliss and Lomo, 1973; Bliss and Collingridge, 1993] mimicking two associative features. Some of hippocampal pyramidal cells in the CA1 and CA3 regions exhibit highly place-specific firing rates (place cells), where the spikes occur at a specific phase of the theta rhythm. Due to the high spatial information content in the place cell firing patterns, the location of an animal can be accurately decoded from the place cell activities [Wilson and McNaughton, 1993]. Place cells form within several minutes after an exposure to a novel environment [O'Keefe, 2007]; and they activate during self-generated explorative movements, but not if an animal was passively carried through space. Hippocampal place cells were initially discovered in rodents [O'Keefe and Dostrovsky, 1971; Ranck, 1973], and were also found in bats and even in goldfish [Canfield, 2004]. The dorsal telencephali of teleost fish are closely related (homologous) to the mammalian hippocampus in terms of the function, anatomy and the evolutionary history [Mueller 2011; Harvey-Girard, 2012]; and ablation of this region similarly impairs the spatial learning abilities in goldfish [Portavella, 2002].

Formation of long-term memories generally requires new protein syntheses that are regulated by the RNA (ribonucleic acid) transcription factors known as the immediate early gene families. The level of RNA expression in the brain can be quantified in a site-specific manner by the use of the in-situ hybridization technique, which creates visible precipitates of a specific RNA in a brain slice after applying a complementary-matching RNA template. By using this technique, several regions in the dorsal telencephali of WEF were identified that selectively express an immediate early gene (EGR-1) when initially presented with novel social stimuli, but the level of EGR-1 expression significantly decreased after animals became fully habituated to the repeated stimuli (see Fig. 1.9) [Harvey-Girard et al., 2010].

All vertebrates including mammals, reptiles, birds and fish share a common basic brain plan [Johnson and Haan, 2011], and the teleost fish have the smallest and simplest neural

architecture of all vertebrates [Mueller, 2011; Butler, 2011]. Thus, WEF offer an anatomically accessible experimental platform for studying the neural basis of memory, and may yield a theoretically tractable model of memory formation (see Fig. 1.10).

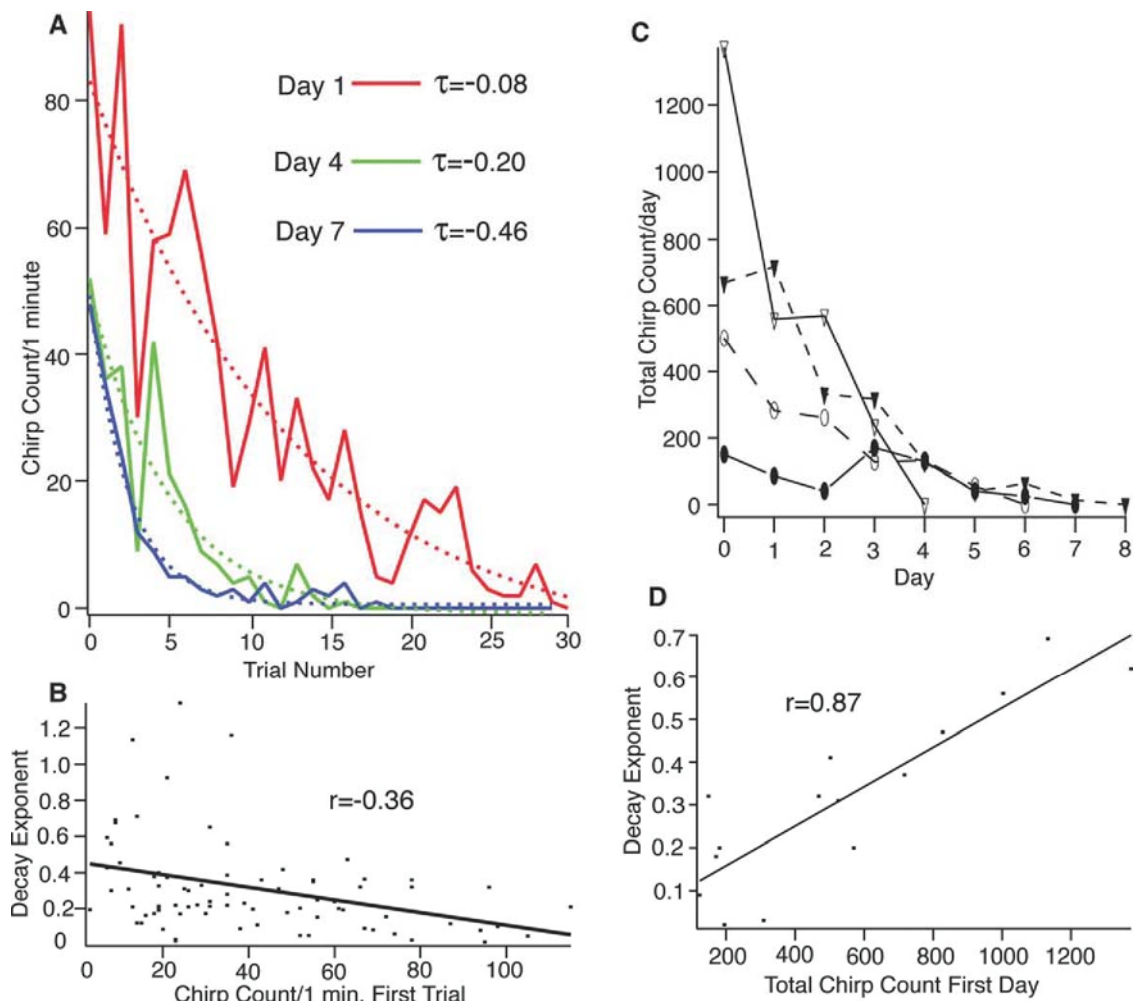


Figure 1.9. Habituation curve in chirp production. Weakly electric fish learned to ignore a conspecific signal (i.e. a communication signal from another fish of the same species) that was repeatedly presented over multiple days. Figure is adapted from Harvey-Girard et al. [2010].

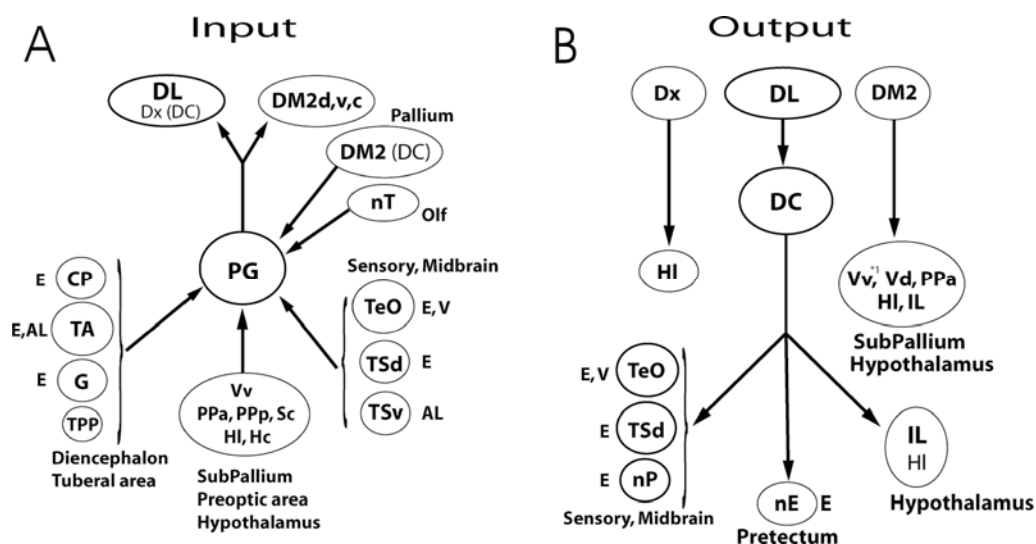


Figure 1.10. Neural basis of learning in teleosts. The forebrain of teleost (including weakly electric fish) follows similar ascending (**A**) and descending (**B**) projection patterns as in mammals that are implicated in novelty detection and learning. Figure is adapted from Giassi et al. [2012b].

1.4 Experimental techniques and data analysis methods

Experimenters often face a unique set of technical challenges when studying previously unexplored phenomena in novel settings. In particular, new technical developments are required to accurately quantify unrestrained animal behaviours. Our experimental aim is to monitor the sensory sampling behaviour during spatial exploration in pulse-type WEF. To this end, we had to measure the location of an animal and its sensory target, and the rate of sensory sampling in a well-controlled environment. A description of the experimental setup can be found in the papers that constitute Chapters 2 and 3, but more details are given here. The spatial information such as the location of fish and its head position were obtained from infrared video recordings and subsequent automated analysis. We used a large tank (1.8 m square) relative to the size of fish (20-30 cm) to provide sufficient area to explore, but confined its vertical movements to a shallow depth (10 ± 2 cm) to facilitate video tracking in two dimensions. The rate of sensory sampling was directly quantified from the EOD pulse timing in darkness, which was measured from multiple, spatially-distributed electrodes attached on the wall. The EOD

signals were synchronized to the video recordings to determine the locations associated with increased sensory sampling. A sensory isolation chamber was built around the tank to block external sources of stimuli such as light, sound and vibration, which could influence the animal's sensory sampling behaviour. Likewise, the water temperature was maintained at a constant level to prevent temperature-induced EOD rate fluctuations. The tank was partitioned into a large central circular arena for the spatial learning tasks and four “living” compartments, one at each corner. The animals did not actually live there between experiments; rather they became acclimatized to the water in those compartments prior to the task. Each compartment was watertight and electrically isolated to prevent EOD rate modulation due to electrocommunication. An animal's entry to the central arena from its “living” compartment was controlled by remotely operated gates in a closed chamber for minimal human intervention. The experimental chamber was under a reverse 12-hour light-dark cycle, such that the nocturnal animal thought that it was night-time during the actual daytime when experiments are conducted. The tank water was aerated and filtered between daily experiments.

1.4.1 EOD recording and signal processing

Physical setup. The EOD rates were determined from the EOD pulses detected by combining signals from multiple electrodes. The major technical challenge was to non-invasively obtain high signal-to-noise ratio (SNR) recordings for all possible animal postures and locations within the tank. The signal amplitudes from a single pair of electrodes vanished whenever the fish's orientation became perpendicular to the electrodes pair, since a WEF is approximately equal to a dipole current source. In order to reliably capture the EOD signals at all possible positions and orientations, we installed eight equally-spaced electrodes on the circular tank wall and paired them in 180° orientations. The initial recording setup was contaminated by 60 Hz mains noise due to the extended physical separation between the electrodes (1.5 m) and the long wires (up to 5 m) carrying unamplified raw signals.

We gradually improved the noise contamination problem by identifying noise sources such as the ventilation fan and ungrounded DC power lines, and all recording wires were replaced by the coaxial cables having sufficient shielding thickness (RG54). All shielding conductors and the Faraday cages were connected to the common ground, and we also

grounded the tank water by placing a graphite electrode at one corner of the tank out of reach from fish to prevent signal saturation. Further, noise present at both electrodes was mostly cancelled by differentially amplifying each pair of electrodes. To prevent animals from detecting the recording electrodes at a distance and using them as spatial landmarks, thin (2 mm in diameter) graphite electrodes were used which have a shorter detection range than the metal or thicker electrodes. We applied heat shrink tubing to firmly join the graphite electrodes to the coaxial cables, and the silicone-coated heat shrink tubing protected the graphite-copper interface from corrosion in a high-humidity environment over extended periods.

Signal processing. We initially developed a real-time electronic EOD detector and recorded only the pulse timestamps to save storage space, but later switched to the raw waveform recordings for more flexible and reliable analysis. The raw EOD signals were filtered (200 Hz high-pass filter) to remove the first and third harmonics of the mains noise, and the amplification gain (30 to 300x gain) was adjusted depending on the size of fish, which was proportional to the EOD strength. The Spike2 software (CED, UK) was used to digitize the data (20 KHz, 16 bits/sample) and to display the instantaneous EOD rate in real-time. A raw EOD pulse waveform contains multiple peaks, and the signal polarities often flipped when the animal orientation changed relative to the orientation of electrode pairs. To sum up multiple channels without cancellation, all channels were AC coupled and rectified before being summed. Afterwards, the summed pulse waveforms were converted to unimodal shapes to assign unique time markers at each peak.

The unimodal filter based on a root-mean-squared operation offered higher temporal precision than a simple threshold crossing method, due to high variations of the relative amplitudes between multiple peaks in the raw waveforms during movements. The EOD rate was then determined by smoothing the instantaneous EOD rate calculated from two successive EOD pulses, and the EOD rate was sampled at constant time intervals (100 Hz) to facilitate data analysis. In addition to the EOD rates, the EOD recordings also provided information about the movement activities since the EOD amplitudes changed when fish moved relative to the electrodes. The activity level was calculated based on the slope of the peak EOD amplitude for each pulse, and this measurement offered a practical way to monitor movements over an extended period of time (10-12 hours per day) without having to record high-speed video for

a long period. In summary, we successfully developed a precise, reliable, non-invasive, and practical EOD measurement technique for quantifying the sensory sampling rates and movement activities from freely swimming WEF over an extended period.

1.4.2 Video recording

Physical setup. Visual recordings is highly effective for studying animal behaviours, since they permit experimenters to review, interpret, and categorize behaviours on a case by case basis. Although humans can intuitively interpret visual scenes, it is not trivial to implement automated algorithm to analyse visual data for tracking animal movements. Commercial software exists for tracking animal movements during standardized behavioural experiments in rodents, but none were appropriate or flexible enough for our experimental settings. We developed custom video tracking software based on the Matlab image processing toolbox, which supported most commonly used image processing operations.

The accuracy and reliability of automated video tracking critically depends on the image qualities such as high contrast backgrounds and homogeneous lighting condition. To enhance the image contrast, non-reflective white paper was placed underneath the glass tank to make darker fish stand out better, and thin grid patterns were printed on it to indicate spatial coordinates for calibration and object placements. The presence of water caused optical complications such as the reflections and glares projected onto the water surface. To avoid reflections casted from above, the top portion the experimental chamber was covered with matt white countertop film, and a white plastic panel was added to hide the cameras and the ventilation hole. Glares were eliminated by directing the eight infrared light sources toward the ceiling, and the matt white surfaces on the top were effective at diffusing the light and creating indirect and uniform illumination.

Video recordings were made by a high-end consumer grade webcam (Logitech C910), and its sensor was sensitive in the near-infrared range (840-880 nm) after removing the infrared cut-off filter. In addition, the built-in lens provided a sufficiently large viewing angle to capture the entire arena, and no noticeable barrel distortions were found on the calibration grids. Video recordings were made using vendor provided software (Logitech Quickcam Software), and were saved in a file format supported by Matlab (wmv) with compression and without quality

loss. The camera offered sufficiently high resolutions (1600 x 1200 pixels) for tracking an animal size much smaller than the entire tracking area, and the frame rate (15 frames/s) was sufficient for smooth motion tracking. The video timing was synchronized to the digitizer time unit by capturing a periodically blinking infrared LED controlled by the digitizer.

Video tracking algorithm. We developed a video tracking algorithm to automatically extract spatial information such as animal position and body posture from raw video recordings. Our spatial analysis was mainly based on the head trajectories since the electroreceptor density is the highest near the mouth; in fact all fish swam by nearly touching the floor with their mouths while searching for food attached to the bottom of the tank. The video tracking algorithm extracted the midline of fish and stored five feature points: the tip of head, midpoint of head, midpoint of body, midpoint of tail, and tip of tail. A head position was manually assigned in the first frame to be distinguished from the tail, and was automatically updated in subsequent frames. Video processing is generally computationally demanding due to the large number of pixels to process.

Thus to speed up the processing, we only analysed a smaller image area around the previously detected animal location, or at a user-defined region in the first frame. We also restricted the range of pixel intensity values to be processed by ignoring intensity fluctuations occurring outside of the range of animals. This intensity filtering operation was effective at rejecting surface ripples and reflections which generally had higher intensity values than the animal images. Five feature points along the midline of fish was extracted by first obtaining a binary image by applying an intensity threshold initially set by a user in the first frame. It is often difficult to determine static parameter values that reliably work well for video tracking due to variations of lighting condition over space and time. Thus we adaptively updated the intensity threshold value for robust tracking by preserving the total area of an animal image. For example, the intensity threshold was increased by one step if the image area increased near the brighter central area, and the threshold was similarly decreased in darker areas near the circular wall.

To reduce intensity fluctuations over time, an automated gain control by the camera was disabled to prevent occasional jitters in the overall image intensity. The binary animal image was then rotated and aligned to its major axis, and was bisected to the head and tail

parts. The head and tail parts were then each rotated and aligned to their major axes, and bounding boxes were fitted to determine the end points. The midline points were found at the intensity median of the perpendicular edges of the bounding boxes. In summary, our video tracking algorithm tracked the head position where electrosensory acuity is the highest, and the tail bending angle to infer the electric field generated by electric organs near the tail.

1.4.3 Spatial analysis of sensory sampling during spatial learning

The spatial learning experiments were initially analysed by pooling the spatial trajectories and EOD rate time-series from each trial to compare the learning performances over the multiple training sessions. To quantify the learning performances per trial, we computed the total distance travelled from the start to the food location, or the total time taken to find food. It was more objective to quantify the learning performance using the total distance travelled per trial, since it does not depend on the swimming speed that varied across days and between individuals. The learning performance improved during the initial part of the training and eventually reached a stable plateau in all animals trained from stable environments, thus we divided the trials into either the early or late learning phases. The learning performances during the early learning phases were compared with the median sensory sampling rate to test whether animals modulate their sensory sampling rates according to their familiarity of the environment. In addition to analysing the learning performances on a per trial basis, we also performed spatially fine-grained analysis to study the evolution of sensory sampling near the food location.

Since the EOD rates exhibited different means and variances across multiple days and between individuals, the EOD rates were converted to rank-based measures to facilitate pooling data. For each trial, the EOD rates were transformed to a scale ranging from -0.5 to 0.5 by computing the their quantiles (ranging from 0 to 1), and were subtracted by 0.5 to quantify the shift of quantile from the median EOD rate per trial. In essence, the EOD rates were self-normalized by their median values and expressed in rank scores. This normalized measure allowed us to test whether the location-dependent sensory sampling modulation exhibited experience-dependent changes. We used four animals at a time to obtain a large number of trials in a timely fashion, and all spatial landmarks were laid out in a rotationally

symmetric manner such that the spatial trajectories from each animal could be pooled by rotating in multiples of 90 degrees.

In addition to quantifying the location-dependent sensory sampling rates, we also quantified how often animals revisit the same location in the tank. The tank was divided into 5 cm grids and the number of visits per grid (visit counts) was counted to measure the animals' spatial sampling behaviours. The visit counts gave a more objective measure of the spatial sampling frequency than the time spent per location measure, since the visit count is independent of the swimming speed. The EOD rates associated with each point along the trajectories were determined after synchronizing the two time-series data. The EOD and trajectory time-series were initially aligned using the first and last synchronization pulses, and each time-series was interpolated at common time intervals and oversampled at 100 Hz. After all animals reached stable learning performances, we randomly removed food in one out of four trials per day (probe trials) to test how unexpected spatial perturbations influence an animal's sensory sampling rates. The visit counts near the food location were compared between the early and late phases and also during probe trials, as well as the normalized EOD rates (quantile shifts) as a function of distance from food. In summary, these measures offered statistically correct methods of pooling data to test whether the location-dependent sensory sampling rates were indeed shaped by past experiences.

1.5 Thesis overview, objectives, and state of originality

This thesis follows a paper-based format, and the contents of papers were directly copied and reformatted for this thesis. Chapters 2, 3 and 4 are three published articles, Chapter 5 is a submitted article (soon to be resubmitted following positive reviews), and Chapter 6 contains unpublished works that were originally presented as a poster [Jun et al., 2012b] and that are in preparation for submission. I am the first author and the principal contributor in all papers appearing in this thesis. In particular, I constructed all the experimental setups and designed the majority of the experiments. I conducted all experiments, data analyses, and wrote the first draft of each paper.

Chapter 2 describes the development of the technique for measuring the EOD pulse timing from freely swimming fish in a non-invasive manner. Movements generated by freely behaving animals contribute to recording artefacts in physiological signals. We implemented a method of spatially summing EOD signals from multiple remotely placed electrodes, and temporally reshaped the EOD pulse waveforms to uniquely determine the EOD pulse timing. The accuracy and reliability of our pulse detection method was tested at many possible locations and orientations within the tank, and also in a freely swimming condition. The precision of the remotely determined pulse timing was high ($\sim 10 \mu\text{s}$) after comparing with the reference timing derived from electrodes locally attached (temporarily) to the fish, and was reliable during free swimming.

In Chapter 3, we introduce a method of tracking multiple WEF using the electrical signals they generate. Animal tracking based on electrical measurements can offer certain advantages over visual tracking methods that often suffer from poor reliability and accuracy due to non-ideal imaging and lighting conditions. WEF can be approximated as an ideal current dipole source when the EOD pulse is at specific phases, since the current source is spatially localized near the head in the initial pulse phase and near the tail in the final phase [Rodríguez-Cattáneo et al., 2008]. We used an ideal dipole approximation to solve the inverse problem of locating a dipole by finding theoretically an ideally matched pair for a given set of multi-electrode measurements from a table of predicted values at many known dipole positions and orientations. The dipole search algorithm was computationally optimized by applying a simplified model of a dipole in 2D that was analytically derived from shallow water boundary

conditions of the tank we used. Our dipole tracking algorithm could rapidly track multiple animals (~ 1000 locations/s), and closely agreed with the concurrent visual tracking.

Chapter 4 presents three main techniques to perform long-term behavioural tracking of freely swimming WEF, which was originally published in a video journal format (Journal of Visualized Experiments) to visually guide other experimentalists to replicate our setup. The chapter describes in more detail the construction of the behavioural observation chamber, EOD signal recording setup, and visual tracking method. The behavioural observation chamber contains an aquarium tank lying on the top of the anti-vibration surface, and was surrounded by sound- and light-proof walls. Second, the chapter describes how to setup electrodes to measure the EOD rates and the movement activities from the raw voltage recordings. Third, the chapter describes how to setup a camera and lighting and subsequent automated visual tracking.

Chapter 5 describes a temporal relationship between the sensory sampling rates and self-initiated movements based on the long-term behavioural observations of spontaneously behaving WEF during active sensing. This paper has gone through a first round of reviews, with a positive outcome; it is in the process of being resubmitted with minor changes. The EOD rates exhibited a clearly bimodal distribution where each modes were associated with resting or active states. Due to the high correlation between the movement activities and the EOD rates, we defined these novel Up-states as periods of active movements and high EOD rates, and the Down-states as periods of resting and lower EOD rates. We found that increases in the EOD rates almost always preceded self-initiated movement onset. Further, the trial-to-trial variability in the EOD rates also increased before self-initiated movement onset, which is a characteristic of neural activity during decision making in higher mammals. The spontaneous state transitions showed no temporal correlations and lacked a characteristic time-scale.

In Chapter 6, we examine how spatial memory guides the sensory sampling in WEF. Animals were trained to retrieve food located at a fixed location using spatial landmarks placed in the aquarium tank. All animals demonstrated learning over repeated training session and reached a stable plateau. The median EOD rate successively increased during learning and showed a significant correlation with the learning performance (distance travelled to find food). The EOD rate shifted to a higher quantile as fish approached food, but the level of the

shift was significantly lower in the late learning phase than the early phase. Interestingly, the EOD rate shifted even higher near the usual food location when food was taken out of the location where it was normally placed. This demonstrates that the sensory sampling rates adapt over repeated exposures to a stable environment but dis-habituate when unexpected novelty occurs (such as removal). Further, we observed significantly enhanced expression of the transcription factor (RNA) associated with long-term memory formation (EGR-1) in the forebrain of WEF after they were exposed to a novel environment.

Chapter 7 is the final chapter of this thesis; I summarise the main findings of this thesis and discuss conceptual connections between chapters. I elaborate on interesting future directions for extending our studies on the memory-guided sensory sampling to further elaborate the general conceptual framework of memory encoding and novelty detection.

Chapter 2

Precision measurement of electric organ discharge timing from freely moving weakly electric fish

This chapter was published as Jun JJ, Longtin A and Maler L [2013]
in the *Journal of Neurophysiology* (**107**, 1996-2007).

2.1 Summary

Physiological measurements from an unrestrained, untethered and freely moving animal permit analyses of neural states correlated to ethologically important naturalistic behaviours. Precise and reliable remote measurements remain technically challenging due to self-motion, which perturbs the relative geometries between the animal and sensors. Pulse-type electric fish generate a train of discrete and stereotyped electric organ discharges (EOD) in order to actively sense their surroundings, and rapid modulation of the discharge rate occurs while free-swimming. The modulation of EOD rates is a useful indicator of the fish's central state. However, the EOD pulse waveforms remotely observed at a pair of dipole electrodes continuously vary as fish swims relative to the electrodes, which biases the judgment of the actual pulse timing. In order to measure the EOD pulse timing more accurately, reliably, and non-invasively from a free-swimming fish, we propose a novel method based on the principles of waveform reshaping and spatial averaging. Our method is implemented using envelope extraction and multi-channel summation, which is more precise and reliable in comparison to other widely used threshold- or peak-based methods, according to the tests performed under various source-detector geometries. Using on the same method, we constructed a stand-alone electronic pulse detector performing an additional online pulse discrimination routine to further enhance the detection reliability. Our stand-alone pulse detector performed with high temporal precision (5 μ s) and reliability (99.9967%), and permits longer recording duration by storing only event timestamps (4 bytes/pulse).

Key words: electric fish, movement, pulse detection, envelope extraction, temporal precision

2.2 Introduction

Study of freely moving animals is becoming an increasingly popular topic in neuroscience despite of technical challenges associated with simultaneous neural and behavioural recordings. Use of restrained or immobilized animals has been generally preferred to study neural correlates of behaviours to minimize movement-induced recording artifacts. Although this approach might be suitable for addressing classical problems of the sensory and motor coding, some of the modern problems in cognitive and behavioural neuroscience can only be addressed by studying freely behaving animal subjects. Naturalistic behaviours such as exploration or foraging can be best observed from non-restrained, freely behaving animals. However a measurement system must correct for the artifacts caused by animals' own movements in order to achieve the precision and reliability. Addressing this challenge, we designed a precise and reliable pulse timing measurement system to observe an active sensory sampling behaviour of a free-swimming pulse-type electric fish.

Weakly electric fish can electrolocate obstacles and prey under conditions of poor visibility (*i.e.* in a murky river or in darkness) by generating an electric field around their body and perceiving electric images of the surroundings [Assad et al., 1999; Chen et al., 2005; Caputi et al., 2005; Babineau et al., 2007]. Weakly electric fish species can be categorized to two types according to their electric waveforms: wave species discharge continuous pseudo-sinusoidal waves, and pulse species discharge trains of discrete pulses [Moller, 1995]. The rate of electric organ discharge (EOD) in pulse fish such as *Gymnotus* sp. is influenced by its self-generated movements and the novelty of the fish's surroundings [Black-Cleworth, 1970]. The EOD rate is increased in situations demanding higher sensory sampling rate [Caputi et al., 2003]. Changes in inter-pulse interval sequences (Δ IPI) occur over a temporal scale on the order of hundreds of microseconds for a free-swimming *Gymnotus* sp. (Figs. 2.1 and 2.2). Thus a sensitive pulse measurement method with a resolution in the order of tens of microseconds is required to reveal fine temporal structures in IPI sequences.

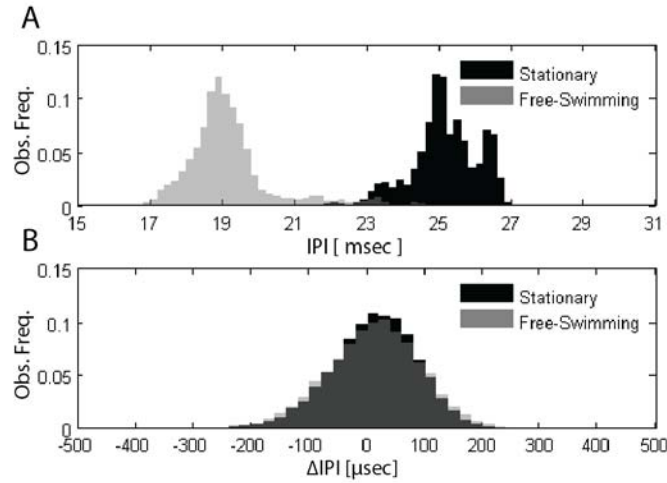


Figure 2.1. Distribution of the interpulse interval (IPI) and IPI change (ΔIPI) under a free-swimming condition. (A) IPI distribution has a long tail and a non-Gaussian shape. (B) cycle-to-cycle ΔIPI occurs on a physiological time scale of tens of microseconds, thus requiring a temporal resolution of 10 μs to capture fine temporal structures. *Obs. Freq.*, observed frequency.

EOD pulses are generated by a population of electrocytes arranged along the body, each discharging with their own stereotyped waveforms and delays from the moment when a spinal electromotor neuron discharge command is generated [Caputi et al., 2005; Rodríguez-Cattáneo et al., 2008]. Therefore, the electric potential observed at a recording electrode is a superposition of spatially distributed electrocyte action potentials, each contributing current whose magnitude is inversely proportional to its distance to the electrode. The electric field generated by a fish is approximated by a current dipole source, since the head and tail ends generate opposite current flows by either sourcing or sinking currents. Thus the observed pulse amplitude depends approximately on the distance from the recording electrodes and the orientation of fish according to the formula [Chen et al., 2005].

$$\Phi(r, \theta) = \frac{Id \cos \theta}{4\pi\sigma r^2} \quad (2.1)$$

where I is the current generated by the dipole; d is the length of the dipole; θ is the angle formed between the lines connecting the current-source end of the dipole, the centre of the dipole, and the detector location; σ is the water conductivity; and r is the distance between the dipole centre to the detector location.

The geometric relation between a fish and recording electrodes defines a source-detector geometry. Fish's motion introduces variations of the source-detector geometry, which in turn changes the observed pulse waveforms and amplitudes. Typical swimming movements such as turning and forward-and-backward scanning maneuvers [Lannoo and Lannoo, 1993] result in large changes in pulse amplitude at each electrode, and distort multiple lobes within a single pulse waveform (roughly tri-phasic in the *Gymnotus* genus [Rodríguez-Cattáneo et al., 2008]), which leads to a poor temporal precision in the pulse detection (Fig. 2.1).

The temporal precision required to capture the physiological scale of IPI variation during free-swimming is in the order of tens of microseconds (Fig. 2.2); but many commonly used basic pulse detection methods fail to meet such requirements due to movement-induced artifacts. A pulse detection method based on a fixed threshold is ill-suited for fast changing amplitudes and waveforms, since the chance of missing a pulse is still high for any choice of thresholds. Even if a portion of pulse was above the threshold, any arbitrary part of a pulse could cross the detection threshold in a haphazard manner, causing artifactual jitter in the IPI sequence. Pulse detection using feature points such as the peak or a zero-crossing point will become unreliable when the relative amplitudes between the constituent lobes vary, which typically occurs when fish come close to a recording electrode. Such movement artifact could be reduced by locally attaching recording electrodes on fish to maintain a constant source-detector geometry; however the tethered wires interfere with the free-swimming motion [Bell, 1974; Graff, 1987].

In this paper, we propose a novel EOD pulse detection method based on the principle of spatiotemporal averaging, which operates by summing the waveforms received and rectified from multiple recording electrodes, and extracting the envelope of the summed pulse. Our spatiotemporal averaging-based pulse detection is highly precise ($\leq 5 \mu\text{s}$) and reliable

($\geq 99.9967\%^1$), according to the tests performs using virtual signal conditioning stages and the electric measurements made at many geometric relations. Furthermore, we implemented a stand-alone electronic pulse detector, featuring an online, noise-tolerant event discriminator, built using a mixed-signal microcontroller. The real-time operation permits a long-duration recording under a high temporal precision, enabled by storing timestamps (4 bytes/pulse) instead of continuously digitizing the whole waveforms.

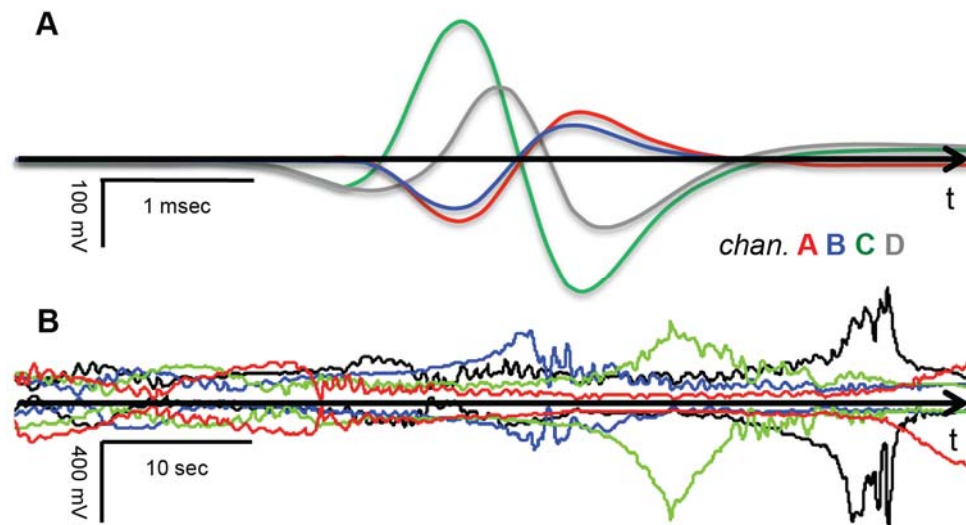


Figure 2.2. Variation of pulse shapes under a free-swimming condition. (A) 4 differently oriented recording dipoles receive different pulse waveforms and amplitudes. (B) Variations of the pulse amplitudes over time observed at 4 differently oriented recording dipoles. The positive and negative curves correspond to the peak positive and negative amplitudes of electric organ discharge (EOD) pulses. At least 1 out of 4 channels (chan. A–D) received strong EOD amplitudes. *t*, Time.

¹ No missing pulses were detected out of 1191371 pulses captured from freely swimming fish for approximately six hours.

2.3 Methods

2.3.1 Overview

The field measurements of fish were made in a custom-built circular aquarium (1.5 m in diameter, filled to 15cm in depth) constructed to study foraging, spatial learning and changes in sensory sampling during the learning that occurs in free-swimming *Gymnotus* sp. (23 cm in length, unknown gender and species). The native tropical environment of central South American fresh water [Lovejoy et al., 2010] was simulated by conditioning the aquarium water with matching conductivity (100 $\mu\text{S}/\text{cm}$, pH 7.0), using a stock salt solution [Knudsen, 1975]. The water temperature is maintained at 25°C by an electrically shielded floor heater (Thermotile; Thermosoft, Buffalo Grove, IL). The whole setup is surrounded by a lightproof Faraday enclosure to block external sources of light and RF noise. All measurements were taken under dimly lit conditions. All experiments described below were approved by the University of Ottawa Animal Care Committee.

Signals were recorded from eight equally-spaced graphite rods (Mars Carbon 2-mm type HB; Staedtler, Germany), glued to the circular perimeter wall using silicone, and electrically coupled to BNC connectors (RG174) using heat shrink tubing. The signals from four opposite-facing electrodes were differentially amplified in order to suppress the common-mode noise (Fig. 2.3). The Faraday cage surrounding the whole aquarium is used as a common ground. No ground electrode was placed in the aquarium for the duration of the recordings because it could distort the field symmetry. However the aquarium water and the Faraday cage made electrical contact shortly before starting the measurement recordings to neutralize the charge build-up caused by evaporation. Raw signals from the four recording dipoles were AC-coupled and amplified without filtering (30 $\times$ gain, Intronix 2015F; Bolton, ON, Canada), then synchronously digitized using a 16-bit ADC sampled at 200 kS/s per channel (CED1401 mkII; Cambridge Electronic Design Ltd., Cambridge, UK). The recordings were processed using Spike2 software (Cambridge Electronic Design Ltd., Cambridge, UK) and MATLAB (The Mathworks, Natick, MA) in order to simulate different signal processing stages and to compare their performances. The waveform was sampled at every 5 μs and the final pulse time-stamps were estimated to 1 μs precision using cubic spline method to interpolate the waveform.

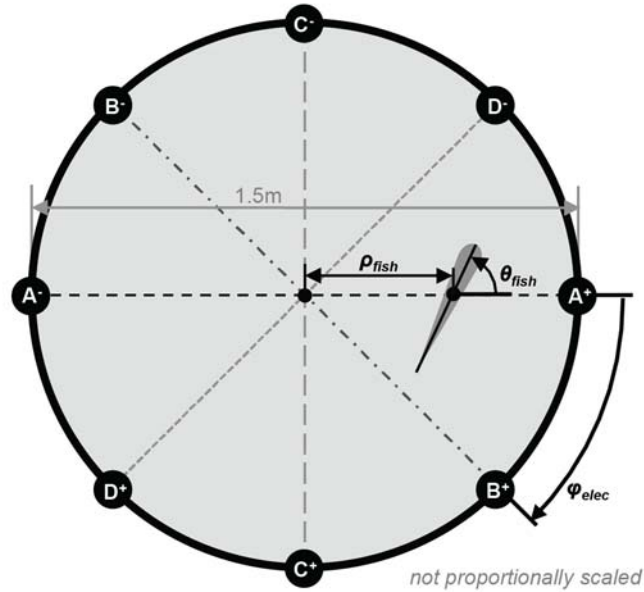


Figure 2.3. Illustration of the source-detector geometry variation. The geometric relation between fish and recording dipoles determines the pulse waveforms and amplitudes, and the source-detector geometry is parameterized by three variables: ρ_{fish} , the distance between the centre of the aquarium to the fish centre of mass, θ_{fish} , the orientation of the fish, ϕ_{elec} , the orientation of the dipole electrodes. Eight recording electrodes are equally spaced along the circular boundary of the aquarium, and opposite facing electrodes are paired off to form four recording dipoles.

In order to reproduce the most commonly occurring variations in the source-detector geometry, we designed a holding frame, inspired by Knudsen [Knudsen, 1975], to fixate the location and orientation of fish. Fish were immobilized in the holding frame by a tightly stretched net, built from a fine nylon mesh pocket surrounded by V-shaped coarse Mylar grids, and submerged at the middle of the tank depth. Floating foam blocks supported the holding frame above the water to minimize the underwater field distortions (Fig. 2.4). In order to simulate a wide range of locations and orientations, the holding frame, guided by the wire attached to the circular fence, travelled from one end to the other end of the recording dipole A, and rotated with respect to the guiding wire. Two recording electrodes were locally installed in the holding frame and maintained at 2.5 cm from the head and tail of the fish respectively,

in order to provide a geometry-invariant reference for the pulses observed at the external recording dipoles A-D attached on the fence. The signal from the local dipole electrodes was amplified ($100\times$ gain, Intronix 2015F) and concurrently recorded along with the signals received from the four external recording dipoles.

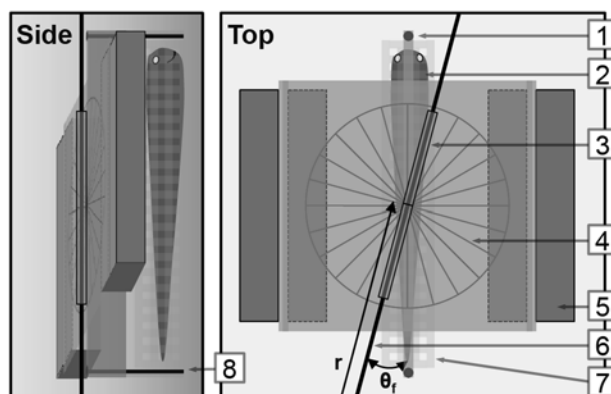


Figure 2.4. The fish holding frame. This holding frame is used to systematically explore different source-detector geometry by translating and rotating the frame along the guiding wire installed across the circular boundary of the aquarium. The frame translated and stopped at the equally spaced markers on the guiding wire; rotated and aligned to the orientation guides marked on the frame itself. (1) head recording electrode; (2) restrained fish; (3) guiding tube; (4) orientation guide; (5) buoyant foam; (6) travel guiding wire, (7) restraining net; (8) tail recording electrode.

2.3.2 Method of spatial averaging by rectified summing

At least one out of the four recording dipoles received strong EOD pulses at any location and orientation of fish; thus summing signals from multiple channels provides a good signal reception at any source-detector geometry, given that the pulses are rectified (Fig. 2.5-2) before summing [Kramer, 1974]. The rectification of signals prevents the cancelling effects from adding signals of opposite polarity; and the polarity reversal occurs when the angle between the fish and the recording dipole approach 180° . Although the pulse detection reliability is

enhanced by the spatial averaging effect of the multi-channel rectified summing, this processing alone is not sufficient to achieve a high temporal precision. The rectified pulse waveform still contains multiple lobes; even though fish produces a stereotyped pulse waveform, these waveforms vary relative to one another under free-swimming conditions. The two most distant peaks within a single pulse from *Gymnotus* sp. are typically separated by 500 μs ; thus a pulse timing detected at the maximum point will contain a timing error up to 500 μs , which is much greater than the natural time scale of ΔIPI ($\sigma_{\Delta\text{IPI}} = 67 \mu\text{s}$, Fig. 2.2B).

2.3.3 Method of temporal averaging by envelope extraction

The envelope extraction transforms a multi-lobed pulse waveform to a unimodal shape by extracting the envelope outline using temporal averaging so that the pulse timing can be unambiguously resolved at the envelope peak (Fig. 2.5-3). Temporal processing is entirely performed in the analogue domain in order to generate precise output in real-time, and to lower the cost of implementation compared to the digital domain processing. Envelope of a pulse waveform is extracted using combination of full-wave rectifier (Fig. 2.5-2) and low-pass filter (Fig. 2.5-3), which is equivalent to performing the Hilbert transformation in the digital domain [Rangayyan, 2002]. Envelope peak is detected in the analogue domain by taking an advantage of a slope-peak relation. First, envelope waveform is differentiated using a band-limited differentiator to prevent amplifying high frequency noise [Clayton and Winder, 2003]. Second, a zero-crossing point which immediately follows a falling threshold crossing is detected using a comparator with hysteresis. Comparator output triggered by background noise, while the signal amplitude is under the detection threshold, is ignored.

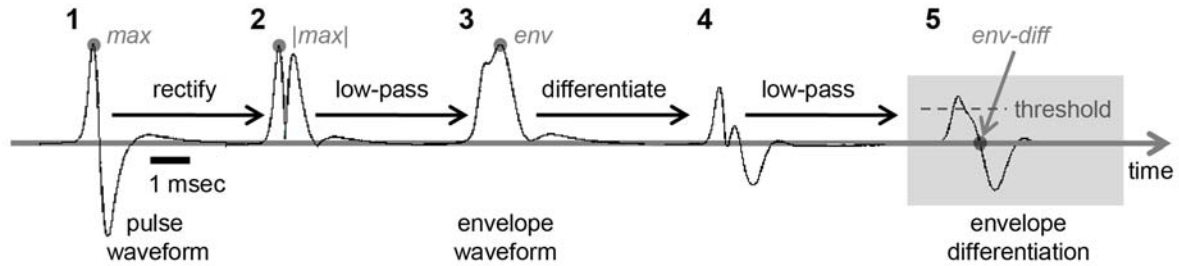


Figure 2.5. Signal conditioning steps. The original pulse waveform is conditioned by the following five steps to improve the detection timing precision. (1) Pulse waveform after filtering out the noise, and *max* marks the maximum amplitude. (2) After the full-wave rectification. $|max|$ marks the absolute maximum amplitude. (3) Pulse envelope is extracted using a low-pass filter, and *env* marks the maximum amplitude of the envelope waveform (4) Differentiated envelope waveform. (5) Reshaped envelope waveform using a low-pass filter, and *env-diff* marks the first zero-crossing point after a falling threshold crossing.

2.3.4 Online pulse discrimination algorithm

Real-time operation must generate highly reliable output since errors cannot be corrected after recording; thus the pulse detector output must be validated in real-time. An event discriminator can selectively discriminate true pulse events from spurious noise spikes. The detection reliability can be enhanced by minimizing the number of false positives, and by maximizing the detection sensitivity. An online event discriminator determines whether to approve or block an incoming pulse in real-time, based on a set of discrimination criteria. The discrimination criteria also need to be updated in real-time, particularly for an input signal with a large dynamic range. Noise spikes are discriminated from true pulses based on clues such as the pulse amplitudes and IPIs. For instance, an output pulse is suppressed if incoming pulse amplitude lies outside of a typical range of true pulses, or when a pulse is observed much sooner than expected from a mean IPI. The discrimination criteria are updated by tracking the running averages of pulse amplitudes and IPIs, which continually change due to free motion of fish.

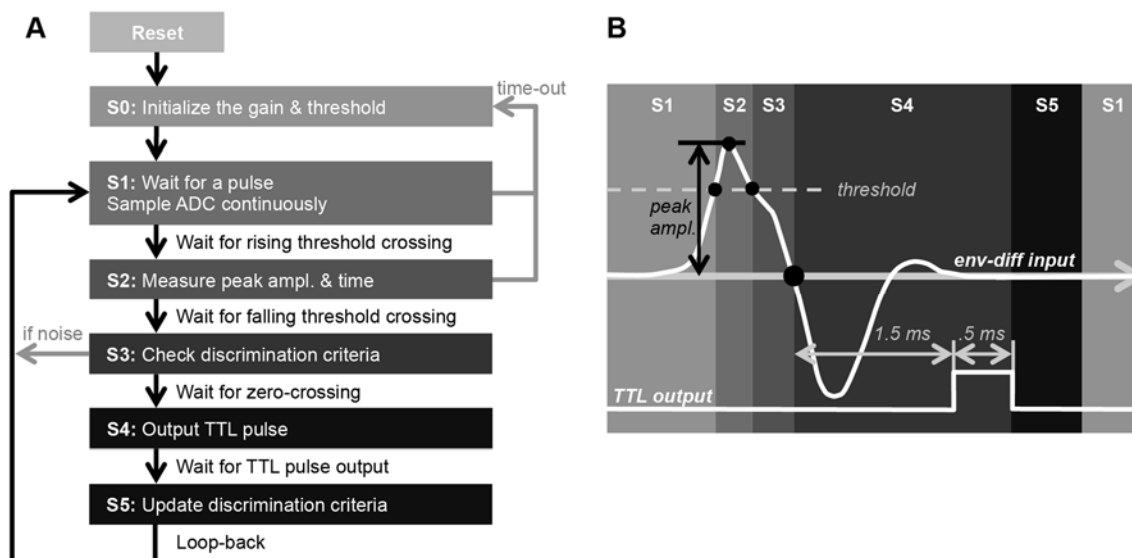


Figure 2.6. The event discrimination algorithm. The algorithm detects valid pulse events and updates the discrimination criteria in real-time. **(A)** The flowchart shows the pulse detection and validation steps; the black arrows represent valid transitions, and the red arrows represent the exception handling. **(B)** A typical pulse detection operation sequence in time, superimposed with a conditioned pulse waveform

The event discriminator receives two inputs: the differentiated envelope waveform sent from the signal filtering stages, and the zero-crossing comparator output. It outputs a TTL pulse with 0.5 ms duration once a valid pulse is detected (Fig. 2.6B). Figure 2.6A illustrates the online pulse discrimination algorithm implemented in the PSoC firmware. The wait-timeout prevents an infinite waiting condition caused by incorrectly setting the threshold too high, or setting the gain too low. If a timeout occurs, the PSoC resets and recalculates the threshold and gain values.

The gain value of the on-chip amplifier (PGA, programmable gain amplifier) is automatically adjusted to maintain the peak amplitude within the range of ADC, thus preventing the signal from saturating the ADC when a fish comes too close to the recording electrodes. We used a signed 8-bit DelSig ADC providing a balance between the sampling resolution (255 levels) and speed (64 μ s/sample). The waveform peak is targeted at around the

half (= 64) of the ADC limit by halving the gain if the upper limit (= 94) was exceeded, and doubling the gain if the peak fell below the lower limit (= 31). The gain is allowed to vary between 1 to 32 in powers of two to prevent over-amplifying the background noise or saturating the digitizer.

The pulse discriminator outputs a TTL pulse precisely 1.5 ms after a rising edge transition of the zero-crossing comparator output. During this 1.5 ms delay, the firmware validates incoming pulse input and suppresses TTL pulse output if a noise spike is detected. In order to generate the time delay with a precision better than a microsecond, the rising edge electronically triggers an on-chip PWM module to generate TTL output, instead of using CPU which is prone to time-jitter.

2.3.5 Electronic Implementation of a real-time pulse detector

This section describes the construction of a stand-alone pulse detector circuit, which performs the signal filtering and the pulse detection and discrimination tasks in real-time. Raw signals from the four recording dipole channels are individually amplified and filtered in parallel, in a manner similar to Crampton *et al.* [2007] (Fig. 2.7A). First, the raw signals from the recording electrodes are capacitively coupled to instrumentation amplifiers (INA128PA, TI) with an initial gain of 100. Second, the amplified signals are high-pass filtered using 2nd order Butterworth filters ($f_c = 650$ Hz) to improve the temporal precision by maximally attenuating the 60 Hz line noise and its higher harmonics. Third, the filtered signals are absolute-value transformed using a precision full-wave rectifier circuit [Clayton and Winder, 2003], and the intrinsic limitations of a diode (finite forward voltage drop and a non-linear I-V relation) are corrected with an opamps-diode combination to prevent signal distortion and clipping.

All analogue stages are constructed using low-noise quad-opamps (TLV2264AIN; Texas Instrument, Dallas, TX) to achieve high precision. Afterwards the rectified signals from the four recording dipole channels are summed using a voltage-adder circuit (Fig. 2.7B) [Clayton and Winder, 2003]. The summed signal is then low-pass filtered ($f_c = 1$ kHz, 2nd order Butterworth) to extract a unimodal envelope, and differentiated by a band-limited differentiator, then low-pass filtered again ($f_c = 1$ kHz) to suppress the high frequency noise amplified by the differentiator.

The envelope-differentiated signal is routed to the PSoC event discriminator, and the external comparator for zero-crossing detection (Fig. 2.7C). An external precision comparator (LM393; National Semiconductor, Austin, TX) offers higher temporal precision ($< 1 \mu\text{s}$ transition jitter) than the on-chip comparators; and the use of a Schmidt trigger further improves the precision by suppressing noise-induced multiple zero-crossing. If enabled by a jumper setting, the PSoC sends text messages to report the discrimination criteria, pulse amplitudes, and IPI via RS232 for the purpose of monitoring and debugging (see Supplemental Material for the serial communication protocol). PSoC 5 chip is only available in surface mount package which requires special soldering equipment. Hence we used an inexpensive, readily-built development board by Cypress (CY8CKIT-014) which offers two rows of pins with 0.1 in spacing for external input and output interface. We added two rows of mounting sockets on our circuit board to exactly fit the PSoC board. We custom-built our circuit board using a toner-transfer method which allowed us to inexpensively fabricate a printed circuit board (PCB) with commonly available tools such as a laser printer and a laminator (see Supplemental Material for PCB layout, bill of materials, and the PSoC firmware). Total cost of implementation is under \$200 USD, and most of the IC could be obtained as free samples for academic institutions from the manufacturers.

The analogue circuits excluding the PSoC board is powered by a miniature lead acid battery instead of the AC supply line to reduce the power noise. A virtual ground IC eliminates a need for two batteries to provide positive and negative supply voltages by splitting a single battery voltage to exactly half. A rail-splitter virtual ground IC (TLE2426IP, Texas Instrument) splits 12V voltage input from a lead-acid battery and produces a signal ground, 6V above the negative terminal of the battery, and dual supply voltages which are $\pm 6\text{V}$ with respect to the signal ground. (Fig. 2.7D). All electronic components except the PSoC board consume less than 10 mA at 12V input voltage. The PSoC board consumed approximately 100 mA at 12V input (5V) and is separately powered by 5V AC adapter to conserve battery without injecting power noise to the signal. The PSoC board is equipped with an internal 3.3V voltage regulator which accepts a voltage input between 5V to 12V. The use of a decoupled power source for the PSoC eliminated occasional contamination of analogue signal by digital signal from the PSoC board.

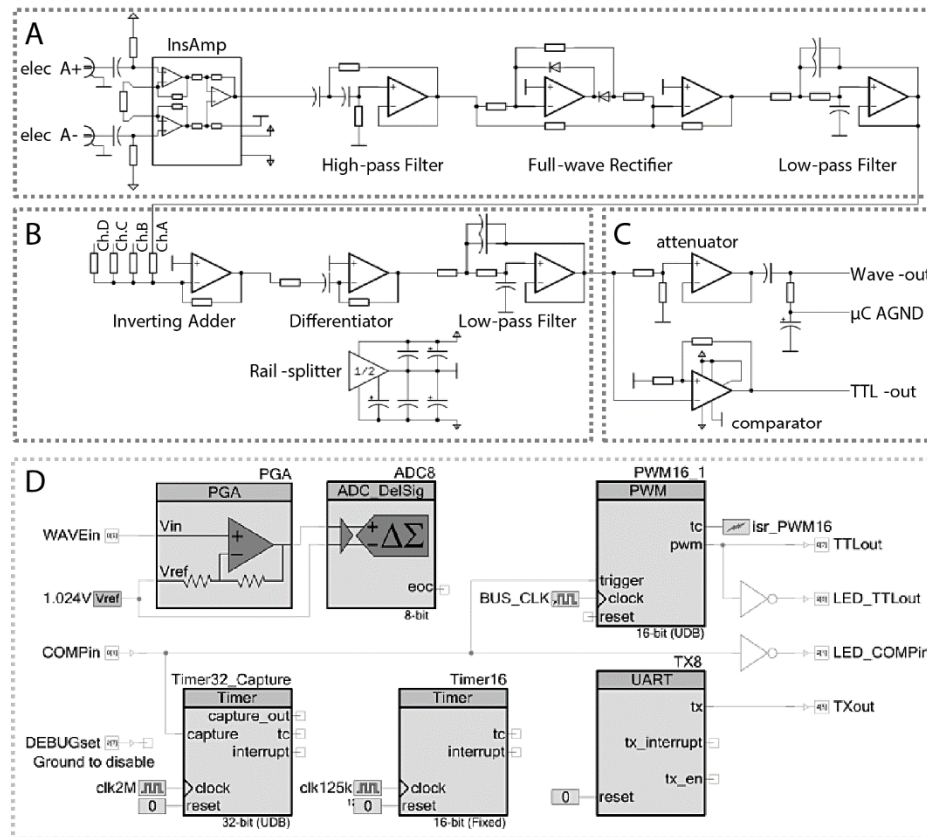


Figure 2.7. Schematics for the electronic pulse detector. (A) The parallel amplification, filter, and rectification stages for each input channel. (B) The signal processing stages in series (adder, envelope extractor, and waveform differentiator). The rail-splitter creates a signal ground at 6V by equally dividing 12V input voltage. (C) The zero-crossing comparator, and the signal translation interface for the PSoC input. (D) The on-chip routing configuration for the PSoC firmware.

2.3.6 Method of testing from free-swimming fish

The real-time pulse detector was tested with fish under restrained and free-swimming conditions. Test with restrained fish allowed us to precisely control the location and orientation of fish to systematically explore wide variety of source-detector geometry. It was also easier to place locally fixed reference electrodes with restrained fish, and the local electrodes remained stable for long duration. In addition to the test with restrained fish, we performed set of tests under a realistic free-swimming condition to ensure correct operation of real-time detector. Pulse waveform during free-swimming varies more dynamically due to body bending, change of depth and pitch angle, touching the recording electrodes, and even jumping in rare cases. These factors could compromise reliability and precision of the pulse detector; hence we designed two tests to independently assess the precision and reliability of our pulse detector.

The precision test was performed to measure the temporal precision by taking difference between time stamps obtained from the external and the local reference electrodes. The local reference electrodes were tethered on fish's body to provide a location- and movement-independent reference for pulse timing. A pair of local electrodes were constructed from short (1 cm) segments of graphite electrode (2mm in diameter) tethered to a pair of twisted (3-4 mm per turn) magnet wire (38 awg). The graphite electrode was wrapped with exposed magnet wire, and then heat shrink was applied to couple them tightly. The graphite electrodes were held to small buttons with four holes (9mm in diameter) by looping a short segment of wire insulation through two holes, and the other two holes were used to suture the button on fish's skin. Fish were anesthetized with bath-applied MS222, and two graphite electrodes were sutured and glued with dental tissue adhesive (PeriAcryl Tissue Adhesive, Citagenix Inc, Laval, QC, Canada) on the fish's back. One electrode was sutured above the gills and another electrode was attached 5 cm caudally. Throughout the suturing procedure fish was artificially respired with oxygenated solution containing light concentration of MS222; and after suturing the fish was awoken by passing oxygenated distilled water through its gills. The signal from the local electrodes was differentially amplified by a custom-built head stage (INA128, 20× gain, TI) located at the centre of the ceiling. Due to frequent rotation of fish, the tethering wire was manually unwound, and the local electrodes were checked for detachment before starting each recording session.

The movement onset and ending times determined by this analysis were verified with a concurrent video recording. All these tests were carried out under infra-red illumination (850 nm) provided by four near-infrared sources (S8100-60-B/C-IR, Scene Electronics, Hong Kong). Infrared illumination allowed observations of fish's swimming behaviour in darkness [Nelson and MacIver, 1999]. The four infrared sources pointed toward the ceiling of the aquarium enclosure to provide even illumination. The ceiling was constructed from a white corrugated plastic sheet with matte finish to reduce reflection and glare on the surface of water, and white sheet was laid underneath the glass aquarium to provide high contrast background. Video recording was made using a near-infrared sensitive camera (Guppy F-036B, Allied Vision Technologies, Stadroda, Germany) with a wide-angle lens (T2616FICS-3, Computar Corp., Tokyo, Japan). The camera was placed right above the centre of the ceiling, and the lens was exposed through a narrow hole. A light guard was installed around the lens to prevent glare from the infrared sources. Images were recorded at 15 frames/s at VGA resolution and stored as image sequences. The frame capture TTL events were captured with the EOD signals for synchronization.

We determined the temporal precisions for stationary and swimming conditions. Onset of movement was determined by tracking changes in the peak pulse amplitudes, which we now refer to as the movement index. The movement index was calculated by the following steps: first, a series of peak pulse amplitudes was smoothed with a Hamming filter (window size of 32); second, differences between adjacent pulse amplitudes were computed; third, binned average was computed (bin size of 200). Once movement index was determined, a threshold was manually set to a value which clearly separated two movement states.

In addition to the temporal precision, we tested pulse detection reliability from an untethered free-swimming fish for extended period of time. The real-time pulse detector output (TTL pulse and envelope-differentiated waveform) were digitized at a slower rate (ADC rate of 20 KS/s and TTL temporal resolution of 10 μ s) to permit longer recording duration (approximately six hours). The TTL pulse output was compared against the continuous waveform recording to check for any missed pulses. The aquarium filters and bubblers were turned off during all recording sessions to minimize electrical interferences. However, the floor

heater remained powered since it did not contribute to noise due to effective electrical shielding between the heater and the aquarium.

2.4 Results

2.4.1 Overview of test from restrained fish under various source-detector geometries

We validated our pulse detection methods by measuring the temporal precision and detection reliability under various source-detector geometries. We digitized raw EOD waveforms and determined EOD pulse timings using several different pulse detection methods. EOD pulse detections were initially performed in the digital domain to find the optimum parameter values such as the cut-off frequencies and gain values, and to compare performances of different pulse detection methods. We implemented digital signal processing stages for different pulse detectors in Spike2, software that drives the ADC hardware. The digitally filtered output closely matched the analogue electronics output with use of equivalent filters and operations. For each filtered pulse waveform, pulse timing was measured at different feature points (zero-crossing, maximum, minimum, and absolute maximum); also, the peak-to-peak amplitudes were measured for the non-rectified waveforms (peak amplitudes for the rectified waveforms).

We physically varied the source-detector geometry by adjusting three parameters: the distance from the centre of the aquarium to the fish (ρ_{fish}), the orientation of the fish (θ_{fish}), and the orientation of the dipole recording electrodes (ϕ_{elec}). The pulse waveforms were recorded for each uniformly spaced sample point in the parametric space. ρ_{fish} was varied from -55 cm to 55 cm in 5 cm steps, θ_{fish} was varied from 0° to 180° in 15° steps, and ϕ_{elec} was chosen at 4 orientations $\{0^\circ, 45^\circ, 90^\circ, 135^\circ\}$, yielding a combined total of 1196 independent observations ($23 \text{ linear steps} \times 13 \text{ angular steps} \times 4 \text{ dipole orientations}$). All angles (θ_{fish} , ϕ_{elec}) were measured with respect to the orientation of dipole A. In order to estimate the unobserved gaps in the parametric space, the raw measurements were interpolated ten times using 2D cubic spline (interp2 function in MATLAB) in the dimensions of ρ_{fish} and θ_{fish} , and converted to polar coordinates (Fig. 2.8).

At each measurement step EOD pulses were recorded for 3 s. We then sorted pulses by their peak amplitudes measured at the local recording dipole, and selected 100 pulses closest to the mean. Pulses far from the mean were discarded, since they were resulted when the restrained fish occasionally moved its gills and fins. The selected pulse waveforms were then filtered and detected by five methods to be compared in the next section. The pulse amplitudes and timings were measured for each pulse detection method, and their statistics (μ , σ) were derived from the interpolated parametric space in polar coordinates. The statistical samples were uniformly chosen from the polar parametric space to fairly represent larger radial distances (ρ_{fish}) covering greater areas.

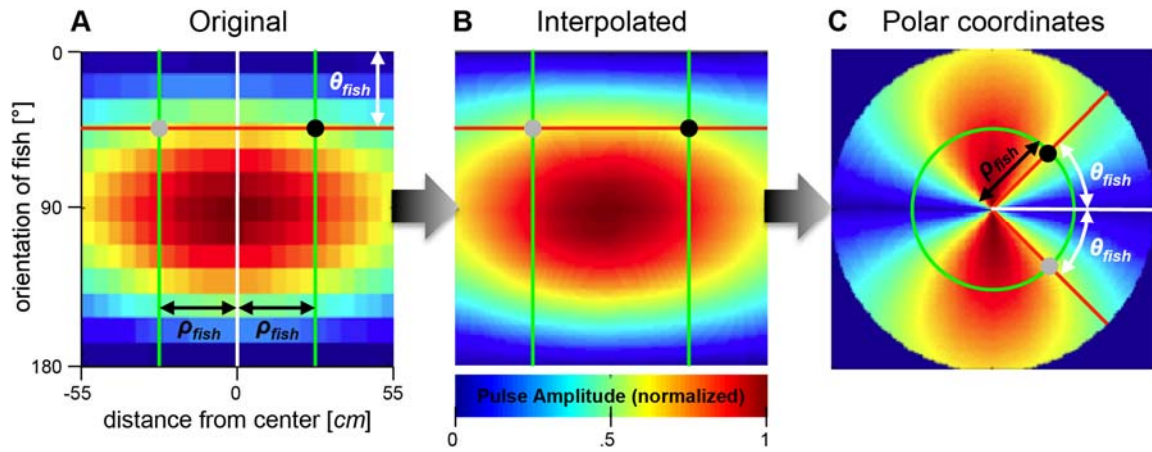


Figure 2.8. Procedures for creating a geometry-dependent plot. A geometry-dependent amplitude observed from the recording dipole C is shown here to demonstrate the procedures for creating a plot. (A) The raw measurements showing 299 data points or pixels (13 values of θ_{fish} in the abscissa, 23 values of ρ_{fish} in the ordinate). (B) Interpolation over the parameter space by a factor of ten using 2D cubic spline interpolation. (C) Transformation to polar coordinates to visually aid comprehension.

2.4.2 Envelope detection offers the best temporal precision

The envelope extraction-based pulse detection was tested and compared with the three commonly used basic pulse detection methods. Results shown here demonstrate the effect of temporal processing based on single channels, instead of using multiple channels to separate the effect of spatial averaging (described in the next section). The effect of the source-detector geometry variation on detection reliability and precision was measured and compared for five pulse detection methods: three basic (*max*, *z-c*, *|max|*), and two envelope detection based (*env*, *env-diff*). Pulse timing markers for each method were selected as indicated by their names: *max* uses a maximum positive peak; *z-c* uses a falling zero-crossing point between two neighbouring peaks of opposite polarity; *|max|* uses an absolute maximum peak; *env* uses an envelope peak; *env-diff* uses a falling zero-crossing point of the differentiated envelope waveform.

Figure 2.9A compares the timing errors of the five pulse detection methods. The bars represent the timing errors which were determined from the SD of the pulse timing difference ($\sigma_{\Delta T}$) between the externally- and the locally determined pulses. The local dipole derived pulse timing served as a geometry-invariant reference. The statistics were pooled over for all geometric variations and for the four recording dipole orientations (channels A-D), and computed separately for five pulse detection methods. In summary, the envelope detection based methods showed the highest temporal precision ($24 \pm 3 \mu\text{s}$ for *env* and $24 \pm 4 \mu\text{s}$ for *env-diff*) among all the methods we tested. Two of the basic pulse detection methods (*z-c* and *max*) performed particularly poorly, since their performance is vulnerable to the polarity reversal of the EOD pulse waveforms.

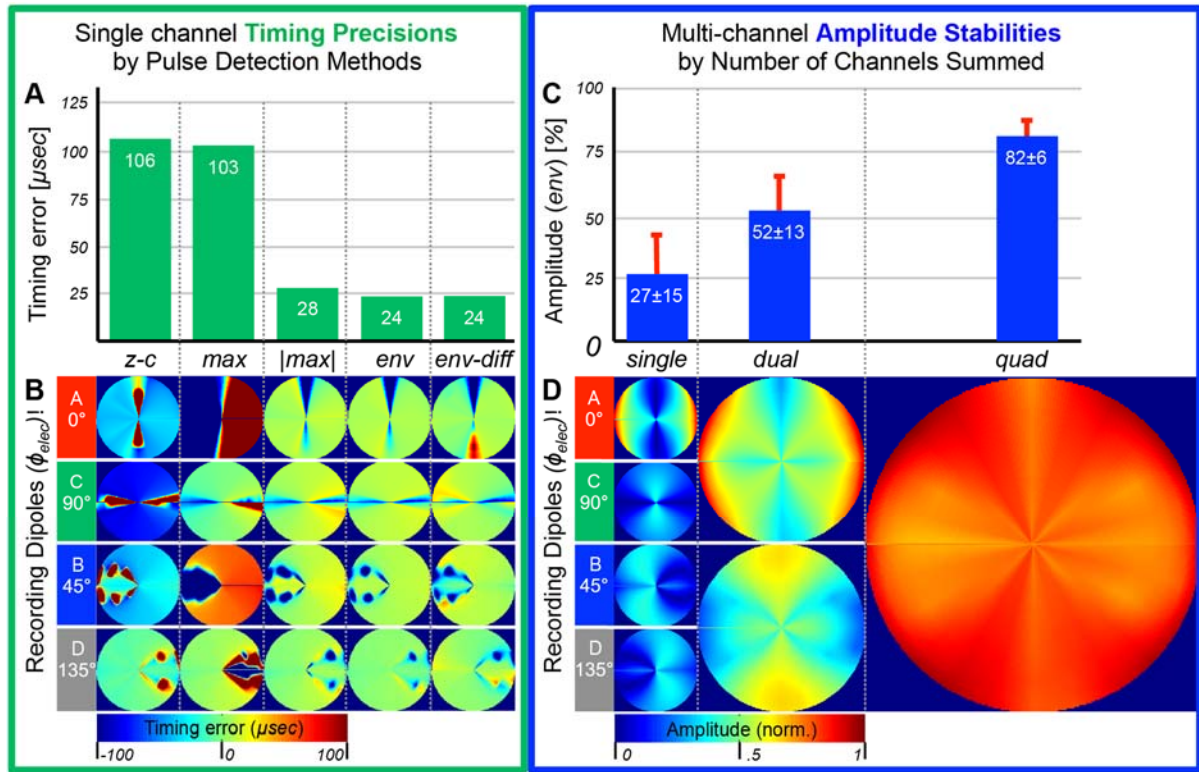


Figure 2.9. Comparisons between the pulse detection methods, and between the channel-summing modes. (A) Timing precisions are compared by five pulse detection methods based on single channel recordings. **(B)** The geometry-dependent timing precisions are tabulated for the four recording dipole orientation, and across the five pulse detection methods. **(C)** The amplitude stabilities are compared by the channel-summing modes. **(D)** The geometry-dependent amplitude stabilities are tabulated for the four recording dipole orientations, and across the three channel-summing modes.

2.4.3 Multi-channel summing offers high detection reliability and temporal precision

The multi-channel summing operation was tested for different numbers of channels recruited for summing. Its effect on the detection reliability and the temporal precision was thus measured. The detection reliability was largely determined by the pulse amplitude stability; thus reliability was indirectly measured from the amplitude variability under geometric changes. For instance, a missing pulse could occur when pulse amplitude drops too quickly

below a threshold, even when the threshold was set adaptively. Three channel-summing modes (single, dual, quad) are compared here and determined by the following: single mode recruits four separate recording dipoles (ch. A,B,C,D), dual mode recruits two sets of two perpendicularly arranged recording dipoles summed together (ch. A+C and B+D), and quad mode recruits four equally spaced recording dipoles summed together (ch. A+B+C+D).

Figure 2.9D shows the geometry-dependent pulse amplitudes as a function of ρ_{fish} and θ_{fish} for the four recording dipoles differently oriented (ϕ_{elec}). The amplitudes were separately normalized by the maximum amplitude observed at each dipole. The four single-channel amplitude plots show the maxima where θ_{fish} becomes parallel to ϕ_{elec} at each dipole A-D, and the minima at the perpendicular orientations as expected from a dipole-like current source. The four single-channel plots approximately appear bilaterally symmetric along the body orientation axis (θ_{fish}), but they are in fact distinct since the fish's head and tail ends generate different waveforms [Rodriguez et al., 2008]. For the same reason, the pulse-timing plots from Figure 2.9B appear more asymmetric along θ_{fish} .

Figure 2.9C compares the magnitude and variation of the summed pulse amplitudes for the three channel-summing modes. The bars represent the mean amplitudes of the summed pulses averaged over various source-detector geometries, each normalized by the maximum amplitudes in its respective channel-summing mode. The bar plot shown was generated from measuring pulse amplitudes after the *env* pulse detection method; other methods (*|max|*, *env-diff*) produced virtually identical results (Table 2.1). Recruiting more channels for summing increased the amplitude stability, or reduced the amplitude variation under geometry changes ($\sigma_{\text{Ampl.}} = 15\%$ in single-, 13% in dual-, and 6% in quad modes); and the mean pulse amplitudes were increased as well ($\mu_{\text{Ampl.}} = 27\%$ in single-, 52% in dual-, 82% in quad modes) (Fig. 2.9C).

Figure 2.10 shows that the temporal precision is further enhanced using additional channels, particularly when the multi-channel summing (Fig. 2.9C) is combined with the envelope detection (Fig. 2.9A). The envelope detection method (*env*) outperformed the best basic pulse detection method (*|max|*) by 57% in dual-, and 65% in quad channel-summing modes; and produced temporal precisions ($\sigma_{\Delta t}$) of $23.5 \mu\text{s}$ in single-, $1.9 \mu\text{s}$ in dual-, and $1.2 \mu\text{s}$ in *quad* channel-summing modes (Table 2.1). *env-diff* performed virtually identically to *env* in *quad* channel-summing mode, but it was easier to implement in analogue electronics.

2.4.4 Real-time electronic pulse detector performance

The real-time electronic pulse detector we constructed was tested for timing error, which employs the *quad* summing and *env-diff* pulse detection methods. The time difference was taken between the TTL pulse output of the electronic detector and the reference pulse derived from the concurrently recorded local dipole, and the timing error was determined from the SD of the time difference over various source-detector geometries. Figure 2.10 shows that the temporal precision of the electronic pulse detector ($2.7\text{ }\mu\text{s}$) is $1.3\text{ }\mu\text{s}$ higher than the precision of the equivalent digitally implemented counterpart (*quad env-diff*). The higher error in the electronic detector could be due to the thermal noise contributed by circuit elements, and non-ideal filter behaviours resulting from non-matched passive component values (resistor precision $\leq 1\%$, capacitor precision $\leq 5\%$). The maximum timing error reported by the electronic detector from restrained fish was $16\text{ }\mu\text{s}$, closely matching the maximum error from the equivalently configured digital pulse detector ($15\text{ }\mu\text{s}$, Table 2.1). Figure 2.11 compares the geometry-dependent timing errors between the electronic detector and the equivalent digital pulse detector. Although they share some common features on the top and bottom of the plots, these two plots generally appear different. This could be due to slight variations in the construction of the four parallel stages (Fig. 2.7A), causing timing offsets dependent on ϕ_{elec} . Manufacturing variability of the components used to construct the electronic detector was the main source of this discrepancy. In summary, our electronic pulse detector performed with a high temporal precision as expected from the equivalent digital pulse detector, while a longer recording duration is made possible by the online operation.

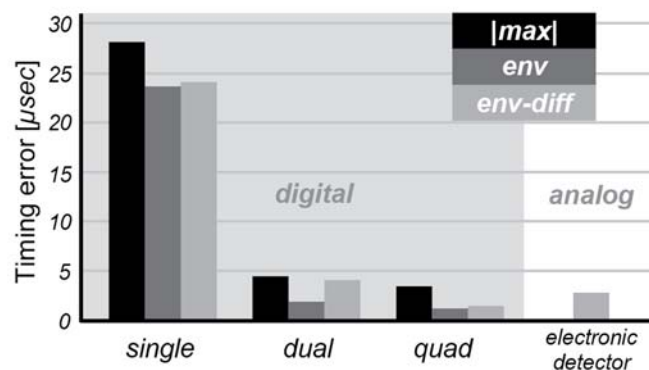


Figure 2.10. Overall comparison of the temporal precisions. The bar plot shows an improved temporal precision from summing multiple channels, and an additional improvement from using the envelope processing (*env*, *env-diff*). The electronic pulse detector (*quad* channel) had a slight increase in timing error ($1.3 \mu\text{s}$) compared to the digitally implemented counterpart (*quad env-diff*), while operating in real-time.

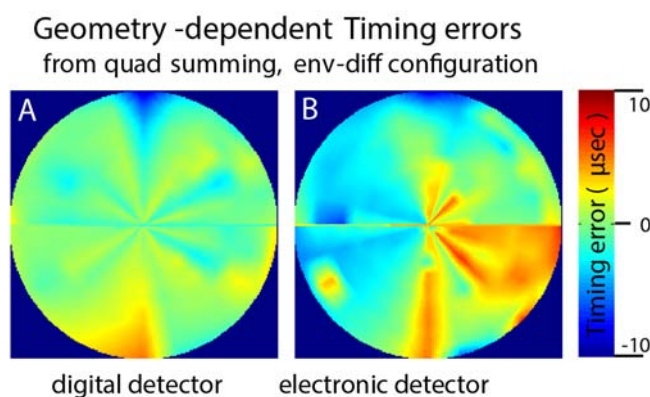


Figure 2.11. Geometry-dependent timing errors compared between the digital and the electronic pulse detectors. The two plots share some common features on the top and bottom parts, but they generally appear different due to manufacturing variability of the components used to construct the electronic detector.

Table 2.1. Table of the test results from restrained fish. This table summarizes the test results from combinations of all pulse detection methods & all channel configurations. The columns show the statistics of pulse amplitudes and timing differences between the externally- and locally derived pulse timing. The results are organized by different pulse detection methods (*z-c*: zero-crossing, *max*: maximum peak, *env*: envelope peak, *env-diff*: differentiated envelope zero-crossing). The mean was computed over all geometric variations, *stdev* denotes the standard deviation, and *range* denotes the maximum observed differences. The rows show different channel configurations by number of channels summed (*local*: local reference channel, *single*: one channel, *dual*: two channels, *quad*: four channels, *detector*: quad channel electronic pulse detector). The local pulse timing difference could not be measured due to lack of a timing reference for the local timing reference themselves. The pulse amplitudes from the electronic pulse detector were not measured.

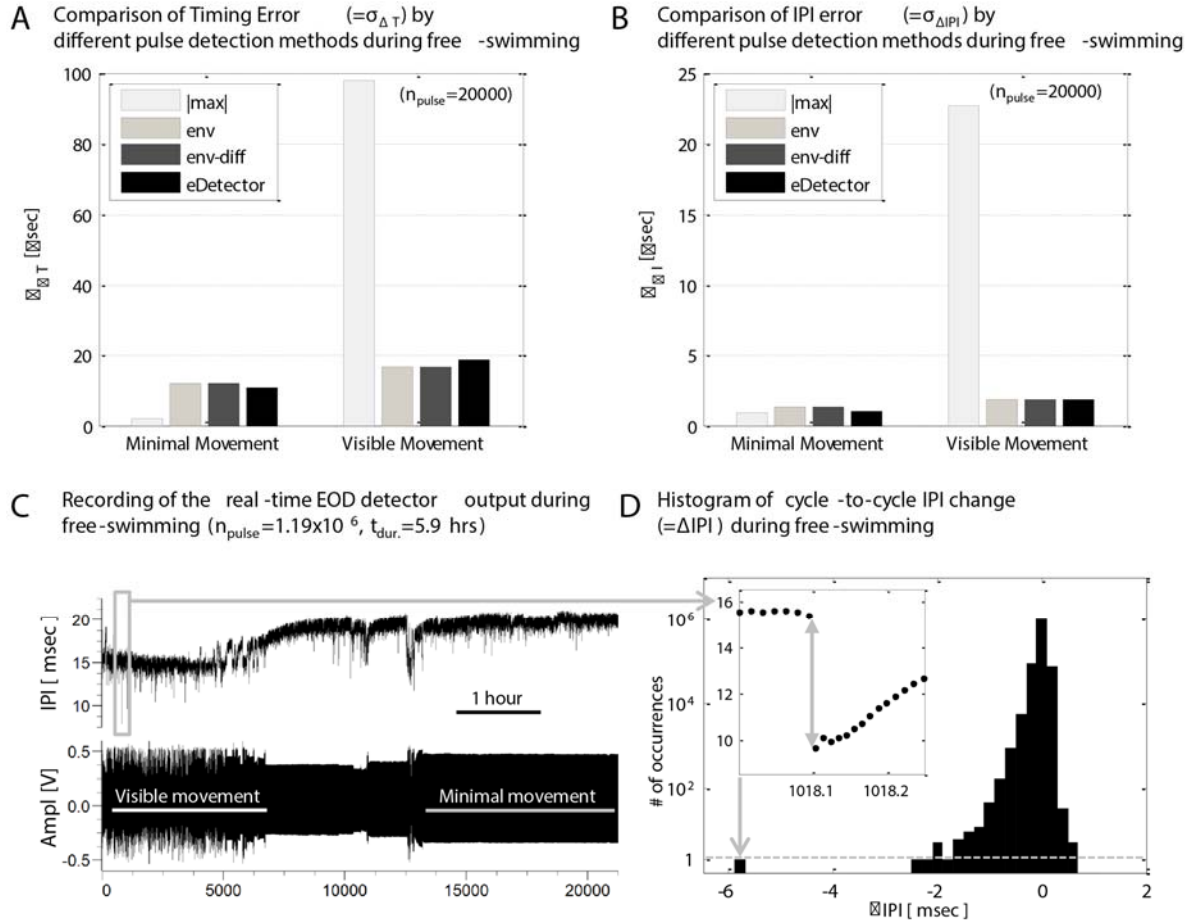
Channel	Method	Amplitude, %			Timing Error, μs	
		Mean	SD	Range	SD	Range
Local	z-c	0.909	0.027	0.168		
	lmaxl	0.866	0.039	0.235		
	env	0.917	0.024	0.147		
	env-diff	0.923	0.024	0.145		
Single	z-c	0.265	0.153	0.992	20.1	1,547.4
	lmaxl	0.255	0.146	0.994	9.3	776.7
	lmaxl	0.268	0.153	0.989	3.5	274.7
	env	0.266	0.152	0.988	3.1	267.2
	env-diff	0.264	0.151	0.989	3.9	292.4
Dual	lmaxl	0.520	0.133	0.824	2.1	35.0
	env	0.516	0.130	0.825	2.0	15.2
	env-diff	0.511	0.130	0.835	1.7	48.3
Quad	lmaxl	0.812	0.059	0.317	2.1	15.1
	env	0.820	0.057	0.308	1.8	6.4
	env-diff	0.807	0.059	0.322	1.4	14.6
Electronic detector	env-diff				2.3	15.7

2.4.5 Pulse detection precision and reliability during free-swimming

Temporal precision of the real-time electronic detector was measured from tethered free-swimming fish, and the body-attached electrodes were used as a timing reference. Timing errors were separately analyzed based on the state of movement. Timing errors increased with movement for all pulse detection methods (Fig. 2.12A), but some pulse detection methods were more resilient to movement compared to others. Envelope-based pulse detection methods (*env*, *env-diff*, *eDetector*) were much less affected by movement (by a factor of 1.4), in comparison to *|max|* method which significantly increased by a factor of 56.5. Timing error increased with visible movement since the depth of fish, body posture, and yaw and pitch angles were simultaneously varied, causing large waveform distortions and fast fluctuations in pulse amplitude. *|max|* method performed particularly poorly during visible movement, since the peak point of a pulse waveform often switches from one lobe to another during free-swimming when externally measured.

The timing errors measured from tethered fish (Fig. 2.12A and Table 2.2) were generally larger than that from fixed fish (Fig. 2.10). However the time difference between the local- and the external recording dipoles drifted more slowly than the EOD pulse interval. Thus pulse interval was more precisely determined compared to the pulse timing due to mutual cancellation of the timing bias (Fig. 2.12B). The pulse intervals determined by the envelope-based methods showed close agreement between the local and external electrodes during free-swimming. The SD of the pulse interval difference ($= \sigma_{\Delta I}$) or the interval errors were below the measurement resolution (ADC sampling interval of 5 μ s) for the envelope-based methods, but *|max|* method produced much greater interval error during visible movements. The maximum interval error (49 μ s) for the electronic detector occurred when fish contacted one of the recording electrodes and saturated an amplifier. This contact occurred rarely and the maximum interval error excluding the gain saturation events was 15 μ s.

Reliability of the real-time electronic pulse detector was tested for an extended time (6 hours) and no missing pulses were detected out of 1191371 pulses captured (< 1 ppm) (Fig. 2.12D). The pulse interval range was between 7.90 ms to 21.14 ms (Fig. 2.12C) indicating no missing pulse or noise spikes. The largest IPI difference (-5.74 ms) occurred during a large novelty event (Fig. 2.12D inset), and was not due to an insertion of a noise spike event.



2.12. Test results from a free-swimming fish. Test results are shown for the temporal precision and reliability of the real-time electronic pulse detector. **(A)** Timing errors during minimal and visible movement for four pulse detection methods, obtained from a tethered free-swimming fish. **(B)** Same as the panel A, except the interval errors are shown. **(C)** Recording traces from an untethered free-swimming fish for 6 hours. **(D)** Histogram of cycle-to-cycle interval change during free swimming indicates no missing pulses over six hour duration. The largest interval change occurred during a large novelty response [Caputi et al., 2003], and was not due to an insertion of a noise spike.

Table 2.2. Table of the free-swimming test results. This table summarizes the test results from a free-swimming fish using different pulse detection methods. Columns show the statistics of interval and timing differences for three pulse detection methods and the real-time electronic detector ($|max|$: absolute peak, env : envelope peak, $env-diff$: differentiated envelope zero-crossing). “ $Stdev$ ” denotes the standard deviation, and “ $worst$ ” denotes the largest observed absolute difference. Statistics were separately derived from a tethered fish for swimming and resting durations, and 20000 pulse events were analyzed for each case. Observe that the envelope-based methods show less variations in the timing and interval differences during motion compared to the peak-based method ($|max|$).

Method	Motion	IPI Error, μs		Timing Error, μs	
		SD	Range	SD	Range
$ max $	No	0.9	15.0	1.7	17.0
	Yes	22.6	668.0	97.8	521.0
env	No	1.3	19.0	11.9	51.0
	Yes	1.9	26.0	16.6	146.0
$env-diff$	No	1.3	19.0	11.9	51.0
	Yes	1.9	25.0	16.6	147.0
Electronic detector	No	1.0	26.0	10.7	43.0
	Yes	1.8	93.0	18.3	251.0

2.5 Discussion

Measuring EOD pulses from free-swimming electric fish requires specialized signal processing to precisely and reliably resolve the pulse timing, because the self-motion of fish alters the geometric relation between the source (fish) and the detectors (recording electrodes), which in turn varies the observed pulse amplitudes and waveforms. Basic pulse detection methods such as threshold crossing or peak detection from the raw pulse waveforms may perform well for stationary fish, but their performance deteriorates once fish swim freely. In contrast, our multi-channel summing envelope detection method performs reliably and precisely even under a free-swimming condition. Our real-time electronic pulse detector achieved temporal resolution under 10 μs from free-swimming fish, and operated with high reliability (error < 1ppm). Such reliability and precision of EOD pulse detection is therefore adequate to study how a fish's sampling rate changes during exploratory behaviour and spatial learning (unpublished observation).

Animals may reveal an interesting repertoire of behaviours (including some rare ones) from a long-term observation. To permit a long-term recording, our real-time electronic pulse detector demands little storage space (4 bytes/pulse) to store the EOD pulse timestamps. In contrast, a full waveform recording demands much larger storage space to offer a comparable temporal precision. To provide 5 μs temporal precision using a non-interpolated peak detection, approximately 2000 times larger storage space is required by sampling at 200 kS/s and 16 bits/sample. Such a high storage requirement only permits short EOD recording durations (< 15 min per 2 GB). Precision of measurement is generally limited by noise, often originating from the AC supply line or radio-frequency coupled sources; thus we physically blocked the noise sources by choosing a battery as a power source instead of the supply line, and by shielding the whole aquarium with a Faraday cage. Furthermore, a range of frequencies exhibiting low signal-to-noise ratio (SNR) was filtered out; the low-and high cut-off frequencies were determined by comparing the power spectra of the aquarium with and without fish.

Modern microcontrollers enable experimentalists to quickly construct high-performance and low-cost instruments by offering numerous analogue and digital capabilities and easy-to-use design software. Inspired by Mavoori *et al.* [2005], we chose the PSoC to

implement an online pulse discriminator, because it offers all the necessary analogue (*e.g.* programmable gain amplifier, ADC, filters) and digital (*e.g.* pulse width modulator, RS232 serial communication, and timer) modules to implement the discriminator. The analogue and digital modules in the PSoC can distribute a task load by performing independently from the CPU, yet can still be reconfigured by firmware during run-time for flexible operations. By taking advantage of these unique capabilities, we constructed a versatile yet inexpensive ($\leq$ \$200 USD, see Supplemental Material, available for this article online at the Journal of Neurophysiology website) pulse detector, which is capable of updating the discrimination criteria in real-time to process a dynamically varying input signal via an adaptive threshold and an automated gain control.

2.5.1 Sources of Error and Limitations

The local recording dipole provided for a geometry-independent timing reference. Since it is fixed with respect to the fish, the local pulse waveform was assumed to be constant over the geometric changes. However, the local pulse amplitudes slightly varied ($\sigma_{\text{Ampl.}} = 3\%$), and decrease as fish approached the circular boundary, because the plastic fences distorted the field. Also, the fish could have slightly repositioned itself within the holding frame for the duration of recordings, thus slowly changing its position with respect to the local electrodes. The spinal pulse-discharge command may be a better reference for pulse timing, since the discharge waveform is independent of the external geometry changes. However, recording this signal requires an invasive surgical procedure which may alter natural course of EOD production and free-swimming behaviour. It is also technically challenging to record from a spinal cord from a freely swimming fish for an extended period of time. Hence we attached electrodes on fish's body to approximate timing of spinal discharge action potential. The attached electrodes did not directly contact fish skin to avoid recording myogenic potential, and were placed sufficiently away from the tail to minimize waveform distortion during tail bending. Although the electrodes were stably attached, pulse amplitude and waveform from the attached local dipole varied during free-swimming (amplitude varied up to 20%), particularly when fish bent its body and closely approached the water surface and the circular fence. The effect of movement on the attached local dipole may have inaccurately exaggerated the actual timing error by corrupting the local reference pulse timing.

The analogue signal filtering stages introduce a constant time delay much greater (100s μs) than the precision of the electronic pulse detector ($\leq 10\text{ }\mu\text{s}$); but the delays can be corrected after the recording session by advancing the pulse timestamps. This post-processing task may not even be necessary when the EOD recording needs to be synchronized with much slower varying signals such as a video recording. And most importantly, the constant time delay does not affect the IPI, which is our main quantity of interest.

Temporal precision of the pulse detection was significantly degraded when fish directly contacted one of the recording electrode and saturating the amplifier. The saturation could be prevented by lowering the gain but this will reduce the signal amplitude when fish is sufficiently far away from the recording electrodes. Alternatively, one could protect the graphite electrodes by surrounding them with fine nylon mesh spaced from the electrodes to prevent direct contact.

Although we simulated and systematically changed the source-detector geometry using three spatial parameters (ρ_{fish} , θ_{fish} , ϕ_{elec}), we could not parametrically study other possible effects such as: body-bending, tilt and yaw, change of fish's depth, fish contacting a recording electrode. This is mainly due to the difficulties in making quantitative measurements. Instead, we observed from tethered (short-term) and an untethered free-swimming fish (long-term) to study their aggregated effects on the performance of our real-time pulse detector.

All measurements from restrained fish were taken from one individual of an unknown species of the *Gymnotus* genus, collected within the same day. Pulse waveforms vary between different individuals, thus a single restrained fish was used to ensure that the pulse waveforms to vary only as a function of the source-detector geometry. It was not possible to exactly determine the species of our fish, due to nearly indistinguishable external appearances and pulse waveforms of numerous *Gymnotus* species [Crampton and Albert, 2003; Crampton et al., 2003; Rodriguez et al., 2008]. The gender could not be determined by external appearance alone, and a dissection was not performed. However our pulse detection method does not depend on particular pulse waveform shapes since the envelope extraction filter converts multi-lobed original waveforms to a unimodal shape. Thus in principle, our method is

applicable to other pulse species of the Gymnotiform and Mormyrid families, after determining the optimal low-pass cut-off frequency for the envelope extraction.

2.5.2 Future directions

The current electronic pulse detector was designed for a lab setting, and operates with a separate timestamp logger. But a future version could incorporate a flash memory-based logging capability for an all-in-one operation suitable for field deployment. A field-deployable EOD pulse detector unit may record from a larger body of water, and modifications to our current design for a scalable deployment may include: an increased number of recording channels, and an optimized spatial distribution of the recording electrodes to increase the signal reception strength. Since each channel contributes noise, inclusion of channels with low SNR deteriorates the measurement precision. Thus to address this issue, an online channel selection algorithm could be implemented to automatically exclude the channels containing low SNR. Social interaction and electric communication between two or more pulse fish (same species) is an active research area in neuroethology [Wong and Hopkins, 2007; Perrone et al., 2009]; and the future version may be able to recognize pulses from different individuals by tracking a spatial location of each fish, using triangulation from multiple channels. It may also be possible to modify our methodology to automate analysis of the frequency modulations used by wave-type gymnotiform fish during naturalistic social interaction [Hupé and Lewis, 2008].

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Chapter 3

Real-time localization of moving dipole sources for tracking
multiple free-swimming weakly electric fish

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3.1 Summary

In order to survive, animals must quickly and accurately locate prey, predators, and conspecifics using the signals they generate. The signal source location can be estimated using multiple detectors and the inverse relationship between the received signal intensity (RSI) and the distance, but difficulty of the source localization increases if there is an additional dependence on the orientation of a signal source. In such cases, the signal source could be approximated as an ideal dipole for simplification. Based on a theoretical model, the RSI can be directly predicted from a known dipole location; but estimating a dipole location from RSIs has no direct analytical solution. Here, we propose an efficient solution to the dipole localization problem by using a lookup table (LUT) to store RSIs predicted by our theoretically derived dipole model at many possible dipole positions and orientations. For a given set of RSIs measured at multiple detectors, our algorithm found a dipole location having the closest matching normalized RSIs from the LUT, and further refined the location at higher resolution. Studying the natural behaviour of weakly electric fish (WEF) requires efficiently computing their location and the temporal pattern of their electric signals over extended periods. Our dipole localization method was successfully applied to track single or multiple freely swimming WEF in shallow water in real-time, as each fish could be closely approximated by an ideal current dipole in two dimensions. Our optimized search algorithm found the animal's positions, orientations, and tail-bending angles quickly and accurately under various conditions, without the need for calibrating individual-specific parameters. Our dipole localization method is directly applicable to studying the role of active sensing during spatial navigation, or social interactions between multiple WEF. Furthermore, our method could be extended to other application areas involving dipole source localization.

Key words: dipole localization, inverse problem, animal tracking, weakly electric fish, pulse-type species, ideal dipole, lookup table, calibration free

3.2 Introduction

Animals must accurately locate signal sources of various kinds [Kramer, 1974; Knudsen, 1979; Fuzessery et al., 1993; Coombs et al., 1996; Aytekin et al., 2004], since this allows animals to quickly respond to prey, predators, or potential mate signals that are very important for their survival. Mechanisms of signal localization depend on particular signal types, but in general, signals attenuate over distance; therefore the intensity information can be useful in signal localization. In fact, many biological and non-biological systems can determine signal locations on the basis of comparing received signal intensities (RSI) from two or more spatially distributed sensors [Knudsen and Konishi, 1979; Nelson and MacIver, 1999; Lewejohann et al., 2009; Rivera et al., 2011]. The signal localization task becomes more complex if the RSI depends additionally on the orientation of the signal source, such as the signals dependent on the orientation of an electric fish dipole. A current dipole consists of two physically separated current sources having opposite polarities, which creates spatially non-uniform field strength as a function of distance and orientation. A dipole source can be approximated as an ideal dipole if the separation between the positive and negative sources is much smaller than the distance between the source and the detector [Knudsen, 1975]. Animals transmit or receive dipole-like signals in the form of sound [Knudsen and Konishi, 1979; Fuzessery et al., 1993], vibration [Coombs et al., 1996; Goulet et al., 2008], and electricity [Kramer, 1974; Chen et al., 2005]. For example, weakly electric fish species can locate other member of species using the electric field they generate [Hagedorn and Heiligenberg, 1985; Davis and Hopkins, 1988; Shieh et al., 1996; Hopkins et al., 1997; Yu et al., 2012].

It is necessary to track free-moving weakly electric fish (WEF) to study their electrolocation or social behaviours under naturalistic conditions. Visual tracking is widely employed for studying animal behaviours, and a typical setup requires an appropriate illumination and a background to produce high contrast images. Animal tracking can be automated by a computer vision algorithm [Spruijt et al., 1992; Winberg et al., 1993; Vatine et al., 1998; Noldus et al., 2001; Windsor et al., 2008; Fontaine et al., 2008; Branson, 2009; Straw 2011], but visual tracking algorithms become less reliable in visually complex scenes [Lewejohann et al., 2009; Vasserman et al., 2012]. Animals' naturalistic habitats often contain objects or other animals, which can cast shadows, obstruct, or confuse the identity of the animal being tracked. Use of visual markers [Spruijt et al., 1992; Winberg et al., 1993; Vatine et al.,

1998] or electronic tags [Lewejohann et al., 2009, Vasserman et al., 2012; Mascanzoni et al., 1986; O’Neal et al., 2004] can improve the tracking reliability during animals’ interaction with objects or with other animals. In particular, electrical tracking methods do not require a direct line of sight, thus the tracking remains reliable while animals are obstructed from view. Here we propose a method of locating current dipole sources in order to track multiple freely swimming electric fish in an environment with objects and/or other electric fish.

WEF are mostly nocturnal and often found in turbid water, thus their visual sensing range is limited in their natural habitat. Instead, they generate an electric field using one or more electric organs in order to perceive their immediate sensory surroundings [Lissmann, 1958; Lissmann and Machin, 1958; Davis and Hopkins, 1988; Emde et al., 1998; Nelson and MacIver, 1999; Babineau et al., 2007], or to communicate between individuals [Kramer, 1974; Hagedorn and Heiligenberg, 1985; Mohres, 1957; Hopkins, 1974; Zakon et al., 2002; Zupanc, 2002; Turner et al., 2007; Hupé and Lewis., 2008]. The geometry of the fish electric field is dipole-like since the rostral and caudal parts of the electric organs generate current flows in opposite directions [Caputi, 1999; Rodríguez-Cattáneo et al., 2008; Castello 2009], and they closely approximate an ideal dipole model at a distance scale greater than fish’s body length [Knudsen, 1975]. The waveform of the electric organ discharge (EOD) is either continuous or pulsatile depending on the species, and the extent of the electric organ is either concentrated or dispersed along the rostro-caudal axis [Sanguinetti et al., 2011]. In the Gymnotid species we study [Lissmann, 1958; Albert et al., 1999; Albert et al., 2001; Albert et al., 2003], the EOD pulses are 1-2 milliseconds in duration, and multiple components of the electric organ activate at different pulse phases to create a complex spatiotemporal pattern [Rodríguez-Cattáneo et al., 2008; Castelló et al., 2009]. When two or more fish are nearby, they discharge so as to actively avoid the collision of their pulses, thus minimizing signal interference between individuals [Bullock et al., 1972; Bastian et al., 1980; Capurro et al., 1998]. Although the localization of dipole sources is complicated by the presence of multiple current sources distributed along the body, and occasional signal interference between animals, it is possible to solve the dipole localization problem by optimizing the EOD pulse measurement and by using the ideal dipole model.

In this paper, we propose a dipole localization method based on the lookup table (LUT) operation, which compares actual received signal intensities from multiple recording channels with theoretically predicted signal intensities at many possible dipole locations. Our LUT search algorithm was computationally optimized for real-time tracking of a freely swimming WEF in a shallow tank to determine its position, orientation, and body-bending angle. In addition, our tracking system reliably dissociated the trajectories of fish dyads and their EOD pulses. Our dipole tracking system could be useful for field studies where long-term visual observation is difficult [Fugere et al., 2011; Henninger et al., 2012]; or it could be used in conjunction with visual tracking to study social interactions in a visually complex environment. Our unique approach to the inverse problem using an ideal dipole model could also be applicable to other dipole localization problems of such as sound or vibration source localizations.

3.3 Methods

3.3.1 Overview

We measured the EOD pulse amplitudes at multiple locations with respect to multiple fixed detectors in shallow water (Fig. 3.1A), and fitted them with an ideal dipole model by varying the position and orientation parameters. The instantaneous slopes at a particular EOD pulse phase were measured from all channels of the detector pairs when the current flow was concentrated near the centre of the fish's body [Rodríguez-Cattáneo et al., 2008]. The RSIs were more accurately measured with the instantaneous slopes than the peak-to-peak amplitudes, because the slope measurements were taken nearly instantaneously (120 μ s) and concurrently from all channels in a phase-locked manner. Due to the brief measurement duration, the slope measurement was less susceptible to the signal interferences between WEFs, and it agreed with the ideal dipole model more closely due to a closer physical separation between the current sources when the measurements were taken. The slope measurements were made relative to the pulse timing reference, which was defined at the peak of the global pulse envelope (Fig. 3.1B). The pulse envelope measures the total power received by all recording channels, and was calculated by first summing all channels after rectification (blue trace in Fig. 3.1B), then smoothing to have a unimodal shape with only a single peak (red trace in Fig. 3.1B) [Jun et al., 2012a]. The measured RSI values were compared with the

theoretical values computed by the two-dimensional ideal dipole model (Fig. 3.1C) at many possible dipole locations, and the closest matching location was found by our search algorithm (Fig. 3.1D). Although it is possible to observe similar normalized RSIs from two different dipole locations, our use of eight recording channels made this highly unlikely. Our dipole search algorithm initially estimated a location in coarse grids using pre-computed values, and it subsequently searched in finer grids around the initial estimate. This two-step search procedure quickly found a dipole location without compromising spatial or angular resolutions. We achieved a further speed increase (~ 1000 localizations/s) after applying a series of optimization techniques.

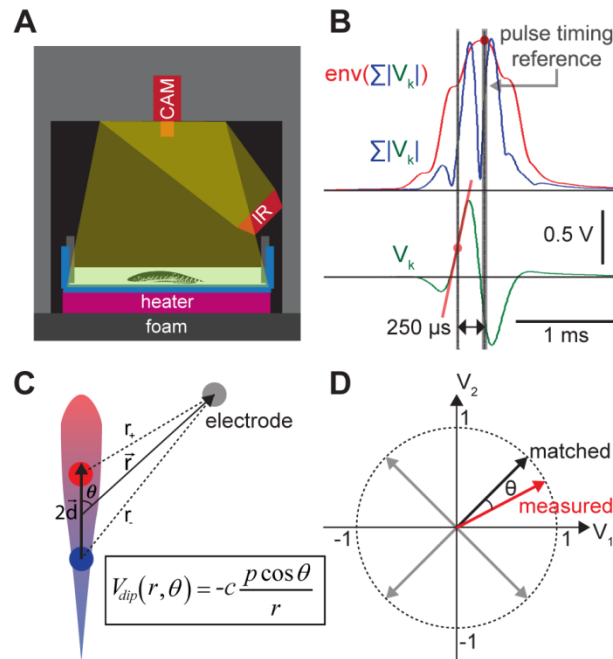


Figure 3.1. EOD signal measurement and ideal dipole approximation. (A) Our experimental setup. Electrodes were attached on the tank wall, and concurrent video recordings were made under infrared illumination. (B) Received signal intensity (RSI) measurement. The original waveforms (green) were rectified then summed from all channels (blue). Signal envelope (red curve) was extracted using an RMS filter, and a pulse timing reference (thick grey) was determined at the peak. An instantaneous slope of the original waveform was measured at $250 \mu\text{s}$ before the reference timing (red line). (C) Ideal dipole voltage (V_{dip}) approximation of an electric fish in two dimensions. (D) Lookup table search using a dot-product to find the best matching vector.

3.3.2 Experimental setup

All experiments described in this paper were approved by the University of Ottawa Animal Care Committee (protocol number: CMM-143). The animal experiments were conducted in a shallow, circular tank (1.5 m diameter, 10 ± 2 cm water depth), which was surrounded by an enclosure to block external sources of light and electrical noise. We obtained South American pulse-type weakly electric fish *Gymnotus* sp. [Lissmann 1958; Albert et al., 1999; Albert and Crampton, 2005a] (species and gender unknown) from a local supplier, and conditioned the aquarium water similarly to their natural habitat (100 ± 20 $\mu\text{S}/\text{cm}$, $\text{pH } 7 \pm 1$, and $25 \pm 1^\circ\text{C}$) using a stock salt solution [Knudsen, 1979] and a floor heater (ThermoTile; ThermoSoft, Buffalo Grove, IL). Animals were active in darkness, thus visual observations were made under near-infrared illumination (850 nm, S8100-60-B/C-IR; Scene Electronics) by a near-infrared sensitive camera (15 frames/s, 640×480 , C910 Logitech, infrared filter removed). The electric organ discharge (EOD) signals were recorded from eight or sixteen vertical graphite electrodes attached on the aquarium wall, and the graphite rods (6 in. long, Mars carbon 2-mm type HB; Staedtler) were coupled to BNC cables (RG54) via heat shrinks. The BNC shielding wires and a signal ground were connected to the Faraday cage which enclosed the whole aquarium (Fig. 3.1A). Raw signals from the four or eight electrodes pairs were differentially amplified and filtered (200x, 200 Hz $\sim$ 2.5 KHz, Intronix 2015F; Bolton, Ontario, Canada) to cancel the common-mode noise, and digitized (40 KS/s per channel at 16 bit, CED 1401 mkII; Cambridge Electronic Design, Cambridge, UK). The video recordings were synchronized to the EOD recordings using infrared light pulses (1 ms duration and 10 s interval), and captured by Spike2 video recorder software (Cambridge Electronic Design, Cambridge, UK).

3.3.3 EOD Pulse measurements

We measured the received signal intensities (RSIs) from the recording channels using the slope of the EOD waveform at particular pulse phases, since the slope is proportional to the RSI. We initially used the peak-to-peak amplitudes to measure the RSI, but the measurements deviated from the ideal dipole model due to distortion of the pulse waveform during active body movements, and the ambiguity of determining the waveform polarity. In order to accurately determine the RSIs from all recording channels, the slope measurements from all channels took place synchronously at a fixed time delay from the pulse timing reference, which was

defined at the peak of the envelope waveform (Fig. 3.1B). The envelope waveform (red trace in Fig. 3.1B) was extracted using a root-mean-square (RMS) filter, ($\tau = 250 \mu\text{s}$) from the summation of all channels after the rectification (blue trace in Fig. 3.1B) to prevent signal cancellation. The envelope waveform provided reliable and precise pulse timing reference [Rangayyan, 2002; Jun et al., 2012a]. In the species we studied, the EOD pulses are initiated near the rostral region and subsequently propagated to the caudal region [Rodríguez-Cattáneo et al., 2008; Caputi et al., 2005]. By using this knowledge, we found the optimal measurement timing when the electric organ (EO) activation was concentrated at the centre of the body ($225 \mu\text{s}$ before the reference timing), which is the location tracked by a typical visual tracking algorithm. We also found another useful measurement timing when the EO activation was concentrated near the tail location ($225 \mu\text{s}$ after the reference timing), as this enabled us to deduce the tail-bending angles by comparing the measurements at the central and the tail regions. The instantaneous slopes were measured within a brief time window using five ADC samples ($125 \mu\text{s}$ duration at 40 KS/s) in order to minimize the probability of the EOD pulses overlapping between different individuals. The slope measurements were performed in Spike2 (Cambridge Electronic Design, Cambridge, UK), and the results were exported to Matlab. A Spike2 script, a sample dataset, and an instruction manual are available in the Supplementary Information (Supplementary Data 3.1, available at [plosone.org](https://doi.org/10.1371/journal.plosone.org)).

3.3.4 Two-dimensional ideal dipole model

Our experimental data were modeled by considering that the WEF is an ideal dipole in a two-dimensional (2D) space, because 1) fish swam in a shallow body of water, and 2) the measurements were taken by vertically oriented electrodes across the full depth of the water column, which amounts to averaging measurements across depth. The dipole in our model consists of a pair of positive and negative current sources separated by the distance d (Fig. 3.1C). According to Gauss' law, the potential at a field location $\vec{r}$ due to such a horizontal dipole $\vec{p}$ is (see Text S3.1):

$$V_{dip}(r, \theta) = -cI(\ln r_+ - \ln r_-). \quad (3.1)$$

where r_+ is the distance between the positive source to the field location, and r_- is the distance between the negative source to the field location. c is the constant of proportionality which we

determined from the three-dimensional boundary condition (see Text S3.1). In the limit of $d \ll r = |\vec{r}|$, Eq (3.1) can be approximated as:

$$V_{dip} \approx -cI \frac{\vec{d} \cdot \vec{r}}{r^2} = -cp \frac{\cos \theta}{r}, \quad (3.2)$$

where θ is the angle between the vectors $\vec{r}$ and $\vec{d}$. See Text S3.1 for the detailed derivation of the two-dimensional ideal dipole formula. Although we assumed a simple two-dimensional space without boundaries, Eq (3.2) closely approximates a more realistic three-dimensional ideal dipole potential computed for a shallow, circular body of water with boundary effects (see Text S3.1). The top interface between the air and the water, and the bottom interface between the water and the glass contain surface charges induced by the ideal current dipole. The method of image charges can simplify our analysis of the two parallel dielectric interfaces by replacing the surface charges with an infinite number of image current sources (Fig. S3.1A) [Griffiths, 1999; Vanderlinde, 2004]. Although the two parallel surfaces generate infinite number of reflections [Zahn, 1976], the net potential converges since each successive reflection produces a weaker image current source at a further location (Fig. S3.1B) [Kumar and Nagabhushana, 1997]. Furthermore, the method of image charges can be extended to determine the potential due to an image dipole. See Text S3.1 for the detailed description of the three-dimensional ideal dipole potential computed for a shallow, circular body of water.

Near field effect correction. The 2D ideal dipole formula did not accurately predict the experimental data when fish were too close to the electrode, where the ideal dipole assumption ($d \ll r$) does not hold. However, animals spent a lot of their time near the wall, and this created non-ideality at the near-field location. The electric field nearest to the fish was distorted by its skin conductance, and a channel became saturated when fish made a direct contact to one of the electrodes. In order to ensure accurate predictions by the 2D ideal dipole model, we excluded a channel from being used for our dipole localization procedure when the fish approached one of its electrode pair closer than a set threshold (13 cm).

Side boundary effect. A current dipole also induces a surface charge at the interface between the water and the tank wall. The method of image charges can be similarly applied to simplify our analysis by replacing the induced surface charge with a pair of image sources, or an image dipole (Fig. S3.2C). The circular side boundary forms an image dipole outside of the circular region, and we determined the effect of the side boundary on the voltage difference between a pair of electrodes (ΔV_{dip}). ΔV_{dip} is the quantity we measured to estimate the RSI, and it is the sum of contributions from the dipole ($\vec{p}$) and the image dipole ($\vec{p}'$): $\Delta V_{dip} = \Delta V_{dip, \vec{p}} + \Delta V_{dip, \vec{p}'}$. It can be shown that the potential due to the image dipole ($\Delta V_{dip, \vec{p}'}$) is proportional to the potential due to the dipole ($\Delta V_{dip, \vec{p}}$) for any dipole location within the circular region (see Text S3.1) [Vanderlinde, 2004]:

$$\Delta V_{dip, \vec{p}'} = \frac{\epsilon_w - \epsilon_p}{\epsilon_w + \epsilon_p} \Delta V_{dip, \vec{p}}, \quad (3.3)$$

where ϵ_w is the permittivity of water, ϵ_p is the permittivity of a plastic wall. Thus, the differential voltage can be simplified to:

$$\Delta V_{dip} = \frac{2\epsilon_w}{\epsilon_w + \epsilon_p} \Delta V_{dip, \vec{p}} = \left[\kappa \left(\frac{\cos \theta_1}{r_1} - \frac{\cos \theta_2}{r_2} \right) \right], \quad (3.4)$$

where κ is a constant factor, $\vec{r}_1$ is the vector from the dipole location to the positive electrode, and θ_1 is the angle between the vectors $\vec{r}$ and $\vec{d}$. $\vec{r}_2$ and θ_2 are similarly defined for the negative electrode. In summary, the presence of the circular boundary simply rescales the differential voltage between a pair of electrodes. This scaling effect of the circular boundary does not affect the performance of our dipole localization algorithm, since it uses relative signal intensities between the recording channels.

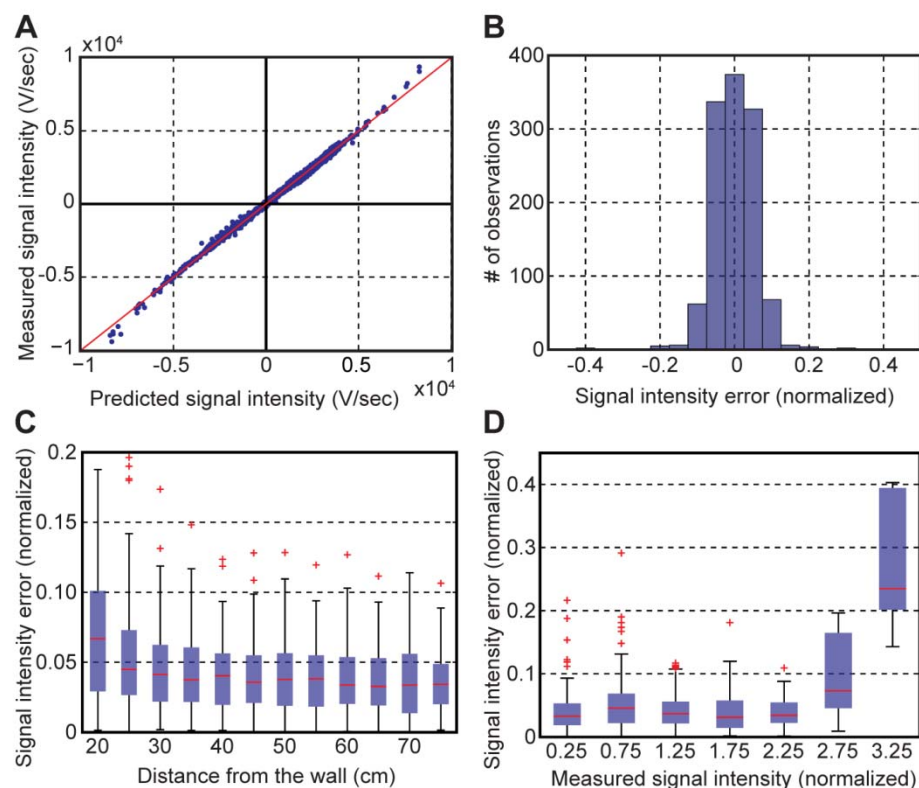


Figure 3.2. Experimental data fitted by the ideal dipole model. (A) The measured RSI values (slopes of differential voltages) closely agree ($\rho_{corr} = 0.9983$) with the RSI predicted by the ideal dipole model. (B) The error distribution of the RSI values normalized to the SD of the measurement averaged across the whole tank. The errors were computed by the measured values minus the predicted values. (C) The RSI error plotted as a function of the distance from the tank wall. The distance was measured to the center of the body. The edges of the boxes represent 25th and 75th percentiles, and the center mark (red line) is the median. Outliers are individually plotted in red. (D) The normalized RSI error as a function of the normalized measured RSI. The RSI values were each grouped within the range of ± 0.25 .

3.3.5 Verification of the 2D ideal dipole model with WEF measurements

In order to experimentally verify our 2D ideal dipole model predictions, we compared the values predicted by the model with differential voltage measurements from a restrained WEF. One fish (23 cm long) was held in a floating platform [Jun et al., 2012a] and positioned 5 cm below water, and the location and orientation of the platform was controlled using a guiding wire installed across the circular wall. We took measurements from four pairs of electrodes with 180° pairing angle at each 5 cm step of translating the animal along the guiding wire from -55 cm to 55 cm, and 15° step of rotating the animal with respect to the guiding wire from 0° to 180°. The position and the orientation of the fish were used to predict the RSI values using the 2D ideal dipole model, and the predicted RSI values were compared against the measured RSI values.

Our 2D ideal dipole model accurately predicted the measured RSI from the position and orientation of the restrained animal. There was a tight correlation ($\rho_{corr} = 0.9983$, $n = 1196$) and a closely linear relationship ($R^2 = 0.9966$) between the predicted vs. measured RSI (Fig. 3.2A). The scaling factor was determined by estimating a linear fit to the data. The RSI error was defined as a difference between the observed and the predicted RSI values, and normalized to the SD of the measurement (2706 V/s). The normalized absolute errors were 0.045 ± 0.038 (mean $\pm$ SD), and most of the errors fell within a narrow range (95% of errors less than 0.1) (Fig. 3.2B). The RSI errors increased when an animal was near the wall (Fig. 3.2C). The centre of the body was always used to measure the distance to the boundary. The greater errors near the boundary were expected from the near field effect, and from the distortion caused by the fish's skin conductivity. As shown in Figure 3.2D, the maximum RSI range (3.25 ± 0.25) had the greatest error, which typically occurred when the fish's head or tail were closest to one of the recording electrodes. In summary, the RSI predicted by the 2D ideal dipole model closely agreed with the actual physiological measurements from WEF at most locations.

3.3.6 Determining optimal electrode configuration by simulation

We simulated four types of electrode configurations and compared their dipole localization performances in the presence of simulated measurement noise. The four electrode configurations we tested varied in the number of channels, the pairing angle, and the geometric layout of the electrodes (see Fig. 3.3A). The three configurations had a circular geometry (1.5 m in diameter) similar to our experimental tank, and one configuration had a square grid layout (2 m in length, 0.5 m grid spacing) similar to the setup used by Henninger et al. [2012]. In our simulation, a dipole location and orientation were randomly assigned at 10 million points, and the RSIs were computed at the recording dipoles according to the 2D ideal dipole model for each electrode configuration. We then simulated the measurement noise by adding random Gaussian noise to the computed RSIs, and provided these values to the dipole localization algorithm. In order to test the effect of noise on the localization accuracy, we varied the noise intensity on a logarithmic scale and normalized the noise intensity to the median of all RSI values across the randomly chosen sampling points. The localization accuracy was quantified by comparing the algorithm-inferred dipole locations with the actual assigned locations.

The localization accuracy was significantly improved when the number of channels doubled from four (*4P90*) to eight (*8P67.5*) as expected from the higher signal-to-noise ratio (SNR) (Fig. 3.3B). While using only four channels, changing the pairing angle alone from 180° to 90° significantly improved the accuracy (Fig. 3.3B). Our algorithm also performed accurately for the square grid configuration (*8Pgrid*), which is suitable for field deployment due to the extendable grid layout [Henninger et al., 2012]. Based on our simulations, we implemented the eight-channel configuration (*8P67.5*) for our circular tank using 16 equally spaced perpendicular electrodes. In comparison, the average noise intensity of our actual measurement system was 0.47% of the SD of measured RSI values (averaged across the tank using all locations visited by fish), and the SNR was 46.5 dB.

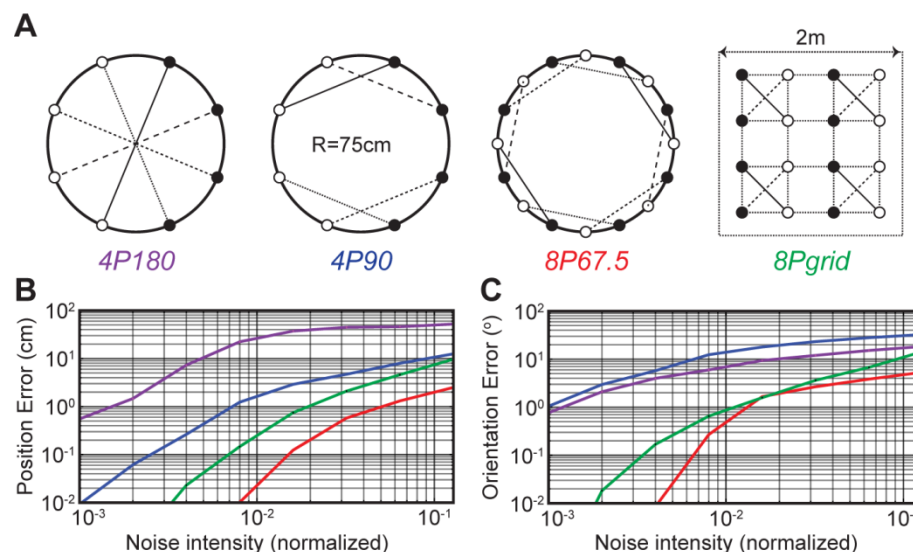


Figure 3.3. Comparison of the simulated noise performances of the four electrode configurations. (A) The four types of electrode configurations tested in simulations. Filled circles represent positive electrodes and open circles represent negative electrodes for the differential voltage measurements. Electrodes pairs are connected with lines. (B) The position errors plotted as a function of the simulated noise intensity (plotted on a log-log scale). The noise intensities were normalized to the SD of the measurement. (C) The orientation errors plotted as a function of the simulated noise intensity (plotted on a log-log scales).

3.3.7 Dipole localization algorithm based on LUT search

We developed an algorithm to deduce the position and orientation of a dipole from a given set of multi-electrode measurements using the 2D ideal dipole model. The measured values were compared with a list of predicted values at many possible locations and orientations of a dipole. The predicted voltages computed by the 2D ideal dipole model were stored in a look-up table (LUT), along with the dipole locations used to compute the predicted voltages. The LUT was constructed once and reused multiple times to increase the search speed. In order to cancel out the constant factor difference between the measured and the predicted RSI values, the RSI measurements from each channel were normalized by the sum of all channels. Thus the LUT stored normalized vectors containing the predicted RSIs at multiple channels. A dipole location was estimated from a given set of measurements by 1) searching for a predicted vector in the LUT having the smallest angle with the given measured vector (Fig. 3.1D), and 2) the dipole location corresponding to the closest matching vector was retrieved. The angle between the

predicted and the measured vectors was determined from their dot product; hence the minimum angle corresponded to the maximum dot product between two unit vectors:

$$\vec{x} \cdot \vec{y} = |\vec{x}| |\vec{y}| \cos \theta \Rightarrow \frac{\vec{x}}{|\vec{x}|} \cdot \frac{\vec{y}}{|\vec{y}|} = \hat{x} \cdot \hat{y} = \cos \theta \quad (3.5)$$

where $\vec{x}$ is the given measured vector, and $\hat{x}$ is the normalized vector of $\vec{x}$. $\vec{y}$ and $\hat{y}$ are similarly defined for the predicted vectors in the LUT. θ is the angle between $\vec{x}$ and $\vec{y}$. The dot products between the given measured vector and all predicted vectors in the LUT were efficiently computed using a matrix multiplication:

$$\vec{x}^T Y = \vec{x}^T [\vec{y}_1 \quad \vec{y}_2 \quad \cdots \quad \vec{y}_n] = [\vec{x} \cdot \vec{y}_1 \quad \vec{x} \cdot \vec{y}_2 \quad \cdots \quad \vec{x} \cdot \vec{y}_n], \quad (3.6)$$

where $\vec{x}$ is the given measured vector, Y is the matrix containing the predicted vectors ($\vec{y}_k$) in the LUT, and n is the number of entries in the LUT.

We tested various LUT search methods (Table 3.1) on the data measured from freely swimming WEF ($n = 11,593$). The dot-product based search was compared against the other widely used distance metrics between two vectors (Euclidean distance, city-block distance, etc.) in terms of the speed and accuracy. The dot product metric yielded the highest speed, while the tracking errors were similar between the different search methods tested.

Table 3.1. Comparison of the LUT search methods. We compared the performances of the five pairwise vector distance metrics used for the LUT search (90%: 90th percentile).

Search methods	speed	Position error (cm)			Orientation error (°)		
	(trials/sec)	mean	median	90%	mean	median	90%
Dot product	1068	3.3	1.4	5.0	4.5	4.6	11.0
Cityblock dist.	208	3.3	1.4	5.1	4.4	4.5	10.5
Euclidean dist.	153	3.3	1.4	5.0	4.5	4.6	11.0
Chevychev dist.	77.7	3.4	1.4	5.1	4.6	4.6	10.8
Correlation	44.5	3.5	1.5	5.2	5.2	4.9	12.0

3.3.8 Optimization of the dipole search algorithm

In order to implement a practical dipole tracking system, we improved our initial dipole search algorithm up to ~ 1000 localizations/s (running on Intel i7-2720QM 2.2 GHz CPU) by applying a series of computational optimizations. The spatial resolution is determined by the size of a LUT, and increasing the spatial and angular resolutions required larger memory and longer search time. In order to increase the search speed without compromising the spatial resolution, we implemented a two-step search procedure. The first step quickly approximated a dipole location in coarse grids using the LUT, and then the second step found a more accurate dipole location by conducting a search around the initial estimate in finer grids. The coarse grid spacing was set to 2 cm and 4° , and the fine grid spacing was set to 0.5 cm and 1° . We doubled the density of the coarse grid spacing near the wall (within 10 cm) in order to improve the localization accuracy near the boundary. The computational results from previous fine grid searches were stored up to 16 previous histories, and the stored results were reused when the coarse grid search returned the same coordinates as previously encountered. Both the positive and negative dot product ranges were used in order to simultaneously search the parallel and anti-parallel dipole orientations. Our dipole algorithm searched only the locations within the circular boundary, and excluded the cases when the head or tail ends lay outside of the boundary. All numbers were computed in the single-precision format instead of the double-precision format to save the computational time and memory requirements, but without compromising the localization accuracy. The LUT entries were partitioned by their strongest channel indices (Fig. 3.4A), such that only a matching region (one eighth of the LUT size) had to be searched by using the channel number having the absolute maximum RSI as a filtering criterion. However in the actual implementation, the search also included a region in the LUT where a given channel index was the second strongest, in order to account for possible order reversals induced by noise.

Figure 3.4B shows cumulative improvements in the search speed after applying each optimization step. The two-step search procedure yielded the highest speed gain, which achieved high localization accuracy without sacrificing speed and memory usage. Our final, optimized search algorithm produced estimates at a rate 20 times the actual EOD pulse rate (~ 50 Hz), thus became fast enough to track multiple electric fish in real-time. The LUT cache was built in 0.40 ± 0.01 s and occupied 30.2 MB of memory using the grid parameters settings

described previously. In summary, our optimized dipole tracking algorithm met our practical needs after improving the search speed by a factor of hundred. For the demonstration purpose, an example Matlab code, a sample dataset, and an instruction manual are available in the Supplementary Information (Supplementary Data 3.1, available at [plosone.org](https://doi.org/10.1371/journal.plosone.0180000)).

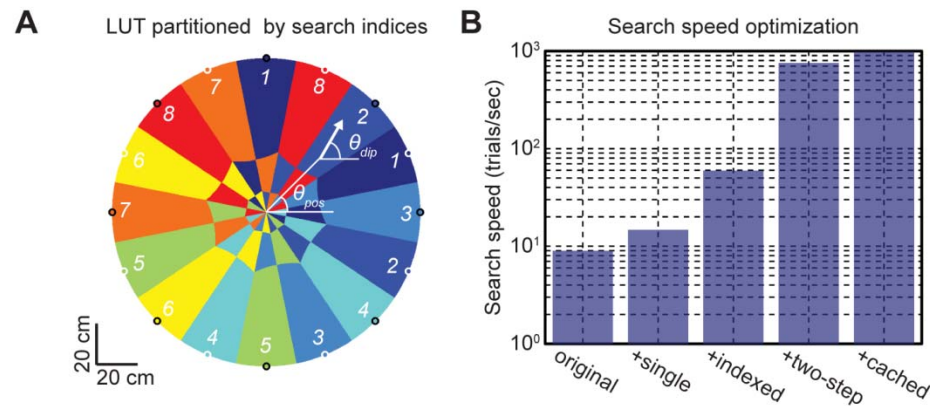


Figure 3.4. Optimization of the dipole search algorithm. (A) The circular tank is partitioned by the LUT indices, which are determined by the absolute maximum channels at each dipole location. In this illustration, the orientation of the dipole (θ_{dip}) was set equal to its angular position (θ_{pos}). The electrodes are shown as black (positive) or white (negative) circles, and their fill colors correspond to their channel indices. The channel numbers 1 to 8 correspond to the colors from blue to red as indicated. (B) Cumulative improvements in the search speed after successively applying the optimization techniques (*original*: single search step, *single*: single numerical precision, *indexed*: LUT indexed by the strongest channel, *two-step*: two-step search procedure, *cached*: fine-grid search was cached with $n_{history} = 16$).

3.3.9 Single fish and fish dyads tracking

Our dipole localization algorithm was then applied to every EOD pulse measurement from a single or two individuals, and their resulting trajectories were filtered to be smoothed. In the case of single fish tracking, we first applied a median filter ($n_{win} = 8$ for position, $n_{win} = 15$ for orientation) to exclude occasional outliers resulting from excess noise, and applied a triangular

filter ($n_{win} = 15$ for position, $n_{win} = 30$ for orientation) to smooth the traces. The orientation traces were unwrapped before applying a filter to prevent jump-associated artifacts. In the case of dyads tracking, we first separated the traces of two individuals before applying the filters. An EOD pulse was associated with an individual by using its previously identified location having the closest position and orientation. Occasionally, the EOD pulses from two individuals temporally overlapped and produced collided pulses; this resulted in a lower tracking accuracy, since the collided pulses poorly matched the RSIs predicted by the 2D ideal dipole model. Hence, the collided pulses were detected by using their dot-product values as an exclusion criterion, and values below 0.9 were removed from the tracked trajectory.

3.3.10 Visual tracking method

Our dipole tracking results were compared with automated visual tracking to quantify the accuracy of our dipole localization. We designed a visual tracking algorithm based on Windsor et al. [2008]. Since fish appeared darker than the background, the background image was subtracted from the recorded images to obtain isolated images of the fish. Binary images were generated by applying an intensity threshold, and the largest blob was chosen after removing speckles by using an image dilation operation. The centre of mass position and the orientation of the blob were found using the *regionprop* function provided by the Matlab image processing toolbox. The tail-bending angle was visually determined from the angle between two lines formed by three feature points at the head, the centre of mass, and the tail. The head and the tail points were determined at the two end points of the midline, which was extracted from the blob using an image skeletonization function [Telea and Wijk 2002]. We determined a correct head orientation by manually assigning the head orientation for the first frame, and the head orientations for the subsequent frames were automatically determined using the previously determined values. The fish's orientation returned by the *regionprop* function was compared with the corrected orientation from the previous frame, and the new orientation was flipped if the angular difference exceeded 90° . In the case of fish dyads tracking, we separately tracked each individual by initially defining a region of interest (ROI) around the fish being tracked, and the ROI locations were updated in subsequent frames by tracking the centre of mass. The ROI served to exclude the other fish's image when they were far apart. If the other fish's image partly appeared in the ROI, it was automatically removed by detecting blobs touching the ROI

boundary. In rare cases, the blobs of two fish merged when they made a direct contact, and we manually deleted the other fish's image by drawing a polygon mask.

3.3.11 Quantification of the dipole tracking accuracy

Our dipole localization accuracy was quantified by comparing the position and orientation of an estimated dipole location with the visual tracking. The position errors were quantified by measuring the distance from the estimated dipole position to the centre of mass point of the image. Similarly, the orientation errors were quantified by taking the absolute difference between the estimated dipole orientation and the orientation of the major elliptical axis from the image blob analysis. The position and orientation errors were quantified using the dipole locations estimated from the earlier part of the pulse phase (225 μ s before the reference timing), when the current source is mostly concentrated at the centre of the body. The dipole locations estimated from each EOD pulse were smoothed and resampled at the image capture times (15 FPS). The number of samples for the dipole tracking error statistics (n) corresponds to the number of image frames used to compute the error. The tail-bending angle errors were quantified by taking the difference between the values determined from the two estimated dipole orientation (the central and the tail regions), and the values determined from the image analysis.

3.4 Results

We applied our dipole localization algorithm to track freely swimming electric fish (20~24 cm in length) in a shallow and circular body of water (10 ± 2 cm depth, 1.5 m diameter). The dipole tracking accuracy was validated by an automated visual tracking protocol, and the positions and orientations determined by the two methods closely agreed (90% within 5cm or 13°) under various test conditions. Our dipole tracking method was accurate and reliable at locations near the tank boundary or an object, and the error remained small when fish bent its tail during turning movements. Our method can therefore be used to estimate the distance between any part of the fish (*e.g.* its head) and an object (*e.g.* a landmark or prey). The tail-bending angles were determined by comparing the orientations of two dipoles at the central and tail regions (see Methods), and they correlated well ($\rho_{corr} = 0.7631$) with the visual observations. Our method was applied to track a fish pair or "dyads", and the localization error remained similar to that for single fish tracking. EOD pulses produced by different individuals

were identified by their dipole source locations, such that the individual identities could be reliably associated with their tracking. Our method can therefore also serve to relate the distances between two fish (*e.g.* between heads, tails, or head and tail) and the electric communication signals they emit [Hupé, 2012].

3.4.1 Single fish tracking accuracy

We applied our dipole-tracking algorithm to track single freely swimming fish in a shallow and featureless tank, and the tracking accuracy was quantified using a visual tracking algorithm (see Methods). Figure 3.5A compares the tracking errors between the four (*4P90*) and the eight channel (*8P67.5*) configurations. The average errors of the eight-channel configuration (2.5 ± 1.2 cm, $5.0 \pm 4.8^\circ$, $n = 10^4$) were significantly lower than the four-channel case (9.4 ± 9.1 cm, $15.7 \pm 14.8^\circ$, $n = 10^4$) as expected from the simulations (Fig. 3.5A, Table 3.2). The position and orientation errors were significantly reduced after excluding a channel if its electrode was within a set exclusion distance from the fish's centre of the body (Fig. 3.5B). The position and orientation errors were minimized at the exclusion distance of 13 cm. The tracking accuracy degraded as animals approached the tank wall (Fig. 3.5C). 90% of all errors fell within 5.3 cm or 13.1° ($n_{\text{samples}} = 2 \times 10^4$, $n_{\text{animals}} = 2$) at all locations (Table 3.2). Figure 3.4D compares the error distributions between the locations near (< 10 cm) and far (≥ 10 cm) from the wall; each fish was observed for 666.7 s to compute this distribution. The average tracking errors (mean $\pm$ SD) at the near locations were 1.4 cm and 3.7° higher than the errors at the far locations (2.2 ± 1.1 cm, $2.7 \pm 2.4^\circ$, $n = 1802$). A short video of a single fish tracking is available in the Supplementary Information (Supplementary Video 3.1, available from *plosone.org*). In summary, our dipole tracking algorithm could accurately track single freely swimming electric fish at most locations within the tank using only eight pairs of recording electrodes, with the electrodes in each pair being at an angle of 67.5° .

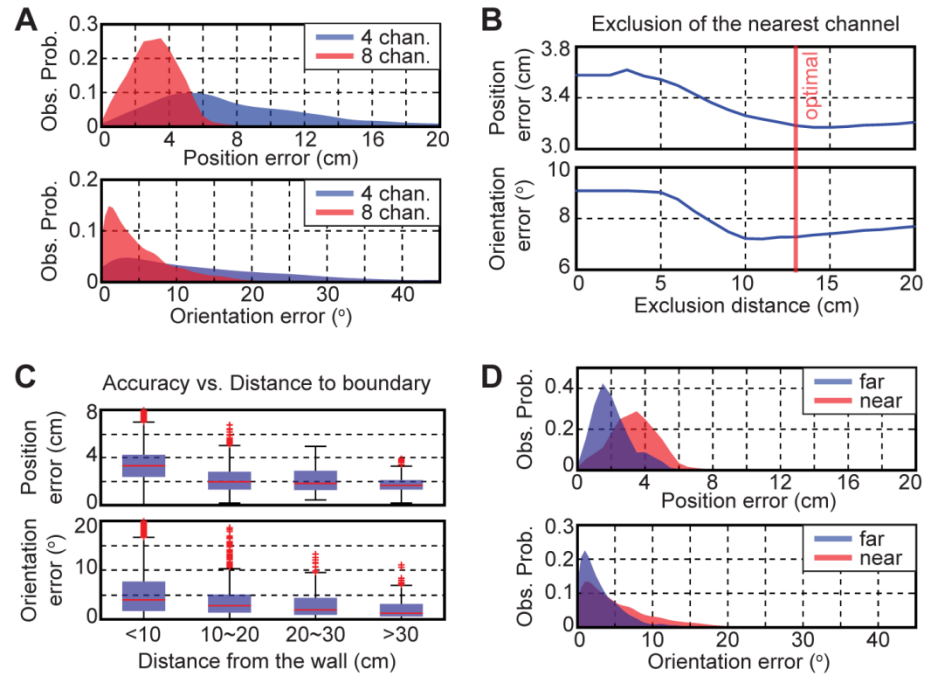


Figure 3.5. Single fish tracking accuracy. (A) The error distribution of the dipole tracking for the four (blue) and eight (red) channel configurations (*Obs. Prob.*: Observation probability). (B) The tracking accuracy improved after excluding the channel nearest to the fish within a set exclusion distance. (C) The tracking errors were plotted vs. the distance from the tank wall. On each box, the central mark (red line) is the median, the edges of the box are the 25th and 75th percentiles, and the whiskers extend to the most extreme data points not considered outliers. Outliers are plotted individually (red markers): a value that is more than 1.5 times the interquartile range away from the top or bottom of the box as determined using the *boxplot* function (Statistics toolbox for Matlab). (D) The error distributions of the dipole tracking near (< 10 cm, red) and far (> 10 cm, blue) from the tank wall.

Table 3.2. Summary of the tracking accuracy under different test conditions. The dipole tracking errors under different test conditions are summarized in this table.

Test			Position Error (cm)			Orientation Error (°)		
Category	conditions	n	mean	SD	90%	mean	SD	90%
Boundary Dist.	far (≥ 10 cm)	1802	2.2	1.1	3.7	2.7	2.4	5.8
	(pooled) near (< 10 cm)	17995	3.6	1.4	5.4	6.4	6.1	13.5
Object Dist.	far (≥ 20 cm)	172	1.4	0.8	2.6	2.7	2.4	5.9
	(larger fish) near (< 20 cm)	110	3.0	1.4	4.6	3.7	3.8	9.4
Tail Bending	small ($< 10^\circ$)	1802	2.2	1.1	3.7	2.7	2.4	5.8
	(pooled) large ($\geq 10^\circ$)	203	2.5	1.0	3.8	7.0	4.5	13.1
Social tracking	single	20000	3.5	1.5	5.3	6.0	5.9	13.1
	(pooled) dyad	20000	3.6	1.4	5.4	6.0	6.6	12.9
No. Channels	4 chan.	10000	9.4	9.1	17.1	15.7	14.8	34.8
	(larger fish) 8 chan.	10000	2.5	1.2	4.1	5.0	4.8	11.5
Fish size	Large (31 g)	1377	2.1	1.1	3.8	3.1	3.0	6.9
	(far from wall) Small (20 g)	628	2.5	0.9	3.7	3.2	2.9	7.1
Fish size	Large (31 g)	10000	3.2	1.4	5.0	5.1	5.0	11.7
	(all locations) Small (20 g)	10000	3.8	1.4	5.6	6.9	6.6	14.2

*1: Data pooled from all animals; *2: Data from larger fish; *3: Far from the wall (>10 cm);
 *4: All locations

3.4.2 Effects of an object

We studied effects of a dielectric object on the dipole localization accuracy by placing a cylindrical plastic object (10 cm diameter, 15 cm height) at different locations (Fig. 3.6A). In theory, the object placed in water will distort the electric field and increase differences between the measured and predicted values; but according to our test, the localization errors were not affected by the object placed at three different locations (Fig. 3.6B). Figure 3.6C illustrates trajectories of a fish when it closely passed by the object, and the false colors represent the position error. The fish images were superimposed every 1 s interval, and a movie version of Figure 3.6C is available in the Supplementary Information (Supplementary Video 3.2, available from *plosone.org*). The electrically tracked traces (color-coded) closely agreed (< 5 cm) with the visually tracked traces (shown in grey) near the object. Figure 3.6D shows the error distributions when the fish was near (< 20 cm) or far (> 20 cm) from the object's surface while the object was placed at the centre of the tank. In order to examine the effect of an object alone, the locations near the wall (< 10 cm) and when the tail-bending angles exceeded 10° were excluded from our analysis. The mean tracking errors when the fish was near the object

were 3.0 ± 1.4 cm and $3.7 \pm 3.8^\circ$ (90% within 4.6 cm or 9.4° , $n = 110$); and the tracking errors when the fish was far from the object were 1.4 ± 0.8 cm and $2.7 \pm 2.4^\circ$ (90% within 2.6 cm or 5.9° , $n = 172$) (Table 3.2). In summary, the presence of an object in water did not significantly decrease the dipole tracking accuracy even when the fish closely passed by the object. Our method permits us to determine the EOD pulse rate as a function of the distance from a fish to an object, which will be relevant for studies such as Pereira et al. [2012] and Hofmann et al. [2012].

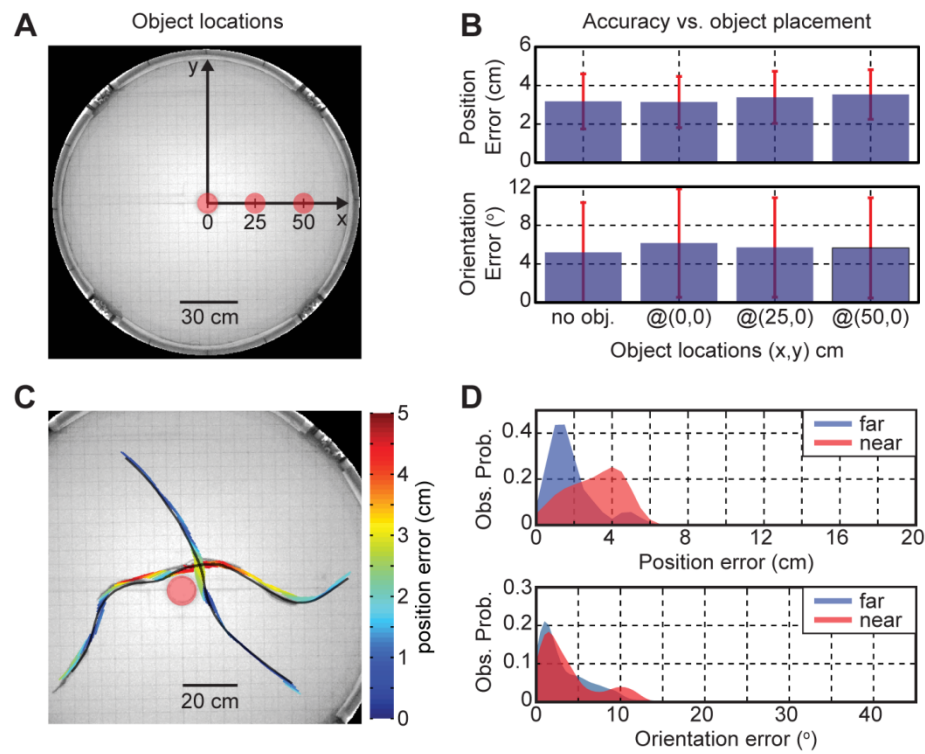


Figure 3.6. Effects of an object on the dipole tracking accuracy. (A) The three object locations tested are marked in red circles, and our coordinates system is shown. (B) The dipole tracking errors for the three object locations and the control (*no obj.*: no object placed) averaged over all locations visited by fish for 666.7 s. (C) A false color representation of the dipole tracking errors when fish passes by an object. The visually determined traces are shown in grey, and the object is marked as a red circle. (D) The error distributions of the dipole tracking near (< 20 cm, red) and far (> 20 cm, blue) from the object's surface.

3.4.3 Effects of tail bending

We quantified the dipole tracking errors during fish's tail-bending behaviour using visual recordings. The tail-bending angle was determined using three feature points (centre of mass, head, and tail ends) from an image; and it was also electrically determined from two dipole orientations at the central and the tail regions (Fig. 3.7A, see Methods). Figure 3.7B shows a linear correlation between the electrically and the visually determined tail-bending angles ($\rho_{corr} = 0.7631$, $n_{samples} = 2005$, $n_{animals} = 2$). Locations near the wall (< 10 cm) were excluded from our analysis due to inaccurate visual tracking near the wall. The absolute tail-bending error was $3.7 \pm 3.9^\circ$, and 90% of all errors were within 7.9° . The orientation error increased linearly with the tail-bending angle, but the position error was not significantly affected by the tail bending (Fig. 3.7C). Figure 3.7D compares the distributions of the tracking errors during small ($< 10^\circ$) versus large ($> 10^\circ$) tail-bending bouts. The mean orientation error increased by 4.3° during the large tail-bending bouts relative to the small tail-bending bouts, while the mean position error increased only by 0.3 cm. In summary, the tail-bending angle could be determined from the electrical measurements alone, and the dipole tracking errors remained reasonably small during large tail bending (90% of errors within 5.8 cm or 13.8°). It has been hypothesized that the tail bending may be used by WEF for active sensing of objects in their surroundings [Heiligenberg, 1975; Sicardi et al., 2000; Sim and Kim, 2011; Hofmann et al., 2012]; our method will thus permit direct tests of this hypothesis.

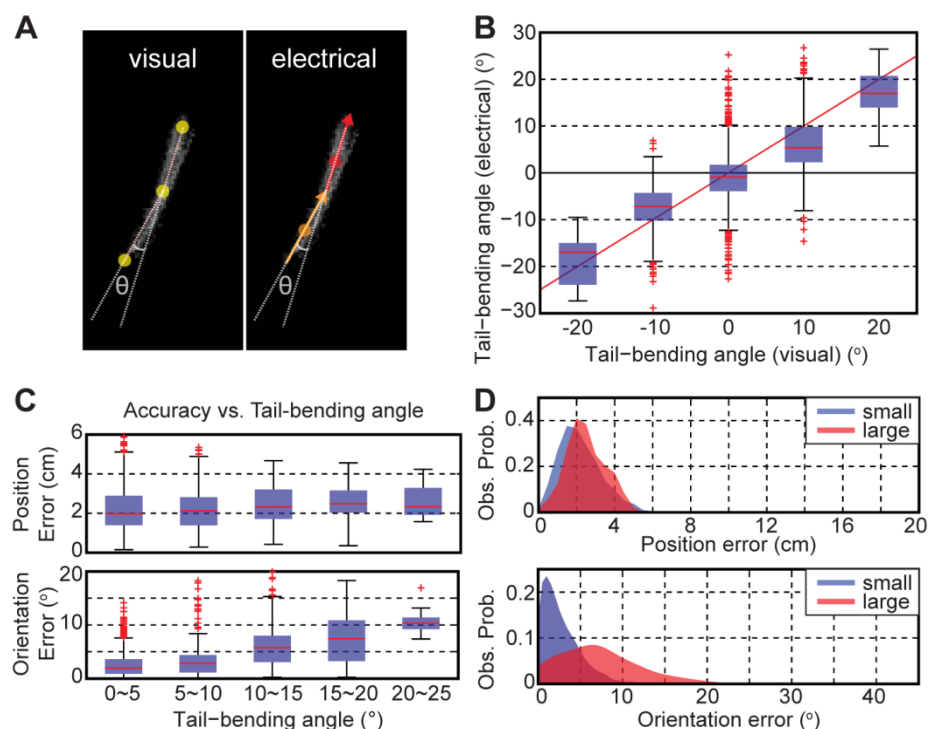


Figure 3.7. Effects of tail bending on the dipole tracking accuracy. (A) Tail-bending angles were visually determined (left) using three feature points (head, tail, and center of the body), and also electrically determined using two dipoles at the central and one in the tail regions (right). (B) The electrically determined tail-bending angles correlated well ($\rho_{corr} = 0.7631$) with the visually determined values. (C) The dipole tracking errors plotted as a function of the tail-bending angles. (D) The error distributions of the dipole tracking for the small ($< 10^\circ$) and large ($> 10^\circ$) tail-bending angles. The locations near the tank wall (< 10 cm) were excluded from our analysis to remove the near-field effect.

3.4.4 Fish dyads tracking

Our dipole tracking system was adapted to track fish dyads both accurately and reliably. First, the dipole localization was carried out on mixed EOD pulses, and the tracked locations were individually identified on the basis of the nearest neighbors (see Methods). The EOD pulses occasionally collided when two fish emitted EOD pulses nearly simultaneously. Nevertheless, the LUT matching scores (dot product values) permitted detection and removal of the collided pulses from the traces (see the outliers of the traces in Fig. 3.8A). The dipole tracking errors of each individual were compared between the single and the dyads tracking (Fig. 3.8B), and the tracking errors were similar in both cases. The larger fish (animal #1, 24.0 ± 0.5 cm, 31 ± 1 g) had 0.6 cm and 1.8° less mean tracking errors than the smaller fish (animal #2, 20.5 ± 0.5 cm, 20 ± 1 g), since the visual tracking accuracy was higher for the larger fish near the wall in particular. Figure 3.8C illustrates the tracking errors during a close encounter between two individuals, and the electrically tracked traces (color-coded) closely agreed (< 5 cm) with the visually tracked traces. The fish images were superimposed every 1 s interval, and the larger fish is shown darker. A movie version of Figure 3.8C is available in the Supplementary Information (Supplementary Video 3.3, available at *plosone.org*). The distributions of the tracking errors when two fish were closer (< 40 cm) or further apart (> 40 cm) are shown in Figure 3.8D. The mean tracking errors when the fish dyads were near were 3.0 ± 1.4 cm and $6.3 \pm 7.6^\circ$ (90% within 4.4 cm or 16.7° , $n = 186$); and the errors when they were far apart were 2.2 ± 0.9 cm and $3.2 \pm 2.4^\circ$ (90% within 3.3 cm or 6.6° , $n = 142$). In summary, our dipole tracking method could simultaneously track fish dyads accurately even during close encounters. Since we time each EOD pulse of each fish to estimate their locations, our method permits determination of communication-associated EOD pulse patterning as a function of distance between the fish, which will be relevant to studies such as Hupé et al. [2012] and Yu et al. [2012].

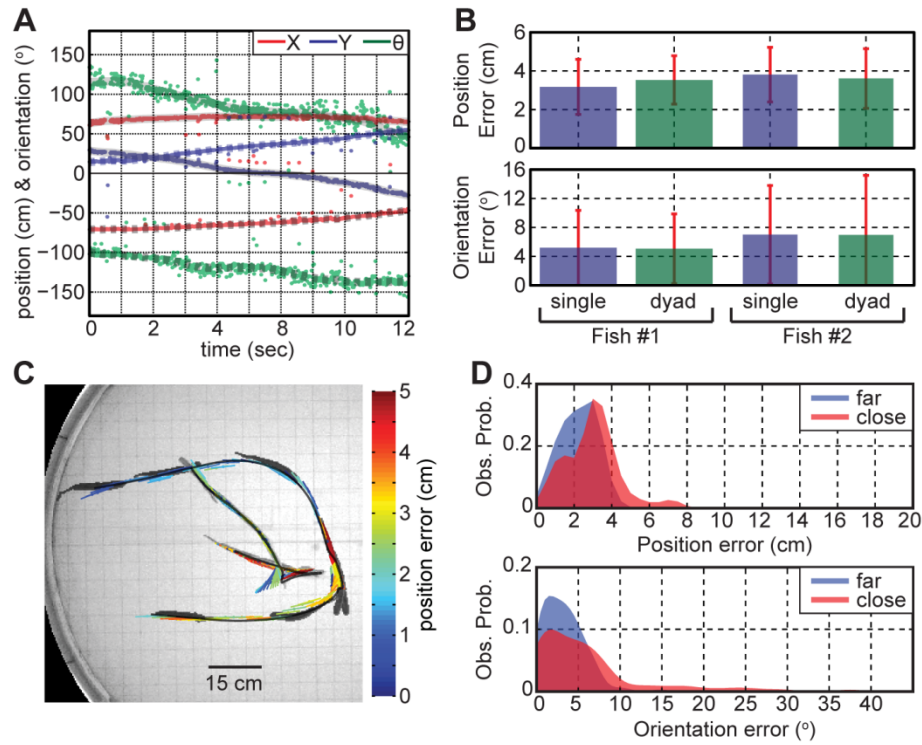


Figure 3.8. Fish dyads tracking accuracy. (A) The traces of two fish were separated after the dipole localization. (B) Comparison of the tracking errors between the single and dyads tracking for each fish. (C) A false color representation of the tracking errors during close encounter between animals. Fish images were superimposed every 1 s interval, and the larger fish is shown darker (Fish #1). (D) The error distributions of the dipole tracking at a close (< 40 cm) and far (> 40 cm) distance between fish. The distances were measured to the center of the body, and the locations near the tank wall (< 10 cm) were excluded from our analysis to remove the near-field effect.

3.5 Discussion

3.5.1 Significance of our paper

In this paper, we introduced a practical real-time electrical tracking system to locate relatively slowly moving dipole sources in a shallow homogeneous medium. Our dipole tracking method was originally developed to study naturalistic animal behaviors where visual observation is difficult to quantify due to the presence of objects or other animals. Our unique approach to the inverse problem was to solve forward problems at many possible locations, and to find a matching solution from a LUT constructed from the ideal dipole model. The forward problems were solved once, stored, and reused multiple times to increase the subsequent search speed. The dipole search operation could be performed in parallel by multiple processors or GPU if the LUT size becomes larger, or when faster search speed is required. In our studies, we tracked three location parameters (x , y , θ) in 2D, but the number of location parameters could be increased to locate dipoles in 3D. The 3D tracking requires at least five location parameters (x , y , z , $\theta_{azimuth}$, $\theta_{elevation}$) to be determined, thus this would require a greater number of recording channels and computational time. Unlike some other electrical tracking methods, our dipole tracking method does not require prior individual specific calibrations, since the dipole search algorithm relies on the relative RSI values between channels. This calibration-free approach of our tracking method would be particularly useful for a field recording setting, where a prior individual-specific calibration is difficult or impossible [Henninger et al., 2012]. Although we used a circular tank, a tank shape is not critical to achieve high tracking accuracy as long as all electrodes are positioned on the tank wall. In the case of the square grid layout, high tracking accuracy could be achieved if the electrodes are sufficiently away from the boundary. The tracking accuracy did not noticeably increase when an object was placed in the tank, but placing too many or too large objects would deteriorate the tracking accuracy, more so if the conductivity differences with the surrounding water were large.

3.5.2 Limitations of visual tracking

Visual tracking provides direct measurements of animal's location, and it is the most widely used method of tracking animals. Automated visual tracking relies on computer vision algorithms to infer an animal's location, but visual algorithms react sensitively to changes in lighting conditions of the environment such as shadows, reflections, and glares [Shapiro and

Stockman, 2001]. Imaging through water introduces additional challenges to visual tracking algorithms such as: glares produced on the water surface when light illumination is projected from the top, ripples triggered by animal's movement, absorption of the infrared spectrum while observing nocturnal animals, and animals' reflections on tank walls. To address these issues, homogenous light illumination could be projected from below a transparent tank to produce high contrast images [Windsor et al., 2008]. However, this solution requires an aquarium to be raised above a light source; thus it is inadequate for a very large aquarium, or if heating is required from underneath the aquarium for tropical fish. Determining an animal's posture such as a tail-bending angle could become a challenge when imaging a small animal in a large environment, due to the limited number of pixels available from an animal. Multiple cameras could be used to track animals in a large or a visually complex environment, but image frames must be synchronized and perspective angles of all cameras need to be calibrated to combine images from multiple cameras. Video recordings generally produce large amounts of data, thus recording durations are limited by available data storage.

Quantitative study of social interactions is an active area of study in behavioral biology, and the spatial aspect of social interactions provides valuable insights into animal communication [Hupé and Lewis, 2008]. Automated visual tracking could be applied to study multiple animals; but the visual algorithm must handle special cases such as when two animals overlap, and still maintain reliable individual identification. Visual markers [Spruijt et al., 1992; Winberg et al., 1993; Vatine et al., 1998] or naturally occurring stripe patterns [Arganda et al., 2012] could be used to identify different individuals. However, fluorescent tags may not be visible under infrared illumination or invasive to use in animals, and the stripe patterns may be absent in some species. A model-based visual tracking method was proposed by Fontaine et al. [2008] to dissociate closely interacting *c. elegans* and zebrafish. Also, mesh models were fitted to images of closely interacting animals by maximizing statistical likelihood using past movement trajectories (Kalman filter); but this tracking method requires high-resolution images of animals and is more computationally demanding.

3.5.3 Advantages of electrical tracking

Electrical tracking of WEF may be more appropriate in circumstances where visual tracking offers limited reliability or accuracy. One of the most significant differences between visual and electrical tracking is that the latter does not require the line of sight between a source and a detector. In naturalistic settings, animals generally prefer to hide, or they often contact other animals during social interactions. In such cases, electrical tracking could provide high reliability and accuracy. In field studies, radar telemetry is widely used to track insects carrying passive radio tags, which reflect microwave signals at their characteristic frequencies [Mascanzoni and Wallin, 1986; O'Neal et al., 2004]. More recently, RFID tags were used to study social behaviors of multiple rats in an enriched environment [Lewejohann et al., 2009; Vasserman, 2012]. However, radar telemetry offers limited detection range in an aquatic environment since water attenuates the radio frequency (RF) spectrum, especially when the conductivity of the surrounding water is high. Also, radio tags can impede with an animals' mobility, or may require invasive surgical implantation of tags.

In contrast, our dipole tracking method makes use of an animal's self-generated electrical signal, thus no tracking tags are required to track or discriminate between individuals. Our method is practically suited for an aquatic environment since the animal's electric field can be detected using a simple setup of inexpensive electrodes, and the lower frequency band (< 10 KHz) of physiological signals travel further in water than the RF spectrum used in radio telemetry. The detection range or accuracy could be enhanced by adding more recording dipole electrodes. It is much simpler to combine signals from multiple electrodes than combining 2D images from multiple cameras. Due to lower data storage demand, long-term behavioral monitoring becomes possible for capturing rarely occurring behaviors. Furthermore, poor visibility conditions in murky water do not affect our electrical tracking accuracy. Our method can also locate an animal obstructed by a shelter, as long as the shelter is electrically transparent such as a porous clay pot or plastic tubing with holes.

3.5.4 Areas to improve

The accuracy of our dipole localization decreased near the tank wall or during the tail bending bouts. When fish was very close to the tank wall, it occasionally contacted one of the recording electrodes and caused amplifier saturation. These occasional amplifier saturations compromised the dipole tracking accuracy near the wall. To improve the accuracy near the wall, the dipole model of the WEF could include the effects of the physically distributed current sources [Chen et al., 2005; Kelly et al., 2008]. This model could explain the near field effect better, but a calibration of the current source distribution would be required [Kelly et al., 2008] for each individual from the visual and electrical measurements. During our dipole tracking, larger tail-bending bouts increased the orientation error because the visual tracking weighs the orientation of the head region more due to its larger area, whereas our dipole tracking weighs more toward the central region (Fig. 3.7B). In order to quantify the dipole tracking errors more accurately, the visual tracking could be performed using infrared-reflective tags. These tags could be sutured or glued on fish's body to serve as visual markers for precisely measuring the position, orientation, and the tail-bending angle of the fish. The reflective markers would produce high contrast images and improve the visual tracking accuracy near the tank wall in particular, where shadows and reflections often decrease the tracking accuracy.

Combining the visual and the electrical tracking. In some cases, the EOD production of WEF is interrupted during social interactions or after a delivery of predatory stimuli [Schuster, 2000; Schuster, 2002]. In *Gymnotus* species, the EOD can pause for several seconds during social interactions, or for up to a few minutes after threatening (predatory) stimuli. Our dipole tracking requires a continuous EOD production, but a WEF may drift away without emitting an EOD. The trajectory during a short EOD interruption may be reconstructed by extrapolating and joining the trajectories before and after the pause. WEF tend to be stationary during longer EOD pauses, but if they move, the movement trajectory could be determined from a concurrent video recording. Combination of concurrent video and electrical recordings will complement the reliability and accuracy of each tracking method. Our dipole tracking can separate different individuals during close social encounters, or locate animals when obstructed from view more reliably than the visual tracking. The dipole tracking could increase the visual tracking speed

if it was is used to define the region of interest in images, or it could direct the viewing angle of a camera in real time if a motorized control is available for monitoring a large area.

Tracking wave-type species. In contrast to the pulse-type species we studied, wave-type species generate their EOD in a continuous and sinusoidal manner. It would require only small modifications to track a single wave-type species. First, the EOD timing reference would need to be determined from the envelope of the rectified and summed waveforms, and the slopes at each channel would be measured at some optimal time separation from the reference timing. The waveform polarity could be determined from the rectified waveforms by using the asymmetry between the positive and negative phases of the waveform. In the case of social tracking, the signal source separation between different individuals of the wave-type species would be difficult in the time domain because the EODs from different individuals constantly interfere. Furthermore, the wave-type species exhibit jamming avoidance responses (JAR) in social settings by shifting their EOD frequencies [Bullock et al., 1972; Bastian and Heiligenberg, 1980]. Therefore, the frequency domain would offer better signal separation between different individuals [Henninger et al., 2012], and the RSIs could be measured after the signal separation for each individual.

3.5.5 Possible future applications

Field studies and 3D tracking. Field studies can offer important new insights, since animals may exhibit different or novel behaviors in their natural settings. Using our dipole tracking method, data already collected from the field [Henninger et al., 2012] could be re-analyzed to determine animals' locations and movement trajectories. This spatial quantification could uncover individual foraging patterns, or social dynamics such as group sizes in their natural environments [Stamper et al., 2010]. Since depth of water is difficult to control in field studies, our two-dimensional (2D) tracking developed for shallow water may need to be modified to include the vertical dimension. The 3D tracking may become necessary if the water depth becomes much greater than the height of fish, or when fish's vertical motion is important for a study (e.g. air gulping or barrel rolls displays) [Nanjappa et al., 2000]. The vertical profile electrodes used in our 2D tracking would not be optimal for the 3D tracking, but instead, a planar grid layout (Fig. 3.3A) would be more appropriate [Henninger et al., 2012]. Similar to the 2D tracking, the 3D tracking would require the LUT to be constructed for each x, y, z

positions and the azimuth and elevation angles. The elevation angle may not be critical for the 3D tracking although changes in the elevation angle lead to the changes in the dipole moment. Our tracking method is not affected by the changes in the absolute dipole moment since it uses the relative, normalized RSIs. The 3D visual tracking was performed in a lab setting by placing one camera above, and another camera in front of a transparent tank for studying prey capture behaviors in WEF [MacIver and Nelson, 2000]. However, this method may be difficult to apply in field settings mainly due to positioning of cameras and illumination sources underwater. In contrast, the 3D dipole tracking would be much simpler to deploy in the field settings for studying the behaviors of WEF in their natural habitats.

EOD amplitude measurement. Changes in EOD amplitude during circadian rhythm or during social encounters were reported by Markham et al. [2009] in a different electric fish species (*Sternopygus macrurus*). In principle, our method could measure the EOD amplitude of freely swimming fish by comparing the ratio of the observed RSIs to the predicted RSIs in order to determine the dipole moment strength. To ensure close match between the predicted and the measured values, the RSI measurements need to be taken when a fish is sufficiently away from the electrodes, and when the tail-bending angle is small. In the studies conducted by Markham et al. [2009], the EOD amplitude measurements were taken when a fish passed through a narrow tube situated between two compartments. Although their method allowed the EOD measurements from unrestrained fish over an extended period of time, the animals were required to spontaneously swim through the measurement tube. In comparison, our dipole tracking method would allow constant monitoring of the EOD amplitudes without requiring the animals to swim through the measurement tube.

The contents below are available at plosone.org

<http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0066596#s5>

Supplementary Video 3.1. This video illustrates dipole tracking results superimposed with an infrared video recording for a single WEF freely swimming in a shallow circular tank.

Supplementary Video 3.2. This video illustrates dipole tracking results superimposed with an infrared video recording, while a single WEF closely swims by a dielectric object placed at the center.

Supplementary Video 3.3. This video illustrates dipole tracking results superimposed with an infrared video recording for WEF dyads during their close encounter.

Supplementary Data 3.1. This dataset includes a software demonstration package for the dipole tracking with a sample dataset and an instruction manual.

3.6 Supplementary information and validity of the 2D ideal dipole approximation in shallow water

In the section 3, we used the two-dimensional ideal dipole formula to predict the voltage difference between a pair of electrodes. The 2D ideal dipole formula was derived by assuming an infinite 2D space without boundaries. In this text, we will derive a more realistic ideal dipole formula by including the depth dimension and the boundary conditions to show that the 3D ideal dipole formula reduces to the 2D ideal dipole formula for a shallow, circular body of water. Our analysis will include the effects of the boundaries and the finite electrode dimension, which influence the voltage difference between a pair of vertically oriented electrodes in the presence of a dipole. The dipole is situated in the circular body of water of a radius R and depth d ; and the water is surrounded by the top, bottom, and side boundaries.

3.6.1 Effects of the top and bottom interfaces

First, let us consider the effects of the top and bottom interfaces on the electric fields, and assume an infinitely wide body of water of a depth d . The current dipole induces surface charges on the two dielectric interfaces, one on the top between the water and the air, and another at the bottom between the water and the glass. The induced surface charges contribute to the electrode potential, but it is difficult to directly determine the surface charge distributions. The method of image charges simplifies our analysis to determine the electrode potential, by replacing the surface charges with image current sources on the opposite side of each boundary [Zahn, 1976; Kumar and Nagabhushana, 1997]. Each dielectric boundary acts as a mirror surface to generate an image source I' on the opposite side of the boundary at an equal distance to the surface, having a magnitude:

$$I' = \underbrace{\left(\frac{\epsilon_w - \epsilon_k}{\epsilon_w + \epsilon_k} \right)}_{\equiv \alpha_k} I = \alpha_k I, \quad (\text{S3.1})$$

where ϵ_w is the permittivity of water, ϵ_k is the permittivity of the neighboring media (air or glass), and α is the attenuation factor. Note that the image source expression does not depend on the conductivity of the media to calculate the electric field inside of the circular region [Kumar and Nagabhushana, 1997]. In our problem, the two parallel surfaces at the top and at the bottom create an infinite number of image sources by creating an infinite number of reflections. Each reflection by a surface k creates a new image source with an attenuation factor α_k . For instance, the first order reflection creates two image sources: I_0 above the top interface at $z_0 = 2d - h$, and I_l below the bottom interface at $z_l = -h$ (see Fig. S3.1A).

$$I_0 = \underbrace{\frac{\epsilon_w - \epsilon_0}{\epsilon_w + \epsilon_0}}_{\equiv \alpha_0} I = \alpha_0 I, \quad I_l = \underbrace{\frac{\epsilon_w - \epsilon_1}{\epsilon_w + \epsilon_1}}_{\equiv \alpha_1} I = \alpha_1 I. \quad (\text{S3.2})$$

where ϵ_0 is the permittivity of air, and ϵ_1 is the permittivity of glass. Similarly, the second order reflections create image sources of I_0 and I_l . For example, the bottom surface creates a second order image source I_{0l} from the first order image source I_l at a height of z_{0l} , where:

$$\begin{cases} I_{01} = \alpha_1 I_0 = \alpha_1 (\alpha_0 I) = \alpha_0 \alpha_1 I, \\ z_{01} = -z_0 = h - 2d. \end{cases} \quad (\text{S3.3})$$

We can describe the reflection operations for each surface recursively. The reflection operations for the top interface (air-water interface, $k=0$) are:

$$\begin{cases} I_{\{k\}0} = f_0(I_k) = \alpha_0 I_k, \\ z_{\{k\}0} = g_0(z_k) = 2d - z_k. \end{cases} \quad (\text{S3.4})$$

Note that the top surface will always reflect an image charge located below the bottom surface ($z_k < 0$), such that the distance to the new image source ($z_{\{k\}0}$) from the original current source will always increase: $|z_{\{k\}0}| > |2d - z_k|$. The reflection operations for the bottom interface (glass-water interface, $k=1$) are:

$$\begin{cases} I_{\{k\}1} = f_1(I_k) = \alpha_1 I_k, \\ z_{\{k\}1} = g_1(z_k) = -z_k. \end{cases} \quad (\text{S3.5})$$

Now, we can express the potential at a field location $\vec{r}$ due to an image source I_k :

$$V(\vec{r} | I_k) = -\frac{cI_k}{|\vec{r} - \vec{r}_k|} = -\frac{cI_k}{\sqrt{r^2 + (z - z_k)^2}}, \quad (\text{S3.6})$$

where $c = (4\pi\sigma_w)^{-1}$ is the constant of proportionality, and σ_w is the conductivity of water.

The net potential at $\vec{r}$ due to the current source I , and its first order reflections is:

$$\begin{aligned} V_{net}(\vec{r}) &= V(\vec{r} | I) + V(\vec{r} | I_0) + V(\vec{r} | I_1), \\ &= -\frac{cI}{|\vec{r} - \vec{r}_I|} - \frac{cI_0}{|\vec{r} - \vec{r}_{I_0}|} - \frac{cI_1}{|\vec{r} - \vec{r}_{I_1}|}, \\ &= -cI \left[\left(r^2 + \left(z - \frac{d}{2} \right)^2 \right)^{-1/2} + \alpha_0 \left(r^2 + \left(z_0 - \frac{d}{2} \right)^2 \right)^{-1/2} + \alpha_1 \left(r^2 + \left(z_1 - \frac{d}{2} \right)^2 \right)^{-1/2} \right]. \end{aligned} \quad (\text{S3.7})$$

The net potential up to the second order reflections is:

$$V_{net}(\vec{r}) = V(\vec{r} | I) + \underbrace{\left[V(\vec{r} | I_0) + V(\vec{r} | I_1) \right]}_{\text{first order reflections}} + \underbrace{\left[V(\vec{r} | I_{01}) + V(\vec{r} | I_{10}) \right]}_{\text{second order reflections}}. \quad (\text{S3.8})$$

Figure S3.1A shows the net potential calculated up to the n^{th} reflection as a function of n . Our numerical calculation indicates that the net potential converges. The convergence of the potential is expected since each reflection produces a weaker image source at a further location from the electrode.

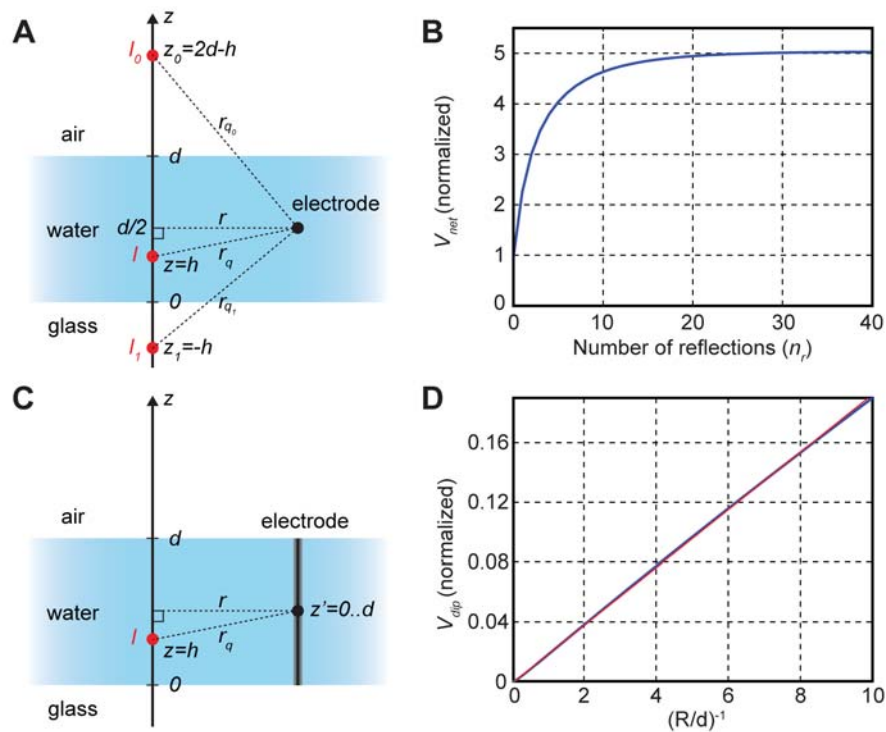


Figure S3.1. The method of image charges applied to the shallow body of water. (A) The image currents of the current source I created by the top and the bottom dielectric interfaces are shown up to the two first order reflections I_0 and I_1 . (B) The net potential (V_{net}) due to the current source and its image currents is plotted as a function of the number of reflections (n_r). V_{net} was measured at the electrode at a distance $r=d$, and normalized to $I / 4\pi\sigma_w d$ (d : depth of water). The current source was located at the height $d/2$. (C) The potential measured at the vertically oriented extended electrode was determined by averaging the potentials measured at different heights. (D) The numerically calculated potential of the vertically oriented electrode

(V_{dip}) is plotted in blue as a function of the normalized inverse distance $(R/d)^{-1}$. The 2D ideal dipole voltage approximation is shown in red.

3.6.2 Finite electrode dimension

Now, let us consider the effect of a finite electrode dimension. The potential at the surface of an electrode is equal throughout its surface since the electrode is a conductor, and the electrode measures the average of potentials at different heights. This could be shown by extending the proof based on connecting two conducting spheres. Thus, the electrode potential can be determined by averaging the potentials at different heights h , where $0 \leq h \leq d$:

$$V_e(\vec{r} | I) = -\frac{1}{d} \int_{z'=0}^d \frac{cI}{\sqrt{r^2 + (z'-h)^2}} dz' = -\frac{cI}{d} \left(\sinh^{-1} \left(\frac{d-h}{r} \right) + \sinh^{-1} \left(\frac{h}{r} \right) \right). \quad (\text{S3.9})$$

If the distance from the current source to the electrode ($= r$) is much greater than the depth of water ($= d$): $d \ll r$, we can apply a Taylor approximation up to the first order:

$$\begin{cases} \sinh^{-1} \left(\frac{h}{r} \right) \approx \frac{h}{r}, \quad \left| \frac{h}{r} \right| \ll 1, \\ \sinh^{-1} \left(\frac{d-h}{r} \right) \approx \frac{d-h}{r}, \quad \left| \frac{d-h}{r} \right| \ll 1. \end{cases} \quad (\text{S3.10})$$

Substitute (S3.9) into (S3.10) to obtain the potential of a rod-shaped electrode:

$$\boxed{V_e(\vec{r} | I) \approx -\frac{cI}{r}}. \quad (\text{S3.11})$$

Therefore, the potential of a vertically oriented extended electrode due to a single current source is approximately equal to the potential of a point-like electrode if the electrode is sufficiently far from the current source relative to the depth of water.

3.6.3 Derivation of the two-dimensional ideal dipole model

Let us derive an expression for the potential due to an ideal dipole in a two-dimensional space. According to Gauss' law, the electric field strength due to a current source (I) in 2D is:

$$\oint_{r'=r} E(r') dr' = \frac{I}{\sigma} \Rightarrow E = \frac{I}{\sigma(2\pi r)} = \frac{cI}{r}, \quad (\text{S3.12})$$

where c is a positive constant, σ is the conductivity of the medium and r is the distance from the current source to the field location. We can obtain the electric potential (V) by integrating the electric field from Eq (S3.12):

$$V(r) = -\int E(a) da = -cI \ln r. \quad (\text{S3.13})$$

The potential due to a dipole is equal to the sum of contributions from the positive and negative current sources:

$$V_{dip} = V_+ - V_- = -cI (\ln r_+ - \ln r_-), \quad (\text{S3.14})$$

where r_+ is the distance between the positive source to the field location, and r_- is the distance between the negative source to the field location. According to the cosine law, the distances from the sources to the field location are:

$$r_{\pm} = r \sqrt{1 \mp \frac{d}{r} \cos \theta + \left(\frac{d}{2r}\right)^2}, \quad (\text{S3.15})$$

where θ is the angle between the vectors $\vec{r}$ and $\vec{d}$ (Fig. 3.1C). Taking the $\ln$ of Eq (S3.4):

$$\ln r_{\pm} = \ln r + \frac{1}{2} \ln \left(1 \mp \frac{d}{r} \cos \theta + \left(\frac{d}{2r}\right)^2 \right), \quad (\text{S3.16})$$

and substituting Eq (S3.16) in Eq (S3.14):

$$V_{dip} = \frac{cI}{2} \left[\ln \left(1 + \frac{d}{r} \cos \theta + \left(\frac{d}{2r} \right)^2 \right) - \ln \left(1 - \frac{d}{r} \cos \theta + \left(\frac{d}{2r} \right)^2 \right) \right]. \quad (\text{S3.17})$$

In the limit of $d \ll r$, Eq (S3.17) can be approximated as:

$$V_{dip} \approx \frac{cI}{2} \ln \left(\frac{1 + (d/r) \cos \theta}{1 - (d/r) \cos \theta} \right). \quad (\text{S3.18})$$

We can apply a Taylor expansion formula:

$$\frac{1}{2} \ln \left(\frac{1+x}{1-x} \right) = x + \frac{x^3}{3} + \frac{x^5}{5} + \dots, |x| < 1, \quad (\text{S3.19})$$

to Eq (S3.18) by setting $x \rightarrow (d/r) \cos \theta$, since $|(d/r) \cos \theta| < 1$ from $d \ll r$. Applying Eq (S3.19) to Eq (S3.18) yields the ideal dipole potential in the two-dimensional case:

$$\boxed{V_{dip}(r, \theta) = \frac{cp \cos \theta}{r}}, \quad p \equiv Id, \quad (\text{S3.20})$$

where p is the current dipole moment.

Now, let us compare the potentials due to a current dipole placed in shallow water determined from the two-dimensional ideal dipole model approximation Eq (3.20) with numerical solutions. The dipole potential can be numerically calculated by estimating a dipole as a pair of closely spaced current source and sink, and summing all potential contributions due to the current sources and their image currents up to the 1000th order. Figure S3.1D shows the potential of a vertically oriented electrode due to a current dipole as a function of the normalized inverse distance. The two current sources were separated by $0.01d$ at a depth of $z = d/2$, and oriented toward the electrode. Our numerical result (blue curve in Fig. S3.1D) confirms that the 2D ideal dipole voltage approximation (red line in Fig. S3.2) is valid for the potential of a vertically oriented extended electrode in shallow water. The 2D ideal dipole

voltage approximation worked very well even when the dipole was located very close to the electrode ($r \sim 0.1d$).

3.6.4 Effect of the circular boundary

A current dipole accumulates a surface charge on the interface between the circular plastic wall and the water, thus we also need to consider the effect of the circular boundary on our measurements. Let us treat our tank as a circle in a two-dimensional space, and use the method of image charges since the 2D approximation is shown to work well in the previous section. Let us consider a current source I oriented inside of the circular region. The inside region of the circular boundary has a conductivity of σ_1 and a permittivity of ε_1 ; and the outside region has a conductivity of σ_2 and a permittivity of ε_2 . The aim is to find the magnitudes and the locations of the image sources corresponding to I . Since image sources cannot be located where the field is evaluated, we must separately find the image sources inside and outside of the circular region. To simplify our derivation, the length unit was normalized to the radius of the aquarium, such that the radius of the aquarium was set to one.

Case 1: Field location inside of the circular region ($r < 1$):

We must place an image source I_1 outside of the region to compute the potential inside of the circular region (see Fig. S3.2A). According to the cosine law, the distance from the current sources to the field point $\vec{r}$ is:

$$r' = \sqrt{r^2 + b^2 - 2rb \cos \theta}, \quad r_1 = \sqrt{r^2 + h^2 - 2rh \cos \theta}. \quad (\text{S3.21})$$

From Eq (S3.17) of the section 3, the net potential due to the current source I and its image source I_1 is:

$$V_{in}(\vec{r}) = -\frac{I}{2\pi\sigma_1} \ln(r') - \frac{I_1}{2\pi\sigma_1} \ln(r_1). \quad (\text{S3.22})$$

Case 2. Field location outside of the circular region ($r > 1$):

We must place an image source I_3 and replace the original current source I with I_2 inside of the circular region [Vanderlinde, 2004] to compute the potential outside of the region (see Fig. S3.2B).

$$V_{out}(\vec{r}) = -\frac{I_2}{2\pi\sigma_2} \ln(r') - \frac{I_3}{2\pi\sigma_2} \ln(r) + \phi. \quad (\text{S3.23})$$

The constant ϕ is required to make the voltage continuous at the circular boundary such that:

$V_{in}(r=1) = V_{out}(r=1)$. Now the electric field must satisfy the boundary conditions below:

$$\begin{cases} E_{in}^{\parallel} = E_{out}^{\parallel} \Rightarrow \frac{d}{d\theta} V_{in} \Big|_{r=a} = \frac{d}{d\theta} V_{out} \Big|_{r=a}, \\ \epsilon_1 E_{in}^{\perp} = \epsilon_2 E_{out}^{\perp} \Rightarrow \epsilon_1 \frac{d}{dr} V_{in} \Big|_{r=a} = \epsilon_2 \frac{d}{dr} V_{out} \Big|_{r=a}. \end{cases} \quad (\text{S3.24})$$

First, let us find the parallel components of the electric field ($E^{\parallel}$). From Eq (S3.22) and (S3.23):

$$\begin{cases} \frac{dV_{in}}{d\theta} \Big|_{r=a} = -\frac{1}{2\pi\sigma_1} \left[\frac{Ib \sin \theta}{b^2 - 2b \cos \theta + 1} + \frac{I_1 h \sin \theta}{h^2 - 2h \cos \theta + 1} \right], \\ \frac{dV_{out}}{d\theta} \Big|_{r=a} = -\frac{1}{2\pi\sigma_2} \left[\frac{I_2 b \sin \theta}{b^2 - 2b \cos \theta + 1} \right]. \end{cases} \quad (\text{S3.25})$$

Equating the above two equations using (S3.24):

$$\begin{aligned} \sigma_2 \left[\frac{Ib \sin \theta}{b^2 - 2b \cos \theta + 1} + \frac{I_1 h \sin \theta}{h^2 - 2h \cos \theta + 1} \right] &= \sigma_1 \left[\frac{I_2 b \sin \theta}{b^2 - 2b \cos \theta + 1} \right], \\ \Rightarrow \frac{\sigma_2 I b}{b^2 - 2b \cos \theta + 1} + \frac{\sigma_2 I_1 h}{h^2 - 2h \cos \theta + 1} &= \frac{\sigma_1 I_2 b}{b^2 - 2b \cos \theta + 1}. \end{aligned} \quad (\text{S3.26})$$

Rationalizing (S3.26) yields:

$$\sigma_2 I b (h^2 - 2h \cos \theta + 1) + \sigma_2 I_1 h (b^2 - 2b \cos \theta + 1) = \sigma_1 I_2 b (h^2 - 2h \cos \theta + 1). \quad (\text{S3.27})$$

Separating cosine dependent and independent terms:

$$\left[\sigma_2 b (h^2 + 1) I + \sigma_2 h (b^2 + 1) I_1 - \sigma_1 b (h^2 + 1) I_2 \right] + 2bh \cos \theta [-\sigma_2 I - \sigma_2 I_1 + \sigma_1 I_2] = 0. \quad (\text{S3.28})$$

The equation above must be satisfied for any choice of θ , thus:

$$-\sigma_2 I - \sigma_2 I_1 + \sigma_1 I_2 = 0 \Rightarrow \boxed{\sigma_1 I_2 = \sigma_2 (I + I_1)}. \quad (\text{S3.29})$$

Substituting (S3.29) into (S3.28):

$$\begin{aligned} \sigma_2 b (h^2 + 1) I + \sigma_2 h (b^2 + 1) I_1 - \sigma_2 b (h^2 + 1) (I + I_1) &= 0, \\ \Rightarrow \sigma_2 I_1 (hb^2 + h - bh^2 - b) &= 0 \Rightarrow b + \frac{1}{b} = h + \frac{1}{h}. \end{aligned} \quad (\text{S3.30})$$

The solution to (S3.30) can be found using the quadratic formula, yielding: $h = \{b, 1/b\}$.

However, image sources cannot be located in the same region of the real current source, thus:

$$\boxed{h = \frac{1}{b}}. \quad (\text{S3.31})$$

Now, let us find the perpendicular component of the electric field ($E^\perp$). From (S3.22) and (S3.23):

$$\begin{cases} \left. \varepsilon_1 \frac{dV_{in}}{dr} \right|_{r=a} = \frac{\varepsilon_1}{2\pi\sigma_1} \left[\frac{I(b \cos \theta - 1)}{b^2 - 2b \cos \theta + 1} + \frac{I_1(h \cos \theta - 1)}{h^2 - 2h \cos \theta + 1} \right], \\ \left. \varepsilon_2 \frac{dV_{out}}{dr} \right|_{r=a} = \frac{\varepsilon_2}{2\pi\sigma_2} \left[\frac{I_2(b \cos \theta - 1)}{b^2 - 2b \cos \theta + 1} - I_3 \right]. \end{cases} \quad (\text{S3.32})$$

Equating the above equations according to (S3.24):

$$\frac{\varepsilon_1}{\sigma_1} \left(\frac{I(b \cos \theta - 1)}{b^2 - 2b \cos \theta + 1} + \frac{I_1(h \cos \theta - 1)}{h^2 - 2h \cos \theta + 1} \right) = \frac{\varepsilon_2}{\sigma_2} \left(\frac{I_2(b \cos \theta - 1)}{b^2 - 2b \cos \theta + 1} - I_3 \right). \quad (\text{S3.33})$$

Substituting (S3.31) into (S3.33):

$$\begin{aligned}
\sigma_2 \varepsilon_1 \left[\frac{I(b \cos \theta - 1)}{b^2 - 2b \cos \theta + 1} + \frac{I_1(b \cos \theta - b^2)}{b^2 - 2b \cos \theta + 1} \right] &= \sigma_1 \varepsilon_2 \left[\frac{I_2(b \cos \theta - 1)}{b^2 - 2b \cos \theta + 1} - I_3 \right], \\
\Rightarrow \sigma_2 \varepsilon_1 I(b \cos \theta - 1) + \sigma_2 \varepsilon_1 I_1(b \cos \theta - b^2) &= \sigma_1 \varepsilon_2 I_2(b \cos \theta - 1) - \sigma_1 \varepsilon_2 I_3(b^2 - 2b \cos \theta + 1), \\
\Rightarrow [-\sigma_2 \varepsilon_1 I - \sigma_2 \varepsilon_1 b^2 I_1 + \sigma_1 \varepsilon_2 I_2 + \sigma_1 \varepsilon_2 (b^2 + 1) I_3] &+ b \cos \theta [\sigma_2 \varepsilon_1 I + \sigma_2 \varepsilon_1 I_1 - \sigma_1 \varepsilon_2 I_2 - 2\sigma_1 \varepsilon_2 I_3] = 0.
\end{aligned} \tag{S3.34}$$

Similarly, the equation above must be satisfied for any choice of θ , thus:

$$\begin{cases} \sigma_2 \varepsilon_1 (I + I_1) = \sigma_1 \varepsilon_2 (I_2 + 2I_3), \\ \sigma_2 \varepsilon_1 (I + b^2 I_1) = \sigma_1 \varepsilon_2 (I_2 + (b^2 + 1)I_3) = 0, \end{cases} \Rightarrow \begin{cases} I_1 = \frac{\sigma_1 \varepsilon_2}{\sigma_2 \varepsilon_1} I_3, \\ I_2 = \frac{\sigma_2 \varepsilon_1}{\sigma_1 \varepsilon_2} (I - I_1). \end{cases} \tag{S3.35}$$

Substituting (S3.35) into (S3.29) yields all the image sources:

$$\boxed{I_1 = \frac{\varepsilon_1 - \varepsilon_2}{\varepsilon_1 + \varepsilon_2} I, \quad I_2 = \frac{\sigma_2}{\sigma_1} \frac{2\varepsilon_2}{\varepsilon_1 + \varepsilon_2} I, \quad I_3 = \frac{\sigma_2 \varepsilon_1}{\sigma_1 \varepsilon_2} \frac{\varepsilon_1 - \varepsilon_2}{\varepsilon_1 + \varepsilon_2} I.} \tag{S3.36}$$

Observe that if $\sigma_1 \gg \sigma_2$, I_2 and I_3 vanishes and we obtain zero potential outside of the circular region, as expected from a body of water surrounded by a circular plastic wall.

3.6.5 Differential voltage due to an image dipole

Previously, we have found the potential due to a current source in 2D with the circular boundary by using the method of image charges. We can apply our previous results to determine the potential due to an ideal current dipole in 2D with the circular boundary condition. The circular dielectric boundary creates an image current dipole outside of the region, and we need to determine the potential difference between two electrodes due to the ideal current dipole (see Fig. S3.2C). The potential due to a current dipole in 2D is:

$$V_{dip} = c \frac{\vec{p} \cdot \vec{r}}{|\vec{r}|^2} = c p \frac{\vec{r} \cdot \angle \phi}{|\vec{r}|^2}, \tag{S3.36-1}$$

where $\angle \phi = (\cos \phi, \sin \phi)$ is the unit vector of the dipole $\vec{p}$. From Eq (S5.1), the voltage difference between the two electrodes e_1 and e_2 (see Fig. S3.2C) is:

$$\Delta V_{dip,p} = cp \left[\frac{\vec{r}_1 \cdot \angle \phi}{|\vec{r}_1|^2} - \frac{\vec{r}_2 \cdot \angle \phi}{|\vec{r}_2|^2} \right], \quad (\text{S3.37-2})$$

where $|\vec{r}_{1,2}'|^2 = 1 - 2r \cos \theta_{1,2} + r^2$. Now, let us compute the potential difference due to the image dipole. The image dipole is created outside of the circular boundary due to the reflection from the dielectric circular wall, and it has a magnitude of [Vanderlinde, 2004]:

$$p' = \left(\frac{\epsilon_w - \epsilon_p}{\epsilon_w + \epsilon_p} \frac{1}{r^2} \right) p \quad (\text{S3.38-3})$$

with the unit vector $\angle(\pi - \phi)$. The voltage difference due to the image dipole is:

$$\Delta V_{dip,p'} = cp \left[\frac{\vec{r}_1' \cdot \angle(\pi - \phi)}{|\vec{r}_1'|^2} - \frac{\vec{r}_2' \cdot \angle(\pi - \phi)}{|\vec{r}_2'|^2} \right] \quad (\text{S3.39-4})$$

where $|\vec{r}_{1,2}'|^2 = 1 - 2 \cos \theta_{1,2} / r + 1 / r^2 = |\vec{r}_{1,2}|^2 / r^2$. It can be shown that $\Delta V_{dip,p'}$ from (S5.4) can be algebraically simplified to:

$$\Delta V_{dip,p'} = \frac{\epsilon_w - \epsilon_p}{\epsilon_w + \epsilon_p} \Delta V_{dip,p} \quad (\text{S3.40-5})$$

for any choice of a dipole location (r, ϕ) and the electrodes locations $\theta_{1,2}$. From Eq (S3.40), the net voltage difference is thus:

$$\Delta V_{dip} = \Delta V_{dip,p} + \Delta V_{dip,p'} = \frac{2\epsilon_w}{\epsilon_w + \epsilon_p} \Delta V_{dip,p} \quad (\text{S3.41-6})$$

In summary, the circular boundary simply rescales the differential dipole potential by a constant factor. The voltage rescaling does not influence our dipole localization algorithm, since it uses the relative signal intensities between multiple channels.

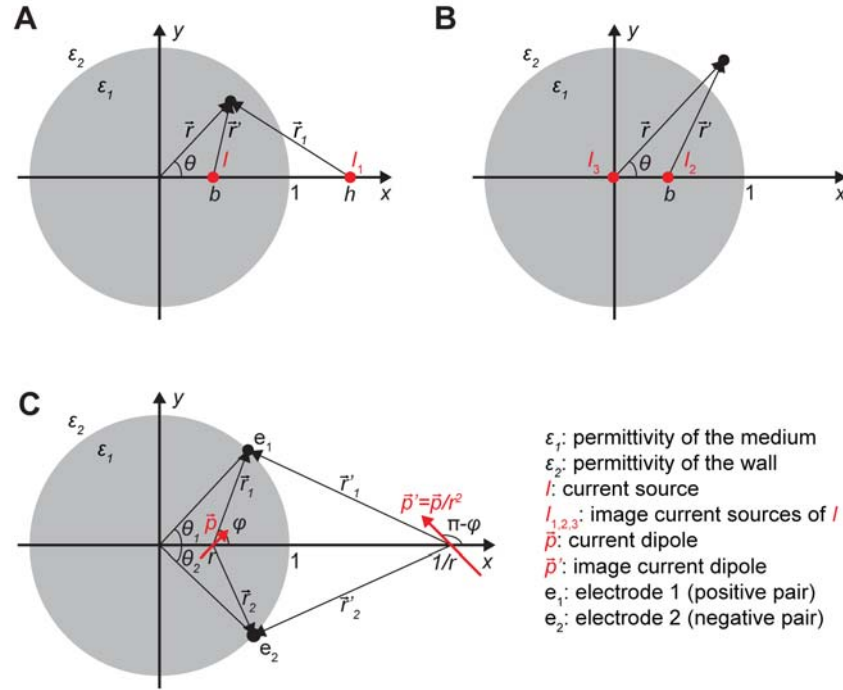


Figure S3.2. The method of image charges applied to the side circular boundary. (A) The current source (I) and its image source (I_1) are shown for the field location inside of the circular region. (B) Two image sources (I_2, I_3) are shown for the field location outside of the circular region. (C) The image current dipole ($\vec{p}'$) location is shown to calculate the differential potential between the electrode pair (e_1, e_2).

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Chapter 4

Long-term behavioural tracking of freely-swimming weakly electric fish

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4.1 Summary

Long-term behavioral tracking can capture and quantify natural animal behaviors, including those occurring infrequently. Behaviors such as exploration and social interactions can be best studied by observing unrestrained, freely behaving animals. Weakly electric fish (WEF) display readily observable exploratory and social behaviors by emitting electrical organ discharge (EOD). Here, we describe three effective techniques to synchronously measure the EOD, body position and posture of a free-swimming WEF for an extended period of time. First, we describe the construction of an experimental tank inside of an isolation chamber designed to block external sources of sensory stimuli such as light, sound, and vibration. The aquarium was partitioned to accommodate four test specimens, and automated gates remotely control the animals' access to the central arena. Second, we describe a precise and reliable real-time EOD timing measurement method from freely swimming WEF. Signal distortions caused by the animal's body movements are corrected by spatial averaging and temporal processing stages. Third, we describe an underwater near-infrared imaging setup to observe unperturbed nocturnal animal behaviors. Infrared light pulses were used to synchronize the timing between the video and the physiological signal over a long recording duration. Our automated tracking software measures the animal's body position and posture reliably in an aquatic scene. In combination, these techniques enable long term observation of spontaneous behavior of freely swimming weakly electric fish in a reliable and precise manner. We believe our method can be similarly applied to the study of other aquatic animals by relating their physiological signals with exploratory or social behaviors.

Key words: behavioural neuroscience, animal tracking, weakly electric fish, electric organ discharge, underwater infrared imaging, behavioural event synchronization, long-term recording, automated image tracking, freely moving animal, sensory isolation chamber, exploratory behaviour, spontaneous behaviour

4.2 Introduction

4.2.1 Background

Quantitative experiments on animal behavior (*e.g.* forced choice, shock avoidance, T-maze etc.) are typically utilized to investigate specific hypotheses concerning sensory-motor skills, learning and memory formation. However, these restrictive experiments miss much of the richness of natural animal behavior, and are likely to result in oversimplified models of the underlying neural basis of behavior. Experiments under more naturalistic conditions are therefore an important complement by which we can explore more fully a species behavioral repertoire. Experiments involving freely moving animals must, however, address unique technical challenges such as movement-induced recording artifacts. Unlike stimulus-evoked responses, spontaneously occurring exploratory behavior cannot be predicted; thus experimental subjects have to be constantly monitored and tracked over an extended period of time. Specific research questions can be best addressed by carefully selected organisms and available technical tools. For example, optical recording and stimulation techniques such as genetically-encoded calcium sensors [Miyawaki et al., 1997] and optogenetics [Boyden et al., 2005] have been successfully applied to freely moving genetic model organisms [Adamantidis et al., 2007; Naumann et al., 2010; Leifer et al., 2011]. Alternatively, miniaturized neural telemetry systems can record and stimulate freely moving small animals [Mavoori et al, 2004; Harrison et al., 2011].

4.2.2 Electric fish

WEF species generate electrical organ discharges (EODs), which allow them to sense their immediate surroundings or to communicate over greater distances. Temporal patterns of EODs vary under different conditions such as self-movements [Jun et al., 2012a; Forlim and Pinto, 2014], sensory stimuli [Caputi et al., 2003; Pluta and Kawasaki, 2008], and social interactions [Heiligenberg, 1974; Capurro et al., 2004]. Pulse-type WEF species produce a train of discrete pulses, as opposed to wave-type species which generate continuous quasi-sinusoidal waveforms. In general, pulse-type species exhibit more variable EOD rate compared to the wave-type species; and animals' EOD rates closely reflect novelty contents of their sensory surroundings [Caputi et al., 2003; Post and Emde, 1999]. Pulse-type species can immediately shorten the inter-pulse interval (IPI) within a single pulse cycle in respond to a novel sensory

perturbation (novelty response [Caputi et al., 2003; Pluta and Kawasaki, 2008; Post and Emde, 1999]). The ongoing electric behavior of these fish can be perturbed by uncontrolled sensory stimuli from external sources; and different kinds of stimuli such as vibration, sound, electricity, and light are known trigger novelty responses. Therefore, special precautions must be taken to block or attenuate external sensory stimuli during a long-term observation of free-swimming WEF. In this way, changes in EOD rate and movement trajectories can be specifically attributed to stimuli presented by the experimenter.

4.2.3 Aquarium tank and isolation chamber

We therefore placed multiple layers of vibration absorbing materials under a large aquarium tank (2.1 m × 2.1 m × 0.3 m), and surrounded the tank with an insulated enclosure to block external sources of light, electrical noise, sound and heat flux. EOD rate depends on the surrounding temperature [Toerring and Serrier, 1978; Ardanaz et al., 2001], thus the water temperature was tightly regulated at a tropical range (25 ± 1 °C) for South American WEF species. We constructed a large and shallow (10 cm water depth) tank to observe spatial exploratory behaviors of WEF mainly restricted in two dimensions (Fig. 4.1A). The tank was partitioned into a central arena to observe spatial behaviors, and four corner compartments to separately house individual fish (Fig. 4.1B). Each compartment was built watertight to prevent electrical communication between individuals. Animals' access to the central arena was controlled from the outside by four motorized gates. The gates were placed between the compartments, and they became watertight when locked by nylon wing-nuts. No metallic parts were used underwater since WEF react sensitively to metals.

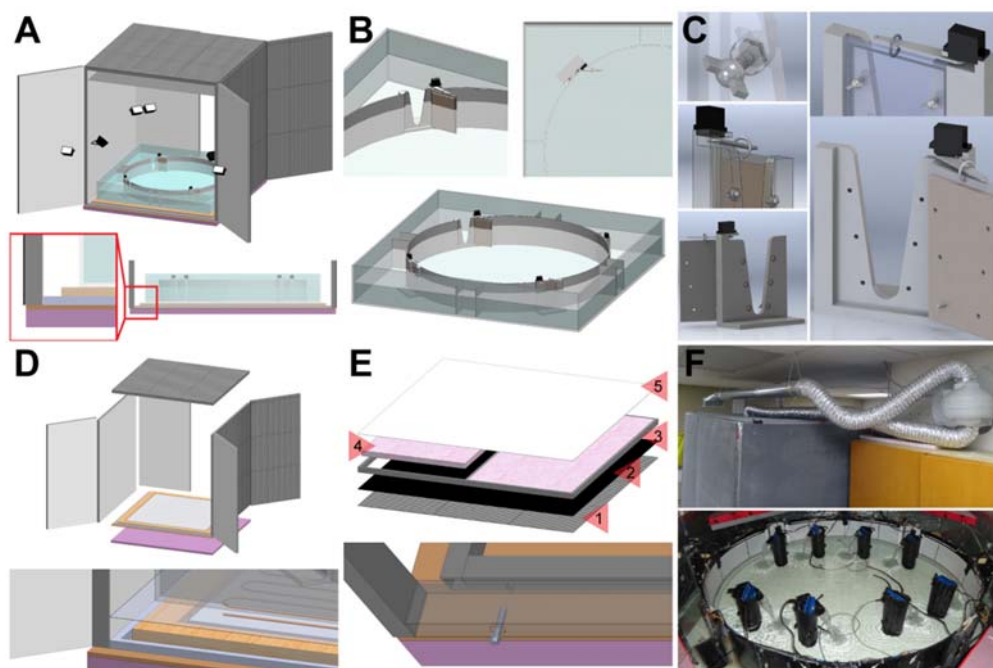


Figure 4.1. Aquarium tank and isolation chamber setup. (A) The experimental chamber consists of an anti-vibration floor, aquarium tank, and an isolation chamber. (B) The aquarium tank was divided into the central arena for running experiments, and four corner compartments for housing individual fish. Each compartment was built watertight to prevent electrical communication between animals. (C) The motorized gate is illustrated at multiple perspective angles. The gate becomes watertight when locked by six wing nuts which compress the rubber gasket (the light brown sheet). Once unlocked, the gate can be remotely operated by the servo motor on the top. (D) The isolation chamber was assembled by joining three wall panels and four door panels, which provide access to the aquarium tank from two sides. The bottom panel illustrates the wooden rails for supporting the tank edges, and the floor heater placement. A layer of aluminum mesh covers the heater to shield its electrical noise. (E) The walls and door panels of the isolation chamber were constructed from aluminum frames for structural support (3). The interior surfaces of the chamber are covered by white plastic panels (5) to reflect internal light sources, and the exteriors are covered by black plastic panels (2) to block external light sources. An aluminum mesh (1) covers the exterior walls to block external electrical noise. The wall is filled with acoustic fiberglass batts (4). (F) The top photo shows the air ventilation setup for removing excess humidity generated from heating; and the bottom photo

shows the water filtration setup for cleaning, diffusing, and aerating the tank water between experimental sessions.

4.2.4 EOD recording

EODs are generated in a stereotyped manner by activation of single (in Mormyrids) or multiple spatially distributed electric organs (in Gymnotiforms) [Bennett, 1971; Rodríguez-Cattáneo, 2008]. Temporal modulations in the EOD rate can reveal higher-level neural activities, since the medullary pacemaker receives direct neural inputs from higher brain regions such as the diencephalic prepacemaker nucleus, which in turn receives axonal projections from the forebrain [Wong and Hopkins, 1997]. However, the EOD timing must be carefully extracted from a raw waveform recording and not biased by the animal's movement-induced distortions. The electric field generated by a WEF can be approximated as a dipole; thus EOD pulse amplitudes at recording electrodes depend on the relative distances and orientations between the animal and the electrodes [Jun et al., 2012a; Jun et al., 2013]. Animal's self-movements change the relative geometry between the animal and the electrodes, thus movements cause the EOD amplitudes at different electrodes to vary over time in a volatile manner (see Fig. 4.2B in Jun et al. [2012]). Furthermore, self-movements also change the shape of recorded EOD waveforms, because relative contributions from different set of the electric organs depend on their locations along the body length and their local curvatures introduced by tail bending. The movement-induced distortions in the EOD amplitudes and shapes can lead to inaccurate and unreliable EOD timing measurements. We overcame these problems by spatially averaging multiple EOD waveforms recorded at different locations, and by adding an envelope extraction filter to precisely determine the EOD timing from a free-swimming WEF. In addition, our technique also measures the EOD amplitudes, which indicate whether an animal is resting or actively moving based on the change of the EOD amplitudes over time (see Fig. 4.2E,F). We recorded differentially amplified signals from the recording electrode pairs to reduce common-mode noise. Since the EOD pulses are generated at irregular time intervals, the EOD event time-series have a variable sampling rate. The EOD time-series can be converted to a constant sampling rate by interpolation if required by an analytic tool of choice.

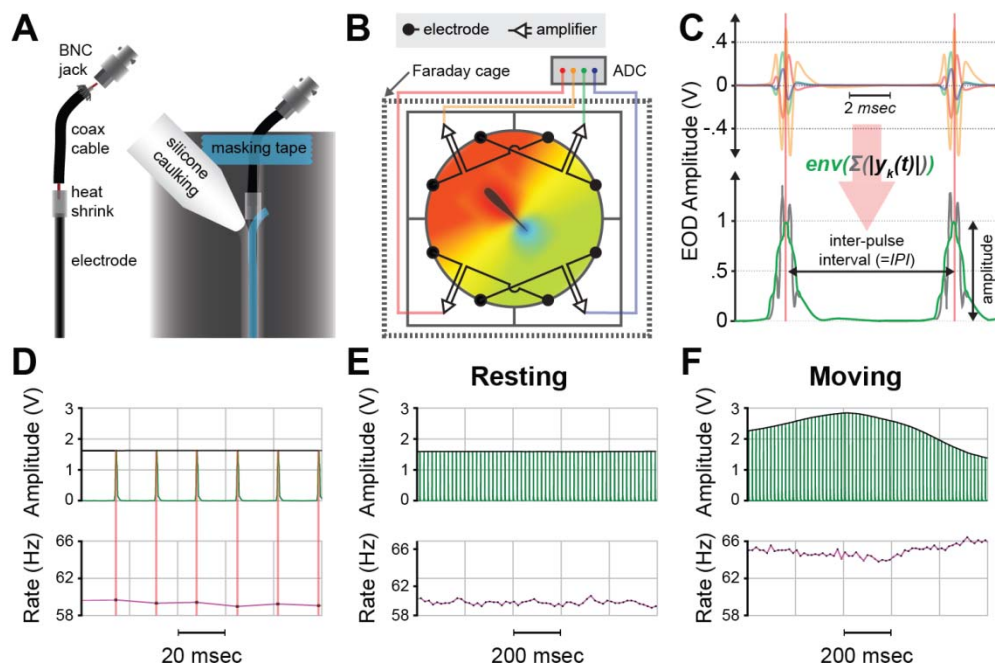


Figure 4.2. EOD recording setup and representative results. (A) The left panel illustrates the electrode assembly consisting of a thin graphite electrode, a short segment of coaxial cable, and a BNC Jack. The right panel demonstrates electrode attachment instructions. Masking tape is used to temporarily position the electrode assembly, and silicone caulking was applied to permanently hold the electrode. (B) The wiring diagram. Two 90° electrodes are paired up, differentially amplified and filtered. Four recording channels were digitized outside of the Faraday cage. (C) Illustration of the EOD signal processing steps. The top traces show raw waveforms from four electrodes pairs, which are rectified and summed to produce the gray trace below. Unimodal envelopes are extracted from the grey waveform using the “*Root-Mean-Square*” (RMS) filter (green trace). The EOD amplitudes and IPIs are determined from the envelope peaks. (D) The time-varying EOD amplitudes (top) and the instantaneous EOD rate (bottom) are shown on a longer time scale than C. The EOD amplitudes and the instantaneous rate ($=IPI^{-1}$) are interpolated at regular time intervals by joining the envelope peaks (black traces). (E) Same as D but plotted on a longer time scale while fish was at rest. (F) Same as E) while fish was actively swimming.

4.2.5 Video recording

Although EOD recording can monitor a gross movement activity of an animal, video recording permits direct measurements of an animal's body position and posture. Near-infrared (NIR) illumination ($\lambda = 800 \sim 900$ nm) permits unperturbed visual observation of freely swimming fish [Rasnow et al., 1997; MacIver and Nelson, 2000], since WEFs are most active in darkness and their eyes are not sensitive to NIR spectrum [Douglas and Hawryshyn, 1990; Ciali et al., 1997]. Most digital imaging sensors (*e.g.* CMOS or CCD) can capture NIR spectrum with the wavelength range between 800 nm to 900 nm, after removing an infrared (IR) blocking filter [Ratledge, 2005]. Certain high-end consumer-grade webcams offer high-definition, wide viewing angle and good low-light sensitivity, which can produce an image quality comparable to, or superior to professional-grade IR cameras available at much greater costs. In addition, certain consumer-grade webcams are bundled with recording software that permits an extended recording duration by compressing video with no quality loss. Most professional-grade cameras offer time synchronization TTL pulse outputs or trigger TTL pulse inputs [Hofmann et al., 2013b] for aligning the timing between the video with the digitized signals, but this feature is generally absent in consumer-grade webcams. However, the timing between a video recording and a signal digitizer can be accurately matched by concurrently capturing a periodically blinking IR LED with the camera and the signal digitizer. The initial and the final IR pulse timing can be used as two time calibration markers for converting the video frame numbers to the signal digitizer time unit, and vice versa.

4.2.6 Lighting & background

Image capturing through water can be technically challenging due to light reflections at the water surface. The water surface can act as a mirror to reflect a visual scene above water, and obscure visual features underwater; thus the scene above water must be rendered featureless to prevent visual interference. In order to image the whole aquarium, a camera needs to be placed directly above the water; and it should be hidden behind the ceiling over a small viewing hole to prevent its reflection on the water surface. Moreover, the water surface can produce glares and non-uniform illumination if light sources are incorrectly projected. Indirect illumination can achieve uniform brightness over the whole aquarium by aiming the light sources toward the ceiling, such that the ceiling and the surrounding walls can reflect and diffuse the light rays before reaching the water surface. Choose an IR illuminator that matches

a spectral response of the camera (*e.g.* 850 nm peak wavelength). Electrical noise from the light sources can be minimized by using LED lights and placing their DC power supplies outside of the Faraday cage. Place a white background underneath the tank, since fish contrasts well in a white background at NIR wavelengths. Similarly, use of matt white color on the inner surfaces of the isolation chamber provides uniform and bright background illumination.

4.2.7 Video tracking

After a video recording, an automated image tracking algorithm can measure the animal's body positions and postures over time. The video tracking can be automatically performed by either ready-to-use software (Viewpoint or Ethovision), or user-programmable software (OpenCV or Matlab *Image processing toolbox*). As the first step of image tracking, a valid tracking area needs to be defined by drawing a geometric shape to exclude the area outside (masking operation). Next, an animal's image needs to be isolated from the background by subtracting a background image from an image containing the animal. The subtracted image is converted to a binary format by applying an intensity threshold, such that the centroid and the orientation axis can be computed from binary morphological operations. In Gymnotiforms [Castelló et al., 2000; Caputi et al., 2002; Pusch, 2008] and Mormyrids [Harder, 1968; Bacelo et al., 2008; Hollmann, 2008], the electroreceptor density is the highest near the head region; thus the head position at any moment indicates a location of the highest sensory acuity. The head and tail locations can be automatically determined by applying the image rotation and bounding-box operations. The head and tail ends could be distinguished from one another by manually defining them in the first frame, and by keeping track of their locations from comparing two successive frames.

4.3 Protocol

This procedure meets the requirements of the University of Ottawa Animal Care Committee. No conflict of interest is declared. Please refer to the Table of Materials and Reagents for the makes and models of the equipment and materials listed below. Custom written Spike2 and Matlab scripts, and sample data are provided in the Supplemental File.

4.3.1 Aquarium tank and isolation chamber setup

1.1) *Anti-vibration floor*. Construct an anti-vibration surface (2.1 m × 2.1 m) by stacking rubber pads, acoustic Styrofoam, marine plywood panel, and polyurethane foam pads from the bottom to the top (Fig. 4.1A). Lay four wooden studs (5 cm × 10 cm) on the plywood panel to support the edges of the aquarium tank.

1.2) *Floor heater*. Lay an electrically shielded heating element over thermally graded foam padding (see Fig. 4.1D bottom). Cover the heating element with a metallic mesh for electrical shielding.

1.3) *Spatial tank*. Construct a wide and shallow aquarium tank (1.8 m × 1.8 m × 30 cm) using 1.3 cm thick tempered glass panels, L-shaped aluminum frame and aquarium-grade silicone (see Fig. 4.1A). Cover the underside of the tank with a large sheet of white background to provide high imaging contrast (see Protocol 3).

1.4) Divide the aquarium tank into a central arena (1.5 m diameter) and four corner compartments (see Fig. 4.1B) by installing walls (22.5 cm tall) made of acrylic sheets (matt white, 0.64 cm thick).

1.4.1) Bend four acrylic sheets (22.5 cm × 102.7 cm) by applying heat to create four curved wall sections, and attach them to the tank bottom using silicone caulk to separate the central arena from the four corner compartments. Leave 20 cm space between the curved sections for the gate installation.

1.4.2) Separate neighboring corner compartments by installing four double walls with 15 cm gaps, which provide extra electrical isolation and places for underwater sensors such as a hydrophone.

1.5) Assemble four motorized gates, and install them between the corner compartments and the central arena.

1.5.1) Assemble four door frames as shown in Figure 4.1C. Create six wells (0.64 cm deep) on each door frame, embed nylon acorn nuts (0.64 cm diameter thread) and secure them with epoxy.

1.5.2) Cut four door panels from acrylic and rubber sheets, and create six holes (0.64 cm diameter) on the acrylic and rubber panels for the locking mechanism. Join the acrylic and rubber panels using silicone caulking.

1.5.3) Install acrylic hinges to join the door panels with the door frames.

1.5.4) Mount swinging arms on servomotors, and install them on the top of the door frames (see Fig. 4.1C). Make loops with cable ties to link the swinging arms to the door panels.

1.5.5) Position the gate assemblies on the gaps created between the curved wall sections, and secure them using silicone caulking.

1.5.6) Connect all servomotors to a servo controller, and connect it to a power source and a computer via an active USB extension cable. Test the gates using control software supplied with the servo controller.

1.5.7) After the silicone hardens, check for water-tightness by locking all gates with nylon screws and filling one compartment at a time.

1.6) *Isolation chamber*. Construct an isolation chamber to surround the aquarium and block external sources of light, sound and electrical noise (see Fig. 4.1D).

1.6.1) Make three wall panels (2 m × 2 m × 5 cm) and four door panels (1.9 m × 0.95 m × 5 cm). For each panel, join aluminum moldings (5 cm × 2.5 cm) to create a rectangular frame; and rivet a white corrugated plastic panel on the aluminum frame. Fill acoustic fiberglass batts in the panels, and close with a black corrugated plastic panel.

1.6.2) Install three wall panels on the anti-vibration floor, and install piano hinges to join the four door panels on the wall panels.

1.6.3) Surround the isolation chamber with aluminum meshes, and ground meshes on all sides to create a Faraday cage.

1.7) *Humidity control.* Install a low-noise exhaust fan (Fig. 4.1F top) to remove excess humidity build-up from heating. Place the exhaust fan at least 2 m away from the recording site, and install an air duct between the isolation chamber and the exhaust fan.

1.8) Routinely monitor and maintain the conditions of the tank water and animals.

1.8.1) Maintain constant water conditions at 10 cm depth, 100 $\mu\text{S}/\text{cm}$ conductivity and pH 7.0 by adding water or salt stock solution (refer to Knudsen³³ for the recipe). Add a bag of crushed coral if the pH drops below 6.5.

1.8.2) Install vertical aquarium filters which can operate from shallow water for cleaning and aerating purposes (Fig. 4.1F bottom). Disconnect the filters and take them out of the central arena during recording sessions.

1.8.3) Deliver live mealworms on the bottom of the tank by attaching them on suction cups with elastics. Avoid free-floating prey such as blackworms to prevent uncontrolled feeding of stray prey during recording.

4.3.2 EOD tracking

2.1) *Electrodes installation.* Assemble eight graphite electrodes, and space them equally on the curved wall of the central arena.

2.1.1) Obtain drawing leads (15 cm in length; Mars Carbon 2-mm type HB) and shave off the outer coating of the leads.

2.1.2) Cut eight 10 cm segments of coaxial cable (RG-174), wrap the cable core around one end of the graphite rods, and apply heat-shrink tubing over them for strong and stable electrical connection. Attach BNC jack connectors on the opposite ends (Fig. 4.2A left).

2.1.3) Position the electrodes on the wall by taping, and apply thin strips of masking tape on the electrode surfaces to protect from silicone. Apply silicone caulking to permanently hold the electrodes, and remove all tape before the silicone hardens (Fig. 4.2A right).

2.2) Build eight cable assemblies by measuring the distance from each electrode to the amplifier unit, and cutting coaxial cables (RG-54) in lengths. Attach BNC plug connectors on both ends of the cables.

2.3) Use the cable assemblies to wire all electrodes to the amplifier unit. Differentially amplify by pairing two 90° oriented electrodes (see Fig. 4.2B), and ground all coaxial shielding wires by connecting them to the Faraday cage.

2.4) Set the amplifier gain below the signal saturation limit, and apply a band-pass filter (200 Hz – 5 KHz) to remove noise. Digitize the four recording electrode pairs at 40 KS/s.

2.5) *Online signal processing.* The instructions are written for the Spike2 software, and the parameter settings are optimized for *Gymnotus* sp. (see Fig. 4.2C for summary).

2.5.1) Add a *DC remove* process ($\tau = 0.1$ s) to all recording channels.

2.5.2) Add a *rectify* process to all recordings channels.

2.5.3) Create a *virtual* channel by summing all four recording channels.

2.5.4) Extract a unimodal envelope per EOD pulse by adding a *RMS* (root-mean-squared, $\sqrt{\langle (x_k - \langle x \rangle)^2 \rangle}$) process ($\tau = 0.25$ ms) to the *virtual* channel, for generating a single peak per EOD cycle to unambiguously determine the pulse timing.

2.5.5) Create a *realmark* channel from the *virtual* channel and record the time and values of the peak amplitudes, after setting an appropriate threshold to capture all EOD pulses without missing a pulse, while avoiding false positives.

2.5.6) Monitor the instantaneous EOD rate in real-time by setting the channel display option of the *realmark* channel to an *instantaneous frequency* mode.

2.5.7) Monitor the fish movement in real-time by duplicating the *realmark* channel, and set the display option to a *waveform* mode.

2.5.8) Quantify an activity level from the RMS of the EOD amplitude slope by creating a *virtual* channel from the *realmark* channel (0.01 s sampling period), and add slope ($\tau = 0.25$ ms) and RMS ($\tau = 0.5$ ms) processes.

2.5.9) Export the *realmark* channel in the Spike2 software to the Matlab format.

4.3.3 Synchronized video tracking

3.1) Create a background scene.

3.1.1) Hide any object that casts a reflection on the water surface by covering with matt white countertop film.

3.1.2) Install a matt white corrugated plastic panel 15 cm below the ceiling to hide the camera and the air vent.

3.1.3) Print grid patterns on a large sheet of white paper for calibrating a camera, and lay it underneath the tank to provide a high-contrast background.

3.2) Install light sources.

3.2.1) Obtain IR LED lights and, remove built-in fans to reduce noise. Drive the LED with a current-regulated DC power supply placed outside of the Faraday cage.

3.2.2) Install IR LED lights for imaging in darkness, and white LED lights for driving a diurnal light cycle in the test fish. Direct all light sources toward the ceiling to achieve indirect and uniform illumination (Fig. 4.3A).

3.2.3) Regulate the diurnal light cycle by driving the white LED lights with a timer-controlled switch (*e.g.* 12 hours on / 12 hours off).

3.3) Install a camera directly above the aquarium.

3.3.1) Obtain a NIR-sensitive camera, or remove an IR blocking filter by breaking a thin sheet of tinted glass at the back of the lens assembly. Make sure the viewing angle is wide enough to image the whole central arena.

3.3.2) Make a small viewing hole in the middle of the ceiling panel, and place the camera directly above the hole.

3.3.3) Install a white ring guard around the lens if the light sources generate glares.

3.4) Make a time-synchronized video recording.

3.4.1) Place an IR LED at one of the four tank corners to generate time synchronization pulses (1 ms duration, 10 s period). Add a load-limiting resistor (1 K Ω) in series, and drive the IR LED from a digital output port of the digitizer hardware.

3.4.1) Use video recording software bundled with the camera if available. Select the highest recording quality (*e.g.* lossless compression) and the highest resolutions supported.

3.4.2) Start the video recording immediately before starting the EOD recording, and stop the video recording immediately after the EOD recording.

3.4.3) After the recording, convert the image frame numbers to the digitizer time unit by linearly interpolating between the first and the last light pulses captured by the signal digitizer and the video recording.

3.5) Automated image tracking

The instructions are written for the Matlab *Image processing toolbox*, and make use of its functions.

3.5.1) *Import video*. Import a video recording file directly to the Matlab workspace using “*Videoreader.read*” function.

3.5.2) Create a composite background image by combining two image frames. Replace the image region occupied by an animal with an unoccupied image of the same region from another frame (see Fig. 4.3B).

3.5.3) Specify an image region to track by drawing a circular mask around the central arena to exclude the area outside (Fig. 4.3B bottom), and multiply by a constant (r_{int}) to set a minimum threshold for intensity difference. For example, setting $r_{int} = 0.85$ will suppress the intensity fluctuations 15% ($= 1 - r_{int}$) below the background.

3.5.4) *Image subtraction*. Subtract an image frame ($= IM_k$) from the background image ($= IM_0$) to obtain the difference image ($= \Delta IM_k$). Use *unsigned integer* numerical precision to store the image intensity values as non-negative integers.

3.5.5) Segment the difference image by applying an intensity threshold determined from the *graythresh* function. Clean the binary image using the *bwmorph* function, and select the largest blob corresponding to an animal after calculating all blob areas using the *regionprops* function.

3.5.6) Determine the centroid and major orientation axis of the largest blob by applying the *regionprops* function, and rotate the image to align the major axis with the x -axis. Divide the image to the head and tail parts at the centroid (Fig. 4.3D top).

3.5.7) Determine the major axis of the head part, and rotate the entire image to align with the x -axis (Fig. 4.3D bottom left). Fit bounding-boxes around the head and tail parts parallel to their major axes using the *regionprops* function.

3.5.8) Determine the median y -coordinates of the blob at the left, center and right vertical edges of the bounding boxes (green dots in Fig. 4.3D bottom); and assign them to five feature points (head-tip, mid-head, mid-body, mid-tail, tail-tip).

3.5.9) Process successive frames after cropping an image frame centered at the animal's centroid determined from its previous frame.

3.5.10) Manually assign the head orientation for the first frame, and use a dot-product between the orientation vectors from two successive frames to automatically determine the head orientation. Inspect the result, and manually flip the head orientation if incorrectly assigned.

3.6) Plot an animal trajectory by joining the head-tips, and smooth using median and average filters ($n=3$) if it has a jittery appearance. Superimpose the trajectory with a background image, and interpolate fish midlines using the five feature points (see Fig. 4.2E).

3.7) Compute the average EOD rate at each image capture time by resampling the instantaneous EOD rate (100 Hz sampling rate) and averaging (0.0625 s time window). Plot the trajectory in pseudo-colors determined from the time-matched EOD rate, and superimpose with a background image (see Fig. 4.2F).

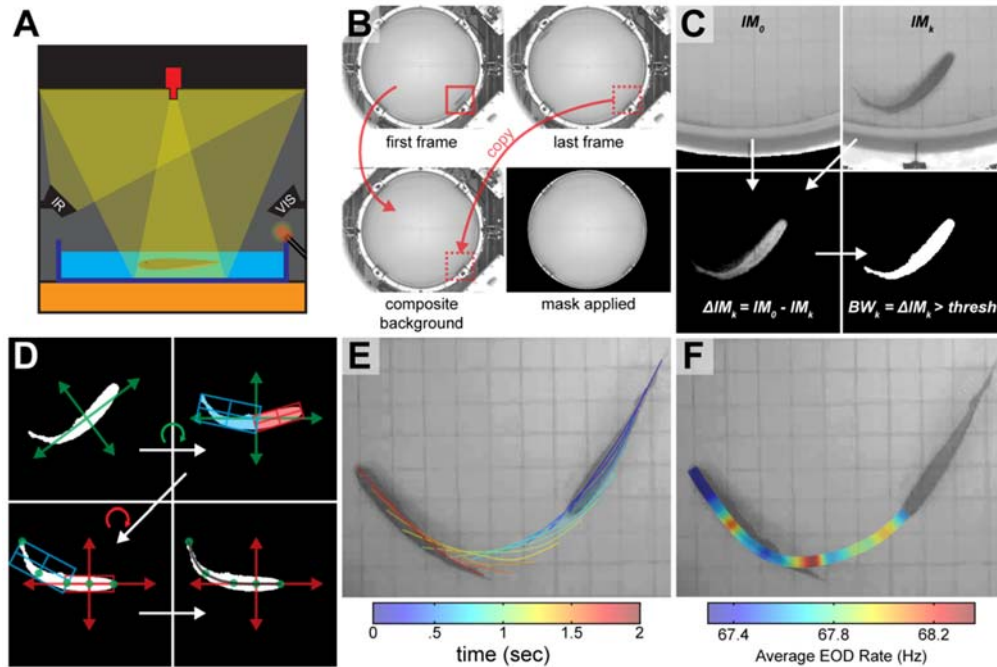


Figure 4.3. Video tracking setup and representative results. (A) The lighting and camera setup is illustrated. The infrared (IR) and visible light sources are attached on the walls and pointed toward the ceiling, such that the ceiling surface reflects and diffuses the light for projecting uniform illumination over the entire tank. The camera is hidden above the ceiling panel to prevent reflection on the water surface. An IR LED is positioned at one of the four tank corners to generate time synchronization pulses. (B) Generating a composite background image is illustrated. Two image frames (images on top) are combined to form the composite background image (bottom left) by replacing the region containing the animal (solid red square) with the region without the animal (dashed red square). Area outside of the central arena is masked out in black (bottom right). (C) Isolating the fish outline. An image frame (top left) is subtracted from the background image (top right) to produce the difference image (bottom left), and converted to the binary image (bottom right) by applying an intensity

threshold. **(D)** Measurements of the body position and posture are illustrated. The binary image of the animal (blob) was rotated to align its major axis with the x-axis (top right), and centered at its centroid. The blob was separated to the head (red) and tail (blue) parts, and each part was separately rotated for determining its bounding-box. The blob was oriented to the animal's frame of reference (bottom left), and five feature points (head-end, mid-head, mid-body, mid-tail, tail-end) were determined from the midpoints of the bounding-box edges. **(E)** Time-lapse image of the fish midlines plotted every 200 ms. The first and last image frames are superimposed during the 2 s turning duration. **(F)** The average EOD rate is represented in pseudo-color and superimposed with the trajectory of the fish head. The same images are used as in **E**.

4.4 Representative Results

4.4.1 EOD tracking results

The recorded EOD waveforms from different electrode pairs varied in amplitudes and shapes as expected from their unique positions and orientations (Fig. 4.2C top). The use of multiple electrode pairs ensured strong signal reception at all possible positions and orientations of WEF within the tank. The envelope waveform (Fig. 4.2C bottom, green trace) always contained a single peak per EOD cycle, which served as a reliable time marker for precisely determining the inter-pulse intervals and the instantaneous EOD rate ($= \text{IPI}^{-1}$). The successive EOD peaks were joined and linearly interpolated at constant time intervals (Fig. 4.2D top, black trace), and the instantaneous EOD rate was similarly interpolated at constant time intervals (Fig. 4.2D bottom, pink trace). The constant-time resampling procedure facilitates the time synchronization between the motion trajectory and the EOD signal, and allows to leverage from a greater number of analytic tools for constantly sampled time-series data. The EOD amplitudes recorded at external electrodes remained constant while an animal was at rest (Fig. 4.2E top), but it varied over time while the animal moved due to changing dipole location and orientation (Fig. 4.2F top). Thus, the fish movement could be inferred from observing the change of EOD amplitudes over time. The baseline EOD rate remained low while fish was at rest (Fig. 4.2E bottom), but the EOD rate became significantly higher while the fish actively

swam (Fig. 4.2F top). Our observation is consistent with the positive correlation between the EOD rate and the fish movement as previously reported [Kramer 1978; Stoddard et al., 2007; Jun et al., 2012a; Forlim and Pinto, 2014].

4.4.2 Video tracking results

The animal's trajectory and midlines are shown in Figure 4.3E with the first and last image frames superimposed. The time-course of posture change was captured while fish was abruptly turning for two seconds, and the fish midlines are plotted every 200 ms. The fish midline correctly started at the head-tip and terminated at the tail-tip of fish. The fish images closely agreed with the automatically tracked midlines despite of the shadows casted by the animal. Figure 4.3F illustrates the time-varying average EOD rate ($\tau = 0.0625$ s) in color, which is superimposed with the time-matched trajectory of fish's head-tip. During the 2 s turning duration, the average EOD rate reached its peak while the animal was in the middle of the turning phase, and the rate decreased toward the end of the turning. This representative result illustrates that our method can be successfully applied to study the relationship between self-guided movements and the EOD rate modulation during free-swimming.

4.5 Discussion

4.5.1 Significance of our techniques

In summary, we first described the construction of a large aquarium tank and an isolation chamber to observe spontaneous exploratory behaviors produced by WEF. Next, we demonstrated the technique of recording and tracking the EOD rate and the movement states from unrestrained fish in real-time using multiple electrode pairs. Finally, we described the infrared video recording technique through water in a time-synchronized manner, and the image tracking algorithm to measure the body position and posture. As an experimental preparation, WEF offers an important advantage for investigating active sensory-guided behaviors by demonstrating readily quantifiable EOD rate, which equals to the active electrosensory sampling rate. Combination of these techniques can enable precise and reliable long-term observation [Jun et al., 2012a] of spontaneous behaviors from unrestrained WEF. Furthermore, the majority of our setup can be constructed from widely available building materials and easily obtainable electronic components. The techniques described here have

been developed and tested to meet our experimental requirements over recent years. Therefore, we recommend these techniques for future studies of spontaneous exploratory behaviors from free-swimming WEF.

4.5.2 Isolation chamber

The isolation chamber provides well-controlled experimental conditions by blocking external sources of light, vibration, sound, and electrical noise with varying degrees of effectiveness. The light blocking performance was tested by placing a motorized camera inside of the dark isolation chamber, and no external light leakage was observed from the camera after scanning all locations using remote pan control. The vibration damping surface installed under the tank provided attenuation against external vibrations channeled from the floor, and stacking of multiple rubber and foam layers was effective for blocking most external vibration events. However, intermittent vibration events such as loud door closing at nearby locations did trigger novelty responses in rare occasions. Although an anti-vibration air table could deliver superior isolation performance from background vibrations, it would be prohibitively expensive to purchase an air table large enough for our aquarium tank. Therefore, we placed a hydrophone underwater to detect and exclude events when large external vibrations triggered novelty responses. To further minimize the influence of noise outside of the laboratory, our experiments were conducted during off-peak hours (after 6 pm). Similarly, external airborne acoustic noise was attenuated via the isolation chamber walls filled with fiberglass batts insulation. Although we did not objectively quantify the sound attenuation performance, most of the background sound in a lab environment did not trigger the novelty responses. In rare occasions, a sudden loud sound from the outside triggered a novelty response; but such an event was detected by the hydrophone recording, and they rarely occurred during off-peak hours. The aquarium tank provided sufficiently large area for our animals to freely swim and explore. The tank size was chosen in proportion to the length of species we used (up to 30 cm), but the tank size can be scaled down if smaller animals were used. We chose *Gymnotus* sp. among different pulse-type species for their large skull size to facilitate electrophysiological recordings during free-swimming [Canfield and Mizumori, 2004]. The electrical recording quality may improve from using more costly copper meshes, and shielding the exhaust fan used for the humidity control.

4.5.3 EOD measurement technique

Our multi-channel EOD recording technique permitted accurate and reliable EOD timing measurement from freely swimming fish. Using our technique, all EOD pulses generated by freely swimming WEF were detected without missing or adding a single pulse for a six-hour long recording duration (see Fig. 12 in Jun *et al.* [2012]). The EOD recording measures not only the EOD rate, but also the activity level from the time varying EOD peak amplitudes recorded at external electrodes. The recorded EOD amplitudes are determined by the relative geometry between an animal and the recording electrodes, thus animal movements induce changes in the EOD amplitudes (Fig. 4.2F). The activity level was computed from the variability (RMS) of the EOD amplitude slope within a moving window (0.5 s). Using this method, video recording would not be required for measuring the activity level over a long time period, and the EOD recording alone may suffice. Instead of using a video recording, the body position and posture of WEF can be inferred from the EOD recording alone based on the electrodes locations, the geometry of a tank, and a theoretical model of a current dipole. Using a similar recording setup, Jun *et al.* [2013] proposed a real-time electrical tracking method for tracking multiple WEFs in presence of an object, which compares measured signal intensities at multiple recording electrode pairs with lookup table entries containing predicted signal intensities at known current dipole locations. The electrical tracking method offers improved tracking reliability in a visually cluttered environment where animals often get obstructed from view, or during tracking multiple animals. WEF's naturalistic habitats contain many visual obstacles such as aquatic plants and roots, where the electrical tracking method could provide more reliable tracking with simpler setup requirements than visual tracking. In principal, our method is directly applicable to the wave-type WEF species after changing filter time constants. The rectification step will introduce two modes per EOD cycle, since the EOD waveform is approximately sinusoidal in wave-type species. In this case, the instantaneous EOD rate can be determined by skipping every other EOD time markers to ignore the negative EOD phase. WEF can detect the recording electrodes when they swim nearby; thus we avoided using large or metallic electrodes which can be sensed from farther away³⁷, and instead used thin graphite electrodes (2 mm diameter). Thinner coaxial cables (RG-174) were used with the electrode assemblies for flexibility; but thicker coaxial cables (RG-54) were used for wiring over extended distances for superior electrical shielding. Longer EOD recording duration can

be achieved by lowering the sampling rate, but at a lower temporal resolution as a trade-off. The mean and variability of the EOD rate varies between species, thus the time window for smoothing the instantaneous EOD rate needs to be adjusted appropriately. A shorter time window is recommended for species having shorter mean and smaller variability in the IPIs (*e.g.* Gymnotiforms), and a longer time window is recommended for species having longer mean and higher variability in the IPIs (*e.g.* Mormyrids).

4.5.4 Lighting and camera setup

Video recordings provide quantitative and qualitative behavioral observations, and here we described the procedures for setting up, recording, and processing the image data. Lighting setup plays an important role in producing high quality images, and the light projection angle is an important factor for imaging underwater animals. Under suboptimal lighting conditions, the water surface can form glares and reflections, which can interfere with the image tracking especially when animals generate surface waves. The glare and reflection problems can be eliminated by projecting light sources from the bottom of a tank. For a small tank, arrays of LED can be placed directly underneath the tank and shine through a diffuser panel to generate uniform light intensity³⁸. Similarly for a larger tank, a light source can be placed below the tank, and uniform light intensity can be achieved by allowing sufficient distance for light to diffuse³⁹. In our setup, we were forced to project the light from above the tank due to space constraints, structural stability, and the heater placement underneath the tank. We avoided the glare and reflection problems by using indirect lighting, such that the light sources were projected toward the ceiling. By rendering the top portion of the chamber featureless and matt white, no reflections were visible on the water surface. To image the whole central arena, a wide angle lens can be mounted on the camera, but some lenses (fish-eye lens) may cause significant barrel distortion. The barrel distortion can be corrected by using a calibration grid sheet underneath the tank to measure the pixel coordinates of the grid locations viewed at the tank center. Together with the corresponding grid locations in centimeters, a transformation matrix can be computed to correct the barrel distortion [Shapiro and Stockman, 2001]. We recommend high resolution cameras if an animal size is much smaller than the tank size, so that sufficient number of pixels can be obtained from the animal to correctly measure its body posture.

4.5.5 Image tracking and time synchronization

The image tracking algorithm described here makes use of the region-of-interest (ROI) operation to quickly measure the body position and posture. The ROI operation reduces the image size to be processed, and constrains the tracking range near the animal location from the previous frame. We extracted the body posture (midline) by using the image rotation and bounding-box operations instead of the usual image skeletonization operation, which sometimes failed to produce a well-defined single midline. The animal's frame of reference was located at the middle of the head bounding-box, which permits egocentric behavioral analysis. The major source of error in the image tracking was due to the optical projection effect at a wide angle. Ideally, animal's vertical movements should not affect the 2D position measurement; but the further away from the central imaging axis, the greater portion of the vertical dimension is projected to the camera. The refraction at the water surface reduced the optical projection effect by 28% in our imaging setup (camera height: 1.8 m, water depth: 10 cm, tank radius: 75 cm); and the worst position error was ± 1.4 cm at the circular fence. The timing between the EOD and video recordings were synchronized using infrared LED pulses to account for the time drift between the video and the signal digitizer clocks, and different recording start-up times. The expected uncertainty in the time synchronization between the video and EOD recordings is proportional to the frame capture interval; for example, 15 frames per second (FPS) frame capture rate will result in the time alignment uncertainty of ± 33 ms. Such degree of time precision is adequate for tracking slower moving fish, but a high-speed camera may be required for tracking faster moving animals. We recommend brighter light intensity with an increased frame rate, since the sensor exposure time is inversely proportional to the frame rate.

4.5.6 Future work

Social interactions between multiple WEFs can be studied by tracking their EOD signals and body locations, and the tracking system must correctly associate the EOD with the location of the same individual. According to the dipole localization method described by Jun *et al.* [2013] using similar setup, the animal locations inferred by their EOD signals received at multiple electrodes can be matched to the visual tracking output for correctly identifying the EOD pulses from different individuals. Image tracking of multiple animals can be performed one individual at a time using the ROI operation. A ROI can be initially defined around an

individual to be tracked, and the ROI will be repositioned at every frame with an updated body position. The other fish will be excluded from the image tracking analysis when it appears outside of the ROI; and if appeared inside, the other fish's image can be automatically removed by checking whether its image touches the ROI boundary. Sometimes, two animals contact each other and their images merge; and if so, a mask can be manually drawn to separate the other fish's image. Another interesting future work is the three-dimensional video tracking to reveal intricate movement sequences during prey capture [MacIver and Nelson, 2000] or social interactions. MacIver and Nelson [2000] used two cameras to view a rectangular aquarium tank from the top and the side for reconstructing a three-dimensional body model. However, this approach would not work in our case, since there are partitioning walls that block side views and the aquarium has much greater width than depth. Instead, it would be more applicable to install multiple cameras on the ceiling at different perspective angles similar to the setup used by Hedrick [2001]. For greater accuracy, the refractive effect introduced by the water and the oblique camera angle would have to be corrected by calibrating images in three dimensions. Our visual tracking method could be applied to study the electric image flow on fish's body surface [Babineau et al., 2007; Sanguinetti et al., 2011] when fish swims nearby an object. As studied by Hofmann *et al.* [2013b], it would be interesting to investigate the object's electric image flow during free-swimming depending on the object distance, shape, size, and material. Ultimately, our methods combined with neural recordings from freely swimming fish [Castelló et al., 1998; Canfield and Mizumori, 2004; Pereira et al., 2005] may reveal novel insights by observations of changes in neural activity and EOD rate while the fish engages in object exploration or social interactions.

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Chapter 5

Enhanced sensory sampling precedes
self-initiated locomotion in an electric fish

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5.1 Summary

Cortical activity precedes self-initiated movements by several seconds in mammals; this observation has led into inquiries on the nature of volition. Preparatory neural activity is known to be associated with decision making and movement planning. Self-initiated locomotion has been linked to increased active sensory sampling; however, the precise temporal relationship between sensory acquisition and voluntary movement initiation has not been established. Based on long-term monitoring of sensory sampling activity that is readily observable in freely behaving pulse-type electric fish, we show that heightened sensory acquisition precedes spontaneous initiation of swimming. *Gymnotus* sp. revealed a bimodal distribution of electric organ discharge rate (EODR) demonstrating Down- and Up-states of sensory sampling and neural activity; movements only occurred during Up-states and Up-states were initiated before movement-onset. EODR during voluntary swimming initiation exhibited greater trial-to-trial variability than the sound-evoked increases in EODR. The sampling variability declined after voluntary movement-onset as previously observed for the neural variability associated with decision-making in primates. Spontaneous movements occurred randomly without a characteristic timescale, and no significant temporal correlation was found between successive movement intervals. Using statistical analyses of spontaneous exploratory behaviors and associated preparatory sensory sampling increase, we conclude that electric fish exhibit key attributes of volitional movements, and that voluntary behaviors in vertebrates may generally be preceded by increased sensory sampling. Our results suggest that comparative studies of the neural basis of volition may therefore be possible in pulse-type electric fish, given the substantial homologies between the telencephali of teleost fish and mammals.

Key words: weakly electric fish; voluntary movement; active sensing; decision making; readiness potential; volition

5.2 Introduction

Volition is generally considered as a defining human faculty; but the outcome of a voluntary decision can be predicted by brain activity even before a subject's conscious awareness [Libet et al., 1983; Soon et al., 2008; Desmurget and Sirigu, 2012], and a gradual increase in neural activity preceding voluntary movement is observed in several species [Kornhuber and Deecke, 1965; Fried et al., 2011; Romo and Schultz, 1987]. Preparatory neural activities for voluntary movements involve movement planning and decision making [Kaufman et al., 2010], and active control of active sensory sampling accompany heightened spatial attention [Winkowski and Knudsen, 2006; Ulanovsky and Moss, 2008]. Here we show that enhanced sensory sampling precedes the voluntary decision to move in an animal model that exhibits a readily observable and quantifiable sensory acquisition rate. In addition to demonstrating preparatory increases in sensory sampling, our results imply close linkages between sensory sampling and neural activity in a weakly electric fish, suggesting that they are also associated with voluntary decision making.

Humans can make conscious decisions to initiate movement [Haggard, 2008] without external sensory stimuli [Sokolov, 1990]. Cortical recordings reveal that neural activity precedes the time when a conscious decision to act is reported [Kornhuber and Deecke, 1965; Libet et al., 1983; Soon et al., 2008], raising questions as to how neural activity relates to conscious decision making and the initiation of voluntary actions. Whereas prior work has shown neural activity preceding voluntary actions in primates capable of advanced cognition [Romo and Schultz, 1987; Kato et al., 1995; Kaufman et al., 2010], here we demonstrate the same pattern in an aquatic vertebrate whose last common ancestor to primates lived more than 450 million years ago. We propose that volition may therefore be a primitive capability of vertebrate brains that precedes the advanced cognitive capacities of primates.

Animals actively sample their environment using e.g., whisking, sniffing, and saccadic eye movements; these active sensing behaviors [Nelson and MacIver, 2006; Kleinfeld et al., 2006; Otero-Millan et al., 2008; Wachowiak, 2011] occur more frequently during periods of active exploration [Poulet and Petersen, 2008; Schroeder et al., 2010]. Gathering of sensory information can guide movement decisions to be made later; but the precise temporal relation between volitional acts and sensory sampling is still not known: e.g., does active sensing

increase before, together with or after self-initiated movements? A general answer is here suggested based on pulse-type weakly electric fish, such as *Gymnotus* sp. These fish emit brief (~1 ms) EOD pulses that stimulate cutaneous electroreceptors; each pulse corresponds to a discrete active sampling event. Objects in the fish's environment distort their EOD-generated electric field resulting in localized electric images that, in the dark, are the primary sensory basis for navigation and prey capture. Unexpected stimuli will cause an increase in the EODR (novelty response) and therefore the putative sampling rate of the tuberous electrosensory system [Caputi et al., 2003; Pluta and Kawasaki, 2008]. Previous work has shown that pulse-type WEF compare the reafferent electrosensory image induced by each EOD pulse with a stored template derived from previous electrosensory input [Heiligenberg, 1980; Hopkins, 1983; Moller, 1995; Post and von der Emde, 1999; Caputi et al., 2003]. Caputi et al demonstrate that a template of the electrosensory input develops over many EOD pulses and that a changed input for only one EOD pulse is sufficient to trigger a novelty response. Further, the magnitude of novelty response undergoes experience-dependent habituation that is inversely proportional to the inter-stimulus interval [Barrio et al., 1991; Post and von der Emde, 1999; Caputi et al., 2003]. We conclude that each EOD constitutes a discrete sampling event and that EODR is therefore a direct measure of sampling rate over some time interval. Pulse-type electro-sensation thus offers a distinct experimental advantage because each sampling event can be readily and non-invasively monitored from an unrestrained animal [Jun et al., 2012; Jun et al., 2014]. In gymnotiform species, EOD pulses are directly driven by a hindbrain pacemaker that is modulated by diencephalic and medullary prepacemaker activity [Heiligenberg et al., 1981; Kawasaki et al., 1988; Dye, 1988; Metzner, 1993; Caputi et al., 1993; Zupanc and Maler, 1997; Wong, 1997; Comas and Borde, 2010]. Previous studies of the electromotor circuitry [Caputi et al., 1993; Wong, 1997; Giassi et al., 2012], functional stimulation of an electromotor thalamic nucleus [Comas and Borde, 2010], and drug injection in the pallium [Santana et al., 2001] all indicate a strong modulation of the EODR from the forebrain. Thus, the net activity of the higher level neural populations projecting to the EOD pacemaker can be inferred from the time derivative of EODR, or EOD acceleration (EODA) [Metzner, 1993; Metzner, 1999; Arnegard and Carlson, 2005; Pluta and Kawasaki, 2008].

5.3 Materials and Methods

Gymnotus sp. were housed in a circular tank surrounded by a sensory-isolation chamber to block external sources of light, sound and vibration. The animals were under 12-hour light cycle, and the recordings were made in darkness during active part of the fish circadian rhythm. Water filtration, aeration, and feeding were performed between recording sessions. EOD pulse times were precisely and reliably recorded as previously described [Jun et al., 2012]. Movement activity level was determined from the slopes of the EOD peak amplitudes [Jun et al., 2013]. During sound-evoked trials, acoustic-stimuli were delivered at random intervals to prevent habituation.

5.3.1 Experimental animals

All procedures including housing and recording protocols were approved by the University of Ottawa Animal Care Committee. We obtained South American pulse-type weakly electric fish (genus *Gymnotus*) of unknown species and gender from a local supplier (Big Al aquarium services, Ottawa, ON, Canada). After arrival, animals were initially housed in a community tank, and fed live blackworms prior to experimental observation. Animals were brought to the experimental tank one at a time, and acclimatized for at least two days before the first recording. Animals were individually housed and maintained on a 12 h light cycle with ad libitum access to food (mealworms) except for the recording duration. Experiments were performed in the dark cycle.

5.3.2 Experimental setup

The experimental tank and isolation chamber were as previously described (Fig. 5.1A) [Jun et al., 2012; Jun et al., 2014]. Underwater sound was recorded during experiments by a hydrophone (TC4013, Reson Inc., Goleta, CA, USA) and amplified (50 dB Gain; VP1000, Reson Inc., Goleta, CA, USA) to check for animal movements triggered by external vibrations. Detailed descriptions of the experimental chamber, the aquarium tank designs and water conditions are provided in Jun et al. [2014].

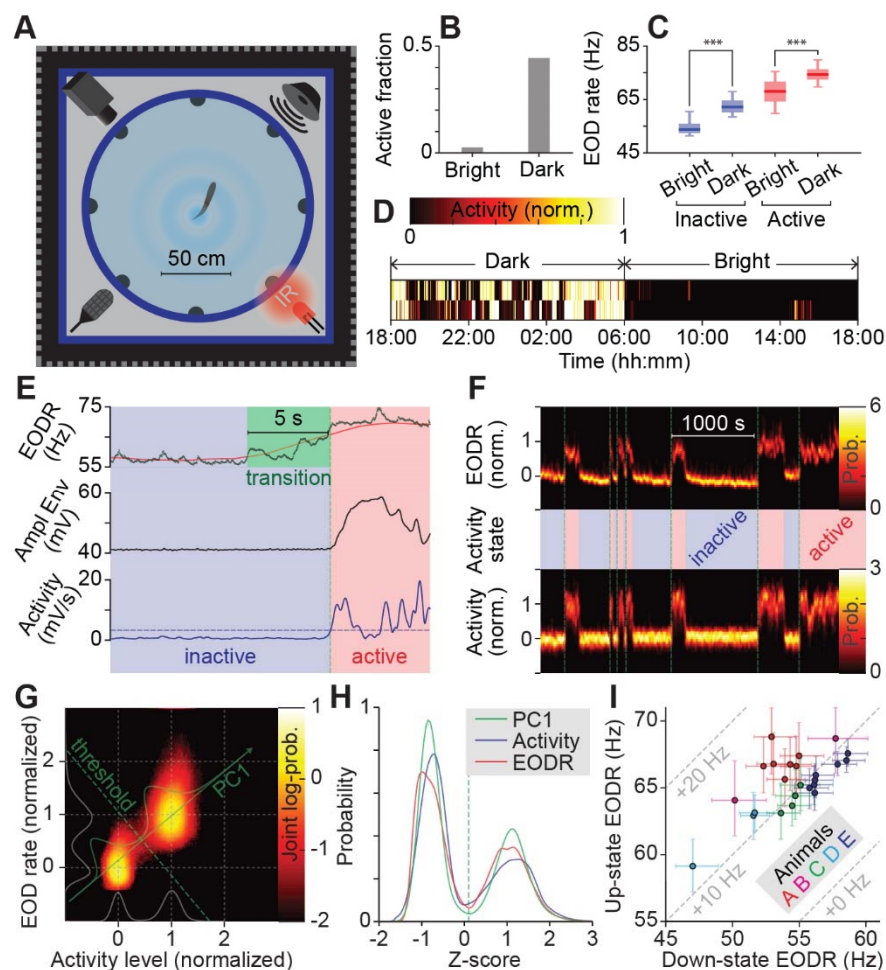


Figure 5.1. Two behavioral and attentional states in *Gymnotus* sp. (A) Experimental tank in a sensory-isolation chamber with infrared (IR) lighting. Electric organ discharge (EOD) signal was captured by eight dipoles symmetrically placed around the edge of the tank to monitor the EOD rate (EODR) and movement activity; a video camera also directly captured movement. Subwoofers delivered random stimuli during sensory-evoked condition and a microphone was used to record possible noise contamination. (B) Fraction of active periods under light and dark conditions. (C) Box plots of EODR distributions (displaying 5th, 25th, 50th, 75th and 95th percentiles) during inactive (blue) and active periods (red) under light and dark conditions. (D) Activity levels recorded for 48 hours indicate predominance of activities in darkness. All data in B-D were obtained from animal A continuously for 48 hours (two rows indicate two consecutive days). Vertical color bars indicate periods of active movements interrupted by resting periods (black). (E) Example traces of the EOD rate (EODR), EOD amplitude envelope (*Ampl Env*), and activity level during a spontaneous movement-initiation. (F) Example traces of the EOD rate (EODR), EOD amplitude envelope (*Ampl Env*), and activity level during a spontaneous movement-initiation. (G) Joint log-probability plot of EOD rate (normalized) and activity level (normalized). (H) Probability distribution of Z-scores for PC1, Activity, and EODR. (I) Up-state EODR (Hz) vs Down-state EODR (Hz) for individual animals (A, B, C, D, E). Dashed lines indicate +20 Hz, +10 Hz, and +0 Hz changes.

Moving average of the EODR (red trace) started to increase five seconds before movement onset (dashed green line); scale bar: 50 cm. **(F)** Probability density (heat map) of the normalized EODR and activity level over time, which randomly switched between two states. Movement onset (dashed green lines) closely coincided with the EODR transition onset. **(G)** Joint log-probability density (heat map) of the normalized EODR and activity level and their first principal component (PC1; green trace) were used to clearly separate the Down- and Up-states by applying a threshold (dashed green line). **(H)** Z-score distributions of the activity level, EODR, and their first principal component (PC1). Dashed green line (local minima of the PC1 distribution) indicates the state-segregation threshold for PC1. **(I)** Scatter plot of the Up- vs. Down-state EODR from multiple days and animals. Black circles indicate the median, and error bars indicate the 25th and 75th percentiles. Each color represents a different individual.

5.3.3 EOD recording and movement activity

The EOD signals were recorded from multiple electrodes, which reliably and accurately captured the animal's EOD pulses at all tank locations (see Jun et al., [2012] for our EOD pulse detection technique). The EODR was determined by smoothing the instantaneous EOD pulse rate (averaging filter, $\tau = 0.0625$ s), and was linearly interpolated at a constant sampling rate (100 Hz) to facilitate data synchronization and subsequent analysis. The EOD amplitudes conveyed movement information, since animal's movements caused fluctuations of the EOD amplitudes at the recording electrodes [Jun et al., 2012]. We quantified the activity level of an animal by integrating the EOD amplitude fluctuations from all recording channels. The activity level computed from the EOD recording accurately quantified the animal's movements without requiring a concurrent video recording, which demands significantly greater storage space due to high-speed and high-resolution imaging for sensitive motion detection (see Jun et al., [2014] for the method of computing the activity level).

5.3.4 State segregation method

The fish's movement activities and sensory sampling rates both followed well-defined bimodal distributions that were highly temporally correlated (Fig. 5.1G). Due to their binary nature, we divided the behavioral time-series into two states in order to characterize the two states and

the state transitions, according to the following procedures. First, principal component analysis was performed to compute the first principal component (PC1) of EODR and activity level. Second, the state segregation was performed based on the PC1 score, since its distribution exhibited greater separation between the two modes than either of the original measures (Fig. 5.1H). To evaluate the goodness of separation, a separation index (SI) was computed for each distribution as follows:

$$SI = \frac{|\text{mode}_1 - \text{mode}_2|}{\sqrt{FWHM_1 \times FWHM_2}} \quad (5.1)$$

where the subscripts 1 and 2 refer to the first and second peaks; FWHM: full width half maximum. Out of the three distributions (EODR, activity level, and PC1), the SI was the highest for the PC1 distribution for 20 out of 23 recording sessions. For each recording day, a threshold level for the PC1 score was determined at the local minimum of its bimodal distribution between the two peaks. Recordings with predominantly Up- or Down-states (i.e., where Up- or Down-states occupied more than 90% of the data) generally occurred during initial acclimation periods, and were excluded from analysis (30% of all recording sessions) due to greater overlap in the bimodal distribution, and inaccurate state segregation. The states were initially divided by applying a threshold; subsequently, transient Up-states lasting less than 1.5 s were merged with their neighboring Down-states, and vice versa for the transient Down-states (Fig. 5.2A). The 1.5 s cut-off was determined from the first local minimum [Romo and Schultz, 1987] of the Up-state duration histogram (Fig. 5.2B, left). After the state segregation, the EODR and activity level were normalized to facilitate pooling data from multiple days and animals, which originally exhibited different baselines (Fig. 5.1I). The median values during Down- and Up-states were mapped to zero and one, respectively, by linearly rescaling both EODR and activity level (Fig. 5.2C). In essence, the normalization procedure expressed the movement activity and sensory sampling rate measures on a standardized scale corresponding to the two states, regardless of their characteristic distributions. We refer to the EODR and activity level in the normalized unit throughout this paper unless otherwise stated.

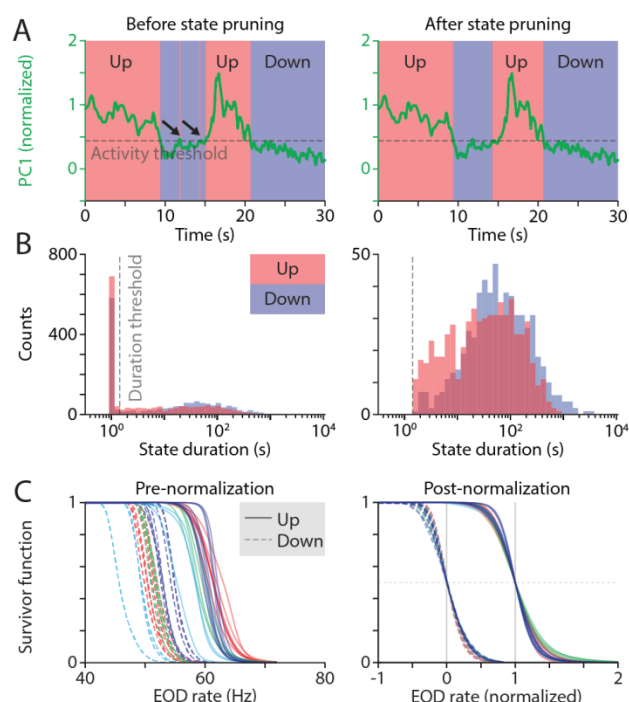


Figure 5.2. State pruning by merging and data normalization. (A) The first principal component of the EODR and activity level (PC1) over time (green trace), and resulting states after the state segregation (red: Up-state, blue: Down-state). Dashed green line represents the PC1 segregation threshold, and black arrows in the left panel indicate transient Up-states (left) to be merged into the Down-state (right). (B) Histogram of the Down- (blue) and Up-state (red) durations before (left) and after (right) the state pruning procedure from the same animal (4 days pooled). Dashed grey lines indicate a threshold for the minimum state duration (1.5 s), which was set at the first local minimum of the Up-state duration histogram (left). (C) Normalization of data across multiple days and animals. Survivor functions of the EODR are shown during Down- (dashed lines) and Up-states (solid lines) before (left) and after (right) the normalization procedure. Each animal is represented by a unique color.

5.3.5 Movement-onset triggered analysis

The Up-transition times determined from the PC1 score did not accurately correspond to the actual movement-onset times, since the PC1 score is also based on EODR which does not directly quantify movements. Therefore, we refined the movement-onset times by using the

normalized activity level that directly quantifies movement. The temporal precision of the EOD-based movement-onset times was improved to the level of a visual detection method (Fig. 5.5A-F) by applying the following procedures [Jun et al., 2014]: First, we selected a subset of Up-transitions exhibiting clearly discernible movement onset and clear Down- and Up-states based on the following criteria. The Down- and Up-state durations had to last longer than 5 s each; the averaged normalized activity level had to be under 0.1 between 0 to 1 s before, and over 0.6 between 0 to 1 s after movement onset for the Down- and Up-states respectively. The first threshold crossing of the activity level within 5 s of the PC1 Up-transition onset (or sound onset times during evoked trials) was taken as the movement-onset time, and the threshold (0.2) was chosen at the mean local minimum of the bimodal distribution of the normalized activity level. Similarly, the EODR rise-onset times were determined at the first threshold crossing (0.4) of the normalized EODR within 5 s of the Up-transition. The transition latency was computed by taking the difference between the EODR rise onset and the movement-onset times.

We did not analyze movement offsets, since it was difficult to determine the exact moment due to the inertia of the fish's body². The movement-onset times determined by the EOD recordings were verified by concurrent visual recordings from animal B, and a mean corrective time offset (25 ms) was subtracted from the EOD-based movement-onset times to minimize the average timing error. In order to check for the effect of external acoustic and vibratory noise on animal movements, the hydrophone recording traces were 1) notch-filtered ($f_c = 60$ Hz, $Q = 10$) and RMS-filtered ($\tau = 0.1$ s) to remove the mains interference, 2) aligned at the movement-onset times (Fig. 5.4C), 3) averaged across trials after subtracting the mean (± 5 s from the movement onset) (Fig. 5.4D). Averaging did not lead to cancellation of the sound waves having different phases, since the RMS-filter extracted the waveform envelope.

5.3.6 Video calibration

In selected trials, we simultaneously recorded infrared videos from animal B to validate and calibrate movement-onset times determined from the EOD recordings (activity level threshold crossing). During video recordings, the experimental chamber was illuminated by eight

² It takes up to a few seconds for fish to come to a complete stop after stopping its fin movements.

infrared LED lights ($\lambda = 880$ nm) invisible to teleost fish [Fernald, 1988; Douglas and Hawryshyn, 1990]. A modified webcam (infrared blocking filter was removed; C910, Logitech, Fremont, CA, USA) captured video (15 frames/s, 640 x 480) from directly above the tank using Spike 2 software (CED, Cambridge, UK). Video recordings were synchronized with the EOD recordings by periodically blinking an infrared LED (10 ms duration, 10 s interval), and the light pulse timing was simultaneously captured by the digitizer. The first and last light pulses captured by the camera were used to convert the image frame numbers to the digitizer time unit, and vice versa. After determining movement-onset times from the EOD recordings, each movement-onset time was independently confirmed by concurrent video recording according to the following procedures. 1) Image frames within 5 s of the EOD-based movement-onset times were loaded to memory. 2) A reference region-of-interest (ROI) was defined by drawing a polygon around an animal image on the first frame. 3) Spatially-averaged Pearson correlation coefficients were calculated for the pixel values in the reference ROI and all the rest of the image frames. 4) An image frame number was manually marked when the averaged correlation coefficient began to decrease significantly, indicating a movement onset (Fig. 5.5A). The differences between the EOD-based and visually determined movement-onset times were calculated to quantify the temporal precision of the movement onset detection (Fig. 5.5B-E), and to calibrate the EOD-based movement-onset times. We quantified the relationship between the activity level and log-speed (Fig. 5.5F). The speed of animal centroid was determined by custom-made Matlab (The MathWorks, Natick, MA, USA) image tracking software [Jun et al., 2014], and the minimum speed was set to 1 cm/s before applying the log transformation.

5.3.7 Acoustic stimuli

In order to compare increase of the EOD rates associated with spontaneous movements with the ones associated with sensory-evoked responses, we delivered loud acoustic stimuli (143 dB-SPL re. 1 μ Pa root-mean-squared) at random intervals (5 ± 1 min) to trigger startle movements. Brief pure-tone acoustic stimuli (0.5 or 1 s duration, 150 Hz) were delivered by two 200 watt subwoofers (Z623, Logitech, Fremont, CA, USA), which rested on foam bases (5.1 cm thick) above the experimental tank and faced upward to minimize acoustic interference between the two speakers. The sound intensity was calibrated by a hydrophone (TC4013, Reson) positioned 3 cm below the water surface at various tank locations, and the spatial

variation of the sound intensity was within 10% from the mean. Delivery of the acoustic stimuli was controlled by the digitizer software (Spike2, CED, Cambridge, UK), and the inter-stimuli intervals were drawn from a uniform random distribution (5 ± 1 min) to prevent habituation [Post and von der Emde, 1999]. The acoustic stimuli always triggered stereotyped novelty responses within a single EOD pulse cycle from the stimulus onset; however, the acoustic stimuli did not always trigger sufficiently large movements ($< 30\%$ of all trials). To generate movement-onset triggered plots (Fig. 5.4A,B), we pooled trials exhibiting significant movements (activity level > 0.325) within 1 s of the stimulus onset; but all trials were pooled for calculating the stimulus-triggered averages and SD (Fig. 5.6A-D; Fig. 5.7A-C) and the coefficient of variation (CV) of EOD intervals (Fig. 5.6G). The acoustic stimuli were delivered in darkness, and we only analyzed trials when animals exhibited clear resting baselines (activity level < 0.1 and EODR < 0.4) between 0 to 1 s before the stimulus onset.

5.3.8 Statistical analyses

The probability distribution of the EODR and EODA (Fig. 5.2A,C) were estimated by applying a kernel smoothing method (*ksdensity* function in Matlab), and all other probability distributions and cumulative distributions were estimated this way (e.g., Fig. 5.8A-C; Fig. 5.9A). The error bars in all probability and cumulative distribution plots indicate 95% confidence intervals, and were estimated by applying a bootstrap sampling method (*bootci* function in Matlab). The error bars in all trial-averaged plots indicate mean $\pm$ SEM (e.g., Fig. 5.4A-D; Fig. 5.6E). Due to the lack of hydrophone recordings, Animal A was excluded from generating the movement-triggered background noise plot (Fig. 5.4D). Animal B was excluded from generating the spontaneous transition statistics (Fig. 5.8A,B,D-G; Fig. 5.9A-D) due to insufficient total recording hours (under 11 hours). We pooled 1319 trials from 5 animals under the spontaneous condition, and 261 trials were pooled from 5 animals under the evoked condition to compute the trial-to-trial variability over time (Fig. 5.6G). During spontaneous and evoked transitions (within 5 s of movement or stimulus onset), trials exhibiting transient EOD-interruptions [Schuster, 2002] were automatically excluded. Trials showing the normalized EODR below the threshold (-1) were removed, which constituted less than 1% of the total number of trials.

Table 5.1. Data summary for the behavioral two states

Animal ID	A	B	C	D	E
Total recording hours	62.1	10.7	34.9	27.4	71.7
Probability of Up-state	26.4%	57.9%	65.8%	71.4%	88.9%
Correlation between EODR and Activity level	0.832, $p < 10^{-12}$	0.815, $p < 10^{-12}$	0.816, $p < 10^{-12}$	0.817, $p < 10^{-12}$	0.726, $p < 10^{-12}$
Up-state EODR ($\mu \pm \sigma$; Hz)	67.1 $\pm$ 3.8	66.2 $\pm$ 5.6	64.2 $\pm$ 3.4	62.5 $\pm$ 3.5	66.3 $\pm$ 2.5
Down-state EODR ($\mu \pm \sigma$; Hz)	53.6 $\pm$ 2.5	48.9 $\pm$ 6.1	54.3 $\pm$ 2.4	48.7 $\pm$ 3.1	56.9 $\pm$ 3.3
KS test ^a for EODR distributions between Down- and Up-states	$p < 10^{-12}$	$p < 10^{-12}$	$p < 10^{-12}$	$p < 10^{-12}$	$p < 10^{-12}$
Up-state EODA ($\mu \pm \sigma$; Hz/s)	-0.1 $\pm$ 6.5	-0.5 $\pm$ 11.7	-0.2 $\pm$ 7.5	-0.2 $\pm$ 7.9	-0.1 $\pm$ 6.0
Down-state EODA ($\mu \pm \sigma$; Hz/s)	-0.0 $\pm$ 4.3	-0.1 $\pm$ 6.9	0.0 $\pm$ 4.9	0.1 $\pm$ 5.0	0.1 $\pm$ 6.0
KS test ^{*1} for EODA distributions between Down- and Up-states	$p < 10^{-12}$	$p < 10^{-12}$	$p < 10^{-12}$	$p < 10^{-12}$	$p < 10^{-12}$

EODR, Electric organ discharge rate; EODA, Electric organ discharge acceleration. ^aKS test refers to the paired Kolmogorov-Smirnov test (*kstest2* function in Matlab).

Table 5.2. Data summary for the transition latency and across-trial variability

Animal ID	A	B	C	D	E
# spontaneous transitions	174	204	323	203	404
# evoked transitions (all)	102	28	89	58	19
# evoked transitions (moved)	86	21	33	49	19
Duration of sound-stimulus (s)	0.5	1	1	1	1
Spontaneous Up-transition latency ($\mu \pm \sigma$; s)	0.67 ± 0.75	0.38 ± 0.48	0.52 ± 0.63	0.33 ± 0.55	0.41 ± 0.46
Evoked Up-transition latency ($\mu \pm \sigma$; s)	0.21 ± 0.13	0.03 ± 0.08	0.09 ± 0.07	0.04 ± 0.05	0.07 ± 0.02
KS test ^a between spontaneous vs. evoked transition latency distributions	$p < 10^{-12}$	$p < 10^{-12}$	$p < 10^{-12}$	$p < 10^{-12}$	$p < 10^{-12}$
Probability of EODR Up-transition preceding spontaneous movement onset	86.4%	95.7%	97.9%	82.3%	93.1%
Across-trial EODR SD ^b during spontaneous transitions	0.27 ± 0.03	0.29 ± 0.03	0.29 ± 0.02	0.26 ± 0.03	0.29 ± 0.02
Across-trial EODR SD during evoked transitions ^a	0.14 ± 0.02	0.17 ± 0.05	0.19 ± 0.03	0.17 ± 0.04	0.14 ± 0.04
Lilliefors test for EODR during spontaneous transitions ^c	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
Lilliefors test for EODR during evoked transitions ^c	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$

<i>F</i> -test for equal variances of EODR between spontaneous and evoked transitions ^c	$p < 10^{-3}$	$p < 10^{-2}$	$p < 10^{-3}$	$p < 10^{-2}$	$p < 10^{-2}$
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EODR, Electric organ discharge rate; SD, standard deviation. ^aKS test refers to the paired Kolmogorov-Smirnov test (*kstest2* function in Matlab). ^bTrial-to-trial EODR SD was computed when the trial-averaged EODR reached the transition threshold (0.4). 95% bootstrap confidence interval is given. ^cThe EODR values were sampled when the trial-averaged EODR reached the transition threshold (0.4).

Table 5.3. Data summary for the spontaneous Up-transitions

Animal ID	A	B	C	D	E
Number of Up-transitions	517	322	595	965	819
Log-normal distribution fitting parameters (μ , σ) for Up-transition log-intervals ^a	5.27 $\pm$ 0.12, 1.42 $\pm$ 0.09	4.35 $\pm$ 0.10, 0.96 $\pm$ 0.07	4.87 $\pm$ 0.08, 1.01 $\pm$ 0.06	3.78 $\pm$ 0.08, 1.22 $\pm$ 0.05	4.88 $\pm$ 0.09, 1.27 $\pm$ 0.06
Correlation between successive Up-transition intervals ^a	0.05 $\pm$ 0.09 $P = 0.2494$	0.09 $\pm$ 0.11 $P = 0.0974$	0.18 $\pm$ 0.08 $P = 0.0000$	0.06 $\pm$ 0.06 $P = 0.0811$	0.02 $\pm$ 0.07 $P = 0.6472$
Correlation between successive Down- and Up-state durations ^a	-0.11 $\pm$ 0.09 $P = 0.0127$	-0.13 $\pm$ 0.11 $P = 0.0210$	-0.14 $\pm$ 0.08 $P = 0.0009$	-0.04 $\pm$ 0.06 $P = 0.1909$	-0.09 $\pm$ 0.07 $P = 0.0079$
Correlation between transition latency and Down-state duration	-0.05 $\pm$ 0.15 $P = 0.4717$	-0.03 $\pm$ 0.14 $P = 0.6544$	-0.04 $\pm$ 0.11 $P = 0.5128$	-0.02 $\pm$ 0.14 $P = 0.8047$	-0.10 $\pm$ 0.10 $P = 0.0493$
Correlation between transition latency and Up-state duration	0.05 $\pm$ 0.15 $P = 0.4972$	0.14 $\pm$ 0.14 $P = 0.0475$	-0.03 $\pm$ 0.11 $P = 0.5780$	0.03 $\pm$ 0.14 $P = 0.6976$	-0.00 $\pm$ 0.10 $P = 0.9294$
Fano factor slope ^a	0.13 $\pm$ 0.01	N/A ^e	0.21 $\pm$ 0.04	0.44 $\pm$ 0.01	0.37 $\pm$ 0.02
Allan factor slope ^c	0.13 $\pm$ 0.02	N/A ^e	0.17 $\pm$ 0.04	0.37 $\pm$ 0.03	0.26 $\pm$ 0.02
Hurst exponent	0.54 $\pm$ 0.01	N/A ^e	0.52 $\pm$ 0.01	0.63 $\pm$ 0.02	0.56 $\pm$ 0.02

^a95% confidence interval is given. ^bLines were fitted to log-log plots between $t = 10^{0.2} - 10^{3.5}$ s for all animals. ^cLines were fitted to log-log plots between $t = 10^{0.2} - 10^{3.5}$ s for animals A, B and E; $t = 10^{2.25} - 10^{3.5}$ s for animal C; $t = 10^{1.75} - 10^{3.5}$ s for animal D. ^dLines were fitted to log-log plots (SD vs. time) between $t = 10^{0.5} - 10^{3.5}$ s for all animals. ^eData from animal B were insufficient to compute the Fano and Allan factors, and the Hurst exponent.

5.4 Results

5.4.1 Behavioral Two States

Here we demonstrate that *Gymnotus sp.* spontaneously switches between two distinct behavioral states: a resting (immobile) Down-state with a low EODR and an active (mobile) Up-state with a higher EODR. We show that the transition to a higher EODR precedes the onset of self-initiated movement.

We studied the fish's spontaneous behaviors in a featureless, dark and quiet environment (Fig. 5.1A) [Jun et al., 2014] devoid of objects. *Gymnotus sp.* is nocturnal and, in a lighted environment, is mostly immobile (Fig. 5.1B) with a low basal level of EOD discharge (Fig. 5.1C). However, in the dark, the basal EODR of stationary fish significantly increased (48 hours of total observation; $p < 10^{-12}$, paired *KS* test). Our analysis was therefore confined entirely to observations taken in a completely dark environment. Despite the absence of external stimuli, fish spontaneously switched between periods of inactivity and of active swimming (Fig. 5.1D); and the EODR of each animal also rapidly switched between lower and higher rates (Fig. 5.1E). During the periods of higher EODR the animal is sampling its environment more frequently than when in the lower EODR state. We therefore considered high and low EODR periods as distinct sensory states with the high EODR state corresponding to a period of higher sensory sampling. Long-term observation (4.5-12 hours/day, total observation time of 207 hours from 5 animals) of spontaneous behaviors revealed that the switching of EODR is tightly coupled to movement (Fig. 5.1F and Movie 5.1), and that the EODR does not simply reflect the circadian cycle [Lisman, 1965]. Two distinct clusters in the joint histogram of the EODR and activity level (Fig. 5.1G), confirm a tight correlation between sensory and behavioral states (Table 5.1). For all animals, movements only occurred in association with higher EODR. Remarkably, the EODR increased up to five seconds before a spontaneous movement (Fig. 5.1E and Movie 5.2). In almost all cases (92.1%) and in all animals tested, the increasing EODR transition preceded the spontaneous movement onset (Table 5.2); the remaining cases (7.9%) are most likely due to the finite temporal precision ($\sigma = 0.1$ s) of the movement onset detection (Fig. 5.5B,C; see Materials and Methods). Because increases in EODR must be due to increased neural activity of neurons providing descending input to the pacemaker nucleus, the increase in EODR implies a change in neural activity.

Thus, as is the case for self-initiated movements in humans [Kornhuber and Deecke, 1965; Libet et al., 1983; Soon et al., 2008], altered neural activity precedes motor activity by up to several seconds. Importantly, we also conclude that heightened active sensory sampling precedes movement.

Due to the binary nature of sampling rate and activity level and their rigid correlation, we refer to an active period with high sampling rate as an Up-state and an inactive period with low sampling rate as a Down-state. We separated the Down- and Up-states (Fig. 5.2A) according to the first principal component (PC1) of the EODR and activity level (Fig. 5.1G,H). As illustrated in Fig. 5.1G, the principle components analysis rotates the axes of the activity level versus EODR variables graph so as to find variables (components) that are statistically independent; further, the method gives a ‘first’ principle component that accounts for most of the variability in both data sets. The PC1 explained $90\% \pm 3\%$ of the total variance; this analysis suggests that a single neural control mechanism is mainly ($\sim 90\%$) responsible for both aspects of the Down-to-Up state transition. We therefore hypothesize that, despite the variable time lag between them, the increase in EODR and onset of movement are triggered by the same neural mechanism. After the separation, transient states were removed by merging (Fig. 5.2A,B, see Methods). The EODR and activity level were normalized across different days and individuals (Fig. 5.1I) by mapping their median values during Down- and Up-states to zeros and ones, respectively (Fig. 5.2C).

The sensory sampling activities showed marked differences between the Down- and Up-states (i.e., without and with movement). In all animals tested, the EODR and EODA distributions significantly differed between the two states ($p < 10^{-12}$, paired Kolmogorov-Smirnov test, $\alpha = 0.05$; 27-72 hours, see Table 5.1). The EODR distributions were clearly separated with minimal overlap (Fig. 5.3A), and the EODR was significantly higher during active periods in all animals tested (Fig. 5.3B; Table 5.1). In both states, the modes of the EODA distributions were negative (Fig. 5.3C, right) as expected from the generally fast rise followed by slow decay of the EODR (Fig. 5.3D). The EODA distribution was wider during Up-states (Fig. 5.3C, left), indicating greater temporal modulation of the sensory sampling rate during active movements. In fact, the EOD pulse train exhibited greater temporal variability during Up-states according to the Fano factor analysis (Fig. 5.3E); the Fano factor quantifies

the mean-normalized count variability of the EOD pulses as a function of the time window and thus clearly demonstrates that the EODR is far more variable during Up-states. These findings suggest that there exist two disjoint states of active sensory sampling, that transitions to the higher sampling rates (i.e., to an Up-state) are tightly coupled to and precede movements; and that the sensory sampling rate is highly modulated when associated with movement.

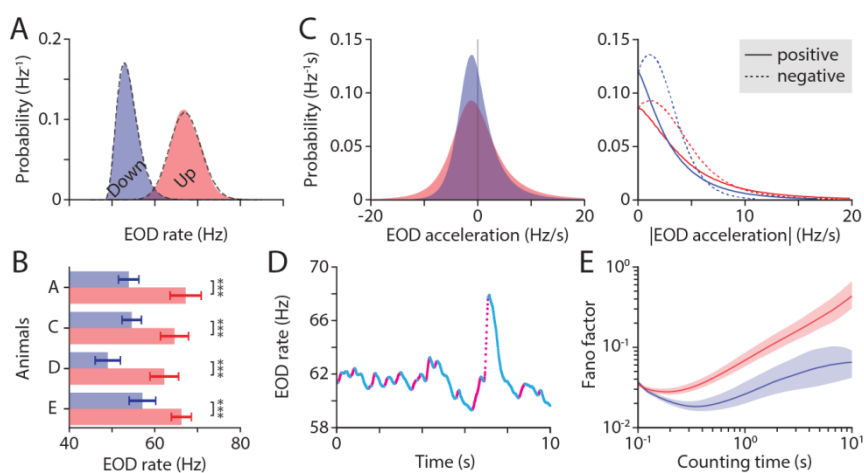


Figure 5.3. State-dependent sensory sampling activities. (A) Distributions of the EOD rate (EODR) showed distinct sensory sampling rates during Down- and Up-states from a spontaneously behaving animal. Red/blue indicates Down/Up-states throughout this figure. Dashed black lines indicate shifted log-normal distribution fitting. (B) Mean EOD rates exhibited significant differences between the two states in all four animals; error bars indicate ± 1 SD; *** $p < 0.001$. (C) Left, Distribution of the EOD acceleration (EODA = $d\text{EODR}/dt$) showed wider spread during Up-states, indicating greater temporal modulations of the sensory sampling. Right, Positive (solid lines) and negative (dashed lines) sides of the EODA distributions were superimposed to depict the asymmetry in the increasing and decreasing phases of the EODR. (D) An example trace of the EODR showed quicker rising (magenta line) and slower falling (cyan line) phases during an Up-state. (E) Fano factors of the EOD pulse counts during a Down-state (200 s long) followed by an Up-state (200 s long); shaded areas indicate 95% bootstrap confidence intervals. All data in A,C-E were obtained from the same animal.

5.4.2 Preparatory increase of sensory sampling rate

Here we compare the increase in EODR that precedes a self-initiated movement versus the increase associated with a stimulus (acoustic)-evoked movement. We show that stimulus-evoked EODR increases are stereotyped and may be reflex responses to a strong, unexpected sound. In contrast, the EODR increases preceding self-initiated movements have highly variable timing and are clearly not reflexes associated with movement.

Sustained preparatory neural activities preceding voluntary movements were reported in humans [Kornhuber and Deecke, 1965; Libet et al., 1983; Soon et al., 2008] and monkeys [Romo and Schultz, 1987; Kato et al., 1995; Kaufman et al., 2010]. Here however, we further demonstrate that the increased neural activity preceding self-initiated movement is also associated with a heightened sensory sampling (EODR) that precedes movement onset. In addition, the temporal relationship between sensory sampling and motor activities exhibited striking differences for spontaneous versus sound-evoked movements. In the sound-evoked condition, loud acoustic stimuli (143 dB-SPL RMS, 150 Hz pure tone, 0.5 s or 1 s duration) were delivered at random intervals (4-6 min inter-stimulus intervals) to trigger startle responses [Korn and Donald, 2005]. We only analyzed evoked trials when the acoustic stimuli triggered significant movements of a resting animal (70% of all trials). The initial rise of EODR preceded a movement onset significantly earlier in the spontaneous condition compared to the evoked condition (Fig. 5.4A; Table 5.2). Likewise, the trial-averaged EODA (slope of the EODR) during spontaneous transitions became positive approximately 1.5 s earlier than during evoked transitions (Fig. 5.4B). Under both conditions, the peak of the trial-averaged EODA coincided with a movement onset (Fig. 5.4B, bottom). To precisely align the trials for averaging, we visually confirmed the movement-onset times determined from the activity level in a subset of trials (Fig. 5.5A-F; see Materials and Methods). The movement-triggered averages of the activity level showed no significant baseline activity before movement onset under both conditions (Fig. 5.4C), thus confirming that the differences in the EODR and EODA between the spontaneous and evoked conditions cannot be attributed to the differences in the baseline activity levels. The differences in the EODR or EODA between the two conditions could not be explained by the differences in movements, because the activity levels under the two conditions did not significantly differ from each other (Fig. 5.4C). We also

observed no significant increase of the background acoustic noise before self-initiated movements (Fig. 5.4D), validating that these transitions were indeed spontaneous.

To quantify the temporal relationship between the sensory and motor transitions, the time intervals between the initial EODR rise and movement onset (transition latency) were compared between the spontaneous and evoked transitions. The transition latency distributions for spontaneous transitions had longer median latencies and wider spreads than for evoked transitions in all animals tested (Fig. 5.4E). The EODR switched to Up-states significantly before self-initiated movements (Table 5.2) with highly variable transition latencies; whereas acoustic stimuli caused an immediate increase of the EODR, and movements quickly followed in a stereotyped (i.e., less variable) manner. Fig. 5.4F shows that animals were more likely to initiate movements as they spent longer time in the EODR Up-state ($\text{EODR} > 0.4$). The probabilistic temporal relationship between the spontaneous switching of the sensory sampling rate and the movement-initiation rules out an automatic coupling between the two. Fig. 5.4G compares two types of trials where spontaneous EODR Up-transitions either lead to movement-initiation (*moved*, $N = 456$) or Down-transitions without movement (*aborted*, $N = 1,198$) from the same animal. The mean trajectory of the aborted transitions reached not much beyond (< 0.5) the EODR threshold (0.4) before falling back, whereas the transitions that led to movement nearly reached the median Up-state EODR (1.0) before crossing the activity threshold (0.2). Fig. 5.4G thus confirms that the EODR Up-transition generally precedes spontaneous movement initiation when they are sufficiently strong and long-lasting. We conclude that external sensory stimuli trigger an immediate stereotyped reflex movement [Korn and Donald, 2005] and a near simultaneous associated increase in the sensory sampling rate [Caputi et al., 2003; Comas and Borde, 2010]; in contrast, self-initiated movements are preceded by a sustained increase in the sensory sampling with highly variable transition latencies and without any apparent sensory trigger.

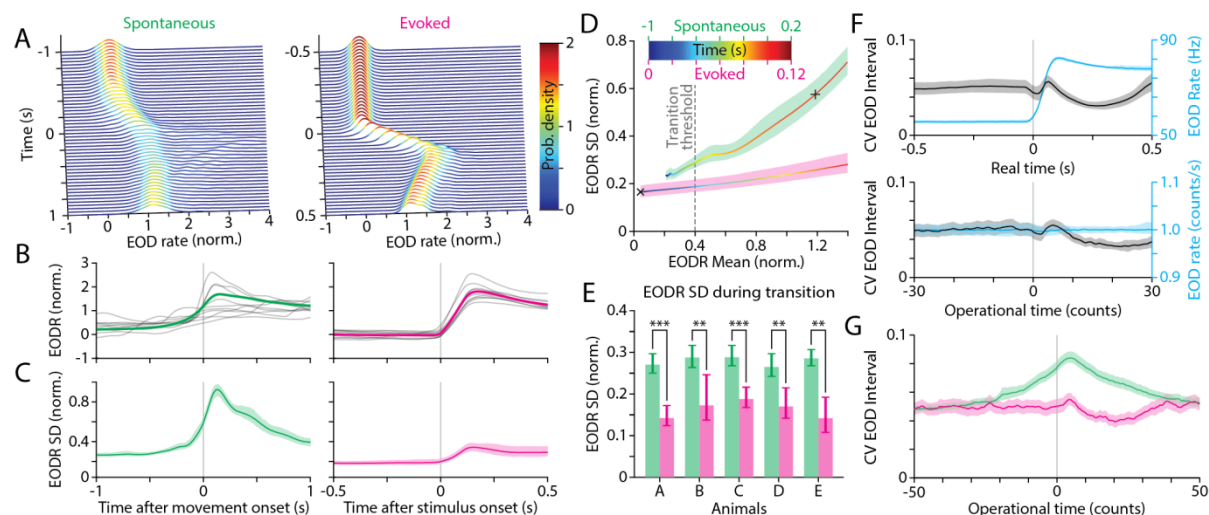


Figure 5.4. Increase in the sensory sampling rate precedes voluntary movement. (A,B) Pseudo-color plots of the normalized EOD rate (EODR, panel A) and the EOD acceleration (EODA, panel B) time courses during spontaneous (top) and sound-evoked (middle) transitions. Time 0 indicates the movement onset, and trials are ordered by their EODR Up-transition onsets. Bottom, Trial-averaged EODR (panel A) and EODA normalized by the peak (panel B) both exhibited striking differences between spontaneous (green traces) and evoked (magenta traces) transitions. (C) Activity level time courses averaged across all trials showed no significant differences between the spontaneous and evoked trials (all animals pooled). Dashed gray line indicates the activity threshold (0.2) for the movement-onset detection. (D) No significant increases in the background noise were observed before spontaneous movement onset; Δ SPL: trial-averaged underwater sound pressure level (SPL) after baseline subtraction. (E) Comparisons of the distributions between spontaneous (green shading) and evoked (magenta shading) transitions demonstrate significantly longer EODR transition latencies for spontaneous compared to sound-evoked transitions in all animals tested (Table 5.2). (F) The probability of initiating movement increased as the duration threshold of the EODR Up-state increased in all animals. (G) Comparison of the two trajectories (moved vs. aborted) averaged across trials that crossed the EODR Up-state threshold (0.4). Dashed horizontal line indicates the movement threshold, and dashed vertical line indicates the EODR threshold. Colors represent the time since the threshold crossing. The error bars or color shading indicate 95% bootstrap confidence intervals. All data in panels A,B,G were obtained from the same animal;

all tested animals were pooled for panel C,D. Solid lines and shaded areas indicate the trial-averaged mean $\pm$ SEM.

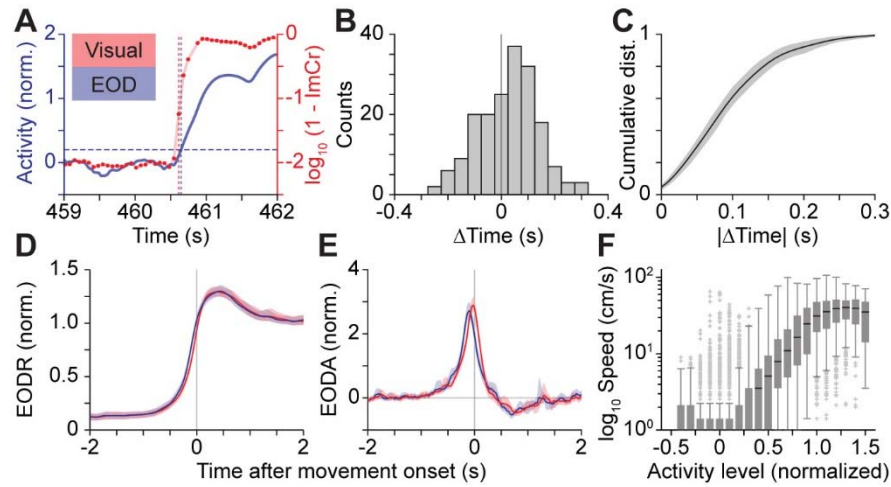


Figure 5.5. Visual confirmation of the EOD-based movement onset detection. (A) Example traces of the EOD-based activity level (blue line) and the image correlation (ImCr) measure (red line) during movement onset. Each image frame is represented by a red dot, and joined together for visual aid. Vertical dashed lines indicate movement onset times determined by the two detection methods, and the horizontal dashed line indicates the EOD-based activity threshold (0.2). (B) Distribution of the time differences (ΔT_{move}) between the two movement onset detection methods. (C) Cumulative distribution of the absolute time differences ($|\Delta T_{\text{move}}|$). Shaded area indicates 95% bootstrap confidence interval. (D-E) Trial-averaged EODR (D) and EODA (E) time courses computed by the EOD-based (blue) and visual (red) movement onset detection methods agree within ~ 0.1 s. Shaded areas indicate ± 1 SEM. (F) Box-plot of the log-speed of the image centroid as a function of the activity level (displaying 5th, 25th, 50th, 75th and 95th percentiles). Medians are indicated by black bars, and outliers are shown as gray crosses. All data in A-F were obtained from animal B.

5.4.3 Preparatory increase in the sensory sampling variability

Here we show that the EODR preceding and accompanying self-initiated movement is very variable across trials. The EODR can be considered as a proxy of the neural activity driving the EOD medullary EOD pacemaker [Comas and Bordes, 2010] and we conclude that, as is the case in primates, neural activity associated with voluntary movement is highly variable

from trial to trial. We also analyze the variability of stimulus-evoked EODR increases and show that they are much less variable, corresponding to their more reflex nature (see above).

Previous studies reported increasing trial-to-trial variability in neural activities leading up to a voluntary movement onset [Steinmetz and Moore, 2010; Churchland et al., 2011], whereas stimulus onset quenches neural variability [Steinmetz and Moore, 2010; Churchland et al., 2010; Litwin-Kumar and Doiron, 2012]. Since the EODR reflects the neural activity associated with sensory sampling, we examined how EODR activity varied between trials during spontaneous and evoked transitions as a proxy for the variation in neural activity.. Figure 6A shows time-evolutions of the normalized EODR distributions. The distributions associated with spontaneous movements (Fig. 5.6A, left) exhibited greater trial-to-trial variability than the sound-evoked responses (Fig. 5.6A, right). EODR traces of ten randomly selected trials from the same animal showed greater deviations from the trial-averaged trace during spontaneous transitions (Fig. 5.6B); and across-trial SD of the normalized EODR confirms greater variability during spontaneous transitions (Fig. 5.6C). All sound-evoked trials having a Down-state baseline were pooled regardless of whether or not movements were elicited, since their initial EODR responses were similar within 0.5 s of the stimulus onset. We separately computed the trial-to-trial variability for each animal (Fig. 5.7A,B) to rule out the variance contributed by individual differences. In all animals tested, increases in the sampling rate variability (SD EODR) preceded self-initiated movements, and decreases followed shortly thereafter (Fig. 5.6C, left; Fig. 5.7A).

During the course of transitions, across-trial SD of the EODR strongly depended on the mean EODR (Fig. 5.6D; Fig. 5.7C). To rule out a possible influence of having different mean values on the across-trial variability, we directly compared the SD of EODR between the spontaneous and evoked transitions when their mean values were both equal to the transition threshold (0.4). For a given trial-averaged EODR, the across-trial SD during spontaneous transitions generally exceeded that of the evoked transitions in all animals tested (Fig. 5.6E; Fig. 5.7C). When the trial-averaged normalized EODR (Mean EODR) crossed the transition threshold, the normalized EODR followed a Gaussian distribution in all animals [Lilliefors test, Table 5.2]; and the across-trial SDs (SD EODR) were significantly greater during spontaneous transitions ($p < 10^{-2}$, F -test for equal variance; see Table 5.2).

The time course of the trial-averaged EODR exhibited large differences between animals and between the spontaneous and evoked conditions (Fig. 5.7A,B). In order to facilitate pooling of data from different animals, the effect of time-varying mean rate on the across-trial variance was removed by applying a time-rescaling procedure [Brown et al., 2002; Nawrot et al., 2008]. This mathematically well-grounded procedure normalizes the time axis of the EODR so that direct statistical comparisons can be made across animals even if their original EODR temporal modulations were different. After applying the procedure, the mean rate became constant in the operational time scale, but the coefficient of variation (CV) of the EOD inter-pulse intervals still exhibited a decline after the stimulus onset (Fig. 5.6F, bottom). Consistent with the previous findings [Steinmetz and Moore, 2010; Churchland et al., 2010; Churchland et al., 2011; Litwin-Kumar and Doiron, 2012], the trial-to-trial variability of the sensory sampling intervals as measured by the CV (5 animals pooled) peaked near the voluntary movement onset, but declined after the stimulus onset (Fig. 5.6G). These findings suggest that the time course of sensory sampling variability mirrors the previously reported increase in neural variability preceding a decision to act [Steinmetz and Moore, 2010; Churchland et al., 2011]; and the trial-to-trial sampling variability associated with voluntary movements significantly exceeds that of the stimuli-triggered responses.

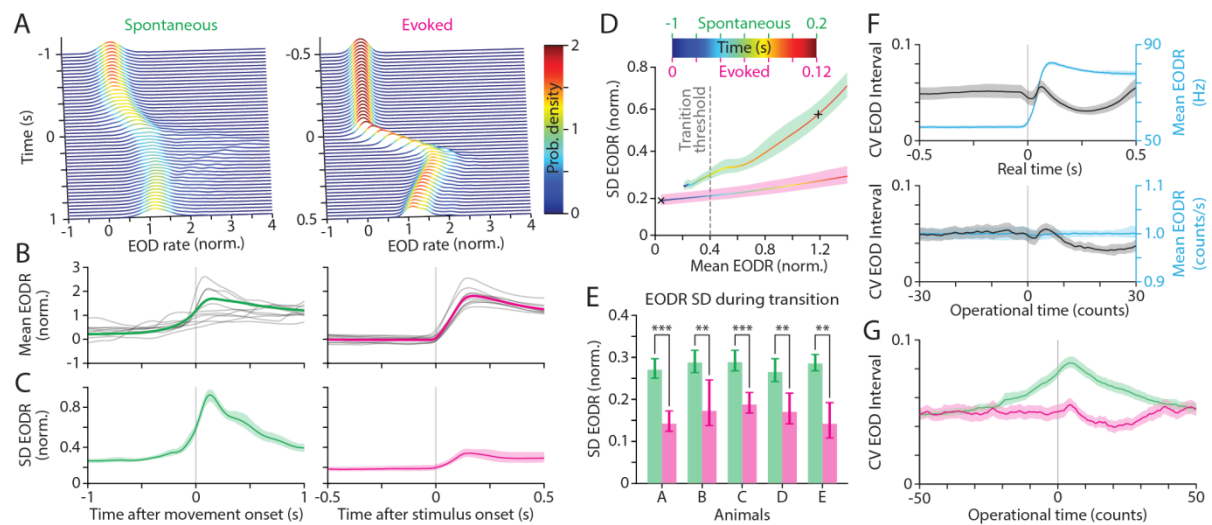


Figure 5.6. Increase in the sensory sampling variability precedes voluntary movement.

(A) Time-dependent distribution of the EOD rate (EODR) during spontaneous transitions (left) exhibited a wider spread than during evoked transitions (Right). Colors represent the probability density of the EODR; time 0 indicates the movement or sound onset. (B) EODR time courses from ten randomly selected trials (gray lines) showed greater deviations from the mean (colored lines) during spontaneous transitions (left). Green/magenta indicates the spontaneous/evoked conditions throughout this figure. (C) Across-trial standard deviations (SDs) of the EODR reveal greater trial-to-trial variability during spontaneous transitions (left). (D) Comparison of the across-trial SDs any given mean EODR confirms greater trial-to-trial variability during spontaneous transitions. Colors represent the time of transition; ‘+’: movement onset; ‘x’: stimulus onset. All data in A-D were obtained from animal C. (E) In all animals tested, the across-trial SDs at the transition threshold (dashed gray line in panel D) exhibited significant differences between the spontaneous and evoked transitions; $**p < 0.01$, $***p < 0.001$. (F) The coefficient of variation (CV) of the EOD intervals (black lines) declined after stimulus onset, but the mean rate (cyan lines) simultaneously increased (top). The CV was recomputed after applying the time-rescaling procedure ($N = 98$, animal A), which produced a constant mean rate (bottom). (G) Trial-to-trial variability peaked near the spontaneous movement onset ($N = 1157$, 5 animals), but declined after stimulus onset ($N = 291$, 5 animals). All shaded areas indicate 95% bootstrap confidence intervals. The CVs were computed after averaging four successive EOD intervals at 0.1 ms resolution.

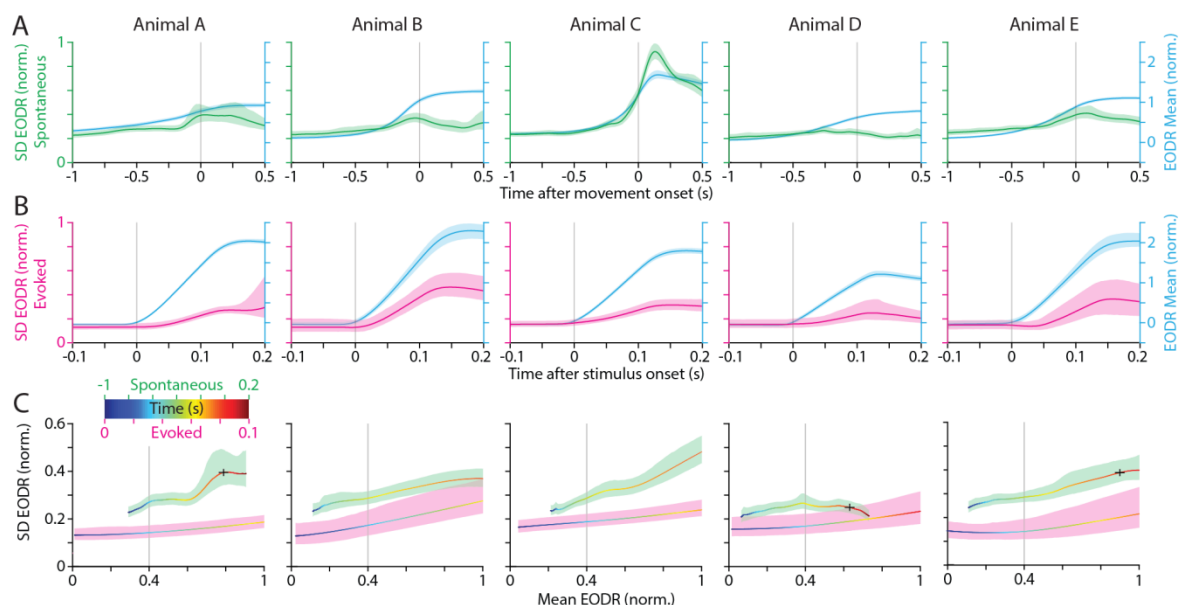


Figure 5.7. Trial-to-trial variability and mean of the EODR for each animal during Up-transitions. (A) Across-trial mean (cyan traces) and SD (green traces) of the EODR for spontaneous transitions as a function of time before and after movement onset. (B) Same as A for evoked transitions as a function of time before and after stimulus onset. The across-trial SDs are shown as magenta traces. (C) Across-trial SDs of the EODR (SD EODR) as a function of the trial-averaged EODR (Mean EODR) during spontaneous (green) and evoked transitions (pink). Shaded areas represent 95% bootstrap confidence interval. Pseudo-color indicates time during transition, and black '+' marks the movement onset. The EODR transition threshold (0.4) is indicated by vertical dashed grey lines.

The time course of the trial-averaged EODR exhibited large differences between animals and between the spontaneous and evoked conditions (Fig. 5.7A,B). In order to facilitate pooling of data from different animals, the effect of time-varying mean rate on the across-trial variance was removed by applying a time-rescaling procedure [Brown et al., 2002; Nawrot et al., 2008]. After applying the procedure, the mean rate became constant in the operational time scale, but the coefficient of variation (CV) of the EOD inter-pulse intervals still exhibited a decline after the stimulus onset (Fig. 5.6F, bottom). Consistent with the previous findings [Steinmetz and Moore, 2010; Churchland et al., 2010; Churchland et al.,

2011; Litwin-Kumar and Doiron, 2012], the trial-to-trial variability of the sensory sampling intervals as measured by the CV (5 animals pooled) peaked near the voluntary movement onset, but declined after the stimulus onset (Fig. 5.6G). These findings suggest that the time course of sensory sampling variability mirrors the previously reported increase in neural variability preceding a decision to act [Steinmetz and Moore, 2010; Churchland et al., 2011]; and the trial-to-trial sampling variability associated with voluntary movements significantly exceeds that of the stimuli-triggered responses.

5.4.4 Spontaneous behavioral transitions

Here we show that transitions from Down- to Up-states (and vice versa) occur in a random manner. This is consistent with the idea that voluntary behaviors are unpredictable [Haggard, 2008]. We use several statistical methods to prove randomness of Down- and Up-state durations and transitions. We also demonstrate that Down/Up transitions were based on an underlying renewal process, i.e. a process with no ‘memory’ so that the duration of a previous Down- or Up-state did not influence the duration of the next state.

It has been previously proposed that voluntary behaviors are initiated in a random or unpredictable manner [Haggard, 2008]. This computational principle indeed held true in the case of spontaneous behavioral state transitions in *Gymnotus* sp., based on the following fractal time-series analyses to uncover their underlying temporal structures from long-term observations. The survivor functions ($= 1 - \text{cumulative density function}$) of the intervals between two successive Down-to-Up state transitions (Up-transitions) showed approximately linear trends on a lin-log scale (Fig. 5.8A); and the durations of Down- and Up-states followed similar trends (Fig. 5.8B). The distributions of Up-transition log-intervals were well fitted assuming an underlying log-normal density for these intervals (Fig. 5.8C; Fig. 5.9A,B; Table 5.3). The power spectrum of spontaneous Up-transition events showed power at all frequencies (Fig. 5.8D), suggesting no clear periodicity features or characteristic time scale [Proekta et al., 2012].

In addition, we found a lack of predictability based on the history of spontaneous transitions. The joint log-interval histogram of spontaneous Up-transitions exhibited no clear structure indicating a lack of temporal correlation (Fig. 5.8E). Indeed, little or no correlations

were found between pairs of Up-transition intervals, state durations, and transition latency (Fig. 5.8F). The Fano factor for spontaneous Up-transition counts increased linearly on a log-log scale (Fig. 5.8G; Table 5.3). To confirm lack of temporal correlation in the spontaneous transitions, we computed the Fano factors for ten randomly shuffled Up-transition intervals, and superimposed them with the original plot (Fig. 5.8H; Fig. 5.9C). Within the limit of our observation time window ($10^{0.2} - 10^{3.5}$ s), the original Fano factors did not significantly differ from that for the shuffled controls in all animals except in one (animal D, $t > 10^2$ s). After removing slow trends in the data by applying Allan factor analysis [Gebber et al., 2006], no significant differences were found between the original and shuffled data in all animals (Fig. 5.9D). The Hurst exponents of the spontaneous Up-transition counts ranged between 0.52-0.63 (Table 5.3), which is indistinguishable from those produced by a random process with no memory [Hardstone et al., 2012]. These metrics all demonstrate that the spontaneous behavioral transitions are not deterministic, but rather due to a renewal process with log-normal characteristics.

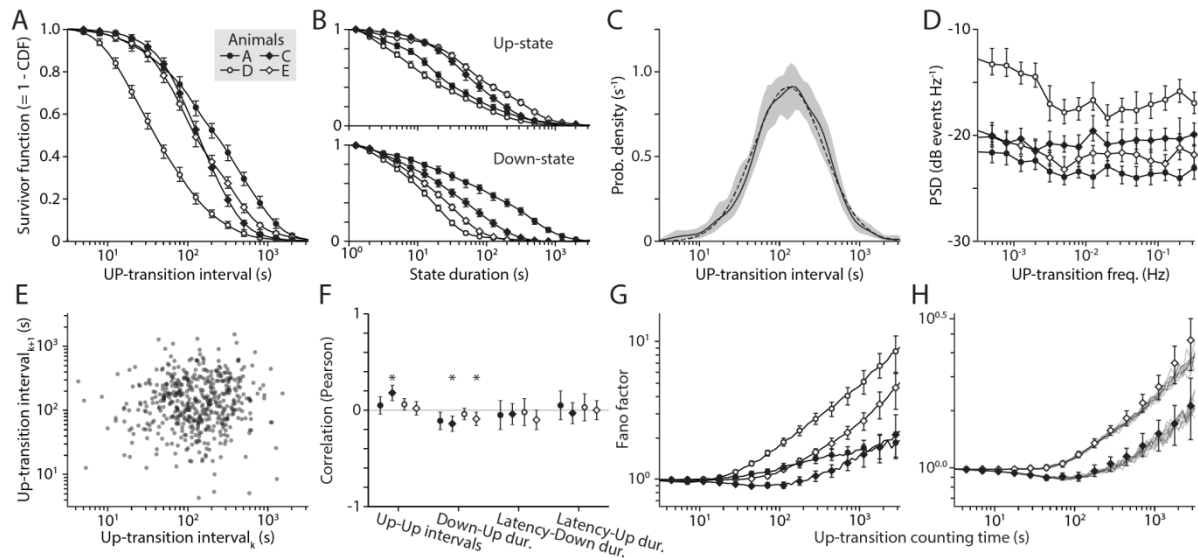


Figure 5.8. Spontaneous behavioral transitions follow a random distribution and lack temporal structure. (A,B) Survivor functions ($= 1 - \text{cumulative distribution function}$) of the log-intervals between two successive Up-transitions (panel A), and log-durations of Up- and Down-states (panel B) showed downward linear trends for all four animals. The symbols for each animal are consistent throughout this figure. (C) Distribution of the Up-transition intervals from the same animal (solid black line) were well fitted by a log-normal distribution

(dashed black line). Shaded areas indicate 99% bootstrap confidence intervals. **(D)** Power spectral density (PSD) of the Up-transition events in the log-frequency domain exhibited approximately equal power at all frequencies, suggesting no characteristic time-scale. **(E)** No significant temporal correlation exists between two successive log-intervals between spontaneous Up-transitions from the same animal. **(F)** Temporal correlations between successive spontaneous transitions were mostly absent or statistically insignificant; $*p < 0.01$; *Up-Up intervals*: two successive intervals between Up-transitions, *Down-Up dur.*: Durations of a Down-state and a following Up-state, *Latency-Down dur.*: transition latency and duration of a preceding Down-state, *Latency-Up dur.*: transition latency and duration of a following Up-state. **(G)** Fano factors for the Up-transition counts showed linearly increasing trends on a log-log scale in all four animals. **(H)** The original Fano factors do not significantly differ from ten randomly shuffled controls from the same animal (gray lines), indicating lack of temporal structure. Errors bars represent 95% bootstrap confidence interval.

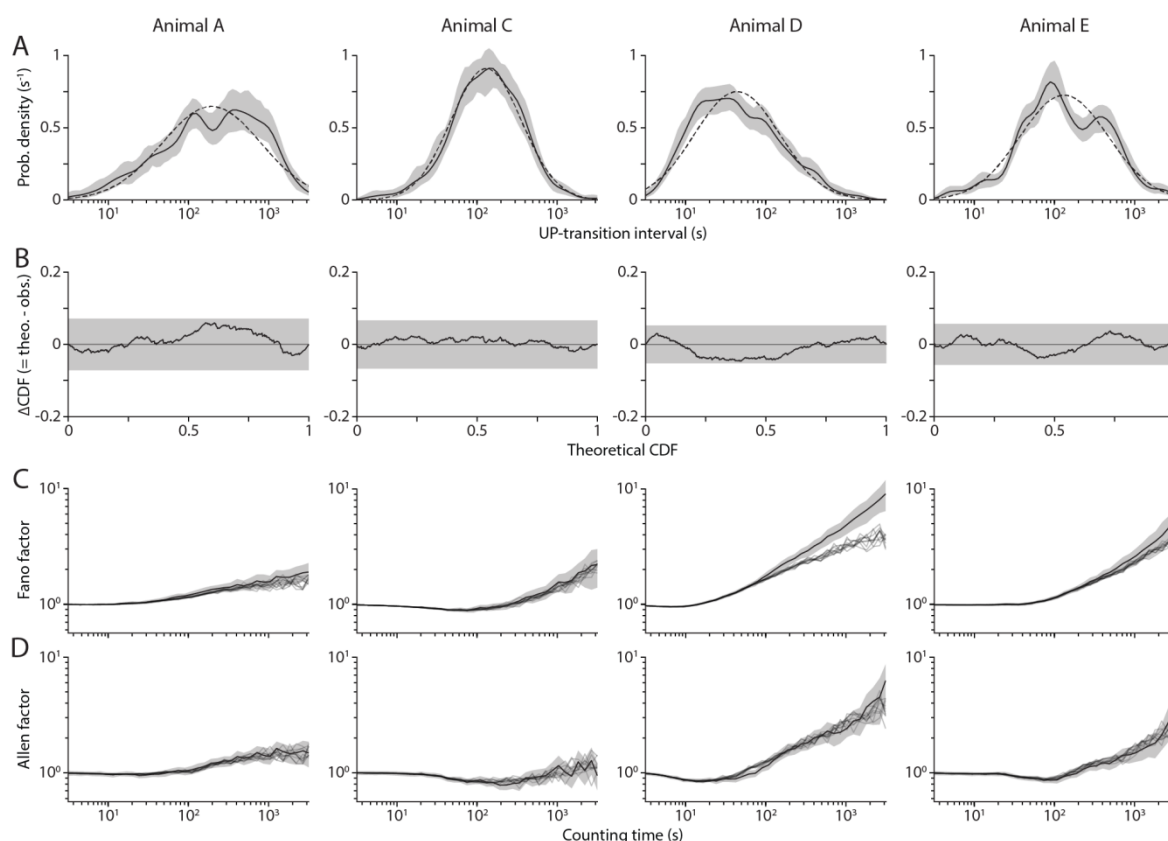


Figure 5.9. Distributions of the intervals between spontaneous Up-transitions for each animal. (A) Distributions of the Up-transition intervals. Shaded areas indicate 99% bootstrap confidence intervals, and dashed black lines indicate log-normal distribution fitting. (B) KS plots of the empirical vs. Δ CDF (= empirical CDF – theoretical CDF). Shaded areas indicate 99% confidence interval for the paired KS test between the empirical and theoretical CDF (log-normal distribution). (C,D) Log-log plots of the Fano (panel C) and Allan factors (panel D) of the Up-transition counts. Shaded areas indicate 95% bootstrap confidence intervals, and gray traces indicate ten randomly shuffled controls.

5.5 Discussion

5.5.1 Summary of our work

Our results reveal that weakly electric fish exhibit preparatory neural activity up to five seconds before spontaneously initiating movement (Fig. 5.1E), and that such movements are associated with enhanced sensory sampling evident by the increased EODR. Through behavioral Down to Up-transitions, such movements are also correlated with the increased variability of neural activity characteristic of voluntary acts in primates. Preparatory neural activity preceding voluntary movements has been shown to be involved in movement planning [Kaufman et al., 2010] and decision making in primates [Steinmetz and Moore, 2010; Churchland et al., 2011]. In *Gymnotus* sp., the higher sensory sampling rates during movement may be attributed to the increased demands on the brain to process the high flow of sensory information induced by self-motion [Nelson and MacIver, 2006]. Yet, this cannot explain our observation of increased sensory sampling rates and sampling rate variability that precedes movement onset; if EODR increases were only required for gathering more information during movement, it would only have to occur during movement and not before movement was initiated. We propose that the increased sensory sampling and associated neural activity preceding movements are not only for gathering of more information, but also involved in the fish's making a decision to move and in planning the trajectory of its movement. We therefore hypothesize a direct analogy of preparatory neural activity in primates and that of *Gymnotus* sp. (as seen in the proxy, EODR).

We established precise temporal relationship between the sensory and motor activities by studying a pulse-type electric fish, which lends itself to the measurement of sensory sampling. The existence of two distinct states in sensory sampling can be explained by high metabolic cost of maintaining the electric organ discharges [Salazar et al., 2013], thus animals save significant energy by switching to lower sensory sampling rates at rest. Unlike motor activities, sensory processing in the brain is normally hidden from outside observers, but in *Gymnotus* sp., transitions between two distinct modes of sensory sampling provide a window to the internal sensory dynamics from spontaneously behaving animals for extended periods. In general, active sensing behaviors such as whisking, touching, and sniffing are coupled to physical movements such as locomotion [Nelson and MacIver, 2006] or respiration cycles [Wachowiak, 2011], which confound the interpretation of exact temporal relationship between the sensory sampling and movements. Electrosensory sampling activity can be modulated in absence of physical movements, which offers a unique opportunity to determine the precise temporal relationship between the two.

5.5.2 The nature of volition from a comparative perspective

The nature of volition has been a long-standing inquiry throughout human history, but the discussions remained largely philosophical until the advent of modern tools to monitor the brain activity. Discovery of the readiness potential preceding voluntary action [Kornhuber and Deecke, 1965; Libet et al., 1983] started quantitative investigation of the brain activity during volitional decision making in humans. Prolonged preparatory neural activities characteristic to human voluntary actions are also found in diverse species including monkeys [Romo and Schultz, 1987; Kato et al., 1995; Kaufman et al., 2010] and rodents [Friedman et al., 2006]; this suggests that the ability to self-initiate movement is a quantifiable biological trait shared across many vertebrate species including teleost fish. The experience of agency is essential for human volitional actions [Haggard, 2008], and the pre-supplementary motor area is shown to be activated when human subjects pay attention to their intention to act [Lau et al., 2004]. However, it is not clear whether non-human animals also share the sense of agency, and if so, how to measure their intention to move. Since the awareness of self exists in relation to the external world, the nature of interactions between enhanced sensory sampling and therefore possibly awareness and motor intention [Liu et al., 2010] may lead to a better understanding of volition.

5.5.3 Functional significance of preparatory sensory acquisition and behavioral variability

Before animals decide to move, they must accumulate sufficient information about their sensory surroundings to minimize predation risks, or to optimize foraging decisions. Since the preparatory sensory acquisition before movement offers substantial evolutionary advantages, it may be a general feature of the vertebrate brain. Recent studies in humans found a link between preparatory visual sampling and voluntary eye movements, by measuring fixational saccades occurring before the stimulus appearance that signalled voluntary gaze [Watanabe et al., 2013]. Sustained period of heightened sensory sampling during movement preparation could improve the detectability of weak or infrequent sensory signals in a noisy background that might be biologically important [Ratnam and Nelson, 2000; Gussin et al., 2007]. Given that the increased sensory sampling almost always preceded voluntary movements (Fig. 5.4E), we propose that heightened sensory acquisition is critical for voluntary decision making.

It has been proposed that variability in spontaneous animal behaviors is an evolutionary necessity, since any form of predictability can be exploited by predators [Jabłoński and Strausfeld, 2000; Catania, 2009]. In support of this idea, the spontaneous transitions between Down- and Up-states exhibited considerable randomness (Fig. 5.8A-D); and the onset of voluntary movement could not be predicted from its past, or from the duration of the preparatory sensory sampling (Fig. 5.8E-H). Furthermore, the time course of sensory sampling preceding voluntary movement showed significantly greater variability across trials in comparison to the sensory-evoked condition (Fig. 5.6D,E). These evidences suggest that although the active sensing behavior exposes the animal's motor intention to external observers, the intrinsic variability in the preparatory sensory sampling impedes accurate prediction of a movement onset.

5.5.4 Potential neural mechanism for preparatory enhancement of sensory sampling

What neural mechanism could explain preparatory increase in the sensory sampling rate and trial-to-trial variability, and what is the link between the behavioral transitions and neural activity states [Gervasoni et al., 2004; Mohajerani et al., 2013]? Voluntary movements in primates are associated with preparatory neural activity in the cortex, whereas external sensory

stimuli can trigger brainstem reflexes such as the orienting reflex [Sokolov, 1990] and novelty responses [Caputi et al., 2003] without necessary cortical activation. Acoustic stimulation likely increases the EODR via brainstem interneurons [Korn and Donald, 2005; Comas and Borde, 2010], whereas the neural substrates of self-initiated movements in *Gymnotus* sp. are not currently known. We suspect that they will include telencephalic regions [Braithwaite, 2005], that are likely homologous with the mammalian pallium and basal ganglia [Wong, 1997; Harvey-Girard, 2012; Harvey-Girard, 2013]. One reason for this idea is that blocking inhibition in the dorsolateral pallium (DL) of *Gymnotus carapo* [Santana et al. 2001] induces striking changes in the patterning of its EOD. DL is known to receive highly processed electrosensory input and might be able to modulate both the EODR and locomotion via its output to midbrain regions including the tectum [Giassi et al., 2012]. However, while the pallium is a reasonable candidate, the ultimate neural basis of enhanced sensory acquisition preceding voluntary movement remains unknown, as well as the nature of interactions between the microcircuits for sensory evaluation and motor planning. It has been shown that forebrain stimulations enhanced visual spatial attention in the barn owl [Winkowski and Knudsen, 2006]; and hypothalamic stimulation triggered head-scanning behaviors followed by locomotion in mice [Sinnamon et al., 1999], suggesting additional candidate regions involved in the initiation of enhanced sensory sampling before movement.

5.5.5 Conclusion

Our study shows that *Gymnotus* sp. can, in the absence of any extraneous input, switch between inactive (Down) to active (Up) states. The self-initiated onsets of Up-states occur randomly and, since they are associated with an increased sensory sampling rate, we hypothesize that they are exploratory behaviors during which the animal is in a heightened sensory state. Haggard [2008] has suggested that voluntary actions in humans are a form of “decision making” with two special characteristics: they are exploratory behaviors that are not directly triggered by sensory input, but rather occur in a random fashion. Our study reveals that the self-initiated movements of *Gymnotus* sp. have these characteristics of voluntary actions. The connection between volition and decision making is reinforced by the fact that neural activity, as reflected in the EODR, precedes self-initiated movements by a time scale comparable to that of the preparatory cortical activity in humans [Kornhuber and Deecke, 1965; Libet et al.,

1983]. Finally, consistent with the studies in mammals reporting that neural variability decreases after voluntary action [Steinmetz and Moore, 2010; Churchland et al., 2011] or stimulus onset [Steinmetz and Moore, 2010; Churchland et al., 2010], the sensory sampling variability in our animals followed the same decline under both conditions. We conclude that *Gymnotus* sp. may not only exhibit volition, but that neural processing preceding the decision to move might also bear some similarity to that in humans according to their similar temporal dynamics. Given the substantial homologies between the telencephali of teleost fish and mammals [Braithwaite, 2005; Harvey-Girard et al., 2012; Harvey-Girard et al., 2013], locating the brain regions that trigger the Up-states in *Gymnotus* sp. may give clues as to the initiation sites of human voluntary actions. In turn, this may expose the circuitry controlling sensory acquisition rates and their possible relation to decision-making mechanisms [Gold and Shadlen, 2007; Heekern et al., 2008].

The contents below are available from the publisher's website.

Movie 5.1. Correlation between the EOD rate and movement activity shown in Fig. 5.1E. This movie shows tight correlation between the EOD rate (EODR) and movement activity of a pulse-type weakly electric fish, *Gymnotus* sp. The colored-region surrounding the circular tank indicates the EODR (increasing scale from blue to red). The playback is 30 times faster than real-time. (MP4; 0.96 MB).

Movie 5.2. Increase in the EOD rate 5 seconds before movement onset shown in Fig. 5.1D. This movie shows tight correlation between the EOD rate (EODR) and movement activity of a pulse-type weakly electric fish, *Gymnotus* sp. The colored-region surrounding the circular tank indicates the EODR (increasing scale from blue to red). The playback is 30 times faster than real-time. (MP4; 0.97 MB).

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Chapter 6

Active sensing is associated with acquisition of
spatial memory in a pulse-type electric fish

This chapter is in preparation to be submitted as Jun JJ, Longtin A and Maler L [2014]

6.1 Summary

Past experiences influence animals' behaviors, and novel features in the surroundings draw their interests leading to active explorations. Active sensing behaviors that outwardly express sensory acquisition events provide unique opportunities to observe how animals' past experiences influence the choice of locations to explore. Weakly electric fish (WEF) are nocturnal animals that navigate by generating electric organ discharges (EODs) to detect electric image distortions caused by the nearby surroundings. Pulse-type WEF species such as *Gymnotus* sp. generate brief EOD pulses (~1 ms), and the fish's sensory sampling rate, in the dark, is simply equal to its EOD rate that varies according to the novelty contents of the surroundings. We studied *Gymnotus* sp. to test whether they can be trained, without visual cues, to learn a location of food using electrically detectable landmarks and, if so, whether they can use their past experiences to optimize their sensory sampling. A live mealworm was presented at a fixed location surrounded by four stable dielectric landmarks until animals reached stable performances in finding food. Upon the completion of learning, the food was removed during probe trials and we simultaneously monitored their trajectories and EOD rates. We found that animals revisited the missing food location with high spatial accuracy, and they intensified their sensory sampling near the expected food location by increasing the number of EOD pulses per unit distance travelled (sampling density). Comparisons with two control groups of animals trained without landmarks, or with randomly placed landmarks revealed that stable landmarks improve the spatial accuracy during food search. The sampling densities were significantly lower in the animal groups trained without stable landmarks, suggesting a random search strategy. Our studies show that *Gymnotus* sp. rely on stable landmarks to choose their spatial search locations and selectively increase the sensory sampling near the expected food location.

Key words: *Spatial learning, active sensing, novelty response, sensory sampling rate, path integration, landmark-based, electroreception*

6.2 Introduction

Animals actively explore their immediate surroundings; the resulting spatial learning then permits them to effectively navigate in this space and thus find food, shelter and mates while avoiding predators. The act of exploration may involve active control of sense organs to orient toward and capture spatial features such as nearby landmarks [Cain and Malwal, 2002; Graff et al., 2004] or distant scenes [Moss and Surlykke, 2010; Philippides et al, 2011]. Active sensing can occur in many modalities: e.g. saccadic eye movements in primates [Otero-Millan et al, 2008; Nakamura et al., 2013] and whisking [Hartmann, 2001; Kleinfeld et al., 2006], sniffing [Wachowiak, 2011] or head scanning [Monaco et al., 2014] in rodents. A subset of active sensing modalities [Nelson and MacIver, 2006] such as echolocation in bats [Lawrence and Simmons, 1982; Jensen et al., 2005; Ulanovsky and Moss, 2007] and electric organ discharges (EOD) in weakly electric fish (WEF) involve emission and detection of energetic pulses that interact with and carry information about their surroundings [Lissmann, 1958; Moller, 1995]. These temporally discrete emissions allow direct quantification of the sensory sampling events from unrestrained freely behaving animals [Jun et al., 2012; Jun et al., 2013; Jun et al., 2014].

Many animals have the mobility ranges that greatly exceed their sensory ranges [Nelson and MacIver, 2006; Snyder et al., 2007], thus the ability to remember a large spatial layout can greatly aid animals to efficiently and rapidly navigate in their habitats; sense input can, in this case, merely confirm the animal's location without requiring time-consuming new spatial learning. Such extensive spatial learning abilities have been observed in many animals including vertebrates (e.g. birds [Clayton et al., 1994; Capaldi et al., 1999], mammals [Bingman, 1992; Bird and Burgess, 2008]) and invertebrates (e.g. bees [Cartwright and Collett, 1983; Dyer, 1996; Capaldi et al., 1999]). Spatial learning in vertebrates is believed to occur in the hippocampal formation [O'Keefe, 1979; Moser et al., 1993; Knierim et al., 1995; Moser et al., 2008] and a recent study has suggested that active sensing (scanning) in rodents is intimately associated with hippocampal spatial memory acquisition (i.e. development of place fields) [Monaco et al., 2014]. In this paper, we analyze the interaction of spatial learning and active electric sensing in a WEF. Specifically, we examine the possible contributions of active sensing to acquisition of landmark memory and memory required for path integration guided

movement towards prey items. We also suggest that similar neural substrates (hippocampal homolog) might underlie spatial learning in these fish.

We use a South American gymnotiform fish, *Gymnotus sp.* for these studies. The *Gymnotus sp.* we used emitted low amplitude (<1 V) short duration (~ 1 ms half width) electric organ discharge pulses (EOD) [Black-Cleworth, 1970; Caputi et al., 2003; Rodríguez-Cattáneo et al., 2007]; during their active exploratory Up states the EOD rate was variable with mean pulse rate ranging from 60-70 Hz [Jun et al, in press]. Each EOD pulse induces an electric field around the fish and objects near the fish perturb this field to generate an electric image on the fish's skin [Caputi et al., 2003; Caputi et al., 2013]. One class of electroreceptors (tuberous receptors) are tuned to the fish's own EOD and respond to each EOD pulse with a variable number of spikes [Castello et al., 2000; Castelló et al., 2008]. Conductive objects, such as the mealworms used as prey in this study, induce a positive electric image (increased local EOD intensity) and therefore increased discharge in the electroreceptors on the affected skin region [Castello et al., 2000; Caputi and Budelli, 2006; Caputi et al., 2008]. In contrast non-conductive objects, such as the plastic landmarks we used, induce a negative electric image (decreased local EOD intensity) and therefore decreased discharge in the electroreceptors on the affected skin region [Castello et al., 2000; Castelló et al., 2008]. All spatial learning trials were run under infrared illumination and we argue that the electroreceptor re-afferent input evoked by each EOD is the main source of spatial information in this learning task. However, the electrosense only operates over short distances (<3 cm) and, unlike the visual or echolocation senses, is not sufficient to encompass the entire extent (1.5 m tank diameter) of our test arena. We show that, despite this limitation, these fish are very capable of spatial learning and that this learning is most efficient when there are stable landmarks available. Most interestingly, in the early stages of learning they actively probe their environment by both increasing their EOD rate as well as by frequently engaging in stereotyped backward swimming maneuvers. These active sensing actions typically occurred near landmarks and food and they are markedly decreased once asymptotic spatial learning is achieved. We therefore have established a direct link between active sensing and spatial learning.

The neural mechanism of the memory formation and recall still remains as a central question in the behavioural neuroscience. Among many types of memories, spatial memory

has been extensively studied by experimentalists and theoreticians, since it lends itself to observation on the basis of navigational choices animals voluntarily make. The spatial memory performances can be quantified by the time and path taken by animals to arrive at goal locations containing food or a hidden platform during, e.g. a Morris water maze test. In mammals, a medial forebrain structure known as the hippocampus is proven to be crucial for the formation of spatial memory, and a large proportion of the hippocampal pyramidal cells, known as the place cells, exhibit place-specific firing rates. From the comparative anatomical perspective, teleost fish (ray-finned fish) collectively possess the most compact forebrain architecture among all vertebrates; while they are still capable of learning spatial tasks [Schwarz and von der Emde, 2001; Rodríguez et al., 2002; Graff et al., 2004; de Perera et al., 2005; Walton and Moller, 2010] that depend on a dorsal telencephalic region, dorsolateral pallium (DL) believed to be functionally and anatomically homologous to the mammalian hippocampus [Mueller 2011; Harvey-Girard, 2012; Giassi et al., 2012a; Giassi et al., 2012b; Giassi et al., 2012c]. Lesions of DL abolish spatial learning capabilities in goldfish [Salas et al., 1996; Portavella et al., 2002; Durán, 2008], and place cells have been found in the same region in goldfish [Canfield and Mizumori, 2004].

6.2.1 Memory-guided active sensing

Since considerable time and energetic costs are associated with sensory sampling, an optimal navigational strategy in a familiar and stable environment would be to rely on the memories of spatial layout instead of constantly scanning every familiar spatial feature. This strategy appears to be used by echolocation bats. When these bats are first introduced to an unfamiliar and cluttered environment, they initially produce calls more frequently to avoid obstacles, but their call rates decrease after repeated exposure to the same environment [Barchi et al., 2013]. Similarly, pulse-type WEF actively capture electric images of the immediate surroundings formed on their body by generating EOD pulses [Caputi et al., 2003; Snyder et al., 2007; Pusch et al., 2008; Hofmann et al., 2013]. In these fish, an increase in the local conductance mimicking a prey-like signal is immediately followed by a transient increase in the EOD rate followed by an exponential decay; but the magnitude of this novelty response undergoes experience-dependent habituation after repeated stimulus presentations [Grau, 1983; Caputi et al., 2003; Pluta and Kawasaki, 2008; Post and von der Emde, 1999].

6.2.2 Possible navigational strategies used by WEF

Similar to the Mexican blind cavefish that rely on the hydrodynamic pressure for detecting nearby objects in a dark cave environment [Coombs et al., 1996; Patton et al., 2010], active electric senses of WEF allow them to detect objects having mismatching impedances in murky water or in darkness. They generate dipole-like electric fields that rapidly decay in strength [Knudsen, 1975; Heiligenberg, 1975], which gives them a limited detection range that extends up to a body length [Belbenoit, 1970; Heiligenberg, 1973; Bastian, 1981; Maciver et al., 2001; Caputi et al., 2013]. Since WEF can only detect nearby objects that quickly become undetectable as they swim away, it is hypothesized that these fish must rely on some form of memory to orient themselves by remembering the locations of previously encountered landmarks. Path-integration is a strategy that permits animals to navigate without landmarks by integrating displacement vectors from a known starting location to keep track of one's position. Such navigational strategy is used in certain desert ant species which travel long distances on featureless terrains where pheromone trails quickly evaporate. A location estimated by path-integration becomes less accurate as a function of path-length because of the accumulation of errors from each displacement vector, thus it needs to be recalibrated using external landmarks. In a natural environment, WEF could rely on landmark-based navigation while swimming near boundaries and objects such as rocks or plant roots, and may switch to a path-integration strategy in open water using proprioceptive senses.

6.2.3 Experience-guided sensory sampling in pulse-type WEF

Here, we investigate how spatial memories shape exploratory sensing by directly observing discrete sensory update events from a freely swimming WEF. *Gymnotus* sp. is a South American pulse-type WEF. It generates brief (~1 ms) EOD pulses at approximately 40-150 Hz that can be readily measured by sets of electrodes in water [Jun et al., 2012a]. The EOD inter-pulse intervals (IPIs) become shorter and more variable during active swimming relative to resting periods [Jun et al., 2014; Forlim and Pinto, 2014]; and transient shortenings of the IPIs occur during sudden body movements or object detection indicative of increased sensory acquisition rates [Jun et al., 2012b; Hofmann et al., 2013]. It has not yet been fully characterized how freely-swimming pulse-type WEF modulate their IPIs as a function of learning, or whether there exists experience-dependent effects on the voluntary sensory scanning in teleost fish. Here, we investigate how the sensory sampling in these fish adapt over

the course of learning by monitoring their movement trajectories and sensory sampling rates (EOD rates) during food searching tasks. To address how these animals use landmarks to navigate at different stages of learning, we trained them to find food placed at a fixed location in a large circular tank (1.5 m diameter) populated with four plastic cylinders having unique shapes and sizes. These objects were placed at fixed locations in shallow water (10 ± 1 cm) to serve as stable landmarks for animals to navigate to the food location. After the task performance reached a stable plateau, we removed food in randomly selected probe trials and monitored the animals' search locations and sensory sampling to observe how sensory scanning is affected by the memory of a food location. To further test how critical stable landmarks are for these animals to locate food, we compared the spatial distribution of the trajectories with two control groups of animals trained without or with unstable landmarks.

6.3 Materials and Methods

6.3.1 Animal handling

All procedures including housing and behavioural experiments were approved by the University of Ottawa Animal Care Committee. We obtained South American pulse-type WEF (genus *Gymnotus*) of unknown species and gender from a local supplier (Big Al aquarium services, Ottawa, ON, Canada). Animals were initially housed in community tanks under 12 hour light cycle, and fed live blackworms. Prior to the behavioural experiments, four animals were transferred to the experimental chamber (Fig. S6.1A-B), and individually housed in the corner compartments for at least a week prior to starting the first trial. Animals were fed live mealworms in their home compartments during the habituation period for familiarization; mealworms were used during the spatial experiments instead of blackworms, since mealworms were easier to restrain on suction cups using elastics. The tank water was maintained at 25 ± 1 °C, pH 6.5-7.5 and 100 ± 10 μ S/cm to closely match the natural habitat for these animals. A mix of salts were added (CaSO_4 6.832 g/L, MgSO_4 0.648 g/L, KCl 0.432 g/L, NaCl 0.115 g/L, NaH_2PO_4 0.144 g/L) [Knudsen et al., 1975] to closely match the freshwater condition of Amazonian river. Air ventilation was installed to expel excess humidity build-up caused by heating. The experimental chamber was under 12-hour reverse light cycle to observe nocturnal behaviours. The chamber was lit by eight timer-controlled white LED lights (DIODER Lighting strip, IKEA, Singapore) during the light cycle. Experiments were performed during

in the dark cycle under infrared illumination (860-880 nm) invisible to these species [Fernald, 1988; Douglas and Hawryshyn, 1990] to permit visual observation. Water filtration was performed in between daily experiments by eight vertical filters (Whisper Internal Power Filter - 40i, Tetra, Germany), and water from all compartments were mixed together during filtration to homogenize odorants. The filters were taken out of the central testing arena during experiments to prevent animals from using them as spatial landmarks.

6.3.2 Experimental setup

We constructed a large glass tank (1.8 m x 1.8 m x 0.3 m) to provide sufficient large for animals to require spatial learning in order to efficiently find food. Video introduction and further technical details are available from our previous publication [Jun et al., 2014]. The tank rested on an anti-vibration surface consisting of multiple layers of vibration-absorbing materials (rubber, polyurethane foam, Styrofoam, sponge) to minimize external vibratory stimuli from influencing the voluntary sensory sampling activity. Hydrophone recordings were made to detect excessive vibratory noise that could not be fully attenuated. Fish swam in shallow water to facilitate video tracking by restricting the animal movements mainly in two dimensions, and the water depth was maintained at 10 ± 1 cm by adding distilled water to replace the evaporative loss. The water temperature was closely regulated by a thermostat controlling a floor heater installed below the tank, to prevent temperature-dependent EOD rate fluctuations [Toerring and Serrier, 1987; Ardanaz et al., 2001]. The heater is equipped with built-in Faraday-shielding (ThermoTile, ThermoSoft Corp., IL, USA), and we added a layer of aluminum mesh above the heater to block potential RF noise. The experimental tank was surrounded by a sensory-isolation chamber to block external sources of light, sound and RF noise. The chamber was equipped with four doors to provide easier access to the experimental tank from the two sides. The experimental tank was partitioned by four sections of curved acrylic sheets (3.2 mm thick) that formed the circular fence, and four acrylic separators between neighbouring compartments (Fig. S6.1A). The partitioning walls were caulked to the glass tank using aquarium-grade silicone. Each compartment was electrically isolated from the others to prevent electrocommunication with neighbours during experiments. To ensure water-tightness, the tank was dried and each compartment was filled one at a time and no leakage was observed.

Once the isolation chamber doors were shut, entries of the animals to the test area were controlled by the remotely operated gates (Jun'13). The gate opened gently (5 seconds to fully open and close) to avoid disturbing the water surface for cleaner imaging. When the animals were not being tested, the gates were shut and locked by nylon wing-nuts that pressed against the rubber gaskets. No metallic parts were used in the water, since our animals reacted sensitively to metals which have higher conductivities than objects found in nature. The landmarks were built from a thin transparent acrylic sheet (1.6 mm thickness) to ensure clear views when animals approach close by. The landmarks were open on top and water filled the inside up to the outside water level to minimize optical refraction and distortion. The landmarks were securely attached to the glass bottom by suction cups mounted at the bottom. Four types of landmarks, small and large squares (5.6 or 9.0 cm/side) and circles (diameters of 6.4cm or 10.2 cm), were built. White grid paper (5 cm spacing) was attached underneath the glass bottom to guide landmark and food placements, and to provide a contrast background for imaging.

6.3.3 EOD an video recording

EOD signals were reliably and accurately captured at all tank locations and orientations by eight graphite electrodes (Mars Carbon 2-mm type HB; Staedtler, Germany) attached on the circular wall at equal spacing [Jun et al., 2012a]. We used heavily-shielded BNC coaxial cable (RG54) and differential amplifiers, which were grounded to the Faraday cage to minimize RF noise pickup. The raw signals were amplified (30-100x gain) and high-pass filtered ($f_c = 300$ Hz, second-order Butterworth) to remove the first and third harmonics of the mains noise. The gain was reduced for larger fish, which produced stronger EOD amplitudes, to prevent amplifier saturation. Four differentially amplified channels were digitized (CED mkII; Cambridge Electronic Design, UK), and the EOD pulse timing was determined in real-time by an online digitizer script (Spike 2; Cambridge Electronic Design, UK). The digitized signals were rectified, summed and RMS-filtered (root-mean-squared) to produce a unimodal pulse shape for accurate pulse timing detection [Jun et al., 2012a]. Video was recorded by an infrared-sensitive camera (C910; Logitech Inc., CA, USA) which provided high resolutions (1600 x 1200 at 15 frames/s) for accurate body tracking. The infrared-blocking filter was removed from the lens unit to enhance IR sensitivity. Video recordings were made using a lossless compression by the manufacturer-provided software. The video recordings were

synchronized to the concurrent EOD recordings by capturing periodically blinking light pulses (10 s intervals and 100 ms duration) emitted by an infrared LED. Automated movement tracking was performed by a custom-built Matlab script [Jun et al., 2014], which reliably discriminated the tip of head from the tail end. The tip of head was tracked since skin around the mouth contains the highest density of electroreceptors, thus serving as an electrosensory fovea [Bacelo et al., 2008; Hollmann et al., 2008]. Video tracking was performed from the moment animal completely entered the test area until it found food. All animals exhibited rapid increases in their EOD rates (novelty responses) when they detected food. Each video tracking result was visually confirmed to check for possible tracking errors. The trajectory data and the EOD rates were resampled at 100 samples/s at regular intervals by using spline interpolation to align the time domain.

6.3.4 Experimental protocol

Animals were trained to find a live mealworm restrained at a fixed location, and four unique landmarks were placed at stable locations to aid navigation. Before each trial, single live mealworm was fastened on a suction cup using elastic harness, and attached to the glass bottom. Trials were repeated up to four times per animal each day, and trials exceeding 15 minutes were aborted when animals failed to find food. After successful feeding or at the end of the trials, we urged animals to get back to their home compartments by blinking visible light using eight bright LED lights attached on the circular wall. Concurrently, the gate was rapidly opened and closed to generate waves to encourage animals to get back. After each trial, remaining food particles were thoroughly removed if found in order to eliminate potential chemical cues as to the food location. Four animals were tested daily per experimental cycle, yielding up to 16 trials per day (4 trials/animal). Landmarks were positioned in a rotationally symmetric manner for all animals except for the random controls. Probe trials were conducted after the learning performance reached a stable plateau by removing the food. The animals were tracked for five minutes during the probe trials, and we ran one probe trial per day in one out of four randomly selected trial. A total of four probe trials were tested per animal. After completing the training using four stable landmarks, we performed two additional control trials with four new animals each. The control experiments were identical except for the landmarks; the *None* group was trained to find food located at a fixed location without any landmarks; the

Unstable group was trained with randomly placed landmarks and food that were randomly shuffled on each trial.

6.3.5 Data analysis and statistical tests

The spatial analysis was conducted in the 80 cm square area centred at the central arena (active zone). The active zone excluded locations near the circular wall containing multiple features such as recording electrodes or gates which could act as landmarks. Map-based analysis was conducted by binning the trajectories falling in the same grids (20 x 20 pixels or 2.7 cm square). We computed various statistics per grid, such as the number of times animals revisited each grid location. We calculated the mean distance between successive IPI per grid to determine the sampling density, which is defined as the average number of sensory samples per unit distance travelled (# EOD pulses/cm). We pooled four animals belonging to the same group unless otherwise stated. The significance tests were conducted using two-sample t-test (two-tailed). The standard errors (SEM) were determined by bootstrap sampling for independently sampled data. The EOD inter-pulse intervals exhibited long-range autocorrelation (~ 1 s), thus we adjusted the SEM by multiplying by $\sqrt{n}$, where n is the correlation length. The correlation length was determined at the first $1/e$ drop-off point in the autocorrelation function. Similarly, the degrees of freedom for the t-tests were divided by the correlation length for fair comparison.

6.4 Results

We have previously demonstrated that, in the dark, *Gymnotus* sp. alternates between behavioural Down and Up states (Jun et al., In press). During Down states the fish are immobile and EOD rate (EODR) is relatively low (60-70 pulses/s). When the fish initiate movement and increase their EODR by ~ 10 pulses/s when they transition into an Up state. The spatial learning described below always occurred when the fish were in an Up state and therefore discharging at a relatively high rate. Our studies, described in detail below, demonstrate that *Gymnotus* sp. can learn the location of food and increase its sensory sampling near the expected food location by actively making use of stable landmarks. First, we present evidences of spatial learning in *Gymnotus* sp. based on improvements in task performances such as distances and time taken to find food. We also show changes in swim trajectories and active sensing motor actions (back swimming) over the course of learning and also quantify

locations frequently visited by animals during food search. Probe trials executed in absence of food were then used to reveal the precision of spatial memory in *Gymnotus* sp. Secondly, we quantify the number of sensory sampling (EOD pulses) per distance travelled (sampling density) near the landmarks and food locations at various stages of learning. We also quantify the temporal pattern of EOD inter-pulse intervals (IPI); they exhibit burst-like temporal patterns (E-scans) that similarly depend on the fish's location and undergo learning-dependent change. Third, we compare the spatial accuracy and electrosensory sampling (EOD pulse rate and inter-pulse interval) during food search between the stable landmark group and two other controls groups either trained without landmarks, or with unstable landmarks placed at random locations; these comparisons suggest that these animals can make an active use of stable landmarks to improve the precision of spatial search.

6.4.1 Evidences of spatial learning in *Gymnotus* sp.

Learning curves. All animals ($n = 4$) reached stable learning performances for food searching tasks measured by either the distance or duration taken to find food (Fig. 6.1A). Both measures reached stable plateaux within four sessions (four trials per session), and up to four trials were performed each day per animal. The distance measures the path-length of the head trajectory starting from the entrance to the food location, and the task duration was measured from the moment of entering the arena until reaching the food location. We compared the task performances between the first two sessions when the fish was unfamiliar with the tank spatial layout (*Early* trials) and the late six sessions (*Late* trials) when performance had reached asymptotic values. Transition periods between the *Early* and *Late* trials were not analysed. Consistent with the learning curve in Fig. 6.1A, the distance travelled and the task duration significantly decreased from the *Early* to *Late* trials (Fig. 6.1B). To prevent outliers from skewing the results, the analysis excluded trials falling in the extreme ranges (below 12.5th or above 87.5th percentiles) in the distance distribution (Fig. S6.2A). The trials excluded are indicated by open bars, and the trials included are indicated by solid bars in the distribution plots for the distance, duration and speed (Fig. S6.2A-C). The average swim-speed significantly increased with learning (Fig. 6.1B, right), indicating that the task durations decreased by travelling shorter distances at higher speeds. The three measures of learning (distance, duration and speed) exhibited high pair-wise correlations (Fig. S6.2D) and agreed closely with each other in quantifying the spatial learning performances. We chose the

distance-travelled measure to represent the overall learning performance, since it did not directly depend on the swim speed unlike the task-duration measure. The variability of task performances (distance and duration), quantified by the coefficient of variation (CV), also decreased with learning (Fig. 6.1C), indicative of more consistent and reliable task performance after learning. These results demonstrate that *Gymnotus* sp. can be quickly trained to reliably perform spatial tasks; we argue below (see Discussion) that these fish are engaged in spatial learning mainly based on the electroreception.

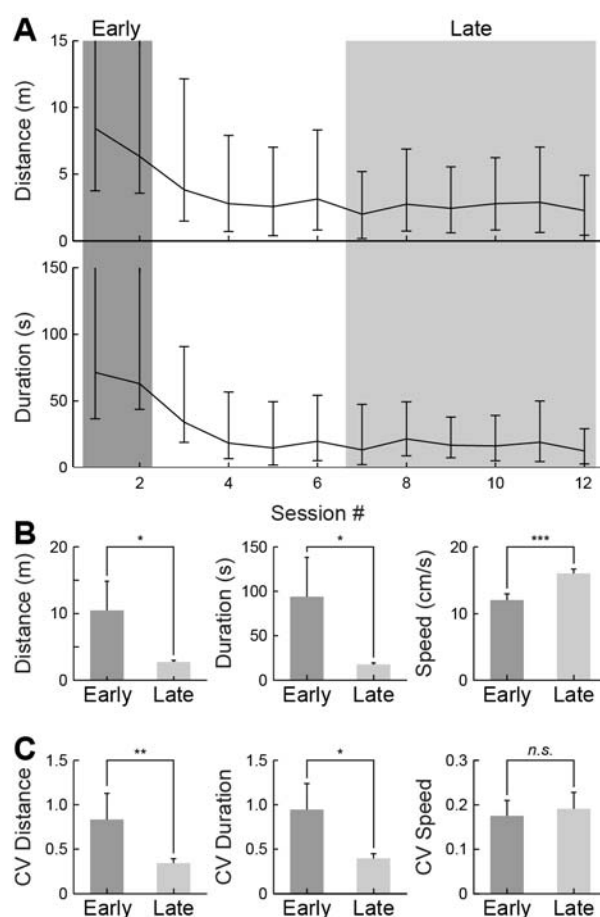


Figure 6.1. Performance improvements in the food searching tasks during learning. (A) Animals learned to retrieve food quickly after about four sessions (sixteen trials) as indicated by the distance travelled (top) and the task duration plots (bottom). The distance and duration plots indicate improvements in the task performances during learning until they reach asymptotic performance during the late learning trials. Each session contains four trials, and four animals were pooled to compute the mean. The first two sessions are defined as *Early*

trials and the last six sessions as *Late* trials (**B**) Task performances (distance, duration, and speed) are compared between *Early* and *Late* trials, indicating significant changes after learning. (**C**) Coefficient of variations (CV) of the task performances are compared between *Early* and *Late* trials. Error bars indicate the bootstrapped SEM. Significant differences in **B** and **C** are indicated by * (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; *n.s.*: not significant).

Food-searching locations. We analysed the swim trajectories from each trial to describe the location-specific searching behaviours. Our video tracking software tracked the tip of head since the skin surrounding the mouth contains the highest density of electroreceptors, thus acting as the electrosensory fovea [Castelló et al., 2000; Caputi et al., 2003; Bacelo et al., 2008]. Further, as animals swam forward, the tip of head generally approached objects first. Our automated video tracking software reliably discriminated the head from the tail end [Jun et al., 2014], and all automated tracking results were visually confirmed. Figure 6.2A shows three example trajectories at various stages of learning (*Early*, *Late* and *Probe* trials). The trajectories are shown from the moment animals entered the active zone (80 cm square zone shown in the background) until the animals found food, or for a 20 s duration for the *Probe* trials. The active zone was defined sufficiently far the tank wall (18 cm from the square corners to the wall) to exclude locations near the wall containing multiple features such as recording electrodes and gates. During *Early* trials, the search trajectories appear less direct and random, but the *Late* trials exhibit more direct trajectories toward the food location. After the learning performances stabilized (after the *Late* trials), *Probe* trials were selected randomly without animals' knowledge, and each animal was allowed in the test arena for five minutes to search for the missing food. The trajectories during the *Probe* trials frequently passed nearby the missing food location, thus directly demonstrating a spatial memory of the expected food location. The lack of possible chemical food cues during the *Probe* trial suggests that these animals can navigate toward the food location without relying on the olfactory senses. The trajectories from four animals were pooled to reveal the frequency of visiting various locations. Figure 6.2B illustrates visit density maps at various stages of learning, and the pseudo colours indicate the number times animals visited each grid location (2.7 cm square per grid). Since each of the four animal entered the test arena from different gates, the landmarks were arranged

symmetrically to produce identical relative positions between the gate and landmarks after rotating by 90, 180 or 270 degrees. The gate is located near the top right corner of the active zone after pooling the trajectories in Fig. 6.2B. The visit density measure is insensitive to the variations in swim speeds, since each grid passing was counted only once per visit regardless of the speed. The visit density maps in Fig. 6.2B are consistent with the example trajectories in Fig. 6.2A. During the *Early* trials, the visit density was initially broad, but it became more concentrated near the food location during the *Late* and *Probe* trials. The visit density during the *Probe* trials in comparison to the *Late* trials is more centred around the missing food location, since the trajectory tracking was terminated as soon as animals found food during the *Late* trials, whereas in the *Probe* trials, animals repeatedly visited the missing food location for the five-minute period allowed. Search area fraction measures the spatial spread of the visit density (Fig. 6.2C, right), by calculating a fraction of the number of grids having visit density values above the threshold ($1/e$ of the maximum visit density). The *Early* trials exhibited significantly greater search area fraction (Fig. 6.2C, left), and the search area fraction significantly decreased ($p = 0.0044$) after learning (*Late* and *Probe* trials). The *Late* and *Probe* trials were not significantly different thus pooled together. In summary, the visit patterns during food search became more defined after learning. The decreased distance and time to find food is therefore associated with and presumably due to significant improvements in the precision of memory of the food location.

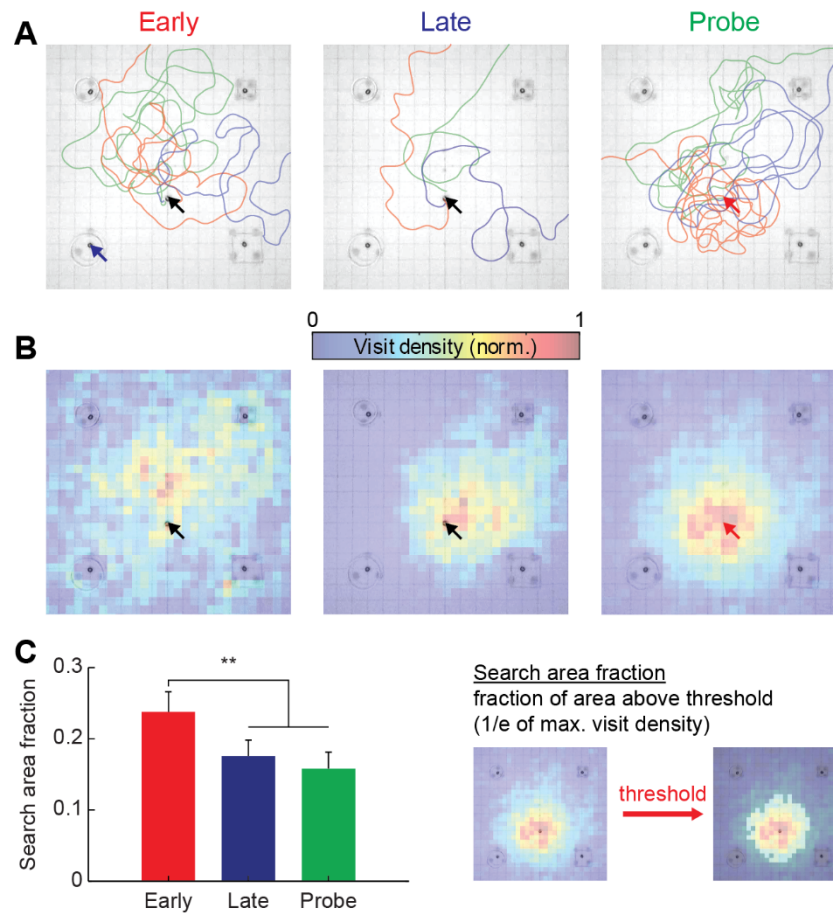


Figure 6.2. Example trajectories and visit density maps from different learning stages.

(A) Three example trajectories are randomly chosen from the *Early*, *Late* and *Probe* trials. No food was placed during *Probe* trials. Trajectories are plotted once the fish has left the edge of the tank. Note that even after asymptotic learning performance (*Late* trials) the fish will take a curved route to the food and that its final angle of approach to the food is very variable. The trajectories during *Probe* trials are clearly centered on, or near the food location of the previous trial. Blue arrow indicates one of the landmarks; black arrows indicate the food location; red arrow indicates the missing food location throughout this figure. (B) Visit density map quantifies the number of times animals visit each grid location, and they are plotted for the early, late, and probe trials. The pseudo colours indicate the visit count density per grid location (2.7 cm square), and was normalized by the maximum pixel value. (C) The search area fraction is defined as the fraction of area above threshold in the visit density map (right). The search

area fraction was computed for each visit density maps and compared between *Early*, *Late* and *Probe* trials (left). Error bars indicate the bootstrapped SEM. Significant difference in **C** is indicated by * (**: $p < 0.01$).

Search time spent at various locations. Animals tend to slow down near the food location, so we quantified the average visit durations at various locations. In Fig. 6.3A, the pseudo-colours represent the average time animals spent per visiting each grid location. For example, the time per visit measure increases when an animal slows down while passing by the grid location. During the *Early* trials, the time per visit was generally higher than the later trials, and was particularly high near the landmarks (Fig. 6.3A, left). The time per visit during the *Late* trials decreased globally (Fig. 6.3A, middle), which is consistent with the increasing swim speed after learning (Fig. 6.1B, right). During the probe trials, the time per visit increased near the missing food location and near the landmarks (Fig. 6.3A, right). This confirms that animals indeed slowed down where they expect to find food, or near the landmarks possibly to orient themselves more precisely (see Discussion). These location-specific variations in the time per visit measure were quantified by the search time fraction, which divides the time spent near the landmarks (within 3 cm) or food (within 15 cm) by the total time spent in the active zone. The search time fraction near the landmarks (Fig. 6.3B, left) drastically decreased after learning (from the *Early* to *Late* trials) in all animals, but consistently increased during the *Probe* trials. The region of interests extends 3 cm from the landmark edges, because the animals began to increase their sensory sampling at this distance (see Fig. 6.5B, to be described below). In contrast, the search time fraction near the food location (15 cm from the centre of food) consistently increased after learning in all animals, and remained high during the *Probe* trials. Together, these evidences suggest that the animals spent less time exploring the landmarks after they learned the food location; but they spent more time near the landmarks when they failed to find food during the *Probe* trials.

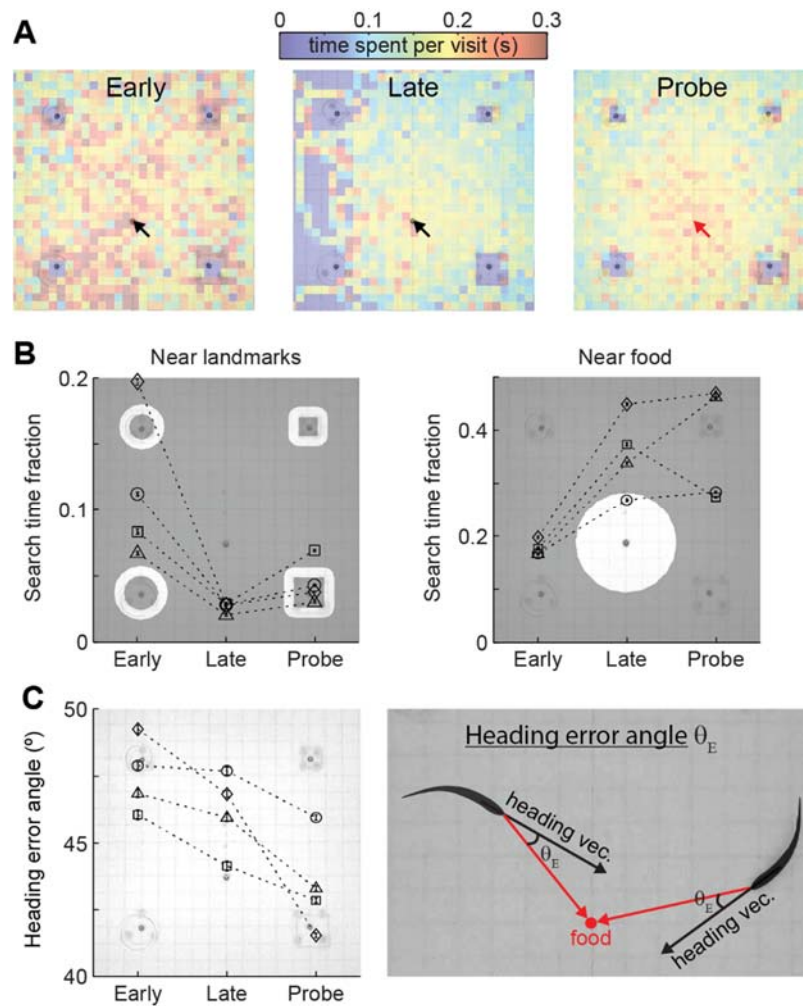


Figure 6.3. Search time fraction and heading error angle changes during learning. (A) Pseudo-colours represent the average time spent per visiting each grid location (time spent per visit), and plotted for the *Early*, *Late* and *Probe* trials. Black arrows indicate the food location and a red arrow indicates a missing food location. (B) Search time fraction is defined as fraction of time spent in a region of interest. Search time fractions were plotted near all landmarks (within 3 cm from the object edges) or near food (within 15 cm from the food center) as indicated by the masked background images. Shapes indicate different individuals. (C) Heading error angle (θ_E) is defined as the acute angle between the heading vector and food vector, and ranges between 0 to 90° (right). The heading error angles in the active zone (background image) decreased after learning in all animals tested. This indicates that animals learned to approach toward a food location more accurately.

Heading direction error. In addition to tracking learning-dependent changes in the locations and durations of visits, we examined how the direction of swimming changed after learning with respect to the food location. The heading error angle measures the acute angle between the heading direction and the direction toward the food location (Fig. 6.3C, right). We took an acute angle ranging between 0-90° to account for the backward swimming and frequent passing away from the food location during the *Probe* trials. The heading error angle in the active zone decreased after learning in all animals no matter from which direction they approached the food (Fig. 6.3C). The improvements in the direction of approach toward the expected food location can be most readily explained by assuming that, after learning, the fish had improved spatial memory of the relation between its own location and that of the food.

Locations of backward swimming. WEF often exhibits forward and backward swimming (*va-et-vient* movements) near novel objects [Toerring and Belbenoit, 1979; Toerring and Moller, 1984; von der Emde, 1990; Lannoo and Lannoo 1993; Nelson and MacIver, 1999; Hofmann et al., 2013a; Hofmann et al., 2014]; these movements are believed to be an active sensory scanning behaviour. We thus characterized the probability of backward swimming at various stages of learning. Pseudo-colour plots in Fig. 6.4A represent the backward swimming probability per grid location in the active zone. Initially, backward swimming occurred more frequently and broadly during the *Early* trials; but the frequency and locations of backward swimming both diminished after learning (*Late* trials). During the *Probe* trials, the animals exhibited backward swimming near the missing food location and near the landmarks. Figure 6.4B shows the backward swimming probability near the landmarks (< 3cm) or food (< 15 cm), and they reveal decreasing trends after learning (from *Early* to *Late*) near the landmarks (all animals), and the food location (3/4 animals). During *Probe* trials, the backward swimming generally increased (3/4 animals) near the missing food location and landmarks. These observations suggest that the animals does not need to sample the landmarks after they form a strong spatial memory, but failures of finding food during the *Probe* trials lead to increased sensory scanning near the landmarks by frequent backward swimming. Further, increased backward swimming near the missing food location is again consistent with the existence of spatial memory in *Gymnotus* sp..

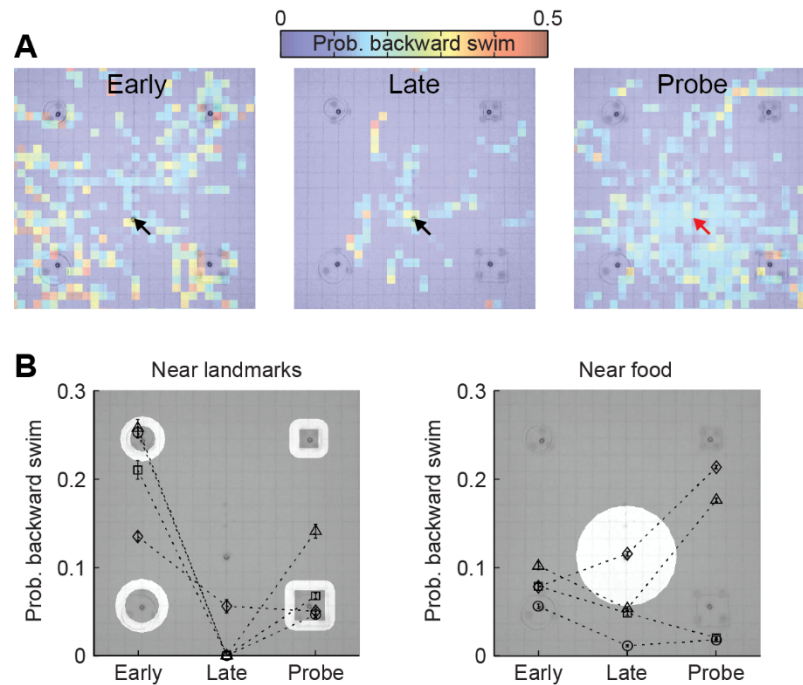


Figure 6.4. Location-specific probability of backward swimming. (A) Probability of backward swimming was computed for each grid location and plotted for the *Early*, *Late* and *Probe* trials. The locations of backward swimming decreased from the *Early* to *Late* trials, but increased markedly during the *Probe* trials. Black arrows indicate the food location, and a red arrow indicates the missing food location. (B) The probability of backward swimming was determined near all landmarks (left, within 3 cm from the edges) or near the food location (right, within 15 cm from the center), and these are plotted across the three type of trials. The probability of backward swimming near landmarks generally decreased with learning (from *Early* to *Late*), but generally increased in the *Probe* trials in 3 out of 4 animals, indicating repeated scanning near the landmarks and the missing food location. Error bars indicate the bootstrapped SEM.

6.4.2 Adaptive sensory sampling

Sampling density. Previously, we described learning-dependent changes in the visit locations based on the movement tracking. We now analyze how the animals modulate their electrosensory sampling rates at various locations based on the synchronized EOD recordings with movement tracking. For a moving animal, there are two ways to acquire more near-sensory information at a given site: by slowing down, or by ramping up the electrosensory sampling rate. The combination of the two results in shorter distance travelled between sequential EOD pulses. We therefore determined the number of sensory capture events (EOD pulses) per unit distance travelled which we define as the sampling density. The sampling density is calculated by taking a reciprocal of the average distances per IPI. In pulse-type WEF, this measure essentially describes the EOD rates normalized by the swim speed.

Factors contributing to the sampling density. Notice that the sampling density maps in Fig. 6.5A closely resemble the time per visit maps in Fig. 6.3A. This is expected since the sampling density is inversely proportional to the speed, thus proportional to the average time spent per visiting each grid location. However the EOD rates also contribute to the sampling density. We thus examined how much the swim speed and IPI duration contributed to the sampling density. Figure S6.3A shows that both factors were positively correlated with the reciprocal of the sampling density (distance / IPI) in all animals and in all trials; but the swim speed had a higher correlation than the IPIs due to a greater dynamic range of the swim speed ($\sim\pm 20\%$) than that of the IPI ($\sim\pm 10\%$). Figure S6.3B shows the variance accounted for (VAF) the distance per IPI by the IPI and speed, and similar patterns were found that the swim speed explained more variance in the sampling density than the IPIs. We conclude that the fish increase their sampling density by both spending more time near important locations (e.g. swimming more slowly near landmarks) and increasing their EOD rate at the same locations.

Figure 6.5A shows the map of sampling density at various learning stages (4 animals pooled). The sampling density underwent location-dependent changes after learning. The sampling density during *Early* trials was generally higher than the later trials (*Late* and *Probe* trials), and it was particularly high near the landmarks. In comparison, the *Late* trials show much reduced sampling density throughout except at the food location and this is due to the feeding-associated increases in the EOD rates. During the *Probe* trials, animals increased their

sampling density near the missing food location, which suggests that the sensory sampling in *Gymnotus* sp. can be influenced by a memory of the expected food location. The sampling densities are plotted as a function of distances from the landmarks, food and the missing food location in Fig. 6.5B. Animals increased their sampling densities within 3 cm from the edges of landmarks, suggesting a perceptual limit of detection in the dark. No significant differences were found between different landmark shapes and sizes, so the sampling densities were pooled. The short detection limit is consistent with a near-sensory nature of the electroreception [Caputi et al., 2013]. The baseline sampling density (> 3 cm) during the *Early* trials (red trace) was significantly higher than the *Late* trials (blue trace), and it also noticeably increased from the *Late* to *Probe* trials (green trace). Similarly, the sampling densities were plotted as a function of distance from the centre of food (Fig. 6.5B, middle), and it drastically increased within the contact distance from food (< 1 cm, dashed line) during the *Early* and *Late* trials. Such increases were absent during the *Probe* trials due to absence of food, but the probe trials still showed gradual increases in the sampling density as approaching toward the missing food at a greater distance scale (Fig. 6.5B, right). This strongly suggests that the spatial memory can drive the active sensing behaviours. In Fig. 6.5C, the sampling densities are plotted near the detection limit of landmarks (< 3 cm) shown above. In all animals, the sampling density near the landmarks decreased with learning (from *Early* to *Late* trials), but increased again in the *Probe* trials. Similarly, the sampling density around the food location (5-15 cm from the centre) decreased after learning. The analysis excluded locations within the food detection limit (< 5 cm from the centre) to exclude drastic increases in the EOD rates during feeding. During the probe trials, the sampling density increased around the missing food location, suggesting an enhanced sensory sampling at the expected food location. In summary, the electrosensory sampling behaviour habituated after repeated trials, but increase once again when the animals failed to locate food during the *Probe* trials.

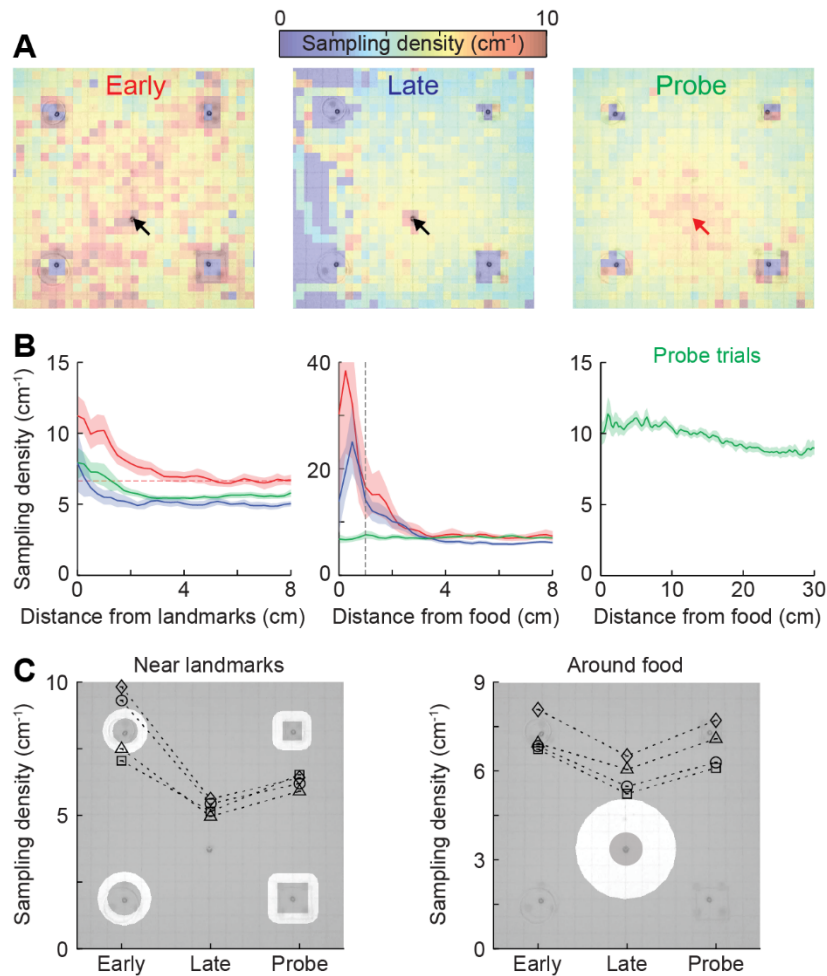


Figure 6.5. Location-dependent sampling density across learning trials. (A) Sampling density quantifies the number of EOD pulses per unit distance travelled. Sampling density map is plotted for the *Early*, *Late* and *Probe* trials. Black arrows indicate the food location, and a red arrow indicates the missing food location. (B) Sampling density is plotted as a function of distance from the landmark edges, and the center of food location (*Early*: red; *Late*: blue; *Probe*: green). Red dashed line (left) indicates the baseline sampling density in the *Early* trials (where the landmarks and food could not be sensed), and the gray dashed line (middle) indicates the edge of food. Sampling density in the probe trials (right) is plotted at a greater distance scale to reveal a trend to increasing sampling as the animal approached a missing food location. Shaded areas represent bootstrapped SEM. (C) Averaged sampling density is plotted for each individuals (indicated by various shapes) and compared across different learning stages. The sampling density decreased from the early to late trials in all animals, but increased in the probe trials near landmarks and food. The background image indicates the areas of

interest near the landmarks or food. We excluded the area of food detection range (within 4 cm from the food center) to rule out feeding-related increases in the EOD rate.

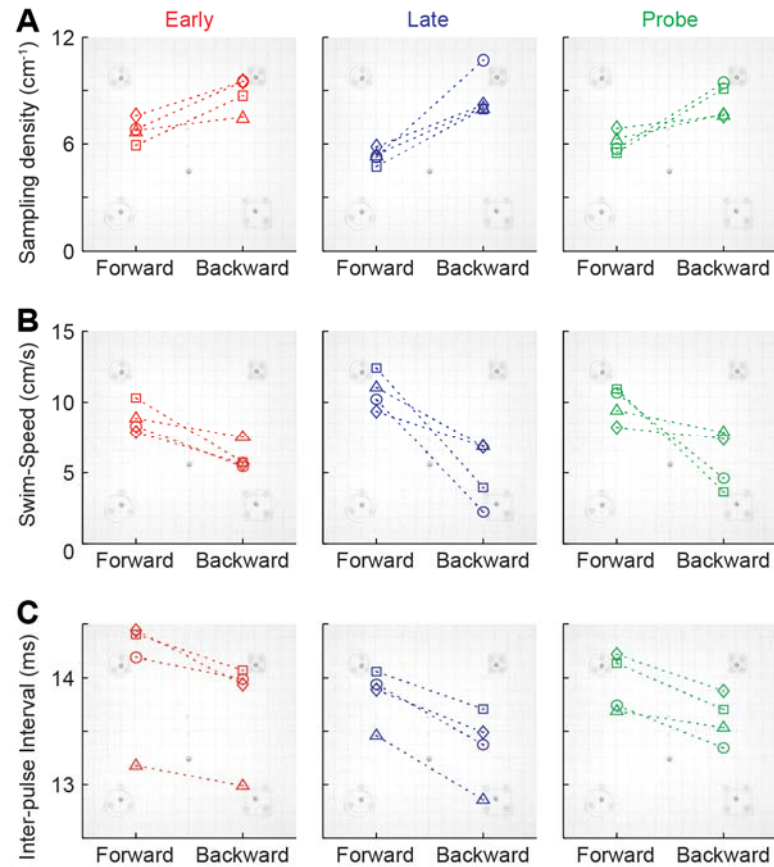


Figure 6.6. Sensory sampling during forward and backward swimming. (A) The sampling density was higher during backward swimming compared to the forward swimming in all three trial types. (B-C) The increases in the sampling density was due to simultaneous decreases in the swim-speed (B) and inter-pulse intervals (C), and the same pattern was observed in all tested animals during all trial types. Each trial types are plotted in different colors (red: early, blue: late, green: probe trials), and different individuals are indicated by various shapes.

Enhanced sampling density during backward swimming. We now compare the sampling density during forward and backward swimming to test whether the sensory sampling increased during backward swimming associated with exploratory behaviours (Fig. 6.4A-B). Indeed, the sampling density during backward swimming consistently increased in all animals (Fig. 6.6A). This was due to simultaneous decreases in the swim speed (Fig. 6.6B) and the inter-pulse intervals (Fig. 6.6C) during backward swimming. The same pattern was observed during all phases of learning (*Early*, *Late* and *Probe* trials), suggesting that animals always increased sensory sampling whenever they swam backward. Given the stereotyped increases in the electrosensory sampling during backward swimming, animals may generate backward swimming to enhance food detection. Furthermore, the correlation of increased electrosensory sampling and backward swimming confirms the importance of backward swimming as an active sensing strategy of WEFs.

Bursts in sensory sampling. Upon receiving external sensory stimuli, pulse-type WEF typically immediately shortens their IPIs; this is known as the novelty responses, which has been studied mainly in restrained animals [Grau, 1983; Caputi et al., 2003; Post and von der Emde, 1999; Pluta and Kawasaki, 2008]. Freely swimming *Gymnotus* sp. spontaneously exhibited similar temporal patterns in the IPIs; these transient IPI shortenings were emitted in bursts (Fig. 6.7A). Since these bursts occurred in the context of active exploration, we define these events as electrosensory scans (E-scans). A threshold was defined to detect transient shortening in the IPIs based on the asymmetry in the ΔIPI ($= \text{IPI}_k - \text{IPI}_{k-1}$) distribution. ΔIPI distribution exhibited a longer tail in the negative domain (Fig. S6.4A), as expected by the rapid IPI decreases followed by slower increases. The survivor functions of the positive and negative ΔIPI were initially similar, but they deviated at further ranges. The ΔIPI value is shown in Fig. S6.4B (green dashed line) when the ratio between the positive to negative survivor functions decreased most rapidly according to the slope of the ratio curve (Fig. S6.4C). Since this value remained stable across individuals and over multiple days, it was used as a threshold to detect E-scan events. The analysis excluded threshold-crossing events that only lasted one cycle below the threshold to remove the false-positives (Fig. 6.7A). Similar to the way sampling density is defined, distances between successive E-scan events were measured to compute the E-scan density, or the number of E-scan events per unit distance travelled. Initially, the E-scan density increased throughout the active zone; but later, it increased only near the food after

learning (*Late* trials). The E-scan density during the *Probe* trials gradually increased toward the missing food location (Fig. 6.7B, right), consistent with the expected food location. The E-scan densities are plotted near the landmarks and food in Fig. 6.7C, and they all decreased after learning with an exception of one animal near the landmarks. In summary, all measures of the sensory sampling activity (sampling density, E-scan density) underwent overall habituation, and developed focused spatial distribution near the food location after repeated exposures to a stable spatial environment. Next, we compare the spatial search strategy of animals trained with stable or unstable landmarks.

6.4.3 Stable landmarks improved the estimation of the food location

In the previous sections, we reported an increased visit density and sampling density near the stable landmarks in the *Early* and *Probe* trials, when animals were less certain about their positions. To directly test whether stable landmarks play a critical role in guiding animals to orient themselves in space, we compared the stable landmark group (*Stable* group) groups with two other controls groups trained without any landmarks (*None* group), or with randomly moving landmarks and food between trials (*Unstable* group). The rest of the experimental conditions were equal in the two control groups other than the presence or absence of landmarks, or the stability of landmark and food locations. The food location was fixed for the *None* group, but it randomly varied between trials for the *Unstable* group. Four animals were trained per group, and data from the same group animals were pooled. Figure 6.8A shows visit density maps from the three groups during *Probe* trials, and the two control groups (*None* and *Unstable* groups) exhibited significantly greater fraction of search area than the *Stable* group (Fig. 6.8B). The *Unstable* group showed increased visit density near the landmarks compared to the *Stable* group, and there was no well-defined centre in the visit density map. The fraction of search area for the *None* group was significantly higher than the *Stable* group, and significantly lower than the *Unstable* group.

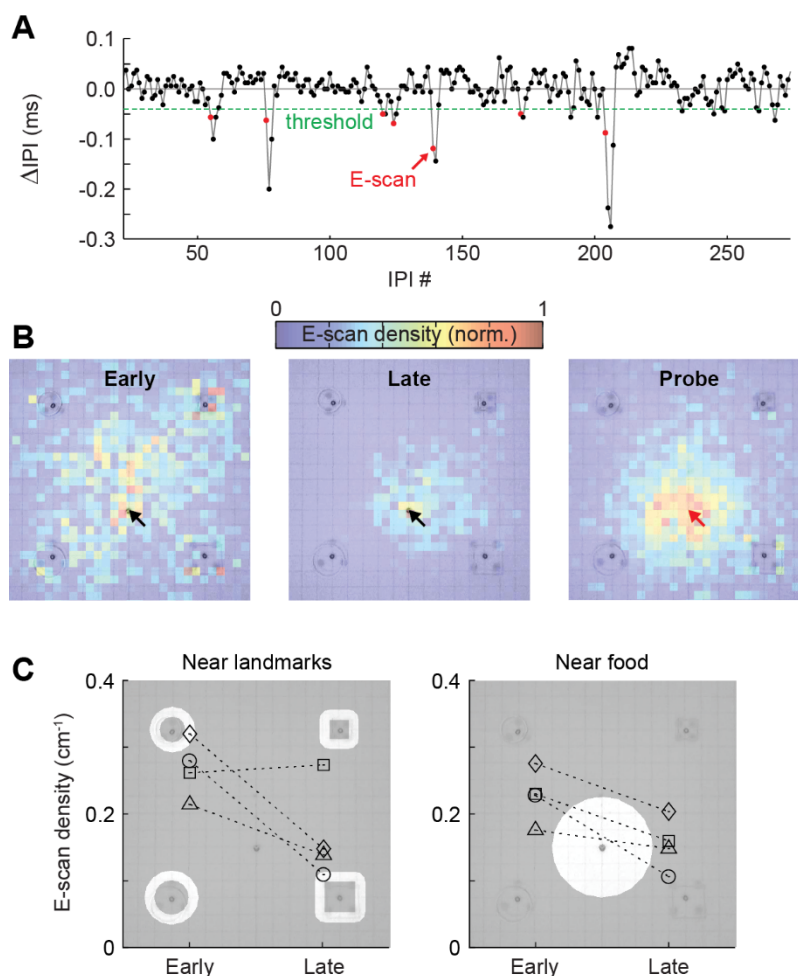


Figure 6.7. Locations and frequency of the E-scan events during spatial learning. (A) E-scan events (red circles) are defined as the transient shortening of the EOD inter-pulse intervals (IPIs), or bursts in the sensory sampling. The cycle-to-cycle changes in the IPIs (ΔIPI s) are plotted in the ordinate, and the IPI sequence orders (IPI#) are plotted in the abscissa. EOD pulse events are indicated by black circles, and the E-scan events are indicated by red circles, which were detected by the onsets of falling threshold (green dashed line) and remaining below for two cycles at least (see Fig. S6.3). (B) E-scan density quantifies the number of E-scans per unit distance travelled, and the E-scan density maps are plotted for the *Early*, *Late* and *Probe* trials. Black arrows indicate the food location, and a red arrow indicates the missing food location. (C) The E-scan density decreased from the early to late trials near the landmarks (3/4 animals, within 3 cm from edges) and food (all animals, within 15 cm from the centre). The background images indicate the area of interests near the landmarks (left) and food (right).

2D Gaussian fitting. To compare the accuracy of the remembered food location between the *Stable* and *None* groups, we fitted a two dimensional Gaussian function described by 6 parameters ($\mu_x, \mu_y, \sigma_x, \sigma_y, \theta, A$) to the visit density maps. Figure 6.8C shows an example of the 2D Gaussian function fitted to a visit density map, which shows the centre of all locations visited (a black dot) and the spread of the spatial distribution shown as a contour plot. Mean estimation error (bias) is defined as the distance between the centre of locations visited, and the actual location of missing food. The mean estimation errors were much lower in the *Stable* group than the *None* group (Fig. 6.8D, left). Similarly, the spread of visit density ($\sqrt{\sigma_x^2 + \sigma_y^2}$) was much higher in the *None* group (Fig. 6.8D, right). The 2D Gaussian fitting analysis confirms that stable landmarks indeed aided animals to estimation the food location more accurately compared to the animals who did not have access to any landmarks in the active zone.

Comparison of the sampling density. Figure 6.8E shows the sampling density maps during *Probe* trials from the three groups. The *None* group did not show increases in the sampling density near the missing food location as seen in the *Stable* group. The sampling density only increased near randomly placed landmarks in the *Unstable* group. The mean sampling density throughout the active zone is compared between the three groups in Fig. 6.8F. The *Stable* group exhibited significantly greater average sampling density compared to the two control groups. No significant difference in the active zone was observed between the *None* and *Unstable* groups. These results suggest that stable landmarks associated with food encouraged animals to increase their sensory sampling during foraging, whereas animals relied on a random search strategy in the absence stable landmarks.

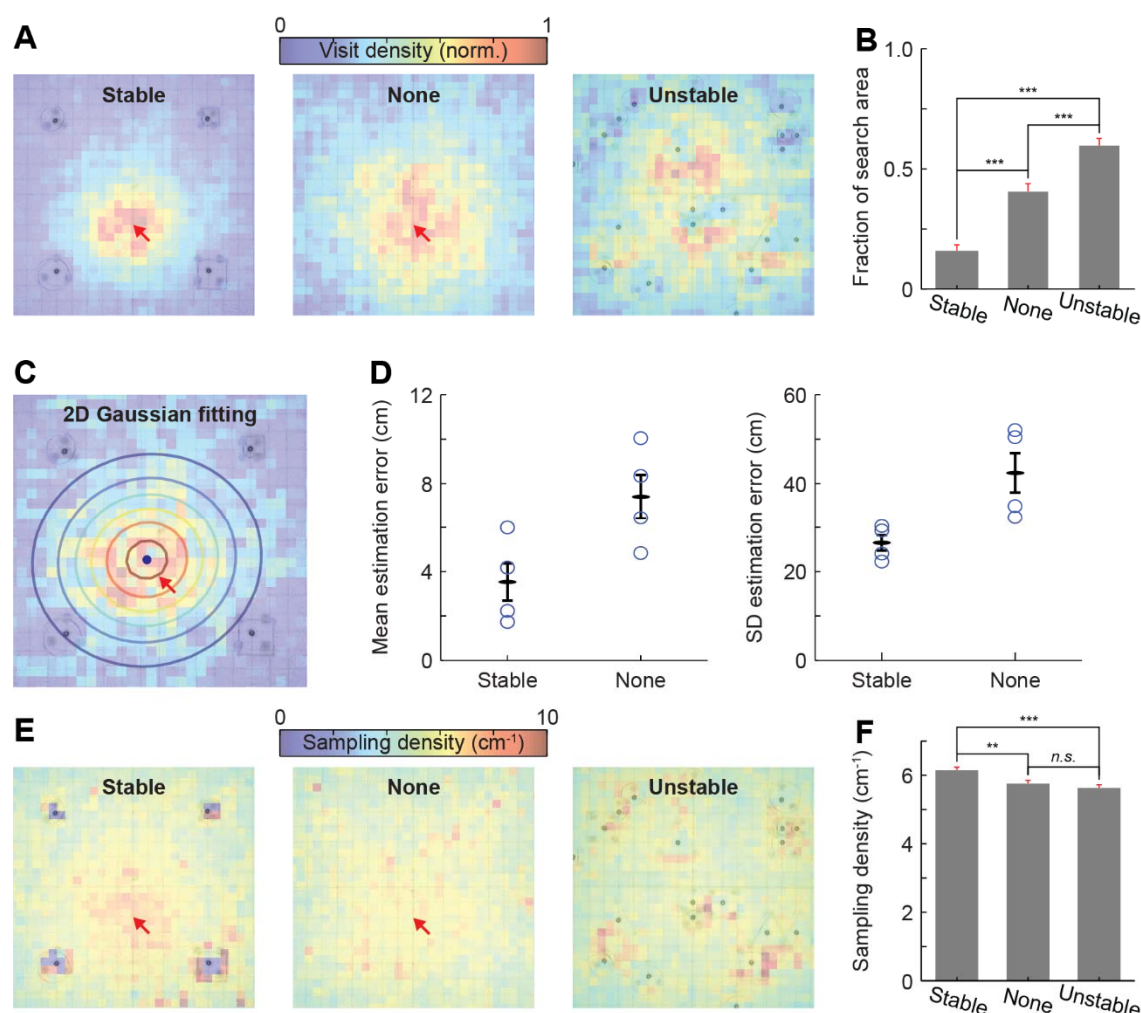


Figure 6.8. Comparisons between animal groups trained with stable, none and unstable landmarks during probe trials. (A) Visit density maps are plotted for the three groups of animals during *Probe* trials. Red arrow indicates a missing food location throughout this figure. *Stable* group animals were trained with four landmarks presented at fixed locations; *None* group animals were trained without any landmarks; *Unstable* group animals were trained with randomly placed four landmarks and food. The background image of the unstable group (right panel) is a composite of four random probe trials. Four animals were pooled in each group. (B) The fraction of search area indicates the most focused search area in the group trained with stable landmarks. (C) Two-dimensional Gaussian function (indicated by the contour plot) was fitted to the visit density map to estimate the centre (blue circle) and spread of the search locations. (D) The mean estimation error quantifies the distance between the missing food location and the center of the 2D Gaussian fitting. The *Stable* group shows smaller mean

estimation error compared to the *None* group (left). The spread of search is quantified by the SD of the 2D Gaussian fit, and this was smaller in the *Stable* group. Fitting results from different individuals are indicated by blue circles. **(E)** Sampling density maps are plotted for the three groups of animals during the *Probe* trials; the *Stable* group exhibits greater sampling density at the usual food location than the *None* group. The *Unstable* group animals increased their sampling density near the randomly placed landmarks. **(F)** The overall sampling density was the highest in the *Stable* group, indicating that the use of stable landmarks during training results in greater and more reliable sensory sampling during food searching tasks. Error bars indicate the bootstrapped SEM. Significant differences are indicated by * (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; *n.s.*: not significant).

6.5 Discussion

We have demonstrated that *Gymnotus* sp. is adept at spatial learning: they rapidly and accurately learn a food location after repeated exposures to a stable spatial environment populated with landmarks. The evidences for spatial memory includes efficient food searching (distance travelled and duration of trials, Fig. 6.1A), focused search locations (Fig. 6.2A-C), and accurate heading direction toward the food location (Fig. 6.3C). Behaviour of animals during the probe trials (absence of food) revealed important signatures of spatial memory, including repeated visits and accurate heading direction toward the missing food location.

6.5.1 Sensory input for spatial learning

Our experiments were all carried out under infrared illumination therefore ruling out vision as contributing to the spatial learning we observed. Olfaction can also be ruled out: we rigorously cleaned out any mealworm debris between trials and eliminating potential chemical cues. Even stronger evidence is provided by the ‘Unstable’ controls – the inefficiency in finding food displayed by the fish strongly suggests that they do not rely on olfaction to find prey. The only other senses available are the lateral line (160 on one side of the body), ampullary electroreceptors (responsive to exogenous electric fields, 360 per side) and tuberous electroreceptors (tuned to the fish’s own EOD, 6500-8500 per side, Carr et al. [1982] in all cases). All three receptors have comparable sensitivity [Nelson et al., 2002]. It may therefore

be that the lateral line receptors also signal a fish's approach to a landmark by detecting bow waves [Montgomery and Coombs, 1998] and then drive the increases in sampling rate; they are, however, unlikely to signal the location of the small mealworms. Likewise, ampullary receptors might well detect weak electric fields emanating from the mealworms and drive the increased sampling rate; these receptors are, however, not activated by the plastic landmarks. We therefore hypothesize that tuberous electroreceptors (driven by the fish's own EOD) are the dominant sensory input underlying spatial learning in these fish. This is based on a) their far greater number than either lateral line and ampullary receptors, and their far greater central representation [Krahe and Maler, 2014], b) the fact that only these receptors will permit detection of both food and landmarks and c) most importantly, the increase in EOD rate near landmarks clearly demonstrate that these fish are actively sampling using their electrosense (tuberous receptors).

6.5.2 Navigation and spatial learning based on a proximal sensing modality

The electrolocation system offers severely limited detection ranges compared to other distant-sensing modalities such as vision or echolocation [MacIver, 2001; Nelson and MacIver, 2006; Caputi, 2013]. We observed ~3 cm detection limit in *Gymnotus* sp. from the edges of dielectric objects, which is similar to the previously reported value of the detection range for daphnia (~2 cm at 100 $\mu\text{S}/\text{cm}$) in a related WEF species [MacIver et al., 2001]. To put this in perspective, drivers experiencing dense fog would be comparable to navigating based on electroreception. A more biological example is the macro vibrissae sense of rodents. These extend out to a similar distance (15-50 mm; Prescott et al. [2011b]), and, in blind rats, the vibrissae sense supports spatial navigation and the formation of place fields in hippocampal cells [Save et al., 1998]. Thus, unlike the visual system, the vibrissae- and electro-sense must 'piece together' multiple local (<3 cm) sense inputs to support navigation of a much large scale.

The limited detection range of electroreception is due to rapid fall-off the dipole potential returned to a receiver as a fourth power of the distance to a target (round-trip). In addition, the electrolocation system receives blurred electric images since it lacks focusing mechanism comparable to a lens in a visual system [Heiligenberg, 1975]. To overcome the limited detection range, WEF evolved an omnidirectional sensing capability enabled by

electroreceptors distributed throughout the body, and highly maneuverable body fully capable of backward swimming. Near-sensory modalities require greater demand for spatial memory to efficiently navigate, since animals need to remember previously encountered landmarks that went out of the detection range. In comparison, distant-sensing modalities can capture multiple landmarks within the field of view, thus possibly lessening the demands on working memory to store different landmarks. Near-sensory modalities need to be efficiently coordinated with the motor systems controlling the position of sensory organs, since precise alignment with a target allows optimal detection. Backward swimming in WEF could be understood as an attempt to orient the animal's sensory surface for an optimal detection [Hofmann et al., 2013], and the increased sensory sampling during backward swimming (Fig. 6.6A-C) supports this view. The vibrissa sense in rodents may use rhythmic whisking movements to similarly allow optimal contacts with object surfaces for improved detection [Mitchinson et al., 2007; Prescott et al., 2011]. An interesting direction for future research will be to determine whether the neural mechanisms used for spatial learning in WEFs are in any way comparable to those used by blind (or in the dark) rodents.

6.5.3 Active sensing and spatial learning

We observed readily quantifiable discrete sensory sampling events that are expressed by EOD pulses and analyzed the learning-dependent changes in pulse rate and pattern (IPI structure). The overall sampling density, defined by the number of EOD pulses per unit distance travelled, decreased after repeated exposures to a stable spatial environment (Fig. 6.5A-C) as previously reported in bats [Petrites et al., 2009; Barchi et al., 2013]. The sampling density selectively increased near the food location after learning (Fig. 6.5A-B), which is consistent with the efficient food searching. The sampling density reflected the spatial knowledge of the animals; enhanced sampling density occurred in the early phase of learning and also during the probe trials when animals were less certain about the food location (Fig. 6.5C). In both cases sampling density was highest near landmarks emphasizing their importance in efficient navigation.

Backward swimming are previously observed during WEF exploratory behaviors [Toerring and Belbenoit, 1979; Toerring and Moller, 1984; von der Emde, 1990; Lannoo and Lannoo 1993; Nelson and MacIver, 20; Hofmann et al., 2013a; Hofmann et al., 2014]

decreased after the animals formed spatial memory (Fig. 6.4A-B). The sampling density consistently increased during backward swimming in all animals during all phases of learning (Fig. 6.6A) that supports the exploratory role of backward swimming [Toerring and Belbenoit, 1979; Toerring and Moller, 1984; von der Emde, 1990; Lannoo and Lannoo 1993; Hofmann et al., 2013a]. The EOD pulses exhibited burst-like temporal patterns (E-scans, Fig. 6.7A) that also habituated after learning (Fig. 6.7B-C). Stable landmarks played a critical role in accurately locating the food according to the comparisons with two control groups either trained without any landmarks or with randomly moving landmarks (Fig. 6.8A-D). Animals trained without stable landmarks exhibited significantly lower sampling density that is indicative of random search strategy (Fig. 6.8E-F). Our results supports our initial hypothesis that *Gymnotus* sp. adapt their sensory sampling in an optimal manner by using past experiences and landmarks for more efficient navigation and spatial search.

6.5.4 Spatial navigation in the dark: landmarks and path integration

Extensive studies [Etienne et al., 1996; Alyan and McNaughton, 1999; Whishaw et al., 2001; McNaughton et al., 2006] have shown that spatial learning depends on both path integration and landmarks. Path integration requires that the animal depend on the memory of internal cues (vestibular, proprioceptive, corollary discharge, [Stackman et al., 2002; Russell et al., 2003; Horri et al., 2004]) and perhaps optic flow [Wylie et al., 1999] to guide them. Errors do accumulate during path integration making it unreliable over long distances. Once the location of landmarks has been learned, it can correct path integration and therefore improve localization of important environmental features.

Spatial learning in WEFs can be readily understood in this framework. However, given the short range of the electrosense (see above), even the location of landmarks must be learned via path integration. In the ‘None’ control, the fish has features at the tank boundaries as landmarks; since its electrosense only extends ~3 cm, it must, from memory, estimate the food location (direction and distance) over a distance of at least 80 cm and traverse this distance by path integration. With landmarks present the distance between edge and landmarks drop to 47-51 cm and from landmarks to food to 36-58 cm. We hypothesize that this is the reason that fish with landmarks do so much better at locating the food than those without. Finding food can be considered a problem in statistical estimation; from this point of view, the fish with

landmarks have far lower mean estimation error and far lower estimation variance. We hypothesize that this is because the path integration errors are lower since the landmarks permits re-calibration after only a short distance from the tank edge.

We further hypothesize that this is the basis of the increased active sensing (backward swimming, increased sampling density, increased E-scans) that is especially prevalent near landmarks and food in the early stage of learning. The fish are, we hypothesize, calibrating their path integration mechanisms by sampling their routes to landmarks and food. Once such calibration is complete, sampling can be reduced as the fish can more efficiently (and rapidly) navigate primarily by path integration. During the probe trials, active sensing again increases. In our view, this is again readily understood as an attempt by the fish to recalibrate its path integration mechanism by increased sampling of the landmarks and their immediate environs.

6.6 Conclusion

Animals must rely on sensory information to navigate in a novel or unstable environment. Sensory scanning occurred more readily when animals were less certain about their locations. Exploratory behaviours were expressed combination of decreased speed, increased sensory capture rate, and frequent backward swimming. These behaviours indicative of sensory-guided navigation occurred more often during the early phase of learning, or during probe trials when animals actively searched for the missing food. In comparison, memory-guided navigation enables animals to efficiently arrive at the target location in a familiar environment. Path-integration strategy updates the location of animal based on the efferent copies of self-motion, and landmarks permit the accumulated errors to be reset. More precise estimation of food location in the stable landmark group compared to the animals trained without landmarks support. Memory-based navigation saves time and energy associated with sensory sampling, and does not require external landmarks. Linear increases in the EOD frequency require exponential increases in the oxygen consumption [Lewis et al., 2014], which suggests that memory-based navigation could offer significant energetic savings. Memory-based navigation is used in echolocating bats when they enter or exit their caves in large groups, since acoustic sensing is impaired by interfering calls from conspecifics in close proximity [Ulanosky and Moss, 2008]. Electroreception ability could be similarly impaired when multiple WEF hunt in close proximity. Barchi et al. (2013) reported stereotyped flying patterns and decreased call

rates in big brown bats, indicative of memory-guided navigation after repeated exposures to a stable environment. Natural environment undergoes dynamic changes such as in freshwater habitats during floods or droughts, thus WEF need to flexibly switch between the memory-based to sensory-based navigation when they detect novelties in their surroundings. Indeed, *Gymnotus* sp. reverted to the sensory-guided navigation when they failed to find food at the expected location during the probe trials. Our observation suggests that these animals use a mixed approach, by adaptively modulating the sampling density based on their locations. The sampling density and the average time per visit increased near the expected food location indicative of sensory-guided navigation; and both decreased at locations further away suggesting memory-guided navigation.

6.7 Supplementary figures

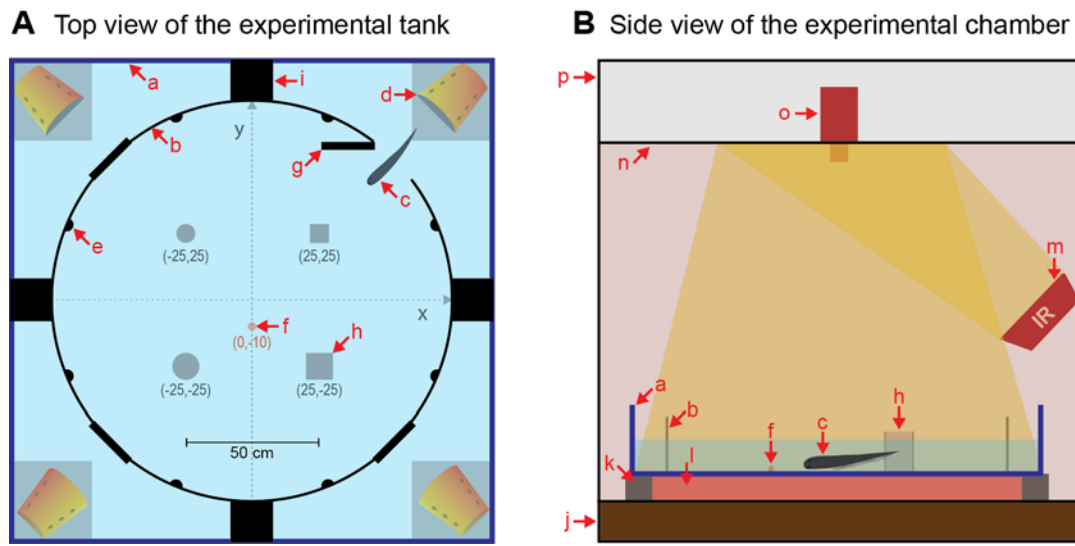


Figure S6.1. Overview of the experimental setup. (A) Top view of the experimental tank is shown. The tank houses four individuals in each corner compartments and the central arena was used as a testing area. Each compartments were made watertight from the neighbouring compartments to prevent electrocommunication. Remotely controlled gate (g) lets an animal to enter the central arena from the corner compartment. Red arrows indicates following objects. a: Glass tank (1.8 m x 1.8 m x 0.3 m); b: circular fence (heat-formed acrylic sheet); c: test fish; d: home base; e: one of eight graphite electrode to capture EOD; f: live mealworm restrained on a suction cup; g: remotely controlled gate; h: one of four landmark; i: compartment separator. (B) Side view of the experimental chamber shows the aquarium tank (a) surrounded by the experimental chamber (p). The chamber wall (p) blocked external sources of light, sound and RF noise. The walls (p) were filled with fibreglass batts for sound-proofing and surrounded by a faraday cage. The chamber rested on multiple layers of vibration absorbing materials (j). The weight of aquarium was supported by aluminum frames on the edges (k), and was uniformly heated from below by a floor heater (l). Eight infrared illuminators (m) uniformly lit the central arena by reflecting off the ceiling (n). The ceiling panel hid an infrared-sensitive camera (o) to prevent its reflection on the water surface.

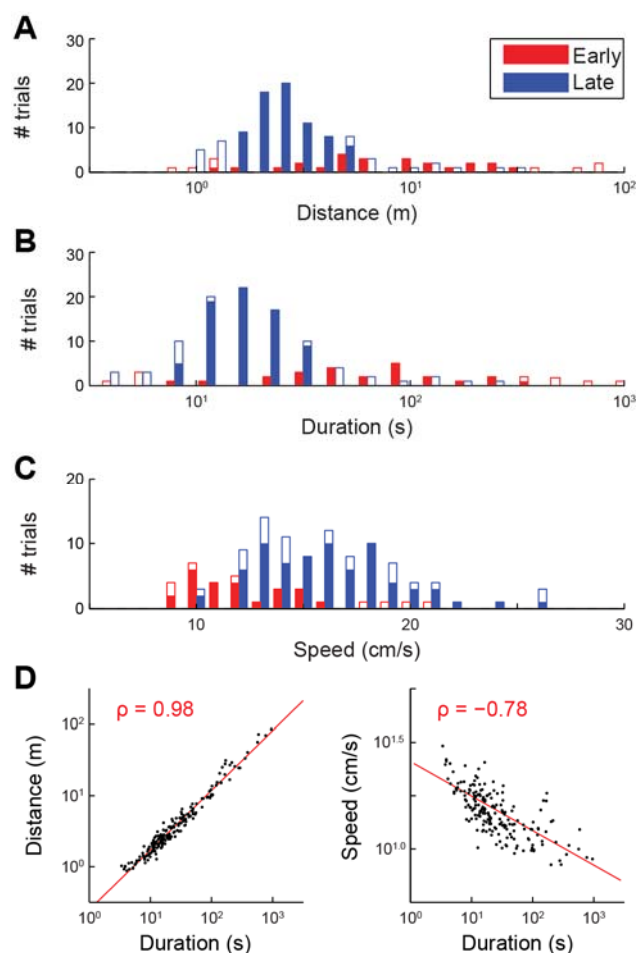


Figure S6.2. Distributions of the learning performances and their correlations. (A-C) Distributions of the task performances from all trials are indicated as open boxes (red: early trials, blue: late trials). We discarded outlier trials based on the distance travelled (below 12.5th or above 87.5th percentiles), and the remaining trials are indicated as filled boxes. (D) The strong correlations between task performances (distance vs. duration on left, and speed vs. duration on right) indicate close agreements between different measures. Lines of the best fit are shown in red.

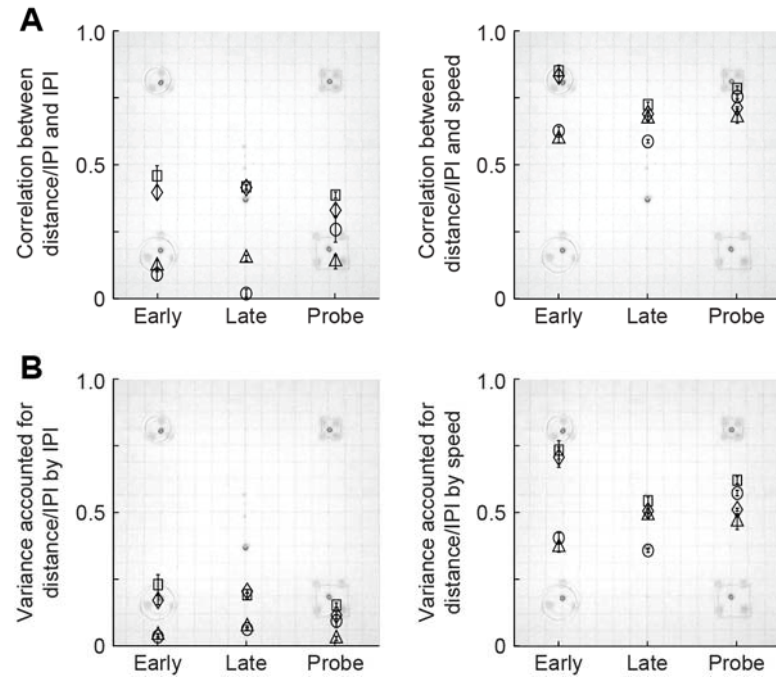


Figure S6.3. Contributions of the IPI and swim-speed to the sampling density. (A) Correlation between the distance travelled between successive IPI (distance/IPI) and IPI (left), or swim-speed (right). Distance/IPI is the reciprocal of the sampling density ($\# \text{ IPI/distance}$), and used here to obtain positive correlations to aid comprehension. (B) Variance accounted for distance/IPI by IPI (left), or swim-speed (right). These plots indicate that both factors (IPI and swim-speed) significantly contribute to the sampling density, and the contribution of the swim-speed is higher. Error bars indicate bootstrapped SEM.

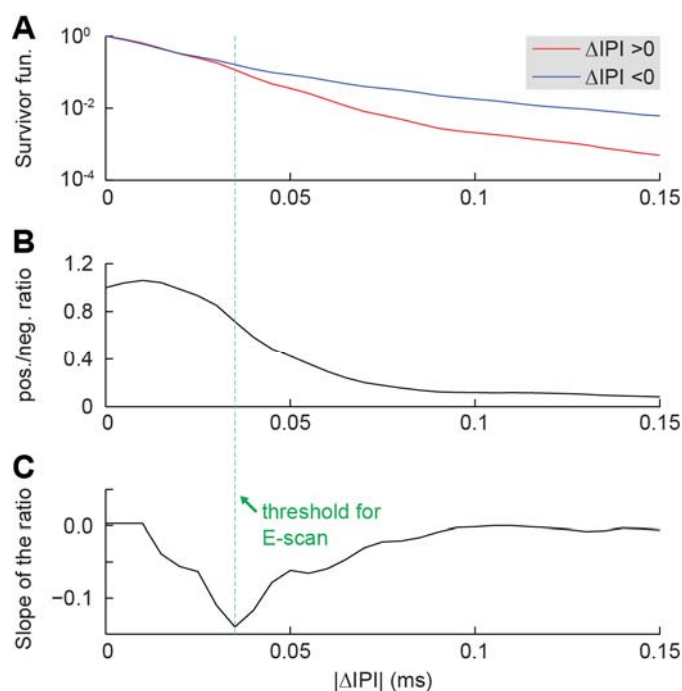


Figure S6.4. Method of determining the E-scan threshold. (A) The survivor functions ($1 -$ cumulative density function) for the positive (red curve) and negative (blue curve) ΔIPI s are plotted in a semi-logarithmic scale. ΔIPI refers to the cycle-to-cycle change in the EOD inter-pulse intervals. (B) Ratios between the positive and negative survivor functions (pos./neg. ratio) is plotted in the ordinate. (C) Slope of the pos./neg. ratio is plotted in the ordinate to determine the minimum, whose ΔIPI value is used as the threshold to detect E-scan events.

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Chapter 7

Conclusion and future directions

7.1 Summary of technical developments

This thesis investigated voluntary sensory sampling activities in freely exploring fish. The pulse-type weakly electric fish as an animal model for voluntary or “active” sensing revealed to us how it controls its sensory sampling during naturalistic behaviours such as spontaneous movements and foraging. Our approach to studying self-generated movements from unrestrained, freely-behaving animals differs from the usual approaches taken in the neuroscience community, where experimenters deliver well-controlled stimuli and study stereotyped behavioural outputs. Study of voluntary behaviours could reveal how animals make decisions in naturalistic contexts, and offer insights to the ecological and evolutionary significance of the behaviour-generating mechanisms. I developed series of experimental techniques to capture and quantify unconstrained behaviours generated by freely swimming *Gymnotus* sp. in a well-controlled spatial environment. Behavioural tracking systems were developed to capture animal movements and sensory capture events in the form of the EOD pulses. The reliability and accuracy were characterized to ensure high-quality measurements uncompromised by unrestrained animal movements.

Chapter 2 described a development of a real-time EOD pulse detector to remotely monitor the EOD rate fluctuations during movements, which accurately determined the EOD pulse timing up to $\sim 10\ \mu\text{s}$ accuracy at all tank locations. We initially observed drastic variations in the EOD waveforms during body movements and at different tank locations that negatively affected the measurement accuracy of a simple threshold-crossing method. The detection accuracy and reliability improved significantly by using a combination of spatial summation and signal-envelope extraction methods. EOD signals from eight recording electrodes were differentially amplified, rectified and summed; and the pulse envelope was extracted to determine a well-defined maximum to be used as a pulse timing reference. The accuracy of our EOD detector was tested by placing a restrained fish at various tank locations and orientations, as well as by recording EOD signals from tethered recording electrodes attached on a freely-swimming fish.

Chapter 3 described a novel electrical tracking technique to locate a dipole-like WEF in shallow water using its self-generated EOD signals. The dipole localization method is based on an accurate forward model for an ideal current dipole in two dimensions that accurately

approximated the EOD signals generated in a shallow body of water. The signal amplitudes from eight recording electrodes pairs were matched with an entry in the look-up table generated by the forward model at many possible locations and body orientations. The animal positions and orientations were efficiently determined by a computationally optimized localization algorithm for real-time tracking. Our tracking system determined the positions up to 5 cm accuracy and orientations up to 15° accuracy according to the simultaneous visual tracking. In addition, we could also determine the tail-bending angles by comparing the dipole orientations generated near the head and tail at different EOD phases. The electrical tracking system simultaneously tracked two fish and successfully discriminated them even during close contacts, which typically leads to visual tracking failures.

Chapter 4 described three separate aspects of our experimental system that was prepared for a video-based journal (*Journal of Visualized Experiments*). The journal editor invited us to describe the construction of our long-term behavioural tracking setup and the data acquisition and analyses techniques. First, we provided instructions for constructing an experimental tank and a surrounding sensory isolation chamber. The tank was built sufficiently large (1.8 m x 1.8 m) for animals to require spatial learning in order to efficiently navigate. The sensory isolation chamber effectively blocked external sources of noise that could trigger novelty responses. Second, we described an electrical recording setup to capture EOD signals from multiple recording electrodes, and to process them in real-time. Third, we described an infrared imaging setup and our video tracking software for an automated animal tracking. The lighting sources and the high-contrast background were carefully arranged to minimize glares and reflections that could interfere with automated tracking, and our video tracking software reliably tracked the head position and body posture without requiring tracking markers. Reliable body tracking enabled us to determine the location of highest sensory acuity at the head, and the geometry of the electric field produced by the electric organ distributed along the body.

7.2 Summary of biological discoveries

The techniques we developed permitted us to study long-term voluntary behaviours in a non-invasive and naturalistic manner. First, we studied spontaneous movements and sensory sampling activities in *Gymnotus* sp. in the dark cycles when they are mostly active. Next we tracked how the sensory sampling and movement trajectories evolved over the course of spatial learning to forage. Long-term behavioural observations that spanned multiple days generated large amounts of raw data that were automatically processed and measured in an objective manner. We uncovered robust patterns in the sensory sampling activities associated with self-initiated movements and acquisition of spatial memory in these animals.

Chapter 5 described spontaneous movement transitions between periods of active and resting periods, and compared temporal relationships between the sensory sampling rate and movements in the voluntary or sensory-triggered contexts. We uncovered two distinct sensory sampling states that were highly correlated with movement states. Up-states refer to periods of active movements coupled with higher EOD rates, and Down-states refer to periods of inactivity coupled with lower EOD rates. Up-state transitions in the EOD rates preceded self-initiated movements by a few seconds (~ 1.5 s), a time-scale similar to that for the preparatory increase in firing rate in mammalian cortical neurons preceding voluntary movements. Further, temporal dynamics of the across-trial EOD rate variability also qualitatively matched those observed for mammalian cortical neurons. Indeed, those neurons also increased the mean and variance of their firing rate before voluntary movement initiation and decreased them after the onset of a stimulus. The switching between the Up and Down-states occurred randomly, and no temporal correlations existed that would reveal an influence of the past history of spontaneous movements. Thus, behavioural transition could not be predicted due to a complete lack of temporal structure in the spontaneous activity time-series. On the basis of these data, we hypothesized that these fish display the main hallmarks of volitional behaviour and that its neural basis might be similar to that in mammals.

Chapter 6 described how the sensory sampling activities during foraging became optimized with acquisition of spatial memory. We trained *Gymnotus* sp. to find food delivered at the same location surrounded by four stable landmarks in the test circular arena (1.5 diameter). Animals quickly learned the food location in approximately five training days (four

trials/day) by demonstrating efficient swimming trajectories to the food location. Even in the absence of food, all animals exhibited accurate spatial memory by swimming near the expected food location. After the acquisition of spatial memory, the active electrosensory activity reflected more efficient sensory sampling near the expected food location by decreasing the distance travelled between successive EOD pulses. The presence and stability of landmarks played a critical role in guiding animals to efficiently locate food, which suggests that stable landmarks were used to reset the distance errors accumulated by a possible path-integration strategy during navigation.

7.3 Future directions

Several unanswered questions arise from this thesis which require further investigations. To address some of these pending questions, a few projects are under way in collaboration with Dr. Maler, Dr. Longtin and their lab members. Various techniques developed during my PhD need to be further refined for wide-spread use in broader settings; these techniques could be applied to uncover novel behaviours of WEF in laboratory or field settings. In principal, the electrical tracking method developed here could be applied to locate any dipole-like signal sources such as extracellular action potentials generated by neural populations. Also, we still do not understand the neural mechanisms behind the voluntary control of sensory sampling activities in an experience-dependent manner. Chronic neural recordings from freely behaving fish from candidate regions, such as the dorsal telencephalon [Giassi et al., 2012b], may reveal important clues on how the memory and volition are expressed by the neural circuits to govern the sensory-motor behaviours.

7.3.1 Behavioural tracking of socially interacting WEF

This thesis described visual and electrical behavioural tracking techniques to remotely monitor the EOD and movement activities in a non-invasive manner. Our visual tracking technique reliably tracked animal locations in a two-dimensional plane, but the tracking needs to be extended to a three-dimensional volume to track a full range of motions including the depth dimension. This will require images to be captured at multiple angles by using multiple cameras [MacIver and Nelson, 2000] or mirrors. Our studies mainly focused on behaviours generated by isolated individuals, and it would be interesting to apply our behavioural tracking techniques to study freely interacting pairs of WEF. Electro-communication studies were

typically conducted in restrained animals by artificially delivering stimuli mimicking communication signals at a fixed location. Recent studies report effects of movements on the signal envelope waveforms in wave-type species [Yu et al., 2012, Fotowat et al., 2013, Marsat, Na Yu, Metzen and Chacron, 2014]. In order to track multiple interacting animals, signals from the animals need to be reliably discriminated. Our dipole tracking method could be applied to study multiple pulse-type WEFs, and in principal, it could be also applied to the wave-type species. Our visual and electrical tracking methods could be combined to achieve accurate body tracking as well as reliably identify different individuals and their EOD signals. Wild WEF populations display a rich repertoire of behaviours not typically observed in artificial laboratory conditions. Henninger et al. [2012] reported electro-communication behaviours in a wild population of wave-type gymnotiform species by using grid-electrode arrays to record EOD signals from extended areas in the Amazonian river. It would be interesting to apply our electrical tracking method to track movements and EOD signals from socially interacting conspecifics in their natural habitats.

7.3.2 Application of dipole tracking technique to locate neurons based on their extracellular action potentials

In addition to tracking freely moving WEF, our electrical tracking system can, in principal, be used to locate any dipole-like signal sources. Extracellular action potentials (EAP) generated by neurons can be approximated by dipole-like current sources [Mechler et al., 2011; Mechler and Victor, 2013]. Although the spatial scale of neurons is much smaller than the scale of WEFs, the same method could be applied if the neuronal signals behave close to an ideal dipole current source. Multiple microelectrodes could be used to simultaneously capture the EAPs at different positions and orientations with respect to the signal source location, which causes variations in the received signal intensity (RSI). The location and orientation-dependent modulation of the RSI can be used to find the three-dimensional position and orientation of the dipole source by efficiently solving the forward model at many possible locations. If the idea of electrically locating neurons based on their EAP is proven feasible, this would allow us to obtain the anatomical information from a deep brain structure. The current optical methods cannot image from a live brain deeper than several hundred micrometres, due to light scattering. High density silicon probes, which are currently being developed in various labs,

offer a large number of active recording sites and provide a controlled electrode geometry, both of which are ideal for the electrical imaging of neurons.

7.3.3 Modelling of the spontaneous transitions between the two behavioural states

The spontaneous transitions between the Up and Down-states did not exhibit temporal correlations, and were well-fitted assuming an underlying log-normal density for the residence time in each state. These properties suggest that it may be possible to model the spontaneous behavioural transitions based on a simple double-well potential model [Deco et al., 2009]. However, Capurro et al., [2001] modelled the spontaneous fluctuations in the EOD rate in a resting *Gymnotus* sp. by applying an autoregressive model driven by Gaussian white noise and shot noise. It might be possible to extend his model to explain the state-dependent changes in the EOD rates during the Down and Up-states. Phenomenological modelling of the temporal dynamics of the EOD rates in a state-dependent manner may confirm or rule out possible mechanisms that drive the EOD pacemaker to generate such uncorrelated behaviour. For example, increases in the mean EOD rates in the Up states may be explained by a constant input to the pacemaker; alternatively, increases in the rate of shot-noise could explain the same phenomena but these models may predict subtle differences that could be tested experimentally. Serial correlations in the EOD inter-pulse intervals (IPI) increased during active movements in Mormyrids [Kramer, 1978] and Gymnotids (unpublished observation), which is an important experimental feature to be reproduced by a possible model.

7.3.4 Cue-based spatial learning abilities in WEF

Chapter 6 investigated the role of stable landmarks on the spatial learning performance. We conducted further experiments to test whether animals can learn to recognize different landmark shapes to locate food. Previous studies have shown that WEF can discriminate different physical shapes [von der Emde, 1990] based on electrolocation abilities, or even different electric field geometries [Graff et al., 2004]. Our preliminary results showed that *Gymnotus* sp. preferably tracked particular object shapes (cues) associated with food location; and they learned to generalize the object shapes independent of the location of the landmarks according to the location-randomized trials. We also observed in our preliminary studies that

the animals tracked the spatial arrangement of landmarks. Their visit densities were displaced by the same amount as the global translation in the landmark positions.

7.3.5 Chronic neural recordings from behaving WEF

I am ultimately interested in the neural mechanisms responsible for spatial memory and voluntary control of sensory sampling and movements in WEF. Although EOD pulses serve as a proxy of neural activities, since the pacemaker receives direct inputs from diencephalic structure and indirect inputs from the pacemaker, it remains to be directly tested whether the neural activities from the higher brain centres, presumably the dorsal telencephalon, is responsible for driving voluntary movements and experience-guided sensory sampling. Further, neural recordings from freely behaving WEF may tell us whether the neural mechanism of the voluntarily controlled movements generalize to all vertebrates. Teleost forebrain exhibit a simpler architecture that is more accessible experimentally and theoretically compared to the mammalian cortex. Tetrode-based recording techniques are commonly used in rodents and primates to record from a large number of neural populations in a behaving animal. The Maler lab is currently developing a wireless tetrode recording technique in a freely swimming *Gymnotus* sp. to observe neural activities associated with self-initiated swimming onset. The dorsocentral (DC) region serves as the common output region of the dorsal telencephalon, known to be homologous to the mammalian cortex and which may be responsible for the motor (i.e. behavioural) output controlled by higher cognitive centres [Giassi et al., 2012b]. It would be also interesting to test whether Up-states could be artificially induced by stimulating these areas in the dorsal telencephalon. Further, the Longtin group is actively working on the theory to explain how WEF form memories to discriminate objects to explain some of our observations during spatial learning.

Finally, place cells are known to be critical for the formation of spatial memory in mammals, and neurons exhibiting place selectivity have been observed in the dorsal telencephalon of goldfish [Canfield and Mizumori, 2004]. Comparison of the hippocampal place cell activities in rodents and echolocating bats revealed interesting similarities but also differences due to a reliance on different sensory modalities for navigation [Ulanovsky and Moss, 2007; Yartsev and Ulanovsky, 2011]. We hypothesize that WEF possess excellent spatial memory modules due to severely restricted range of electrolocation, which requires

them to internally keep track of their locations by means of path-integration, i.e. by remembering the movements to get to a target. One expects valuable insights from the comparison of how the spatial memory modules in different animals interact with their sensory modalities exhibiting near or far detection ranges.

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Curriculum Vitae

Jaeyoon James Jun

Education

University of Ottawa	2007-present
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Ph.D. Physics (Neurophysics, Supervisors: Dr. Leonard Maler and Dr. Andre Longtin)

- Thesis: Memory-guided sensory sampling during self-guided exploration in pulse-type electric fish
- GPA: 3.9/4.0

Marine Biology Laboratory	2011
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Neural Systems & Behavior course (Directors: Paul Katz and James Knierim)

- Learned theories and techniques of studying neural correlates of animal behaviors from wide variety of animals including leech, mice, zebrafish, and rabbit.

University of Calgary	2005-2007
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B.Sc. Physics (First class Honours) with Applied Mathematics Minor

- Honour's Thesis: Implementations of DEP based neuronal guidance methods using microelectrode arrays (Supervised by Dr. Naweed Syed)
- GPA: 3.7/4.0

University of Calgary	2002-2005
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BSc Computer Science

- CPSC 571Project: Association-Rules Mining Based Broadcasting Approach for XML Data (Supervised by Dr. Reda Alhajj and published in a book chapter)
- GPA: 3.4/4.0

Technical certifications	2005
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- Comptia Network+ Certification (N10-003)
- Comptia A+ Certification: OS & Core (220-301 , 220-302)

Awards and Scholarships

• Best PhD (3+years) Poster Award, Brain Health Research Day, University of Ottawa	2014
• 10 th Intl. Congress of Neuroethology Travel Award (\$325 USD)	2012
• University of Ottawa Student Mobility Award (\$4,000)	2011

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|---|------------------------|
| • University of Ottawa FGPS Travel Grant (\$550) | 2011 |
| • Lola Ellis Robertson Endowed scholarship (\$3,980 USD) | 2011 |
| • NSERC PGS-D award (\$63,000) | 2009-2012 |
| • 5 th Intl. Workshop Statistical Analysis of Neuronal Data Travel Award (\$475 USD) | 2010 |
| • University of Ottawa Excellence scholarship | 2009 |
| • University of Ottawa Dean's Scholarship (\$1,500) | 2009 |
| • University of Ottawa Admissions award (\$50,000) | 2007 |
| • NSERC Undergraduate Summer Research Award (\$4,500) | 2007 |
| • University of Calgary Dean's List | 2002, 2003, 2006, 2007 |
| • AHFMR summer research award (\$5,200) | 2007 |
| • Wilfred Archibald Walter Bursary (\$2,500) | 2007 |
| • Louise McKinney Scholarship (\$2,500) | 2006 |
| • Jason Lang scholarship (\$1,000) | 2003 |
| • Alexander Rutherford Scholarship (\$2,100) | 2002 |
| • Calgary Youth Science Fair (Bronze medal) | 2001 |

Positions held

Graduate Research Assistant	2007-2014
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- Systems neurodynamics laboratory under supervision of Dr. Len Maler and Dr. Andre Longtin
- Designed and constructed a controlled environment for spatial learning experiments, and developed video tracking software and electrical tracking system for behavioral monitoring

Teaching Assistant	2007-Present
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- Physics help centre tutor for introductory physics classes
- Introductory physics laboratory demonstrator and marker at the University of Ottawa

Undergraduate Research Assistant	2006-2007
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- Dr. Naweed Syed's Neurobiology Laboratory at the University of Calgary
- Designed computer vision tracking software and custom electronics for microelectrodes array

Summer Student Research Assistant	2006
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- Dr. Michael Colicos' Neurobiology Laboratory at the University of Calgary
- Designed experimental automation software for Olympus Light microscope

Private Tutor	2002-Present
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- Taught subjects covering introductory level calculus, statistics, linear algebra, physics, and computer programming courses. Taught all high school level science and mathematics courses

Jun IT Solutions

2002-2004

- Sole proprietor, independent computer technician
- Built customized computer systems, troubleshoot and repaired hardware and software problems for homes and businesses

Publications

Peer-reviewed Journal

Jun JJ, Longtin A, Maler L (2014) Spatial memory guides sensory sampling in a pulse-type electric fish. (In preparation).

Jun JJ, Longtin A, Maler L (2014) Sensory attention precedes self-initiated locomotion in an electric fish. *J Exp Biol* (In press).

Jun JJ, Longtin A, Maler L (2013) Long-term behavioral tracking of freely swimming weakly electric fish. *J Vis Exp* **82**, e50962.

Jun JJ, Longtin A, Maler L (2013) Real-time localization of moving dipole sources for tracking multiple free-swimming weakly electric fish. *PLoS One* **8**, e66596.

Jun JJ, Longtin A, Maler L (2012) Precision measurement of electric organ discharge timing from freely moving weakly electric fish. *J Neurophysiol* **107**, 1996-2007.

Book Chapter

Chenier C, Jun JJ, Zhang J, Özyer T, Alhajj R (2006) Association-Rules Mining Based Broadcasting Approach for XML Data. *Lecture Notes in Computer Science* **4243**, 207-216.

Conferences Contributions

Jun JJ, Longtin A, Maler L (2014) Spatial learning and adaptive sampling in a pulse-type Gymnotiform fish. Electrosensory Meeting, Mont St-Hilaire, Quebec, Canada.

Jun JJ, Longtin A, Maler L (2013) Sensory attention precedes self-initiated movements in an electric fish. Gordon Research Conference on Neuroethology, West Dover, VT, USA.

Jun JJ, Longtin A, Maler L (2013) Sensory sampling rate predicts spontaneous swimming initiation in an electric fish. Electrosensory Meeting, Mont St-Hilaire, Quebec, Canada.

Kiar G, Mamatjan Y, Jun JJ, Maler L, Adler A (2013) Electrical localization of weakly electric fish using neural networks. 15th International Conference on Electrical Bio-Impedance & 14th Conference on Electrical Impedance Tomography, Heilbad Heiligenstadt, Germany.

Jun JJ, Harvey-Girard E, Longtin A, Maler L (2012) Spatial learning in Gymnotus sp.: Spatial memory shapes active sensory sampling in pulse-type electric fish. 10th Intl. Congress of Neuroethology, College Park, MD, USA.

Jun JJ, Longtin A, Maler L (2011) Spatial learning in Gymnotus sp.: Up states and the E-saccade. Electrosensory Meeting, Mont St-Hilaire, QC, Canada.

Jun JJ, Longtin A, Maler L (2010) Causal State Modeling on the EOD Pulse Rate of Weakly Electric Fish. 5th Intl. Workshop on Statistical Analysis of Neuronal Data, University of Pittsburgh, PA, USA.

Jun JJ, Longtin A, Maler L (2010) Precision EOD Pulse Timing Detector for Freely Swimming Fish. Electrosensory Meeting, Mont St-Hilaire, QC, Canada.

Jun JJ, Longtin A, Maler L (2009) Navigation and spatial learning in Gymnotus Carapo - Novelty response as a probe of spatiotemporal memory. Electrosensory Meeting, Mont St-Hilaire, QC, Canada.

Jun JJ, Longtin A, Maler L (2008) Spatial memory experiment in Gymnotus Carapo. Electrosensory Meeting, Merrickville, ON, Canada.

Courses taken

Neuroscience

- **Graduate level:** Computational Neuroscience, Systems Neuroscience, Cellular & Molecular Neuroscience

Engineering

- **Graduate level:** Biomedical Instrumentation

Physics

- **Graduate level:** Computational Physics (I/II), Nonlinear Dynamics, Statistical Mechanics
- Classical Mechanics (I/II), Modern physics (I/II), Quantum Mechanics(I/II), Optics, Electromagnetics (I/II/III), Thermodynamics, Statistical mechanics, Relativity

Mathematics

- Calculus (I/II/III/IV), Linear algebra (I/II/III), Discrete Mathematics, Statistics (I/II), Mathematical methods for Physics, Fourier Analysis

Computer Science

- Computer architecture and low-level programming (I/II), Operating systems, Computability (I/II), Information Structures (I/II/III), Distributed processing, Computer Communications, Software engineering (I/II), Database(I/II), Digital circuit design, Advanced Programming Techniques, Quantum Computation

Technical Skills

Computer skills

- Specialized in developing video tracking algorithm and analyzing large physiological dataset
- Programming (15 years of experience): C/C++, Java, Matlab, JSP, PHP, Visual Basic, Fortran, ASM, SQL, Pascal
- Software: Solidworks, Cadsoft Eagle, Maple, Mathematica, Microsoft Office, Comsol Multiphysics, Adobe Illustrator & Photoshop, ACDsee Canvas, LaTeX, MathType, EndNote, NI Circuit Design Suite
- Operating systems: Windows, Mac, Linux

Electronics skills

- Microcontrollers: AVR, PSoC, TI MS430, Arduino
- PCB layout design and fabrication
- Analog circuit design and fabrication

Language skills

- Fluent in English and Korean

Professional Membership

- International Society for Neuroethology (since 2012)
- Canadian Association of Physicists (since 2007)
- The Association of Korean-Canadian Scientists and Engineers (since 2012)

Personal Activities

- Full Marathon (Ottawa 2011, 2013)
- Half Marathon (Calgary 2007, Ottawa 2008, 2009, 2012)
- Soldiers of Fitness (Ottawa 2013)

Volunteer Experiences

Rollercoasterology at Calaway park

2007 Summer

- Department of Physics, University of Calgary
- Assisted Dr. Langill in helping highschool students with physics worksheets regarding amusement park rides

Research Assistant

2001 Summer

- The Health Science Centre, University of Calgary
- Assisted Dr. Liu (Microbiology specialist) in bacterial genome mapping