Using a classic paper by Bell as a platform for discussing the role of corollary discharge-like signals in sensory perception and movement control

Aaron L. Cecala
Department of Biology, Elizabethtown College, Elizabethtown, Pennsylvania

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Using a classic paper by Bell as a platform for discussing the role of corollary discharge-like signals in sensory perception and movement control. —Decades of behavioral observations have shown that invertebrate and vertebrate species have the ability to distinguish between self-generated afferent inputs versus those that are generated externally. In the present article, I describe activities focused around the discussion of a classic American Physiological Society paper by Curtis C. Bell that lays the foundation for students to investigate the neural substrate underlying this ability. Students will leave this activity being able to 1) describe the technical aspects and limitations of an electric fish preparation commonly used to acquire single unit (extracellular) neurophysiological data, 2) provide physiological evidence showing that the activity of principal cells in the posterior lateral line lobe of the electric fish brain reflects that of a reafference comparator that could be used in dissociating self-generated versus externally generated sensory signals, and 3) knowledgeably discuss hypotheses concerning the role of corollary discharge and cerebellar-like structures in vertebrate and invertebrate species. The skills and background knowledge gained in this activity lay the platform for advanced study of scientific investigations into sensory, motor, and cognitive processes in undergraduate, graduate, or medical school curricula.

Corollary Discharge and Electric Fish: a Brief Historical Perspective

Before describing the results from the Bell’s (3) classic study with your students, it is worth reviewing some common terminology in the behavioral neuroscience literature used to describe external versus self-generated sensory input to peripheral sensory receptors. Most modern neurobiologists use the nomenclature provided by von Holst and Mittelstaedt (43) to describe sensory receptor inputs from an external source (exafference) versus that which is self-induced (reafference). As stated above, individual sensory receptor cells are indifferent to the cause of their activation. Therefore, they will convey both exafference and reafference signals equally such that, if left unmodified, these signals would result in identical processing in subsequent sensory, cognitive, or motor structures in the neuraxis. von Holst and colleagues (39, 43) recognized that this would undoubtedly result in a reafference signal being confused for an exafference signal and vice versa. They proposed that the sensory processing system of an organism could account for reafferent input if a copy of a motor command [an efference copy (43) or corollary discharge (39), hereafter only referred to as corollary discharge] was sent from the motor system to the sensory system. In this schema, portions of sensory structures receiving a corollary discharge would act as “reafference comparators” whose output would be the difference between the corollary discharge and all the relevant sensory information collected by the organism’s sensory apparatus (an “error signal,” as shown in Fig. 1). If the output of the reafference comparator is zero, then the organism had both an accurate internal model of the sensory consequences of its
movement and all the input to the sensory system was reafference. In contrast, a nonzero error signal could suggest that 1) the organism’s internal model is accurate, in which case the error signal represents solely exafferent input to the sensory system; or 2) the organism lacked an accurate internal model, in which case the error signal represents some combination of unaccounted for reafference and true exafference. In the latter case, the reafference comparator would benefit from being plastic or “modifiable” such that, with experience, it would be able to adjust its output over time such that it would represent solely exafference. Regardless, von Holst et al.’s theoretical framework suggested that the output of a reafference comparator could be used by downstream neural structures to differentiate self-generated from externally generated signals.

An inability to differentiate these signals would be particularly detrimental for animals such as the weakly electric fish and echolocating bats, which use active sensory systems for navigation and communication. Weakly electric fish (family: Mormyridae) are nocturnal animals that navigate and communicate in the murky, fresh waters of South America and Africa using an electroreceptive system (12, 17, 25). The electroreceptive system emits signals from skeletal muscle-derived electrocytes housed in an electric organ located in the fish’s tail region (3). The output of the electric organ is referred to as an electric organ discharge (EOD). When emitted, a portion of the EOD signal will reflect off of animate and inanimate objects in the fish’s surrounding aquatic environment before returning back to the fish. In the fish’s natural environment, reafferent electrical signals are intermixed with exafferent signals from a variety of other sources (e.g., a species’ conspecific using its EOD for communication). In order for the sensory system of the fish to process solely exafferent information crucial for navigation, communication, reproduction, and predator avoidance, its nervous system must have a mechanism to filter out reafferent signals related to the EOD discharge and self-generated movement. The results of the classic paper by Bell (3) suggest that a cerebellar-like structure in the electric fish exhibits the properties of a reafference comparator and that it is modifiable with experience.

Corollary Discharges, Their Proposed Functions, and Neural Substrate

The reafference comparator framework suggested by von Holst and colleagues in the 1950s has influenced several generations of behavioral neurobiologists during their study of sensory, motor, and cognitive systems. Although a comprehensive review of corollary discharge-like signals across the animal kingdom is beyond the scope of this report, it is worth taking some time out of your lesson plan to impress on your students that corollary discharge-like signals have been identified in a number of animals other than the electric fish using a variety of techniques ranging from genetic to functional neural imaging and have been shown to be required for optimal sensorimotor behaviors in these species (10). For example, the nematode worm *Caenorhabditis elegans* requires a corollary discharge signal to inhibit reflex activity that would otherwise prohibit escape behaviors triggered by noxious stimuli. The *C. elegans* avoidance response consists of either forward or backward motion away from an environmental stimulus. These behaviors are antagonistic: when a nematode moves forward in response to a tail stimulus, reafference from receptors on its head sends a signal to initiate a reflexive response in the backward direction. Luckily for the worm, inhibitory neurons rapidly silence the latter pathway, thereby ameliorating the effect of the reafference. The worm would otherwise be locked in a perpetual reflex loop that would render it essentially immobile (7, 28).

Sommer and colleagues have identified a corollary discharge pathway in another popular animal model, the rhesus macaque oculomotor system, using single unit neuronal recording and temporary chemical inactivation (9, 34, 35, 36, 37, 38). In their inactivation experiment, monkeys were asked to make sequential rapid eye movements (saccades) to two briefly flashed targets that were presented before the onset of the first saccade. In this type of “double-step” task, the second saccade must be made without the help of visual feedback since the two targets are flashed before the onset of the first movement and never reilluminated (16). Therefore, the monkey’s oculomotor system must be able to monitor the size of their first saccade, presumably through the use of a corollary discharge signal, to produce an accurate second saccade to obtain their reward. Disruption of the corollary discharge signal carrying information about the first movement would therefore cause the subject to produce inaccurate movements. Sommer and colleagues (36) were able to show that inhibition of the mediodorsal nucleus (MD) of the thalamus impairs a monkey’s ability to produce an accurate second movement while not impairing its ability to produce an accurate first movement in the double-step task. The authors’ interpretation of this result was that the corollary discharge used to assess the accuracy of the first movement was disrupted by inactivation of the MD, which caused the second saccade to be inaccurate compared with control movements with an intact MD. These are but two of the many examples that you or your students can use to help place the results of Bell’s experiments (3) into a much larger context, and, in the *Sample Questions for Discovery Learning*, I invite you to encourage your students to think of other neuroetho-
logical examples where corollary discharge signals might be crucial for optimal behavior.

As noted above, a corollary discharge signal has been observed in the simple C. elegans nervous system and the relatively more complex thalamocortical circuitry of the rhesus macaque. It should therefore already be apparent that the neuronal implementation of a reafference comparator could take on many different forms and several corollary discharge signals may be found within the brain at any given time. The classic paper by Bell describes recording from the ventral lateral zone of the posterior lateral line lobe (PLLL), which is a cerebellum-like structure (5) found within the electric fish brain (Fig. 2). Cerebellum-like structures, which can be found in most vertebrates and whose anatomic characteristics are similar to the cerebellum, are “sensory structures that receive input from the periphery in their deep layers and parallel fiber input into their molecular layers” (5). Over the course of the last century, evidence accumulated from a variety of clinical, experimental, and theoretical studies support a plethora of hypotheses that state that the primary function of these structures is to generate predictions about expected sensory inputs or states (2). This suggests that the cerebellum could function as a reafference comparator (Fig. 1). Furthermore, the cerebellum and cerebellum-like structures have been shown to be required for motor learning in operant conditioning tasks (2, 5, 19, 20, 31) and have been models for the cellular/molecular aspects of synaptic plasticity (29). Bell’s elegant study (3) can therefore be used as a jumping off point for discussions of the anatomy, physiology, and functional role of reafference comparators and cerebellar function.

**Teaching Points**

The following teaching points illustrate some of the important physiological principles that can be explored using this classic paper.

**Teaching point 1.** Individual neurons in the ventral lateral portion of the PLLL of a curarized weakly electric fish responded to artificially generated electrical stimuli and exhibited properties consistent with that of a corollary discharge.

A. A given PLLl neuron was responsive to either a negative or positive electrical current in the aquatic environment surrounding the fish (Fig. 3).

B. A PLLL neuron’s firing rate was low and relatively constant in response to receiving a corollary discharge that was primarily composed of motor information from the electric organ (a “command signal”) for a prolonged period of time (~5 min; Fig. 4, A and E).

C. When an electrical stimulus in the environment was initially paired with the command signal, a transient increase in firing rate occurred (Fig. 4, B and F) before the cell fell silent.
for a brief period of time. This response is consistent with the cell producing an error signal [difference between expected (command only) and actual (command + external electrical stimulus) input to the receptors] in the reafference comparator schema (Fig. 1).

D. The firing rate of a PLLL neuron would return to baseline after several minutes of pairing the electric organ command signal with a consistent external electrical stimulus (Fig. 4, C and G), which suggests the output of the PLLL neuron is modifiable based on changes to the command and/or sensory inputs.

E. When the external electrical signal was removed (Fig. 4, D and H), a transient decrease in firing rate occurred before the cell’s firing rate increased for a brief period of time. The magnitude and timing of this response were approximately a “negative image” of the cell’s previous responses to the command and the external electrical stimulus just after the cell had received only the command input for a prolonged period of time. This response is consistent with the cell producing an error signal of the opposite sign [difference between expected (command + external electrical stimulus) and actual (command only) input to the receptors] in the reafference comparator schema (Fig. 1).

Teaching point 2. Using a common technique (single unit neurophysiology), Bell (3) was able to describe a corollary discharge-like signal in a cerebellum-like structure in an awake but reduced preparation (the curarized fish). The clear results gained from his simple experimental design provide an opportunity to discuss the anatomic structure, basic physiology, and potential role(s) of the cerebellum and cerebellum-like structures in sensorimotor control (2). Undoubtedly, this discussion will include segments on the limitations of the single unit recording (for reviews, see Refs. 6, 8, 12, and 21), the simplicity of the artificial electrical stimuli used to drive responses in PLLL cells, and the pros and cons of using restrained versus unrestrained organisms in neuroscience experiments.

Even with these limitations, and possibly because of them, this classic paper affords the opportunity to discuss the use of electric fish as a model organism to address complex questions regarding the possible modulation of neural activity during sensorimotor processing (4), navigation (18), and communication (1, 40) as well as the effects of modulating external and internal environmental states (15, 23, 33, 32) on behavior and basic neuronal function (29). This classic paper sits at a pivotal point in the history of the electric fish literature where neuroethologists were beginning to make great strides linking field work observations with wet (literally) laboratory data collection.

Teaching point 3. Over the last century, a great deal of prose has been devoted to describing how an aberrant corollary discharge could be the substrate underlying a neurological disease that affects the initiation, accuracy, or precision of a movement (37, 38). More recently, it has been proposed that a disrupted corollary discharge could result in the sensory anomalies described by patients with psychiatric diseases such as schizophrenia (13, 22, 42). Therefore, any discussion of corollary discharge should attempt to bridge the theoretical gap between nonhuman animal studies and human neurological and psychiatric disorders. Such a wide-ranging and dynamic discussion will allow both students and faculty members to realize that alterations in corollary discharge-like signals may be at the root of many other disorders. This discussion will undoubtedly lead to the development of experiments to probe the integrity of corollary discharges in various impaired populations. Even if these experiments are not carried out by you or your students, the thought exercise will be useful for both parties.
Seminar Characteristics and Assignments

Before delving into Bell’s paper (3), a student in your course should have knowledge of the cellular mechanisms underlying the generation of an action potential, the technology used to record extracellular action potentials (for reviews, see Refs. 6, 8, 12, and 21), and the general characteristics of the active electrosensory system (for reviews, see Refs. 5 and 24), including the neuroanatomic characteristics of the cerebellar-like electrosensory lobe (see Fig. 2 or Figs. 1 and 3 in Ref. 5). Although the majority of these topics are typically covered in physiology, neuroscience, neuroethology, or other upper-division seminar courses involving animal behavior, specifics on the electrosensory system will need to be covered in any seminar discussing Bell (3). The target audience for the use of this particular activity is an upper-division behavioral neuroscience course with a neuroethological emphasis.

The majority of the class meeting time should be devoted to the Sample Questions for Discovery Learning (questions 2–6), in which the students must search for flaws in the classic paper’s design, apply the knowledge gained from reading this material to the generation of novel scientific experiments, and discuss how the results of Bell’s study generalize to other sensorimotor systems in a variety of species (45–55 min). I have used similar assignments and lecture questions successfully in my Neural Control of Movement (Bio 373) seminar while I was a visitor at Franklin and Marshall College (Lancaster, PA) and my Behavioral Neurobiology (Bio 314) course at Elizabethtown College (Elizabethtown, PA); therefore, I believe that this lecture outline could be modified to fit introductory and upper-division undergraduate and graduate seminars in biology, psychology, neuroscience, biomedical engineering, or related fields.

Figures for Discovery Learning

Figure 2 in the present article illustrates the experimental setup (A) used to generate the data shown in Figs. 3 and 4 as well as the gross anatomic organization of the PLLL of the mormyrid brain (B). In brief, a mormyrid species (Gnathonemus petersii) was anesthetized with tricaine methanesulfonate, a commonly used anesthetic in aquatic preparations, and fixed to a wax block, which was used to suspend the fish such that only the head was above the surface of the water. Access to the ventrolateral zone of the PLLL was made possible by an incision in the posterior portion of the fish’s skull and reflection of the valvula cerebelli. After the surgery was completed, a dose of curare was injected into the fish and then constantly perfused in the aerated water surrounding the fish for the duration of the experiment. Curare is a nicotinic acetylcholine receptor blocker that eliminates synaptic transmission at the neuromuscular and neuroelectrocyte junctions. Constant perfusion of curare therefore allowed Bell to remove tricaine methanesulfonate, which allowed the fish to awaken while also preventing both active locomotion and electrical discharge from the fish. Interestingly, the motor neurons directly associated with the EOD remain active and regularly produce action potentials at a rate of 2–4 Hz. A multunit command signal was recorded during the experiment and used to trigger the production of an artificial electrical current pulse in the water surrounding the fish. The location, timing, magnitude, and probability of the artificial electrical stimulation in the environment could be experimentally controlled by the investigator (see Ref. 3 for specific stimulation parameters). Finally, the timing of the pulse was catalogued so that the experimenter could judge the delay in principal cell response as well as know when the command occurred even when no electrical pulse was provided to the fish.

Figure 3 shows an example single unit extracellular neuronal recording taken from an “outside negative” principal cell in the ventrolateral zone of the PLLL. As shown in the top traces in Fig. 3A, this type of cell initially responded to a step change from “neutral” to a negative environmental current with a short burst of action potentials followed by a prolonged increase in mean firing rate (relative to the neutral environment) as long as the negative stimulus was present. This type of behavior is very similar to the “pulse-step” response of sensory [e.g., slowly adapting afferents (41)] and motor neurons [e.g., oculomotor neurons in the cranial nerve nuclei (30)] in other vertebrate systems. A change in the opposite direction (Fig. 3A, bottom trace), from neutral to positive current, resulted in a reduction in mean firing rate, often to the point of silencing the cell completely. This cell also rebounds such that the firing rate of the cell when exposed to the neutral environment is higher than before the negative pulse.

Figure 4 shows the primary evidence for a corollary discharge signal being sent to the principal cells of the ventrolateral zone of the PLLL and that the response of these cells is what would be expected if they were acting as a reafference comparator. In brief, Fig. 4 shows the response of an outside negative cell to the EOD command alone (A and E), its initial response to a pairing of the command and artificial electrical current in the fish’s surrounding environment (B and F), the response of the cell after 5 min of command/current pairing (C and G), and the cell’s response to only the EOD command after either 5 min (D and H) or 15 min (I) of receiving the EOD command. As shown in Fig. 4, the cell responds very little to the command alone (A and E) or after 5 min of pairing the command with a constant external current pulse (C and G). However, two interesting observations can be made when one compares the response of the cell at the onset of the command/current pulse pairing (Fig. 4, B and F) and the onset of the command-only condition after 5 min of command/current pulse pairing (Fig. 4, D and H). First, the principal cell firing rate clearly increases (Fig. 4, B and F) or decreases (Fig. 4, D and H) in response to the transition from one condition to another compared with the aforementioned conditions (Fig. 4, A, E, C, G, and I). Second, the changes in firing rate are almost “negative images” (named after the negative image obtained in film photography) of each other. Combined, these two images would each other cancel out, which is what one would expect if the fish’s corollary discharge and reafference signals were equal and opposite, producing a constant output from the cell. The output of the cell is probably nonzero, which one might expect when the two signals cancel out, so that the circuitry downstream from the principal cell can recognize both a positive (assumed increase in the principal cell firing rate) and negative (assumed decrease in the principal cell firing rate) output from the reafference comparator. Furthermore, when one of the two inputs is larger or smaller than expected, the output of the principal cell would be nonzero, as shown in Fig. 4, B, D, F, and H.
Student Learning Outcomes

After completing this activity, students will be able to:

1. Critically assess the use of artificially generated electrical signals and single unit neural recordings as tools to address questions in the field of neuroethology.
2. Provide physiological evidence showing that the activity of principal cells in the PLLL of the electric fish brain reflects that of a reafference comparator that could be used in dissociating self-generated versus externally generated electrical signals.
3. Knowledgeably discuss hypotheses concerning the role of corollary discharge(s), cerebellum, and cerebellar-like structures in vertebrate and invertebrate species.

Sample Questions for Discovery Learning

The following question should be addressed by the student before arriving to the lecture.

**Question 1.** Compare the architecture of the mormyrid cerebellum-like electroreceptive lobe and mammalian cerebellum to the essential components of a reafference comparator. Are the principal output cells of these brain regions in a position to act as reafference comparators? (Instructors and students should refer to Ref. 5 for an indepth discussion of this question.)

The following questions could be discussed in small groups during the lecture period.

**Question 2.** Describe Bell’s use of artificial environmental stimuli and single unit neuronal recordings in curarized electric fish to test the hypothesis that the electroreceptive lobe contains cells that produce a corollary discharge-like signal. Extracellular neuronal recording under these circumstances is often described as a “correlative technique” (for reviews, see Refs. 6, 8, 12, and 21). What does this mean? What other common neurobiological methods could be considered correlative techniques? What common neurobiological methods could be considered “causative” techniques? In an organism of your choice, design a series of experiments to test the hypothesis that a group of neurons produces a corollary discharge-like signal that is required for the animal’s normative behavior. You must use one correlative technique and one causative technique in the design of your experiments [see Sommer and Wurtz (38) for examples of such experiments].

**Question 3.** Using the data provided by Bell, describe, in detail, the evidence for or against each of the following statements.

A. Principal cells in the ventrolateral zone of the PLLL are solely reactive to a positive electrical stimulus artificially produced in the environment (see Fig. 2 in Ref. 3 for the answer).
B. The response of a principal cell in the PLLL is malleable and dependent on repeated pairing of a command input from the electric organ and sensory input from electroreceptors (see Fig. 3 in Ref. 3 for the answer).
C. The magnitude of the principal cell response to a command input from the electric organ alone was largest when the command and sensory inputs were paired for 8 min or more (see Fig. 5 in Ref. 3 for the answer).
D. The magnitude of the principal cell response to a command input from the electric organ alone was largest when the command and sensory inputs were paired for 8 min or more (see Fig. 5 in Ref. 3 for the answer).
E. The magnitude and response profile of principal cell responses did not depend on the magnitude of the artificially generated electrical signal (see Fig. 7 in Ref. 3 for the answer).
F. Principal cell responses are dependent on the position of the electrical stimulus in the environment (see Fig. 8 in Ref. 3 for the answer).

**Question 4.** List as many problems/limitations with the experimental design, data analysis, or interpretation in the Bell study (3) that you can think of.

The following is a short list of potential issues to discuss.

A. For pragmatic reasons, the fish in Bell’s study was prevented from moving, which allowed the author to acquire single unit neural recordings while presenting simplistic (constant current) electrical stimuli. However, this reductionist approach has its limitations. For example, it is reasonable to expect that the principal cells that the author recorded typically would receive command input from the motor control system used to produce undulations of the body plan for locomotion, proprioceptive input from the muscles and vertebral column, and even auditory information (11, 14) that can help the fish generate a sense of self and an image of the world surrounding it. Modern telemetry techniques (26) could be used to record neuronal activity while the electric fish is freely moving alone in an open tank with or without inanimate objects, alone with simple or complex (e.g., communication-like stimuli) artificial electrical and acoustic stimuli controlled by the experimenter, together with another freely behaving conspecific or any combination thereof. The latter, more neuroethologically relevant, situation would allow investigators to draw firmer conclusions regarding the role of cerebellum-like structures in the generation of electric fish behavior.

B. Where is the plasticity happening in the system? Bell acknowledges that the changes in cellular activity he observed could be the result of changes in the weight of the corollary discharge or sensory inputs to the principal cells he recorded or at some other extra-PLL location. This acknowledgement opens up the opportunity to discuss the limitations of using single unit electrophysiological techniques to address hypotheses concerning sensorimotor learning. The challenge for students will be to come up with a feasible experiment to investigate the locus of change in this system that may occur at the synaptic or subcellular level, a challenge that has been met with the aid of modern molecular, cellular, and imaging tools not available to Bell in the early 1980s (for a recent review of the cellular mechanisms underlying plasticity in cerebellum-like circuitry, see Ref. 23).

C. Electroreceptors are located along the extent of the lateral line of the fish as well as in its head region. In brief, although the author altered the spatial location of the electrical probe in the fish’s environment, he did not describe the response (or lack thereof) of principal cells found in the ventral lateral zone of the PLLL to electrical stimuli presented to the rostral portion of the fish. Are cells in the ventral lateral zone responsive to electrical stimuli presented in this region of the body plan? Are cells in other segments of the PLLL responsive to electrical stimuli presented to this region of the body plan? Are these regions of the brain also working as a reafference comparator or do they play some other role?
D. No statistics were used in this study. Would statistics be necessary to support Bell’s conclusions if this study were being published today? Why or why not? If you were to reanalyze Bell’s data, how would you do it? What would you compare and what statistical tests would you use?

Question 5. Electric fish had been used for electrophysiological and behavioral experiments for a number of years before Bell’s study and continue to be used as a model organism today. What are the advantages and limitations to using nonprimate models, particularly those with highly specialized sensorimotor capabilities like electric fish, to study nervous system functions? Do you believe we can learn general principles from looking at the properties of such a specialized system or should neuroscientists restrict themselves to looking at “more relevant” animal models?

Conclusions

The use of electric fish as a model for the study of the neural substrates underlying sensory, motor, and cognitive behaviors has continued to grow since the early 1980s, when Bell performed his landmark study describing the plasticity of a corollary discharge signal in the PLL of curarized electric fish. Although Bell’s work was not the first to describe a corollary discharge signal, his approach to describing a modifiable corollary discharge signal laid the foundation for others to explore the use of corollary discharge in sensorimotor systems across the animal kingdom, many of which can be found in American Physiological Association journals such as the Journal of Neurophysiology (e.g., Refs. 35, 36, and 27). Therefore, students can benefit from discovering Bell’s work and can use it to decipher modern studies that use more complex physiological (single unit or multunit neural recording, functional magnetic resonance, etc.), molecular, and behavioral techniques to study the corollary discharge in motor control, learning and memory, decision making, and etiology of neurological disease. This makes the Bell study (3) an essential read for any student interested in pursuing advanced study in the biomedical sciences.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: A.L.C. prepared figures; A.L.C. drafted manuscript; A.L.C. edited and revised manuscript; A.L.C. approved final version of manuscript.

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